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Title: Genetic Epistasis Regulates Amyloid Deposition in Resilient Aging

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Abstract: INTRODUCTION: The brain-derived neurotrophic factor (BDNF) interacts with important genetic Alzheimer's disease (AD) risk factors. Specifically, variants within the SORL1 gene determine BDNF's ability to reduce A $\beta$  in vitro. We sought to test whether functional BDNF variation interacts with SORL1 genotypes to influence expression and downstream AD-related processes in humans.

METHODS: We analyzed postmortem brain RNA-sequencing and neuropathological data for 441 subjects from the Religious Orders Study/Memory and Aging Project, and molecular and structural neuroimaging data for 1 285 subjects from the Alzheimer's Disease Neuroimaging Initiative.

RESULTS: We found one SORL1 RNA transcript strongly regulated by SORL1-BDNF interactions in elderly without pathological AD, and showing stronger associations with diffuse than neuritic A $\beta$  plaques. The same SORL1-BDNF interactions also significantly influenced A $\beta$  load as measured with [18F]Florbetapir PET.

DISCUSSION: Our results bridge the gap between risk and resilience factors for AD, demonstrating interdependent roles of established SORL1 and BDNF functional genotypes.

January 26, 2017

Dr. Ara S. Khachaturian  
Executive Editor, Alzheimer's & Dementia

Dear Editor,

We are pleased to re-submit our revised manuscript entitled "Genetic Epistasis Regulates Amyloid Deposition in Resilient Aging" for publication in Alzheimer's & Dementia. We have made changes in accordance with the editor's suggestion, and the limitations section of our discussion has been enhanced as a result.

Again, we greatly appreciate the time taken by editors and reviewers to re-evaluate our manuscript, and will await further notice regarding our submission.

Sincerely,

**Aristotle N. Voineskos**

Head, Kimel Family Translational Imaging-Genetics Laboratory, Centre for Addiction and Mental Health,  
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Response to Editor

“Please incorporate your response to Reviewer #1 into the discussion, perhaps in the paragraph that describes the limitations. 2-4 sentences should suffice and read like, "it has been suggested...(reviewer #1 comments)". "Unfortunately...(your response)." And the third (or fourth) sentence should propose some future research/experiments to address this limitation.”

As requested, we have incorporated a brief summary of this exchange regarding the measurements of SORL1 proteins as a limitation in our paper’s discussion:

(Pg. 15, paragraph 2): “Finally, we acknowledge that mRNA levels of *SORL1* may not be reliable indicators of protein levels. Unfortunately, we did not measure protein directly in our experiments. Future studies will need to measure protein directly in the tissues. In fact, proteomic projects are currently underway in the ROS/MAP cohort that will include both *SORL1* and BDNF. However, there are also limitations to targeted proteomics; for example, it cannot capture individual post-translational modifications. Thus, ultimately, a variety of approaches, including the mRNA approach used in the present study, are useful for identifying genes and proteins important in the pathogenesis of AD.”

Again, we thank the editor for their time and consideration.

## Research in Context

*Systematic review:* Authors reviewed relevant literature using traditional sources (e.g., Pubmed) and meeting abstracts and presentations. SORL1 is a major risk factor for Alzheimer's disease and several studies have demonstrated its interactions with other genes and proteins within neuroplastic and canonical Alzheimer's-related pathways.

*Interpretation:* We demonstrate that there is a genetic interaction between *SORL1* and *BDNF* that influences SORL1 isoform expression and amyloid deposition both postmortem and *in vivo*. This result is convergent with previous reports of *SORL1*-dependent aspects of Alzheimer's disease biology.

*Future directions:* We find that one SORL1 isoform, SORL1-005, is an interactively regulated transcript and should be a focus of future studies. In addition, the relative impact of SORL1- and BDNF-related mechanisms on diffuse and neuritic amyloid accumulation should be explored further.

## Genetic Epistasis Regulates Amyloid Deposition in Resilient Aging

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†Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database ([adni.loni.usc.edu](http://adni.loni.usc.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.usc.edu/wp-content/uploads/how\\_to\\_apply/ADNI\\_Acknowledgement\\_List.pdf](http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf)

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## Highlights:

- BDNF Val66Met interacts with *SORL1* variants to impact expression of SORL1-005.
- SORL1-005 may influence diffuse rather than neuritic amyloid pathology in postmortem brain.
- The *BDNF-SORL1* interaction effect is present in individuals without confirmed Alzheimer's disease.
- *In vivo* amyloid, measured by PET imaging, is also impacted by the BDNF-SORL1 interaction.

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4 **Abstract**

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6 INTRODUCTION: The brain-derived neurotrophic factor (BDNF) interacts with important genetic  
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8 Alzheimer’s disease (AD) risk factors. Specifically, variants within the SORL1 gene determine BDNF’s  
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10 ability to reduce A $\beta$  in vitro. We sought to test whether functional BDNF variation interacts with SORL1  
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12 genotypes to influence expression and downstream AD-related processes in humans.  
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16 METHODS: We analyzed postmortem brain RNA-sequencing and neuropathological data for 441  
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18 subjects from the Religious Orders Study/Memory and Aging Project, and molecular and structural  
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20 neuroimaging data for 1 285 subjects from the Alzheimer’s Disease Neuroimaging Initiative.  
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24 RESULTS: We found one SORL1 RNA transcript strongly regulated by SORL1-BDNF interactions in elderly  
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26 without pathological AD, and showing stronger associations with diffuse than neuritic A $\beta$  plaques. The  
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28 same SORL1-BDNF interactions also significantly influenced A $\beta$  load as measured with [18F]Florbetapir  
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30 PET.  
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34 DISCUSSION: Our results bridge the gap between risk and resilience factors for AD, demonstrating  
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36 interdependent roles of established SORL1 and BDNF functional genotypes.  
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43 *Keywords:* Alzheimer’s disease, epistasis, RNA sequencing, amyloid, BDNF, SORL1, PET imaging  
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## 1. Background

Genetic epistasis may be a major contributor to the “missing heritability” of late-onset Alzheimer’s disease (AD) [1], and recent efforts have demonstrated the importance of evaluating gene-gene interactions among AD risk variants using integrative approaches [2]. Variants within the sortilin-related receptor (*SORL1*, *SORLA*, *LR11*) gene are among the most highly-replicated genetic risk factors for late-onset Alzheimer’s disease (AD); they have been associated with AD diagnosis in candidate studies [3], genome-wide association studies [4], and meta-analyses [5]. While studies have implicated *SORL1* genotypes independently in gene expression [6], the transcriptional control of *SORL1* also depends on extragenous factors, particularly levels of the brain-derived neurotrophic factor (BDNF) [7]. Accordingly, it was recently shown that BDNF administration in iPSC-derived neuron cultures up-regulates *SORL1* expression in a *SORL1*-genotype dependent manner [8]. The *BDNF* Val66Met polymorphism determines the activity-dependent secretion of BDNF [9] and also the function of the BDNF pro-peptide in facilitating neuroplasticity (LTD) [10]. As such, *BDNF* Val66Met may serve as a functional assay for BDNF activity in the brain. Effects of *BDNF* Val66Met have been shown on early AD phenotypes, such as structural [11] and functional [12] neuroimaging, and cognition [13]. These effects may be downstream consequences of BDNF’s stimulation of *SORL1* activity [14], and therefore may be subject to modulation by both *BDNF* and *SORL1* genotypes interdependently. Studying the interaction of functional *BDNF* and *SORL1* genotypes in large, well-characterized samples may provide insight into the nature of this transcriptional regulatory mechanism and risk vs. resilience for AD.

We have previously shown a main effect of *SORL1* genotype on levels of prefrontal *SORL1* mRNA in postmortem brain [15] using microarray technology that was unable to detect specific *SORL1* transcript isoforms. Since previous reports show differential *SORL1* transcript expression both in AD [16] and as a result of *SORL1* genotype [6], microarray analyses may have missed crucial transcript-specific information. RNA-sequencing (RNA-seq) offers distinct advantages over probe-based methodologies as

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4 it allows for the alignment of assembled transcript reads to any sequence template and the estimation  
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6 of isoform expression based on these reads. We have also previously shown age-dependent effects of  
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8 the *BDNF* Val66Met polymorphism on white matter microstructure, cortical thickness, and episodic  
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10 memory performance in healthy adults [17], suggesting that as-of-yet unidentified factors may act to  
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12 influence *BDNF*'s protective effects on neurodegeneration and cognitive aging.  
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17 Given the regulatory interaction of BDNF protein with *SORL1* genotype in human iPSC-derived neurons  
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19 [8], we hypothesized that common *SORL1* gene variants may interact with *BDNF* Val66Met to influence  
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21 the expression of *SORL1* transcripts. Further, given the functions of *SORL1* within the amyloidogenic  
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23 cascade, we hypothesized that genetic interactions predicting altered *SORL1* expression may affect  
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25 amyloid neuropathology as well as brain structures at risk in the early stages of AD. To test this, we  
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27 performed an unbiased locus-wide gene-gene interaction analysis of *SORL1* SNPs with *BDNF* Val66Met  
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29 to model the expression of multiple *SORL1* transcripts, quantified by RNA-seq of postmortem brain  
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31 tissue, in 441 subjects from the Religious Orders Study and Memory and Aging Project (ROS/MAP).  
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36 Transcripts showing significant evidence for regulation by *SORL1*-*BDNF* interactions were also tested for  
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38 effects on postmortem neuropathology in the same subjects. We then tested significant SNP-SNP  
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40 interactions for effects on *in vivo* frontal amyloid load, as measured by [18F]Florbetapir PET, in 710  
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42 subjects from the Alzheimer's Disease Neuroimaging Initiative (ADNI). Finally, to explore potential  
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44 downstream effects of these SNP-SNP interactions on brain structure, we examined 1 285 subjects from  
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46 ADNI and 172 subjects from ROS/MAP with MRI estimates of entorhinal cortex volume, and 185 subjects  
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48 from ADNI 2 with diffusion tensor imaging (DTI) data for tracts implicated in AD.  
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## 2. Methods

### 2.1 Religious Orders Study and Memory and Aging Project (ROS/MAP)

#### 2.1.1 Study Participants

A total of 441 subjects with genomic, RNA sequencing, and neuropathological data were included in the present study. All participants were from ROS [18] and MAP [18]; two large ongoing cohort studies enrolling non-AD subjects at baseline, centered at the Rush Alzheimer's Disease Center at Rush University in Chicago, IL. Both studies were approved by the Institutional Review Board of Rush University Medical Center.

#### 2.1.2 Genetics

Genotyping of all subjects was performed using the Affymetrix (Santa Clara, CA, USA) Genechip 6.0 platform. *APOE* (rs7412 and rs429358) genotypes were imputed from MACH (version 1.0.16a) and HapMap release 22 CEU (build 36), as previously described [19]. Common variants within 10kb of the *SORL1* locus (chr. 11, position 121,312,912 – 121,514,471; GRCh37 coordinates) were extracted using PLINK (v1.90b) [20]. Variants were pruned for minor allele frequency (MAF>0.1) and Hardy-Weinberg Equilibrium (HWE  $p>0.001$ ), resulting in a final set of 160 for analysis.

