Published in final edited form as:
Neurobiol Aging. 2013 May ; 34(5): 1518.e9–1518.e18. doi:10.1016/j.neurobiolaging.2012.09.020.

Genetic interactions associated with 12-mo atrophy in hippocampus and entorhinal cortex in ADNI

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Abstract

Missing heritability in late onset Alzheimer disease (LOAD) can be attributed, at least in part, to heterogeneity in disease status and to the lack of statistical analyses exploring genetic interactions. In the current study, we use quantitative intermediate phenotypes derived from MRI data available from the Alzheimer's Disease Neuroimaging Initiative (ADNI), and we test for association with gene-gene interactions within biological pathways. Regional brain volumes from the hippocampus (HIP) and entorhinal cortex (EC) were estimated from baseline and 12 month MRI scans. Approximately 560,000 SNPs were available genome-wide. We tested all pairwise SNP-SNP interactions (~151 million) within 212 KEGG pathways for association with 12-month regional atrophy rates using linear regression, with gender, *APOE* ε4 carrier status, age, education and clinical status as covariates. A total of 109 SNP-SNP interactions were associated with right HIP atrophy, and 125 were associated with right EC atrophy. Enrichment analysis indicated significant SNP-SNP interactions were overrepresented in the calcium signaling and axon guidance pathways for both HIP and EC atrophy and in the ErbB signaling pathway for HIP atrophy.

Keywords

Alzheimer's disease; *biological pathway*; *brain atrophy*; *epistasis*; *gene-gene interaction*; *GWIA*; *GWAS*; *KEGG*; *MRI*; *neuroimaging*; *GWIA*; *GWAS*; *pathway-based analysis*

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

Disclosure Statement The authors have no actual or potential conflicts of interests to declare.

1. Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder associated with aging, and is clinically characterized by a progressive decline in memory and other areas of cognition, with considerable clinical heterogeneity among persons with the disease. Neuropathological hallmarks of AD include extracellular accumulation of amyloid beta (Aβ) plaques and intracellular accumulation of neurofibrillary tangles containing phosphorylated tau protein. AD also has a complex genetic etiology and likely multiple, as yet unconfirmed, environmental risk factors.

The last several decades of research have yielded only one genetic risk factor of large effect for late-onset AD (LOAD)—apolipoprotein-E (*APOE*)—with two copies of the e4 allele conferring about 6–30 fold risk for the disease (1). More recent genome-wide association studies (GWAS) have identified and replicated nine additional AD susceptibility genes, including BIN1, CLU, ABCA7, CR1, PICALM, MS4A6A, CD33, MS4A4E and *CD2AP[3, 9, 20, 23, 32, 45]*. However, all of these have low effect sizes (odds ratios of 0.87–1.23) and cumulatively account for approximately 35% percent of population attributable risk[32].

A large portion of the “missing heritability” can be attributed to two common attributes of genetic association studies. One is the use of discrete disease status as the phenotypic trait of interest despite the fact that LOAD is a clinically heterogeneous disorder. A second reason the field has not explained more of the heritability in LOAD yet is that most genetic association studies compute statistics at the single marker level and fail to address the underlying biological interactions that contribute to the development of disease.

One way to address the issue of clinical heterogeneity in LOAD is to use intermediate quantitative traits such as clinical or cognitive features, biochemical assays, or neuroimaging biomarkers as phenotypes of interest for genetic association testing. These endophenotypes often are measured as continuous variables and thus exhibit a higher genetic signal-to-noise ratio. Further, most intermediate phenotypes are more proximal to their genetic effect than is disease status. Thus, the incorporation of intermediate quantitative traits serves to increase statistical power to detect disease-related genetic associations(4). An ancillary benefit of using intermediate phenotypes is they can serve as effective biomarkers for monitoring disease progress or treatment response in clinical practice or drug trials.

