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Featured Article

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 amyloid β (Aβ) 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 1285 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 Aβ plaques. The same *SORL1-BDNF* interactions also significantly influenced Aβ load as measured with [^{18}F]Florbetapir positron emission tomography.

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. Introduction

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]. Although studies have implicated *SORL1* genotypes independently in

[1]Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (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.

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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 induced pluripotent stem cells (IPSCs)-derived neuron cultures upregulates SORL1 expression in an *SORL1* genotype–dependent manner [8]. The *BDNF* Val66Met polymorphism determines the activity-dependent secretion of BDNF [9] and also the function of the BDNF propeptide in facilitating neuroplasticity (hippocampal long-term depression) [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 versus resilience for AD.

We have previously shown a main effect of *SORL1* genotype on levels of prefrontal *SORL1* messenger RNA (mRNA) in postmortem brain [15] using microarray technology that was unable to detect specific SORL1 transcript isoforms. Because 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. Furthermore, given the functions of SORL1 within the amyloidogenic cascade, we hypothesized that genetic interactions predicting altered *SORL1* expression may affect amyloid neuropathology and 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* single-nucleotide polymorphisms (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 [^{18}F]Florbetapir positron emission tomography (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 1285 subjects from ADNI and 172 subjects from ROS/MAP with magnetic resonance imaging (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

2.1.1. Study participants

A total of 441 subjects with genomic, RNA-seq, and neuropathological data were included in the present study. All participants were from ROS [18] and MAP [19] and 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 [20]. Common variants within 10 kb of the *SORL1* locus (chromosome 11, position 121,312,912–121,514,471, GRCh37 coordinates) were extracted using PLINK (v1.90b) [21]. Variants were pruned for minor allele frequency (MAF $>$ 0.1) and Hardy-Weinberg Equilibrium (HWE, $P > .001$), 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 after the construction of complementary DNA libraries, as previously published [22]. 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: midfrontal neuritic plaques and diffuse plaques, total amyloid, paired helical filament tau, and neurofibrillary tangles (see Supplementary Methods).