#### 2.1.3 Postmortem *SORL1* Isoform Expression

RNA-seq data (50 million paired-end reads of 101 bp) were generated from frozen dorsolateral prefrontal cortex tissues following the construction of complementary DNA libraries, as previously published [21]. Expression abundance was calculated as fragments per kilobase of exon per million reads mapped (FPKM) (See Supplementary Methods).

#### 2.1.4 Postmortem Neuropathology

A board-certified neuropathologist blinded to age and all clinical data established neuropathologic diagnoses for each subject. Five types of AD pathology were quantified for ROS/MAP subject samples:

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4 mid-frontal neuritic plaques and diffuse plaques, total amyloid, paired helical filament tau, and  
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6 neurofibrillary tangles (see supplementary Methods).  
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#### 10 **2.1.5 *In Vivo* Structural MRI**

11 High resolution structural T1 images were acquired for a subset of n=172 ROS/MAP subjects (overlap of  
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13 n=5 with subjects from expression dataset) [22] and entorhinal cortex volumes (mm<sup>3</sup>) were estimated  
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15 for each subject using Freesurfer (<http://surfer.nmr.mgh.harvard.edu>) (see Supplementary Methods).  
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## 22 **2.2 Alzheimer’s Disease Neuroimaging Initiative (ADNI)**

### 23 **2.2.1 Study Participants**

24 The Alzheimer’s Disease Neuroimaging Initiative (ADNI; phases 1, GO, and 2 – see Supplementary  
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26 Methods) is a multi-center collaboration enrolling elderly subjects at various stages of cognitive  
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28 impairment. All subjects are administered clinical evaluations at time of study enrollment by trained  
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30 physicians as previously described [23]. See supplementary Methods for details.  
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### 36 **2.2.2 Genetics**

37 ADNI subjects were genotyped using the HumanOmniExpress BeadChip (Illumina Inc., San Diego, CA).  
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39 Genetic quality control was conducted using PLINK (v1.90b). Imputation was performed using IMPUTE2  
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41 (v2.3.1) [24], with the 1000 Genomes Phase1 integrated haplotypes as the reference panel. SNPs with  
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43 an IMPUTE2 info score of less than 0.5, HWE  $p < 0.001$ , and MAF  $< 0.01$  were excluded from further  
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45 analyses.  
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### 50 **2.2.3 *In Vivo* Structural MRI and DTI**

51 A total of 1 285 subjects from ADNI 1, GO, and 2 underwent structural MRI protocols to generate  
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53 estimates of entorhinal cortex volume. Entorhinal cortex and total intracranial volumes were estimated  
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55 using FreeSurfer (v4.3) [25]. Diffusion-weighted images were acquired for a subset of 185 subjects from  
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57 ADNI 2 using an optimized protocol and rigorous quality control [26]. Estimates of fractional anisotropy  
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4 (FA) were generated for specific white matter tracts according to the JHU “Eve” white matter atlas labels  
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7 (see Supplementary Methods).  
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#### 10 **2.2.4 In Vivo A $\beta$ [ $^{18}$ F]Florbetapir PET**

11 PET imaging data were available for 710 subjects from ADNI 2. Details of brain A $\beta$  [ $^{18}$ F]Florbetapir PET  
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13 imaging and preprocessing in ADNI have been described elsewhere [27]. Signal from [ $^{18}$ F]Florbetapir  
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15 within cortical subregions were not standardized and so we co-varied for signal from a composite  
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17 reference region (see Supplementary Methods).  
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### 24 **2.3 Statistical Analysis**

25 Analyses were performed using R (v3.1.1) statistical software (<http://www.r-project.org/>) [28].

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27 Haploview (v4.2) [29] was used for calculations of LD structure. Based on lack of expression, data for  
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29 three transcripts could not be analyzed: SORL1-003, SORL1-004, SORL1-007 (See Figure S1 for size and  
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31 position of all transcripts). All 10 remaining transcripts showed heavily right-skewed FPKM distributions  
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33 (skewness ranging from 0.71-5.3, all D’Agostino test  $p < 1.3 \times 10^{-5}$ ) that could not be coerced to normal  
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35 (using Box-Cox power transformations) and thus were evaluated as binary outcomes (expressed above 0  
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37 FPKM vs. 0 FPKM, or median split where the median > 0). Each of 160 SNPs within the *SORL1* locus were  
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39 tested for interaction with *BDNF* Val66Met, with each transcript as outcome using logistic regression,  
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41 co-varying for technical and demographic factors.  
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48 To correct for multiple testing accounting for LD structure across *SORL1* SNPs, we first calculated the  
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50 effective number of independent SNPs across the *SORL1* locus (as in Replogle et al. [30]); of the 160  
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52 *SORL1* tested, six independent SNPs captured the haplotypic diversity at this locus (strong LD structure  
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54 shown in Figure S2). Considering 10 *SORL1* transcripts, the final experiment-wise Bonferroni corrected  
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56 significance threshold was  $p < 8.33 \times 10^{-4}$  ( $\alpha = 0.05 / 6$  independent SNPs / 10 transcripts).  
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4 Transcripts that showed significant evidence for interactive regulation by *SORL1* variants and *BDNF*  
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6 Val66Met were analyzed for effects on neuropathology. Pathology levels were square-root transformed  
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8 and analyzed using linear regression, and, since *SORL1*-005 expression was evaluated as a continuous  
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10 predictor, influential observations (evaluated using *dfbetas* and Cook's distance) were removed from  
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12 these analyses (final n=439).  
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17 SNP-SNP interactions from expression analyses that remained significant after correction for multiple  
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19 testing were carried forward to test for effects on *in vivo* A $\beta$  [18F]Florbetapir PET. To maintain regional  
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21 specificity, average amyloid loads across seven bilateral frontal cortical regions of interest were analyzed  
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23 as outcomes for each gene-gene interaction using linear regression. Interaction *p*-values were corrected  
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25 for multiple testing using the False Discovery Rate (FDR) procedure ( $q=0.05$ ).  
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30 Finally, to examine potential downstream consequences of altered amyloid pathology on brain  
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32 structure, the same set of SNP-SNP interactions identified by expression analyses were explored for  
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34 effects on entorhinal cortex volume (one of the earliest brain regions to be affected by AD pathological  
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36 lesions and atrophy [31]), and white matter FA across five bilateral tracts.  
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40 See Supplementary Methods for details.  
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### 3. Results

#### 3.1 SORL1 Transcript Expression and Postmortem Neuropathology

Sample demographics for ROS/MAP are summarized in Table 1. Out of a total 1 600 tests, 36 remained significant after correction for multiple testing and all of these 36 modeled the same transcript, SORL1-005 (ENST00000534286), as the outcome (Figure 1). The SNP showing the strongest interaction effect with *BDNF* Val66Met was rs12364988 (Wald  $X^2_1=19.09$ ,  $p=1.25 \times 10^{-5}$ ,  $n=441$ ), where the rs12364988<sup>T</sup> allele reduced likelihood of SORL1-005 expression in the *BDNF*<sup>Val</sup> homozygotes ( $OR_{TT:CC}=0.38$ , C.I.<sub>.95%</sub>=[0.18,0.80]), but greatly increased likelihood of expression among *BDNF*<sup>Met</sup> carriers ( $OR_{TT:CC}=7.03$ , C.I.<sub>.95%</sub>=[2.42,20.46]) (see Figure 2A). Rs12364988, within the 5' region of *SORL1*, was in moderate to strong linkage disequilibrium (LD) with the remaining 35 SNPs that showed significant interaction with *BDNF* Val66Met in the ROS/MAP sample ( $D'$  range=0.80-1,  $r^2$  range=0.35-1).

Post-hoc tests in diagnostic subgroups revealed different patterns of effect in non-AD vs. pathologically-confirmed AD subjects. In the non-AD subgroup ( $n=179$ ), 44 tests showed interaction  $p$ -values below our threshold for multiple testing. As in the overall sample, all significant models predicted SORL1-005 expression as outcome, though with much stronger effect sizes observed for the top interacting *SORL1* SNP (rs676759, Wald  $X^2_1=19.27$ ,  $p=1.14 \times 10^{-5}$ ) in the *BDNF*<sup>Val</sup> homozygote ( $OR_{CC:TT}=0.093$ , C.I.<sub>.95%</sub>=[0.026,0.34]) and *BDNF*<sup>Met</sup> carrier groups ( $OR_{CC:TT}=23.12$ , C.I.<sub>.95%</sub>=[3.04,175.36]) (see Figure 2B). In the pathologically-confirmed AD subset ( $n=262$ ), no test survived correction for multiple testing (for rs676759, interaction  $p=0.16$ , see Figure 2C), suggesting that the interaction effect is specific to individuals without confirmed AD.

Since all interactions showed regulatory effects on the same transcript, SORL1-005, we evaluated the effect of SORL1-005 expression on five measures of postmortem neuropathology in the ROS/MAP sample. Across diagnostic groups, there was a modest effect of SORL1-005 on diffuse plaques, whereby

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4 increased levels of SORL1-005 were associated only with increased number of plaques SORL1-005  
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6 ( $F_{1,428}=4.91$ ,  $p=0.027$ ,  $n=439$ ), though this result did not survive correction for multiple testing (see  
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8 Supplementary Results). No effects were observed for any other pathology at  $p_{\text{raw}} < 0.05$ .  
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### 10 11 12 13 14 **3.2 In Vivo Neuroimaging**

15 Sample demographics for ADNI subjects are summarized in Table 2. A total of 36 SNP-SNP interactions  
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17 surviving correction for multiple testing in *SORL1* expression analyses were analyzed across seven  
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19 bilateral frontal cortical regions of interest for *in vivo* A $\beta$  using [ $^{18}\text{F}$ ]Florbetapir PET, resulting in a total of  
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21 252 significant tests before correction. After FDR correction, 44 models remained significant. Between  
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23 these 44 models, 18 different *SORL1* SNPs demonstrated significant interaction with *BDNF* Val66Met to  
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25 predict levels of frontal A $\beta$  across five regions of interest (see Supplementary Results). The top SNP  
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27 showing interaction was rs618874 ( $F_{1,649}=12.12$ ,  $p_{\text{raw}}=5.3 \times 10^{-4}$ ,  $n=710$ ); the rs618874<sup>T</sup> allele was  
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29 associated with decreased amyloid burden in *BDNF*<sup>Val</sup> homozygotes, but increased amyloid in *BDNF*<sup>Met</sup>  
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31 carriers (see Figure 3). This is in alignment with effects observed on gene expression and  
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33 neuropathology in ROS/MAP, whereby the rs12364988<sup>T</sup> allele, which is strongly linked to rs618874<sup>T</sup>  
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35 (ADNI  $r^2=0.73$ ; ROS/MAP  $r^2=0.74$ ), resulted in *BDNF*<sup>Met</sup>-dependent increases in SORL1-005.  
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43 Structural imaging analyses revealed similar patterns of *SORL1*-*BDNF* interaction at  $p < 0.05$ ; however,  
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45 effects did not survive correction for multiple testing (see Supplementary Results; Figure S3).  
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#### 4. Discussion

We found that linked *SORL1* variants interacted with *BDNF* Val66Met to regulate prefrontal expression of one *SORL1* transcript, SORL1-005 (ENST00000534286), and that this isoform was weakly associated with increased diffuse amyloid plaques in midfrontal tissue from the same subjects. We then demonstrated that the same genetic interactions regulating SORL1-005 expression determined frontal amyloid deposition *in vivo* using PET imaging. The gene variant that most significantly interacted with *BDNF* Val66Met to influence SORL1-005 expression, rs12364988, is part of the same highly-linked haplotype block recently shown to interact with BDNF in human iPSC-derived neurons to regulate *SORL1* mRNA expression [8]. By combining layers of genetic, transcriptomic, and neuroimaging data, we have shown that *BDNF* Val66Met status may determine the background on which *SORL1* risk variants exert their effects (results across phenotypes are summarized in Table S1). Through this lens, BDNF's modulation of resilience via cognitive reserve [32] is also clarified; depending on *SORL1* genotype, the effects of Val66Met may influence protection against AD by promoting diffuse plaque deposition preferentially over neuritic.