Over the past 10–15 years, studies have identified robust and predictive biomarkers for AD including assays quantifying levels of tau, ubiquitin and Aβ peptides in CSF, selective measures of brain atrophy using magnetic resonance imaging (MRI), imaging of glucose hypo-metabolism and amyloid using positron emission tomography (PET) [17]. However, out of the above biomarkers, MRI derived measures are the least invasive, thereby conferring a major advantage over other methodologies. Cortical atrophy of the medial temporal lobes, as measured using MRI in both transgenic mouse models and in humans, has shown to be a valid and reliable biomarker for early detection of LOAD [16, 17, 22, 42] During early stages of the disease, in subjects with mild cognitive impairment and early AD, atrophy is seen primarily in the entorhinal cortex and hippocampus and can reliably predict MCI conversion to AD [15, 35]

Recent genetic studies in LOAD have employed quantitative MRI phenotypes [36, 44], and others have implemented pathway based analysis [1, 24]. However, to our knowledge, no study has combined the two analytic strategies. A GWAS using hippocampal volume as the quantitative outcome detected association with multiple novel candidate genes, including *TOMM40, EFNA5, CAND1, MAGI2, ARSB* and *PRUNE2*, in LOAD and cognitively normal controls [36]. A more recent GWAS investigated single-marker associations between LOAD and gray matter density, volume and cortical thickness at baseline in multiple regions

of interest (ROIs) across the whole brain [44]. This study identified multiple SNPs close to the *EPHA4, TP63* and *NXPH1* genes, along with *APOE* and *TOMM40*, in significant associations with multiple brain-ROI metrics. In particular, the study found that *NXPH1* gene was associated with right hippocampal gray matter density in LOAD. Both these studies, however, did not use longitudinally measured atrophy rates to test for genetic associations in LOAD.

Addressing the second challenge for finding the missing heritability in LOAD, we can effectively explore epistatic interactions in GWAS by using *a priori* statistical and/or biological evidence to generate a reduced genome-wide marker set for interaction testing. Pathway level analysis incorporating prior biological knowledge is one such approach offering an alternative way to explore GWAS of complex diseases such as LOAD [21]. Using a computational bioinformatics approach, Liu et al., (2009) [29] investigated spatial pathway clusters in different AD brain regions and the crosstalk among pathways by integrating protein-protein interaction and gene expression data. The study identified 77 biological pathways that most closely interact with the primary AD pathway.

The present study utilized INTERSNP software [21] to evaluate how genetic interactions tested within known biological pathways influence 12-month atrophy rates in hippocampus and entorhinal cortex, which as stated above, are brain regions known to be affected most significantly, consistently and earliest in LOAD [31, 40].

2. Methods

2.1. Subjects and Data

Data used in the preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.ucla.edu). The ADNI was launched in 2003 by the National Institute on Aging (NIA), the National Institute of Biomedical Imaging and Bioengineering (NIBIB), the Food and Drug Administration (FDA), private pharmaceutical companies and non-profit organizations, as a $60 million, 5-year public-private partnership. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early Alzheimer's disease (AD). Determination of sensitive and specific markers of very early AD progression is intended to aid researchers and clinicians to develop new treatments and monitor their effectiveness, as well as lessen the time and cost of clinical trials. The Principal Investigator of this initiative is Michael W. Weiner, MD, VA Medical Center and University of California – San Francisco. ADNI is the result of efforts of many co-investigators from a broad range of academic institutions and private corporations, and subjects have been recruited from over 50 sites across the U.S. and Canada. The initial goal of ADNI was to recruit 800 adults, ages 55 to 90, to participate in the research, approximately 200 cognitively normal older individuals to be followed for 3 years, 400 people with MCI to be followed for 3 years and 200 people with early AD to be followed for 2 years. For up-to-date information, see www.adni-info.org.

We applied for and were granted permission to use data from the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort (http://www.adni-info.org/) to conduct genetic interaction analyses. Study subjects gave written informed consent at the time of enrollment for imaging and genetic sample collection and also completed questionnaires approved by each participating site's Institutional Review Board (IRB). After applying quality control procedures (detailed below), the study sample included 156 controls, 281 mild cognitive impairment (MCI) and 140 LOAD Caucasian subjects. Demographic and imaging characteristics of the sample are listed in Table 1.