*SORL1* is a member of the Vps10p-domain (Vps10p-D) family of neuronal receptors [33], several of which have been shown to interact directly with BDNF. Sortilin, another Vps10p receptor, is responsible for intracellular trafficking of newly synthesized proBDNF via its physical interaction with the region of *BDNF* pro-domain in which the Val66Met substitution resides [34]. *BDNF* Val66Met has been associated with risk for AD [35] and AD-related intermediate phenotypes [36], albeit inconsistently, and is thought to be an important factor in modulating neuroplasticity [10]. Our results may provide insight into the inconsistent literature on the effects of *BDNF* Val66Met (i.e. why it has not been identified by GWAS for AD); the vast majority of studies in this area have not accounted for *SORL1* genotype and thus may be missing crucial information determining the direction and magnitude of *BDNF*'s effects. The mechanisms via which *BDNF* Val66Met influences downstream risk for AD are complex and not yet understood;

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4 recently it was shown that *BDNF* Val66Met alters the expression of miR-146 in humanized *BDNF* knock-  
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6 in mice [37], suggesting that this variant may influence the expression of multiple target genes  
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8 simultaneously.  
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11 SORL1-005 is a putative protein-coding transcript (UniProt ID: E9PP43) of 3 501bp, containing 25 exons  
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13 (compared to 48 in the full length transcript), and lacking the Vps10p domain found in full length SORL1  
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15 protein. The lack of this domain could potentially interrupt the function of SORL1-005 protein within the  
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17 amyloid cascade and contribute to the accumulation of pathogenic A $\beta$  species [3], consistent with our  
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19 observations of concomitant increases in diffuse plaques postmortem and amyloid burden *in vivo*. A  
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21 second mechanism via which increased SORL1-005 may exert pathological effects on brain structure is  
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23 regulated intramembrane proteolysis [38]; SORL1 has been shown to undergo sequential cleavage by  
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25 alpha and gamma-secretase enzymes [39], liberating protein fragments that are internalized to the  
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27 nucleus and play roles in gene regulation. It is possible that altered recognition of SORL1-005 by gamma-  
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29 secretase results in the absence of SORL1 COOH-terminal fragments that may preserve pathologically-  
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31 protective gene regulation; such absence is also seen in cells co-transfected with FAD-linked *PS1*  
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33 mutations [39]. In either case, given SORL1's dual roles in recycling APP [3] and lysosomal targeting of  
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35 A $\beta$  [40], alternative splicing causing loss-of-function would be expected to influence amyloid levels in  
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37 the direction observed in our study.  
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46 While functional annotations of SORL1 variants within our top interacting SORL1 haplotype block (which  
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48 lie approximately 50kb 5' exon 25 of the full length transcript; Figure S4) do not directly reveal an  
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50 underlying mechanism related to alternative splicing (See Supplementary Materials), it has been shown  
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52 that a functional SORL1 rare variant (rs117260922, E270K), which is located only one base pair from our  
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54 top SNP, rs12364988, is responsible for a conformational change in SORL1 that results in failure to  
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4 interact with APP at the cell surface [41]. In addition, another nearby variant in high LD with rs12364988  
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6 – rs923893 – is a cis-eQTL for SORL1 expression in human brain tissue [42].  
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10 Our observation that *SORL1-BDNF* interactions influenced SORL1-005 expression in non-pathological AD  
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12 subjects may be due to the interruption or masking of transcriptional mechanisms by AD-related  
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14 neuropathology: it has been shown that A $\beta$  oligomers are capable of inducing gene expression changes  
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16 across diverse functional classes in human brain tissue [43], and that genes involved in intracellular  
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18 trafficking specifically show marked down-regulation in postmortem AD brain [44]. These findings may  
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20 have implications for identifying gene regulatory mechanisms in AD brain, as the effects of  
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22 transcriptional machinery may be altered depending on levels of cellular pathology.  
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27 The weak association of SORL1-005 with only diffuse plaques of midfrontal cortex may suggest a  
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29 differential contribution of the *SORL1-BDNF* interaction to diffuse vs. neuritic plaque pathology. Diffuse  
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31 plaques account for the majority of plaque pathology in human brain [45], are associated with AD  
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33 diagnosis [46], but are also found frequently in so-called “normal” aging [47], suggesting that they may  
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35 be indicative of the early, pre-symptomatic stages of disease or even just a non-pathological form of  
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37 aging. It has been shown that A $\beta$  plays crucial roles in neuroplasticity [48], and may be produced as part  
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39 of a neuroprotective response to synaptic pathology in AD [49]; A $\beta_{1-28}$  has been shown to promote  
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41 growth and survival of hippocampal neurons [50]. As a result, it is possible that the regulatory action of  
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43 *SORL1* and *BDNF* may act on amyloid pathways in such a way that influences both neuritic plaque  
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45 (neurotoxic) and diffuse plaque (neuroprotective) pathologies. Particularly, the interactive effects of  
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47 variants identified by our study may influence the latter pathway to the greatest degree; hence why  
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49 genetic effects are only observed in the non-AD group. Further, [18F]Florbetapir has been shown to  
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51 measure both neuritic and diffuse plaque burden in the brain [51], meaning that our *in vivo* A $\beta$  findings  
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53 may be reflective of SORL1-005’s effect on diffuse plaques in postmortem frontal cortex. Taken  
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4 together, we suggest that the interaction between *BDNF* and *SORL1* may provide links between AD risk  
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6 and the healthy aging process by influencing the expression of a transcript that is not related to AD risk,  
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8 but nonetheless modulates diffuse amyloid deposition postmortem and *in vivo*.  
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11 The present study has several limitations. First, the decision to analyze gene expression as a binary  
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13 outcome necessarily introduces a level of bias into the analyses; it is possible that by splitting the  
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15 distributions of transcript expression into expressed vs. not expressed, we missed quantitative  
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17 information that here we would have been unable to test without violating statistical assumptions.  
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19 Second, the expression of *SORL1* has been shown to be cell-type specific [52], whereby some individuals  
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21 with AD have loss of expression in neurons, but not glia. We are unable to test this directly in our  
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23 sample, as the ROS/MAP expression data are derived from tissue homogenate of the prefrontal cortex.  
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25 We took steps to maintain regional specificity in our analyses by analyzing frontal pathology, which  
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27 should help mitigate some concerns over differences between regions. Third, as with any RNA  
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29 sequencing experiment, alignment error must be considered as a potential confounder. Fourth, BDNF's  
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31 effects on amyloid pathology [8] as well as TrkB-dependeng trophic signaling [14] have been shown to  
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33 depend on *SORL1*, and in this study we used the functional Val66Met variant as an indirect proxy for  
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35 brain BDNF activity [9]. However, due to inconsistency in the literature surrounding the influence of  
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37 Val66Met on BDNF protein and mRNA expression in blood and brain tissue, we acknowledge the  
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39 uncertainty in this assumption. Finally, we acknowledge that mRNA levels of *SORL1* may not be reliable  
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41 indicators of protein levels. Unfortunately, we did not measure protein directly in our experiments.  
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43 Future studies will need to measure protein directly in the tissues. In fact, proteomic projects are  
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45 currently underway in the ROS/MAP cohort that will include both *SORL1* and BDNF. However, there are  
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47 also limitations to targeted proteomics; for example, it cannot capture individual post-translational  
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4 modifications. Thus, ultimately, a variety of approaches, including the mRNA approach used in the  
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6 present study, are useful for identifying genes and proteins important in the pathogenesis of AD.  
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10 In conclusion, we have demonstrated a novel interaction between two AD-associated genes that  
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12 determines the isoform-specific expression of *SORL1*, and impacts levels of amyloid deposition in two  
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14 large samples. We believe that this interaction may provide insight into the convergence of prototypical  
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16 neurotoxic A $\beta$  deposition and the brain reserve found in aged individuals who are resilient to AD. This  
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18 work has implications for the way that genetic association studies of *SORL1* and *BDNF* are interpreted  
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20 and may be of use in determining specific groups of genetically at-risk individuals in future clinical trials  
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22 of novel therapies directed toward amyloidogenic and neuroplastic mechanisms.  
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## Figure Legends

**Figure 1.**  $-\log(p\text{-values})$  for interaction terms of SNPs across the *SORL1* locus with *BDNF* Val66Met in logistic regression models for expression of *SORL1*-005 (ENST00000534286). The top interacting SNP was rs12364988 (Wald  $X^2_1=19.09$ ,  $p=1.25 \times 10^{-5}$ ,  $n=441$ ). Colour coding shows LD structure in the region (according to 1000 Genomes hg19 EUR reference), with red indicating high LD ( $r^2 > 0.8$ ) and dark blue indicating low LD ( $r^2 < 0.2$ ) with respect to rs12364988. Plot was generated with LocusZoom [53].

**Figure 2.** Top interaction effects of *SORL1* variants (rs12364988 and rs676759) and *BDNF* Val66Met on prefrontal mRNA expression of *SORL1*-005 (ENST00000534286) in A) the whole ROS/MAP sample (Wald  $X^2_1=19.09$ ,  $p=1.25 \times 10^{-5}$ ,  $n=441$ ), B) only non-pathologically confirmed AD (non-pathoAD) subjects (Wald  $X^2_1=19.27$ ,  $p=1.14 \times 10^{-5}$ ,  $n=179$ ), and C) pathologically confirmed AD (pathoAD) subjects (Wald  $X^2_1=1.99$ ,  $p=0.16$ ,  $n=262$ ).

**Figure 3.** *SORL1*-*BDNF* interaction effect on *in vivo*  $A\beta$  in the pars orbitalis measured by [ $^{18}F$ ]Florbetapir PET in the ADNI 2 sample ( $n=710$ ). A) Residual amyloid load according to *SORL1* rs618874 and *BDNF* Val66Met groups. B) Effect of *SORL1* rs618874 within *BDNF*<sup>Val</sup> homozygote and *BDNF*<sup>Met</sup> carrier groups separately, adjusted for co-variates, with 95% confidence intervals. Rs618874<sup>T</sup> was associated with decreased amyloid burden in *BDNF*<sup>Val</sup> homozygotes ( $\Delta A\beta_{TT:CC} = -0.067$ , C.I.<sub>95%</sub> = [-0.13, -0.01]), but increased amyloid in *BDNF*<sup>Met</sup> carriers ( $\Delta A\beta_{TT:CC} = 0.12$ , C.I.<sub>95%</sub> = [0.03, 0.21]). Results were not impacted by removal of observations lying beyond 1.5\*interquartile range of mean binding.

## Tables

**Table 1.** ROS/MAP Sample Demographics

ROS/MAP expression (n=441)	Non-AD (n=179)	PathoAD (n=179)	Diff ( $p$ ) <sup>1</sup>		
<b>Sex (F/M)</b>	107 F, 72 M	171 F, 91 M	0.027		
<b>Age at death (y(SD))</b>	86.6 (7.2)	89.8 (5.9)	<0.0001		
<b>Education (y(SD))</b>	16.3 (3.6)	16.6 (3.4)	0.47		
<b>MMSE (SD)</b>	25.1 (6.8)	19.1 (9.4)	<0.0001		
<b>RIN (SD)</b>	7.2 (1.0)	7.1 (0.9)	0.21		
<b>PMI (SD)</b>	6.8 (4.1)	7.3 (5.4)	0.25		
<b>APOE <math>\epsilon</math>4 status (-/+)</b>	153-, 26+ (15%+)	168-, 94+ (36%+)	<0.0001		
<b>BDNF genotype (valval/met carrier)</b>	116Val, 63M	170Val, 92M	1		
ROS/MAP MRI (n=172)	CN (n=112)	MCI (n=41)	AD (n=13)	Other <sup>2</sup> (n=6)	Diff ( $p$ ) <sup>1</sup>
<b>Sex (F/M)</b>	81 F, 31 M	30 F, 11 M	12 F, 1 M	3 F, 3 M	0.25
<b>Age at scan (y(SD))</b>	83.3 (6.7)	85.3 (5.1)	85.8 (3.8)	86 (2.8)	0.072
<b>Education (y(SD))</b>	15.6 (3.3)	15.22 (3.1)	15.9 (2.4)	14.7 (2.4)	0.82
<b>MMSE (SD)</b>	28.3 (1.5)	26.9 (2.1)	19.2 (6)	22.7 (4.5)	<0.0001
<b>APOE <math>\epsilon</math>4 status (-/+)</b>	95-/17+ (15%+)	29-/12+ (29%+)	8-/5+ (38%+)	5-/1+ (17%+)	0.084
<b>BDNF genotype (valval/met carrier)</b>	77/35	29/12	7/6	4/2	0.81

Note: <sup>1</sup> $p$ -values are two-sided and derived from Fisher's exact test (for sex, APOE  $\epsilon$ 4 status, and BDNF genotype) and either two-sample t-tests (in expression dataset for age at death, education, MMSE, RIN, and PMI) or ANOVA (in imaging dataset for age at scan, education, and MMSE). ROS/MAP = Religious Orders Study / Memory and Aging Project; CN = cognitively normal; non-AD = non-neuropathologically-confirmed Alzheimer's disease; pathoAD = neuropathologically-confirmed Alzheimer's disease; MMSE = Mini Mental Status Exam score at last visit before death; Val = Val/Val homozygotes; Met = Met allele carriers; F = female; M = male; y = years; SD = standard deviation; R = right; L = left.

**Table 2.** ADNI Sample Demographics

<b>ADNI 1/GO/2 MRI (n=1 285)</b>	<b>CN (n=335)</b>	<b>SMC (n=82)</b>	<b>EMCI (n=235)</b>	<b>LMCI (n=407)</b>	<b>AD (n=226)</b>	<b>Diff (p)<sup>1</sup></b>
<b>Study phase (1, GO, 2)</b>	193 (1), 142 (2)	82 (2)	111 (GO), 124 (2)	291 (1), 116 (2)	131 (1), 95 (2)	<0.0001
<b>Sex (F/M)</b>	159 F, 176 M	51 F, 31 M	101 F, 134 M	155 F, 252 M	102 F, 124 M	0.0012
<b>Age (y(SD))</b>	74.7 (5.4)	71.7 (5.4)	71.0 (7.1)	73.3 (7.4)	74.3 (8.1)	<0.0001
<b>Education (y(SD))</b>	16.2 (2.7)	16.7 (2.6)	16.1 (2.6)	15.8 (2.9)	15.2 (2.9)	<0.0001
<b>MMSE (SD)</b>	29 (1.1)	29 (1.2)	28.4 (1.6)	27.1 (1.8)	23.1 (2.1)	<0.0001
<b>APOE ε4 status (-/+)</b>	251-/84+ (25%+)	53-/29+ (35%+)	132-/103+ (44%+)	175-/232+ (57%+)	68-/158+ (70%+)	<0.0001
<b>BDNF genotype (valval/met carrier)</b>	231/104	54/28	159/76	271/136	154/72	0.96
<b>ADNI GO/2 Amyloid Sample (n=710)</b>	<b>CN (n=136)</b>	<b>SMC (n=88)</b>	<b>EMCI (n=244)</b>	<b>LMCI (n=121)</b>	<b>AD (n=121)</b>	<b>Diff (p)<sup>1</sup></b>
<b>Study phase (GO, 2)</b>	136 (2)	88 (2)	106 (GO), 138 (2)	121 (2)	121 (2)	<0.0001
<b>Sex (F/M)</b>	66 F, 70 M	55 F, 33 M	107 F, 137 M	55 F, 66 M	50 F, 71 M	0.024
<b>Age (y(SD))</b>	73.9 (5.9)	72.2 (5.8)	71.4 (7.4)	72.5 (7.5)	74.3 (8.5)	0.001
<b>Education (y(SD))</b>	16.5 (2.5)	16.8 (2.6)	16 (2.6)	16.5 (2.6)	15.8 (2.6)	0.016
<b>MMSE (SD)</b>	29.1 (1.2)	29 (1.3)	28.3 (1.6)	27.7 (1.8)	23.1 (2.1)	<0.0001
<b>APOE ε4 status (-/+)</b>	104-/32+ (24%+)	60-/28+ (32%+)	130-/114+ (47%+)	51-/70+ (58%+)	43-/78+ (64%+)	<0.0001
<b>BDNF genotype (valval/met carrier)</b>	95/41	58/30	167/77	89/32	79/42	0.66

Note: \**p*-values are two-sided and derived from Fisher's exact test (for study phase, sex, *APOE* ε4 status, and *BDNF* genotype) and ANOVA (for age, education, and MMSE). ADNI = Alzheimer's Disease Neuroimaging Initiative; CN = cognitively normal; SMC = some memory concern; EMCI; early mild cognitive impairment; LMCI = late mild cognitive impairment; AD = Alzheimer's disease; MMSE = Mini Mental Status Exam score; Val = Val/Val homozygotes; Met = Met allele carriers; F = female; M = male; y = years; SD = standard deviation.

Figure 1

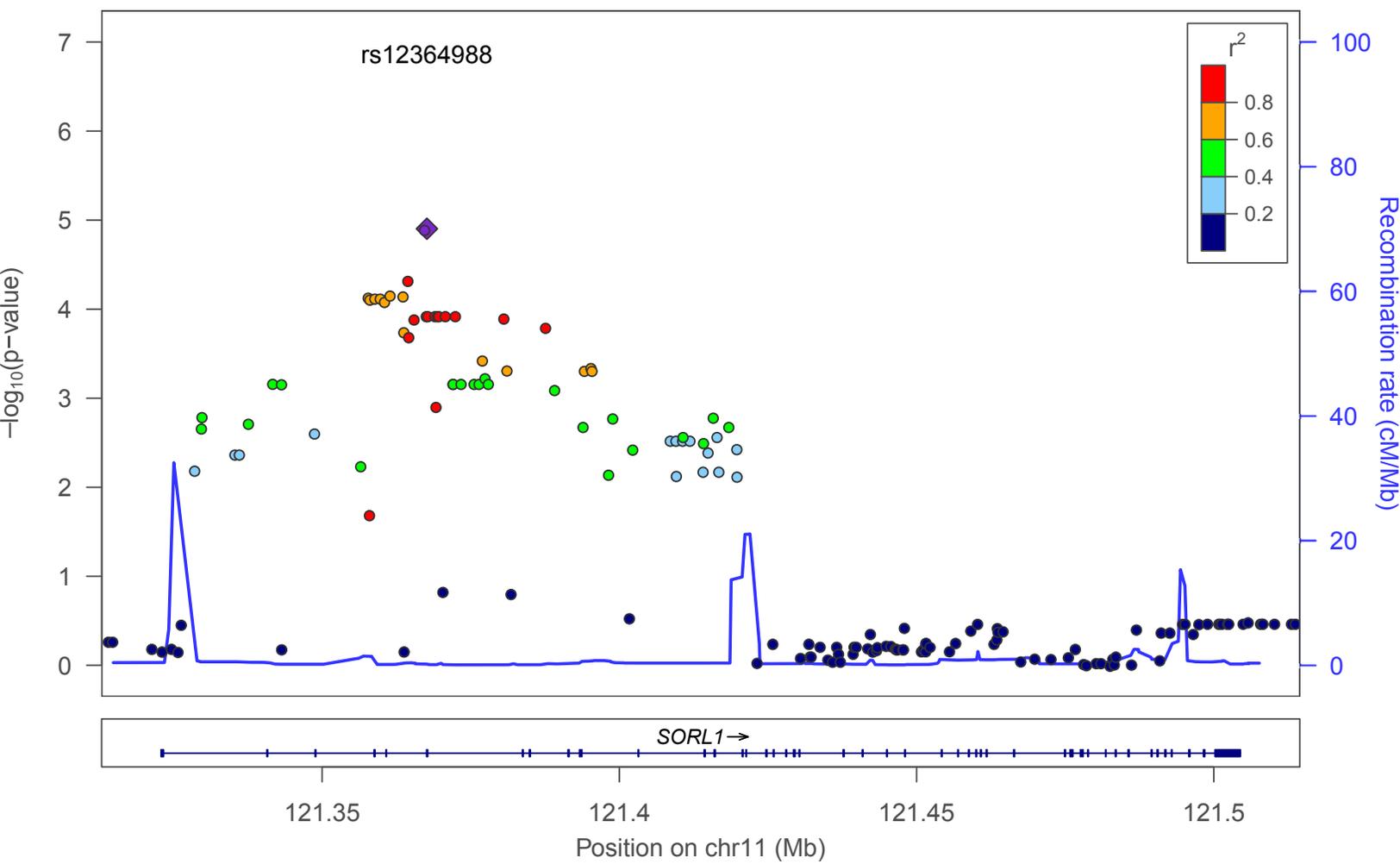
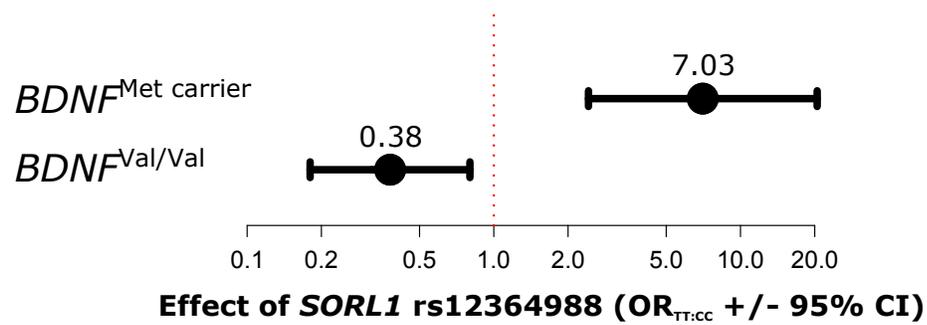
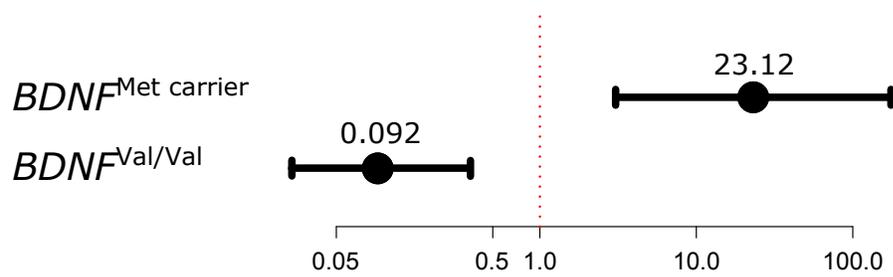


Figure 2

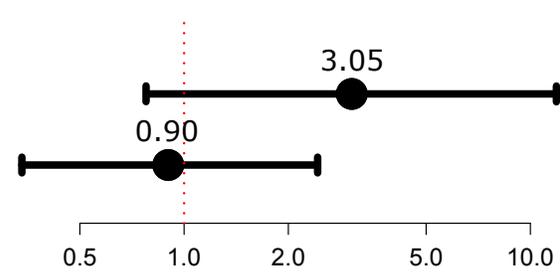
**a Whole Sample (n=441)**



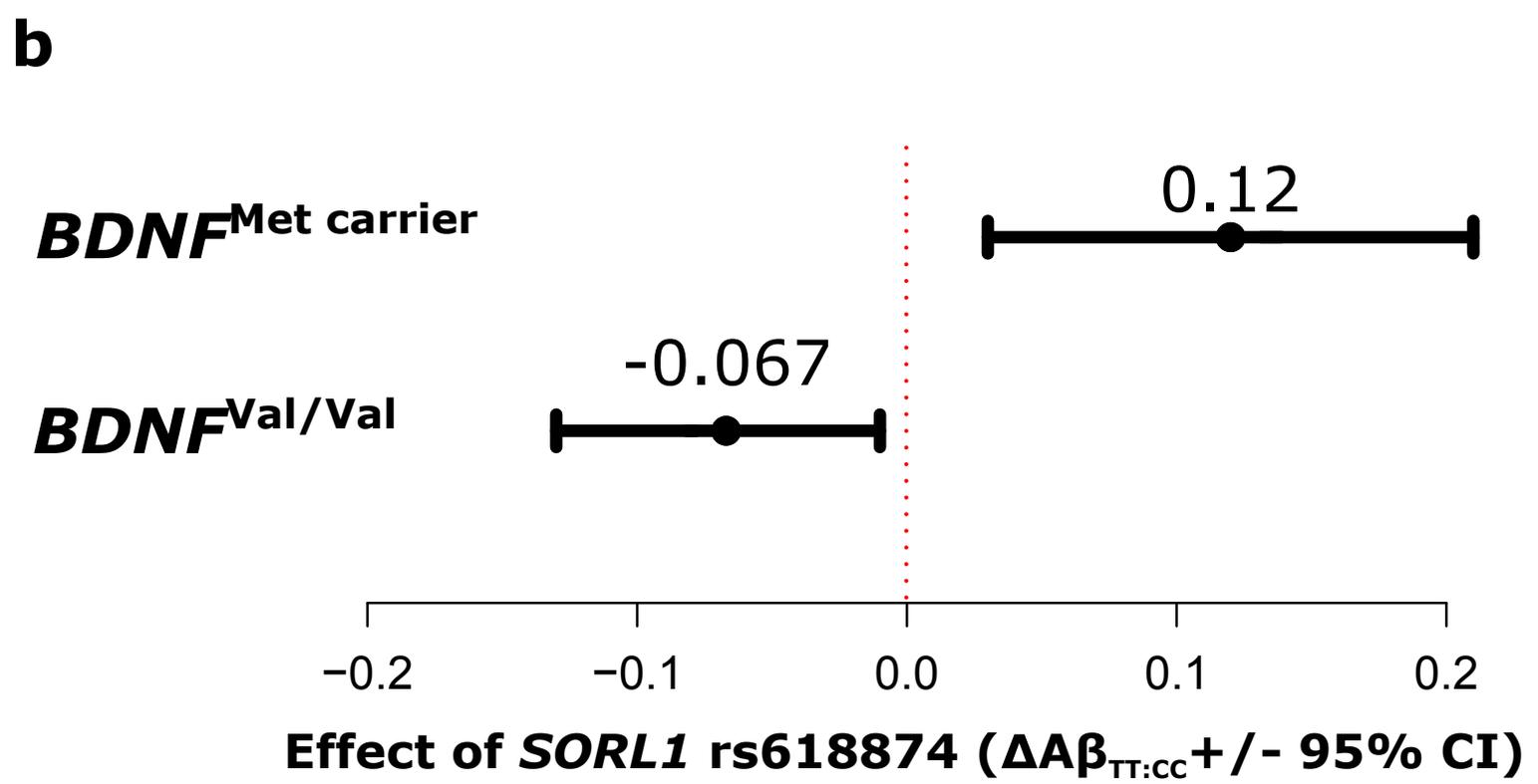
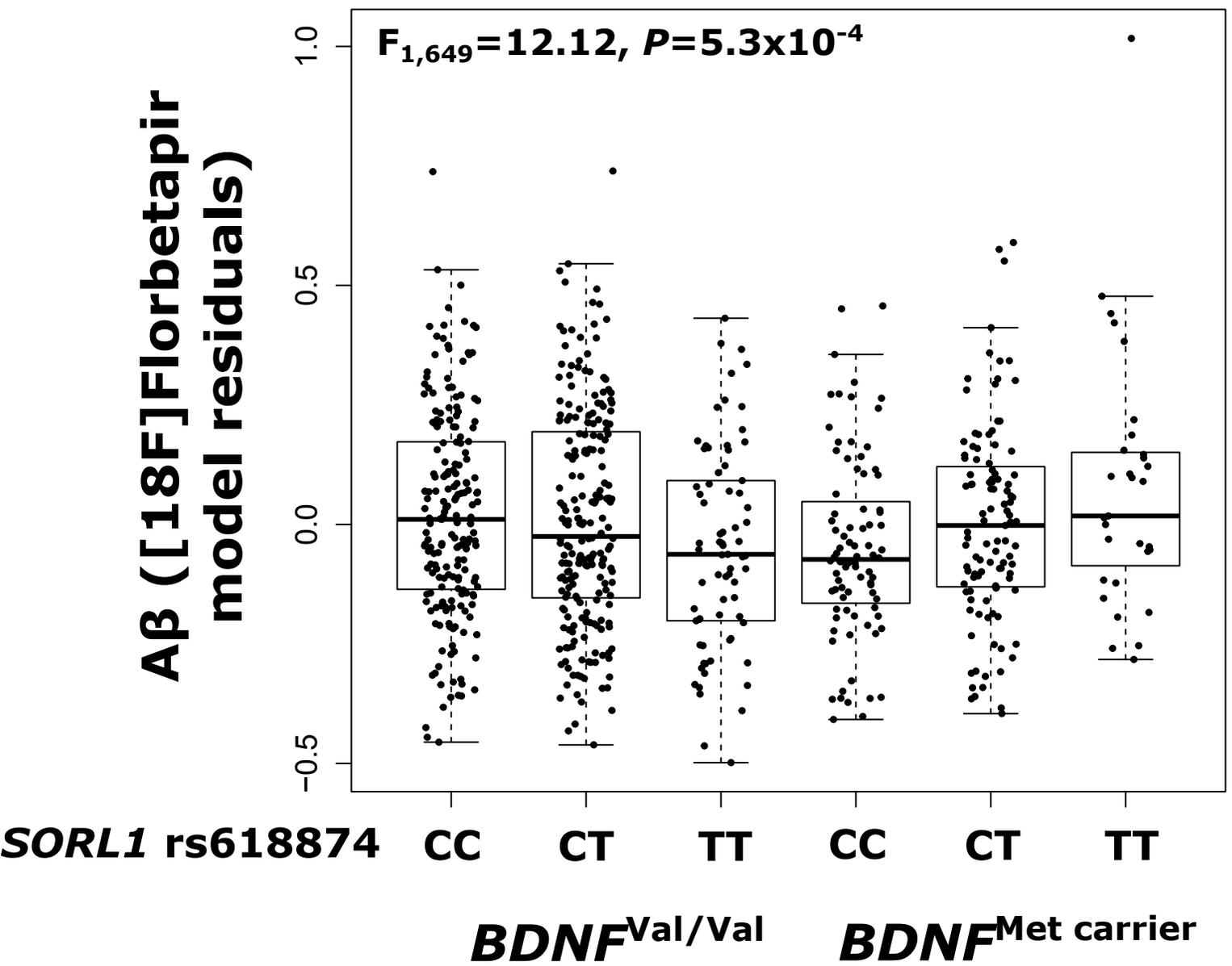
**b Non-pathoAD (n=179)**



**c PathoAD (n=262)**



Effect of *SORL1* rs676759 ( $OR_{CC:TT}$  +/- 95% CI)



## Genetic Epistasis Regulates Amyloid Deposition in Resilient Aging

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†Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database ([adni.loni.usc.edu](http://adni.loni.usc.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.usc.edu/wp-content/uploads/how\\_to\\_apply/ADNI\\_Acknowledgement\\_List.pdf](http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf)

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## Highlights:

- BDNF Val66Met interacts with *SORL1* variants to impact expression of SORL1-005.
- SORL1-005 may influence diffuse rather than neuritic amyloid pathology in postmortem brain.
- The *BDNF-SORL1* interaction effect is present in individuals without confirmed Alzheimer's disease.
- *In vivo* amyloid, measured by PET imaging, is also impacted by the BDNF-SORL1 interaction.

## Abstract

**INTRODUCTION:** The brain-derived neurotrophic factor (BDNF) interacts with important genetic Alzheimer's disease (AD) risk factors. Specifically, variants within the SORL1 gene determine BDNF's ability to reduce A $\beta$  in vitro. We sought to test whether functional BDNF variation interacts with SORL1 genotypes to influence expression and downstream AD-related processes in humans.

**METHODS:** We analyzed postmortem brain RNA-sequencing and neuropathological data for 441 subjects from the Religious Orders Study/Memory and Aging Project, and molecular and structural neuroimaging data for 1 285 subjects from the Alzheimer's Disease Neuroimaging Initiative.

**RESULTS:** We found one SORL1 RNA transcript strongly regulated by SORL1-BDNF interactions in elderly without pathological AD, and showing stronger associations with diffuse than neuritic A $\beta$  plaques. The same SORL1-BDNF interactions also significantly influenced A $\beta$  load as measured with [18F]Florbetapir PET.

**DISCUSSION:** Our results bridge the gap between risk and resilience factors for AD, demonstrating interdependent roles of established SORL1 and BDNF functional genotypes.

*Keywords:* Alzheimer's disease, epistasis, RNA sequencing, amyloid, BDNF, SORL1, PET imaging

## 1. Background

Genetic epistasis may be a major contributor to the “missing heritability” of late-onset Alzheimer’s disease (AD) [1], and recent efforts have demonstrated the importance of evaluating gene-gene interactions among AD risk variants using integrative approaches [2]. Variants within the sortilin-related receptor (*SORL1*, *SORLA*, *LR11*) gene are among the most highly-replicated genetic risk factors for late-onset Alzheimer’s disease (AD); they have been associated with AD diagnosis in candidate studies [3], genome-wide association studies [4], and meta-analyses [5]. While studies have implicated *SORL1* genotypes independently in gene expression [6], the transcriptional control of *SORL1* also depends on extragenous factors, particularly levels of the brain-derived neurotrophic factor (BDNF) [7]. Accordingly, it was recently shown that BDNF administration in iPSC-derived neuron cultures up-regulates *SORL1* expression in a *SORL1*-genotype dependent manner [8]. The *BDNF* Val66Met polymorphism determines the activity-dependent secretion of BDNF [9] and also the function of the BDNF pro-peptide in facilitating neuroplasticity (LTD) [10]. As such, *BDNF* Val66Met may serve as a functional assay for BDNF activity in the brain. Effects of *BDNF* Val66Met have been shown on early AD phenotypes, such as structural [11] and functional [12] neuroimaging, and cognition [13]. These effects may be downstream consequences of BDNF’s stimulation of *SORL1* activity [14], and therefore may be subject to modulation by both *BDNF* and *SORL1* genotypes interdependently. Studying the interaction of functional *BDNF* and *SORL1* genotypes in large, well-characterized samples may provide insight into the nature of this transcriptional regulatory mechanism and risk vs. resilience for AD.

We have previously shown a main effect of *SORL1* genotype on levels of prefrontal *SORL1* mRNA in postmortem brain [15] using microarray technology that was unable to detect specific *SORL1* transcript isoforms. Since previous reports show differential *SORL1* transcript expression both in AD [16] and as a result of *SORL1* genotype [6], microarray analyses may have missed crucial transcript-specific information. RNA-sequencing (RNA-seq) offers distinct advantages over probe-based methodologies as

it allows for the alignment of assembled transcript reads to any sequence template and the estimation of isoform expression based on these reads. We have also previously shown age-dependent effects of the *BDNF* Val66Met polymorphism on white matter microstructure, cortical thickness, and episodic memory performance in healthy adults [17], suggesting that as-of-yet unidentified factors may act to influence *BDNF*'s protective effects on neurodegeneration and cognitive aging.

Given the regulatory interaction of BDNF protein with *SORL1* genotype in human iPSC-derived neurons [8], we hypothesized that common *SORL1* gene variants may interact with *BDNF* Val66Met to influence the expression of *SORL1* transcripts. Further, given the functions of *SORL1* within the amyloidogenic cascade, we hypothesized that genetic interactions predicting altered *SORL1* expression may affect amyloid neuropathology as well as brain structures at risk in the early stages of AD. To test this, we performed an unbiased locus-wide gene-gene interaction analysis of *SORL1* SNPs with *BDNF* Val66Met to model the expression of multiple *SORL1* transcripts, quantified by RNA-seq of postmortem brain tissue, in 441 subjects from the Religious Orders Study and Memory and Aging Project (ROS/MAP). Transcripts showing significant evidence for regulation by *SORL1*-*BDNF* interactions were also tested for effects on postmortem neuropathology in the same subjects. We then tested significant SNP-SNP interactions for effects on *in vivo* frontal amyloid load, as measured by [18F]Florbetapir PET, in 710 subjects from the Alzheimer's Disease Neuroimaging Initiative (ADNI). Finally, to explore potential downstream effects of these SNP-SNP interactions on brain structure, we examined 1 285 subjects from ADNI and 172 subjects from ROS/MAP with MRI estimates of entorhinal cortex volume, and 185 subjects from ADNI 2 with diffusion tensor imaging (DTI) data for tracts implicated in AD.

## 2. Methods

### 2.1 Religious Orders Study and Memory and Aging Project (ROS/MAP)

#### 2.1.1 Study Participants

A total of 441 subjects with genomic, RNA sequencing, and neuropathological data were included in the present study. All participants were from ROS [18] and MAP [18]; two large ongoing cohort studies enrolling non-AD subjects at baseline, centered at the Rush Alzheimer's Disease Center at Rush University in Chicago, IL. Both studies were approved by the Institutional Review Board of Rush University Medical Center.

#### 2.1.2 Genetics

Genotyping of all subjects was performed using the Affymetrix (Santa Clara, CA, USA) Genechip 6.0 platform. *APOE* (rs7412 and rs429358) genotypes were imputed from MACH (version 1.0.16a) and HapMap release 22 CEU (build 36), as previously described [19]. Common variants within 10kb of the *SORL1* locus (chr. 11, position 121,312,912 – 121,514,471; GRCh37 coordinates) were extracted using PLINK (v1.90b) [20]. Variants were pruned for minor allele frequency (MAF>0.1) and Hardy-Weinberg Equilibrium (HWE  $p>0.001$ ), resulting in a final set of 160 for analysis.

#### 2.1.3 Postmortem *SORL1* Isoform Expression

RNA-seq data (50 million paired-end reads of 101 bp) were generated from frozen dorsolateral prefrontal cortex tissues following the construction of complementary DNA libraries, as previously published [21]. Expression abundance was calculated as fragments per kilobase of exon per million reads mapped (FPKM) (See Supplementary Methods).

#### 2.1.4 Postmortem Neuropathology

A board-certified neuropathologist blinded to age and all clinical data established neuropathologic diagnoses for each subject. Five types of AD pathology were quantified for ROS/MAP subject samples:

mid-frontal neuritic plaques and diffuse plaques, total amyloid, paired helical filament tau, and neurofibrillary tangles (see supplementary Methods).

### **2.1.5 *In Vivo* Structural MRI**

High resolution structural T1 images were acquired for a subset of n=172 ROS/MAP subjects (overlap of n=5 with subjects from expression dataset) [22] and entorhinal cortex volumes (mm<sup>3</sup>) were estimated for each subject using Freesurfer (<http://surfer.nmr.mgh.harvard.edu>) (see Supplementary Methods).

## **2.2 Alzheimer's Disease Neuroimaging Initiative (ADNI)**

### **2.2.1 Study Participants**

The Alzheimer's Disease Neuroimaging Initiative (ADNI; phases 1, GO, and 2 – see Supplementary Methods) is a multi-center collaboration enrolling elderly subjects at various stages of cognitive impairment. All subjects are administered clinical evaluations at time of study enrollment by trained physicians as previously described [23]. See supplementary Methods for details.

### **2.2.2 Genetics**

ADNI subjects were genotyped using the HumanOmniExpress BeadChip (Illumina Inc., San Diego, CA). Genetic quality control was conducted using PLINK (v1.90b). Imputation was performed using IMPUTE2 (v2.3.1) [24], with the 1000 Genomes Phase1 integrated haplotypes as the reference panel. SNPs with an IMPUTE2 info score of less than 0.5, HWE  $p < 0.001$ , and MAF  $< 0.01$  were excluded from further analyses.

### **2.2.3 *In Vivo* Structural MRI and DTI**

A total of 1 285 subjects from ADNI 1, GO, and 2 underwent structural MRI protocols to generate estimates of entorhinal cortex volume. Entorhinal cortex and total intracranial volumes were estimated using FreeSurfer (v4.3) [25]. Diffusion-weighted images were acquired for a subset of 185 subjects from ADNI 2 using an optimized protocol and rigorous quality control [26]. Estimates of fractional anisotropy

(FA) were generated for specific white matter tracts according to the JHU “Eve” white matter atlas labels (see Supplementary Methods).

#### **2.2.4 In Vivo A $\beta$ [ $^{18}$ F]Florbetapir PET**

PET imaging data were available for 710 subjects from ADNI 2. Details of brain A $\beta$  [ $^{18}$ F]Florbetapir PET imaging and preprocessing in ADNI have been described elsewhere [27]. Signal from [ $^{18}$ F]Florbetapir within cortical subregions were not standardized and so we co-varied for signal from a composite reference region (see Supplementary Methods).

### **2.3 Statistical Analysis**

Analyses were performed using R (v3.1.1) statistical software (<http://www.r-project.org/>) [28].

Haploview (v4.2) [29] was used for calculations of LD structure. Based on lack of expression, data for three transcripts could not be analyzed: SORL1-003, SORL1-004, SORL1-007 (See Figure S1 for size and position of all transcripts). All 10 remaining transcripts showed heavily right-skewed FPKM distributions (skewness ranging from 0.71-5.3, all D’Agostino test  $p < 1.3 \times 10^{-5}$ ) that could not be coerced to normal (using Box-Cox power transformations) and thus were evaluated as binary outcomes (expressed above 0 FPKM vs. 0 FPKM, or median split where the median > 0). Each of 160 SNPs within the *SORL1* locus were tested for interaction with *BDNF* Val66Met, with each transcript as outcome using logistic regression, co-varying for technical and demographic factors.

To correct for multiple testing accounting for LD structure across *SORL1* SNPs, we first calculated the effective number of independent SNPs across the *SORL1* locus (as in Replogle et al. [30]); of the 160 *SORL1* tested, six independent SNPs captured the haplotypic diversity at this locus (strong LD structure shown in Figure S2). Considering 10 *SORL1* transcripts, the final experiment-wise Bonferroni corrected significance threshold was  $p < 8.33 \times 10^{-4}$  ( $\alpha = 0.05 / 6$  independent SNPs / 10 transcripts).

Transcripts that showed significant evidence for interactive regulation by *SORL1* variants and *BDNF* Val66Met were analyzed for effects on neuropathology. Pathology levels were square-root transformed and analyzed using linear regression, and, since *SORL1*-005 expression was evaluated as a continuous predictor, influential observations (evaluated using *dfbetas* and Cook's distance) were removed from these analyses (final  $n=439$ ).

SNP-SNP interactions from expression analyses that remained significant after correction for multiple testing were carried forward to test for effects on *in vivo* A $\beta$  [18F]Florbetapir PET. To maintain regional specificity, average amyloid loads across seven bilateral frontal cortical regions of interest were analyzed as outcomes for each gene-gene interaction using linear regression. Interaction *p*-values were corrected for multiple testing using the False Discovery Rate (FDR) procedure ( $q=0.05$ ).

Finally, to examine potential downstream consequences of altered amyloid pathology on brain structure, the same set of SNP-SNP interactions identified by expression analyses were explored for effects on entorhinal cortex volume (one of the earliest brain regions to be affected by AD pathological lesions and atrophy [31]), and white matter FA across five bilateral tracts.

See Supplementary Methods for details.

### 3. Results

#### 3.1 SORL1 Transcript Expression and Postmortem Neuropathology

Sample demographics for ROS/MAP are summarized in Table 1. Out of a total 1 600 tests, 36 remained significant after correction for multiple testing and all of these 36 modeled the same transcript, SORL1-005 (ENST00000534286), as the outcome (Figure 1). The SNP showing the strongest interaction effect with *BDNF* Val66Met was rs12364988 (Wald  $X^2_1=19.09$ ,  $p=1.25 \times 10^{-5}$ ,  $n=441$ ), where the rs12364988<sup>T</sup> allele reduced likelihood of SORL1-005 expression in the *BDNF*<sup>Val</sup> homozygotes ( $OR_{TT:CC}=0.38$ , C.I.<sub>.95%</sub>=[0.18,0.80]), but greatly increased likelihood of expression among *BDNF*<sup>Met</sup> carriers ( $OR_{TT:CC}=7.03$ , C.I.<sub>.95%</sub>=[2.42,20.46]) (see Figure 2A). Rs12364988, within the 5' region of *SORL1*, was in moderate to strong linkage disequilibrium (LD) with the remaining 35 SNPs that showed significant interaction with *BDNF* Val66Met in the ROS/MAP sample ( $D'$  range=0.80-1,  $r^2$  range=0.35-1).

Post-hoc tests in diagnostic subgroups revealed different patterns of effect in non-AD vs. pathologically-confirmed AD subjects. In the non-AD subgroup ( $n=179$ ), 44 tests showed interaction  $p$ -values below our threshold for multiple testing. As in the overall sample, all significant models predicted SORL1-005 expression as outcome, though with much stronger effect sizes observed for the top interacting *SORL1* SNP (rs676759, Wald  $X^2_1=19.27$ ,  $p=1.14 \times 10^{-5}$ ) in the *BDNF*<sup>Val</sup> homozygote ( $OR_{CC:TT}=0.093$ , C.I.<sub>.95%</sub>=[0.026,0.34]) and *BDNF*<sup>Met</sup> carrier groups ( $OR_{CC:TT}=23.12$ , C.I.<sub>.95%</sub>=[3.04,175.36]) (see Figure 2B). In the pathologically-confirmed AD subset ( $n=262$ ), no test survived correction for multiple testing (for rs676759, interaction  $p=0.16$ , see Figure 2C), suggesting that the interaction effect is specific to individuals without confirmed AD.

Since all interactions showed regulatory effects on the same transcript, SORL1-005, we evaluated the effect of SORL1-005 expression on five measures of postmortem neuropathology in the ROS/MAP sample. Across diagnostic groups, there was a modest effect of SORL1-005 on diffuse plaques, whereby

increased levels of SORL1-005 were associated only with increased number of plaques SORL1-005 ( $F_{1,428}=4.91$ ,  $p=0.027$ ,  $n=439$ ), though this result did not survive correction for multiple testing (see Supplementary Results). No effects were observed for any other pathology at  $p_{\text{raw}} < 0.05$ .

### 3.2 *In Vivo* Neuroimaging

Sample demographics for ADNI subjects are summarized in Table 2. A total of 36 SNP-SNP interactions surviving correction for multiple testing in *SORL1* expression analyses were analyzed across seven bilateral frontal cortical regions of interest for *in vivo* A $\beta$  using [ $^{18}\text{F}$ ]Florbetapir PET, resulting in a total of 252 significant tests before correction. After FDR correction, 44 models remained significant. Between these 44 models, 18 different *SORL1* SNPs demonstrated significant interaction with *BDNF* Val66Met to predict levels of frontal A $\beta$  across five regions of interest (see Supplementary Results). The top SNP showing interaction was rs618874 ( $F_{1,649}=12.12$ ,  $p_{\text{raw}}=5.3 \times 10^{-4}$ ,  $n=710$ ); the rs618874<sup>T</sup> allele was associated with decreased amyloid burden in *BDNF*<sup>Val</sup> homozygotes, but increased amyloid in *BDNF*<sup>Met</sup> carriers (see Figure 3). This is in alignment with effects observed on gene expression and neuropathology in ROS/MAP, whereby the rs12364988<sup>T</sup> allele, which is strongly linked to rs618874<sup>T</sup> (ADNI  $r^2=0.73$ ; ROS/MAP  $r^2=0.74$ ), resulted in *BDNF*<sup>Met</sup>-dependent increases in SORL1-005.

Structural imaging analyses revealed similar patterns of *SORL1*-*BDNF* interaction at  $p < 0.05$ ; however, effects did not survive correction for multiple testing (see Supplementary Results; Figure S3).

## 4. Discussion

We found that linked *SORL1* variants interacted with *BDNF* Val66Met to regulate prefrontal expression of one *SORL1* transcript, SORL1-005 (ENST00000534286), and that this isoform was weakly associated with increased diffuse amyloid plaques in midfrontal tissue from the same subjects. We then demonstrated that the same genetic interactions regulating SORL1-005 expression determined frontal amyloid deposition *in vivo* using PET imaging. The gene variant that most significantly interacted with *BDNF* Val66Met to influence SORL1-005 expression, rs12364988, is part of the same highly-linked haplotype block recently shown to interact with BDNF in human iPSC-derived neurons to regulate *SORL1* mRNA expression [8]. By combining layers of genetic, transcriptomic, and neuroimaging data, we have shown that *BDNF* Val66Met status may determine the background on which *SORL1* risk variants exert their effects (results across phenotypes are summarized in Table S1). Through this lens, BDNF's modulation of resilience via cognitive reserve [32] is also clarified; depending on *SORL1* genotype, the effects of Val66Met may influence protection against AD by promoting diffuse plaque deposition preferentially over neuritic.

*SORL1* is a member of the Vps10p-domain (Vps10p-D) family of neuronal receptors [33], several of which have been shown to interact directly with BDNF. Sortilin, another Vps10p receptor, is responsible for intracellular trafficking of newly synthesized proBDNF via its physical interaction with the region of *BDNF* pro-domain in which the Val66Met substitution resides [34]. *BDNF* Val66Met has been associated with risk for AD [35] and AD-related intermediate phenotypes [36], albeit inconsistently, and is thought to be an important factor in modulating neuroplasticity [10]. Our results may provide insight into the inconsistent literature on the effects of *BDNF* Val66Met (i.e. why it has not been identified by GWAS for AD); the vast majority of studies in this area have not accounted for *SORL1* genotype and thus may be missing crucial information determining the direction and magnitude of *BDNF*'s effects. The mechanisms via which *BDNF* Val66Met influences downstream risk for AD are complex and not yet understood;

recently it was shown that *BDNF* Val66Met alters the expression of miR-146 in humanized *BDNF* knock-in mice [37], suggesting that this variant may influence the expression of multiple target genes simultaneously.

SORL1-005 is a putative protein-coding transcript (UniProt ID: E9PP43) of 3 501bp, containing 25 exons (compared to 48 in the full length transcript), and lacking the Vps10p domain found in full length SORL1 protein. The lack of this domain could potentially interrupt the function of SORL1-005 protein within the amyloid cascade and contribute to the accumulation of pathogenic A $\beta$  species [3], consistent with our observations of concomitant increases in diffuse plaques postmortem and amyloid burden *in vivo*. A second mechanism via which increased SORL1-005 may exert pathological effects on brain structure is regulated intramembrane proteolysis [38]; SORL1 has been shown to undergo sequential cleavage by alpha and gamma-secretase enzymes [39], liberating protein fragments that are internalized to the nucleus and play roles in gene regulation. It is possible that altered recognition of SORL1-005 by gamma-secretase results in the absence of SORL1 COOH-terminal fragments that may preserve pathologically-protective gene regulation; such absence is also seen in cells co-transfected with FAD-linked *PS1* mutations [39]. In either case, given SORL1's dual roles in recycling APP [3] and lysosomal targeting of A $\beta$  [40], alternative splicing causing loss-of-function would be expected to influence amyloid levels in the direction observed in our study.

While functional annotations of SORL1 variants within our top interacting SORL1 haplotype block (which lie approximately 50kb 5' exon 25 of the full length transcript; Figure S4) do not directly reveal an underlying mechanism related to alternative splicing (See Supplementary Materials), it has been shown that a functional SORL1 rare variant (rs117260922, E270K), which is located only one base pair from our top SNP, rs12364988, is responsible for a conformational change in SORL1 that results in failure to

interact with APP at the cell surface [41]. In addition, another nearby variant in high LD with rs12364988 – rs923893 – is a cis-eQTL for SORL1 expression in human brain tissue [42].

Our observation that *SORL1-BDNF* interactions influenced SORL1-005 expression in non-pathological AD subjects may be due to the interruption or masking of transcriptional mechanisms by AD-related neuropathology: it has been shown that A $\beta$  oligomers are capable of inducing gene expression changes across diverse functional classes in human brain tissue [43], and that genes involved in intracellular trafficking specifically show marked down-regulation in postmortem AD brain [44]. These findings may have implications for identifying gene regulatory mechanisms in AD brain, as the effects of transcriptional machinery may be altered depending on levels of cellular pathology.

The weak association of SORL1-005 with only diffuse plaques of midfrontal cortex may suggest a differential contribution of the *SORL1-BDNF* interaction to diffuse vs. neuritic plaque pathology. Diffuse plaques account for the majority of plaque pathology in human brain [45], are associated with AD diagnosis [46], but are also found frequently in so-called “normal” aging [47], suggesting that they may be indicative of the early, pre-symptomatic stages of disease or even just a non-pathological form of aging. It has been shown that A $\beta$  plays crucial roles in neuroplasticity [48], and may be produced as part of a neuroprotective response to synaptic pathology in AD [49]; A $\beta_{1-28}$  has been shown to promote growth and survival of hippocampal neurons [50]. As a result, it is possible that the regulatory action of *SORL1* and *BDNF* may act on amyloid pathways in such a way that influences both neuritic plaque (neurotoxic) and diffuse plaque (neuroprotective) pathologies. Particularly, the interactive effects of variants identified by our study may influence the latter pathway to the greatest degree; hence why genetic effects are only observed in the non-AD group. Further, [18F]Florbetapir has been shown to measure both neuritic and diffuse plaque burden in the brain [51], meaning that our *in vivo* A $\beta$  findings may be reflective of SORL1-005’s effect on diffuse plaques in postmortem frontal cortex. Taken

together, we suggest that the interaction between *BDNF* and *SORL1* may provide links between AD risk and the healthy aging process by influencing the expression of a transcript that is not related to AD risk, but nonetheless modulates diffuse amyloid deposition postmortem and *in vivo*.

The present study has several limitations. First, the decision to analyze gene expression as a binary outcome necessarily introduces a level of bias into the analyses; it is possible that by splitting the distributions of transcript expression into expressed vs. not expressed, we missed quantitative information that here we would have been unable to test without violating statistical assumptions. Second, the expression of *SORL1* has been shown to be cell-type specific [52], whereby some individuals with AD have loss of expression in neurons, but not glia. We are unable to test this directly in our sample, as the ROS/MAP expression data are derived from tissue homogenate of the prefrontal cortex. We took steps to maintain regional specificity in our analyses by analyzing frontal pathology, which should help mitigate some concerns over differences between regions. Third, as with any RNA sequencing experiment, alignment error must be considered as a potential confounder. Fourth, BDNF's effects on amyloid pathology [8] as well as TrkB-dependeng trophic signaling [14] have been shown to depend on *SORL1*, and in this study we used the functional Val66Met variant as an indirect proxy for brain BDNF activity [9]. However, due to inconsistency in the literature surrounding the influence of Val66Met on BDNF protein and mRNA expression in blood and brain tissue, we acknowledge the uncertainty in this assumption. Finally, we acknowledge that mRNA levels of *SORL1* may not be reliable indicators of protein levels. Unfortunately, we did not measure protein directly in our experiments. Future studies will need to measure protein directly in the tissues. In fact, proteomic projects are currently underway in the ROS/MAP cohort that will include both *SORL1* and BDNF. However, there are also limitations to targeted proteomics; for example, it cannot capture individual post-translational

modifications. Thus, ultimately, a variety of approaches, including the mRNA approach used in the present study, are useful for identifying genes and proteins important in the pathogenesis of AD.

In conclusion, we have demonstrated a novel interaction between two AD-associated genes that determines the isoform-specific expression of *SORL1*, and impacts levels of amyloid deposition in two large samples. We believe that this interaction may provide insight into the convergence of prototypical neurotoxic A $\beta$  deposition and the brain reserve found in aged individuals who are resilient to AD. This work has implications for the way that genetic association studies of *SORL1* and *BDNF* are interpreted and may be of use in determining specific groups of genetically at-risk individuals in future clinical trials of novel therapies directed toward amyloidogenic and neuroplastic mechanisms.

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## Figure Legends

**Figure 1.**  $-\log(p\text{-values})$  for interaction terms of SNPs across the *SORL1* locus with *BDNF* Val66Met in logistic regression models for expression of SORL1-005 (ENST00000534286). The top interacting SNP was rs12364988 (Wald  $X^2_1=19.09$ ,  $p=1.25 \times 10^{-5}$ ,  $n=441$ ). Colour coding shows LD structure in the region (according to 1000 Genomes hg19 EUR reference), with red indicating high LD ( $r^2>0.8$ ) and dark blue indicating low LD ( $r^2<0.2$ ) with respect to rs12364988. Plot was generated with LocusZoom [53].

**Figure 2.** Top interaction effects of *SORL1* variants (rs12364988 and rs676759) and *BDNF* Val66Met on prefrontal mRNA expression of SORL1-005 (ENST00000534286) in A) the whole ROS/MAP sample (Wald  $X^2_1=19.09$ ,  $p=1.25 \times 10^{-5}$ ,  $n=441$ ), B) only non-pathologically confirmed AD (non-pathoAD) subjects (Wald  $X^2_1=19.27$ ,  $p=1.14 \times 10^{-5}$ ,  $n=179$ ), and C) pathologically confirmed AD (pathoAD) subjects (Wald  $X^2_1=1.99$ ,  $p=0.16$ ,  $n=262$ ).

**Figure 3.** *SORL1*-*BDNF* interaction effect on *in vivo*  $A\beta$  in the pars orbitalis measured by [18F]Florbetapir PET in the ADNI 2 sample ( $n=710$ ). A) Residual amyloid load according to *SORL1* rs618874 and *BDNF* Val66Met groups. B) Effect of *SORL1* rs618874 within *BDNF*<sup>Val</sup> homozygote and *BDNF*<sup>Met</sup> carrier groups separately, adjusted for co-variables, with 95% confidence intervals. Rs618874<sup>T</sup> was associated with decreased amyloid burden in *BDNF*<sup>Val</sup> homozygotes ( $\Delta A\beta_{TT:CC}=-0.067$ , C.I.<sub>95%</sub> = [-0.13,-0.01]), but increased amyloid in *BDNF*<sup>Met</sup> carriers ( $\Delta A\beta_{TT:CC}=0.12$ , C.I.<sub>95%</sub> = [0.03,0.21]). Results were not impacted by removal of observations lying beyond 1.5\*interquartile range of mean binding.

## Tables

**Table 1.** ROS/MAP Sample Demographics

ROS/MAP expression (n=441)	Non-AD (n=179)	PathoAD (n=179)	Diff ( <i>p</i> ) <sup>1</sup>		
Sex (F/M)	107 F, 72 M	171 F, 91 M	0.027		
Age at death (y(SD))	86.6 (7.2)	89.8 (5.9)	<0.0001		
Education (y(SD))	16.3 (3.6)	16.6 (3.4)	0.47		
MMSE (SD)	25.1 (6.8)	19.1 (9.4)	<0.0001		
RIN (SD)	7.2 (1.0)	7.1 (0.9)	0.21		
PMI (SD)	6.8 (4.1)	7.3 (5.4)	0.25		
<i>APOE</i> ε4 status (-/+)	153-, 26+ (15%+)	168-, 94+ (36%+)	<0.0001		
<i>BDNF</i> genotype (valval/met carrier)	116Val, 63M	170Val, 92M	1		
ROS/MAP MRI (n=172)	CN (n=112)	MCI (n=41)	AD (n=13)	Other <sup>2</sup> (n=6)	Diff ( <i>p</i> ) <sup>1</sup>
Sex (F/M)	81 F, 31 M	30 F, 11 M	12 F, 1 M	3 F, 3 M	0.25
Age at scan (y(SD))	83.3 (6.7)	85.3 (5.1)	85.8 (3.8)	86 (2.8)	0.072
Education (y(SD))	15.6 (3.3)	15.22 (3.1)	15.9 (2.4)	14.7 (2.4)	0.82
MMSE (SD)	28.3 (1.5)	26.9 (2.1)	19.2 (6)	22.7 (4.5)	<0.0001
<i>APOE</i> ε4 status (-/+)	95-/17+ (15%+)	29-/12+ (29%+)	8-/5+ (38%+)	5-/1+ (17%+)	0.084
<i>BDNF</i> genotype (valval/met carrier)	77/35	29/12	7/6	4/2	0.81

Note: <sup>1</sup>*p*-values are two-sided and derived from Fisher's exact test (for sex, *APOE* ε4 status, and *BDNF* genotype) and either two-sample t-tests (in expression dataset for age at death, education, MMSE, RIN, and PMI) or ANOVA (in imaging dataset for age at scan, education, and MMSE). ROS/MAP = Religious Orders Study / Memory and Aging Project; CN = cognitively normal; non-AD = non-neuropathologically-confirmed Alzheimer's disease; pathoAD = neuropathologically-confirmed Alzheimer's disease; MMSE = Mini Mental Status Exam score at last visit before death; Val = Val/Val homozygotes; Met = Met allele carriers; F = female; M = male; y = years; SD = standard deviation; R = right; L = left.

**Table 2.** ADNI Sample Demographics

<b>ADNI 1/GO/2 MRI (n=1 285)</b>	<b>CN (n=335)</b>	<b>SMC (n=82)</b>	<b>EMCI (n=235)</b>	<b>LMCI (n=407)</b>	<b>AD (n=226)</b>	<b>Diff (p)<sup>1</sup></b>
<b>Study phase (1, GO, 2)</b>	193 (1), 142 (2)	82 (2)	111 (GO), 124 (2)	291 (1), 116 (2)	131 (1), 95 (2)	<0.0001
<b>Sex (F/M)</b>	159 F, 176 M	51 F, 31 M	101 F, 134 M	155 F, 252 M	102 F, 124 M	0.0012
<b>Age (y(SD))</b>	74.7 (5.4)	71.7 (5.4)	71.0 (7.1)	73.3 (7.4)	74.3 (8.1)	<0.0001
<b>Education (y(SD))</b>	16.2 (2.7)	16.7 (2.6)	16.1 (2.6)	15.8 (2.9)	15.2 (2.9)	<0.0001
<b>MMSE (SD)</b>	29 (1.1)	29 (1.2)	28.4 (1.6)	27.1 (1.8)	23.1 (2.1)	<0.0001
<b>APOE ε4 status (-/+)</b>	251-/84+ (25%+)	53-/29+ (35%+)	132-/103+ (44%+)	175-/232+ (57%+)	68-/158+ (70%+)	<0.0001
<b>BDNF genotype (valval/met carrier)</b>	231/104	54/28	159/76	271/136	154/72	0.96
<b>ADNI GO/2 Amyloid Sample (n=710)</b>	<b>CN (n=136)</b>	<b>SMC (n=88)</b>	<b>EMCI (n=244)</b>	<b>LMCI (n=121)</b>	<b>AD (n=121)</b>	<b>Diff (p)<sup>1</sup></b>
<b>Study phase (GO, 2)</b>	136 (2)	88 (2)	106 (GO), 138 (2)	121 (2)	121 (2)	<0.0001
<b>Sex (F/M)</b>	66 F, 70 M	55 F, 33 M	107 F, 137 M	55 F, 66 M	50 F, 71 M	0.024
<b>Age (y(SD))</b>	73.9 (5.9)	72.2 (5.8)	71.4 (7.4)	72.5 (7.5)	74.3 (8.5)	0.001
<b>Education (y(SD))</b>	16.5 (2.5)	16.8 (2.6)	16 (2.6)	16.5 (2.6)	15.8 (2.6)	0.016
<b>MMSE (SD)</b>	29.1 (1.2)	29 (1.3)	28.3 (1.6)	27.7 (1.8)	23.1 (2.1)	<0.0001
<b>APOE ε4 status (-/+)</b>	104-/32+ (24%+)	60-/28+ (32%+)	130-/114+ (47%+)	51-/70+ (58%+)	43-/78+ (64%+)	<0.0001
<b>BDNF genotype (valval/met carrier)</b>	95/41	58/30	167/77	89/32	79/42	0.66

Note: \**p*-values are two-sided and derived from Fisher's exact test (for study phase, sex, *APOE* ε4 status, and *BDNF* genotype) and ANOVA (for age, education, and MMSE). ADNI = Alzheimer's Disease Neuroimaging Initiative; CN = cognitively normal; SMC = some memory concern; EMCI; early mild cognitive impairment; LMCI = late mild cognitive impairment; AD = Alzheimer's disease; MMSE = Mini Mental Status Exam score; Val = Val/Val homozygotes; Met = Met allele carriers; F = female; M = male; y = years; SD = standard deviation.

**Revised Supplementary Material**

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