2.2. Genotyping and quality control

Single nucleotide polymorphism (SNP) genotyping for 620,901 target SNPs covering the entire genome was completed on all subjects using the Illumina Human-610-Quad BeadChip per the standard ADNI protocol. Specifics of genotypic methods are detailed in Shen et al. (2010) [44, 44]]. The following quality control (QC) procedures were employed using PLINK software (http://pngu.mgh.harvard.edu/~purcell/plink/). SNPs not meeting the following criteria were excluded from further analysis: (1) call rate per SNP ≥90%, (2) minor allele frequency (MAF) ≥1% (N=63,950 SNPs were excluded based on criteria 1 and 2), and (3) Hardy–Weinberg equilibrium test of $p \geq 10e{-}6$ (N=906 SNPs were excluded) using control subjects only. Participants were excluded from the analysis if any of the following criteria were not satisfied: (1) call rate per participant ≥90% (3 participants were excluded); (2) gender check inconsistency (2 participants were excluded); and (3) identity check for related pairs (3 sibling pairs were identified with PI_HAT over 0.5; one participant from each pair was randomly selected and excluded). Population stratification analysis performed in EIGENSTRAT[37] (and confirmed in STRUCTURE[38]) showed 79 study participants did not cluster with the remaining subjects and with the CEU HapMap samples who are primarily of European ancestry (non-Hispanic Caucasians). Due to inadequate power to detect interaction effects in the remaining samples, these 79 participants were excluded from analysis, as in previously published analyses of these data [44], instead of including them and using principal components to adjust for population substructure.

2.3. Analysis of MRI data

A structural MRI scan was acquired on all subjects during their baseline and 12-month follow up visit per the ADNI protocol (http://adni.loni.ucla.edu/research/protocols/). We obtained pre-computed volumetric estimates of hippocampus and entorhinal cortices from ADNI derived from brain parcellations performed using QUARC software [22]. QUARC employs unbiased non-linear registration techniques for longitudinal MRI scans, enabling the detection of small, early anatomical changes, with improved performance over other software packages, including Freesurfer and tensor based morphometry [22]. The computed ROI segmentation estimates from the baseline and 12 month follow up scans for each subject were used to calculate an annual percent change (APC) score according to the following formula; APC = (12 month ROI value − Baseline ROI value)/ Baseline ROI Value [41]. These APC values were then used as the quantitative phenotype in subsequent analyses. Subjects with a missing or artifactual scan exceeding image quality standards (as determined by the ADNI quality control pipeline) at one or both time points were excluded from further analysis. After the genotyping and MRI QC procedures, 577 out of 818 participants and 556,045 out of 620,903 markers remained in the analysis, resulting in a 99.5% genotyping rate.

2.4 SNP-by-SNP interaction analysis

We performed genome-wide interaction analysis (GWIA) using INTERSNP software (http://intersnp.meb.uni-bonn.de/). An explicit test for genotypic interaction (full model including both additive and dominance effects + interaction term versus reduced model that does not contain interaction terms) was performed on all SNP pairs using the multi-marker routine thus allowing for detection of non-linear effects in the data [21]. The computation was conducted in a linear regression framework using the respective APC estimates of both EC and HIP as the QTs in two separate univariate analyses. Further, we used relevant baseline demographic data, including age at baseline scan, education level, *APOE* ε4 carrier status, sex and clinical diagnosis at baseline as covariates in the analysis. We also employed the pathway based option in the software as a prior to restrict testing of SNP pairs between genes belonging to a common biological pathway, thereby reducing the total number of statistical tests and improving the interpretability of results. The biological pathways used in

the analyses were derived from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.genome.jp/kegg/). This resulted in a total of approximately 151 million unique pairs of SNPs to be tested from the ADNI dataset.

2.5 Annotation and visualization

In an effort to interpret significant interactions in the context of existing biological knowledge of AD, all SNPs in significant SNP-SNP interactions from the GWIA analysis were first annotated to their corresponding genes by querying against the dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP/). Next we identified significant interactions containing genes previously associated with LOAD (Genotator software, cutoff gene score > 0;[48]), which might implicate specific mechanisms based on current biological knowledge. To visualize interactions with genes identified in the GWIA analysis, we used the search tool for retrieval of interacting genes/proteins (STRING; http://tools.autworks.hms.harvard.edu [47]) to identify interrelated genes based on prior knowledge from multiple sources and to construct interaction network diagrams shown in Figures 1–2. STRING sources include Neighborhoods: Synteny derived from Swiss Prot and Ensembl; Co-occurrence: phylogenetic profiles derived from COG database; Coexpression: coregulation of genes measured using microarrays imported from ArrayProspector; Experiments: protein-protein interaction inferred or confirmed by experiments; Databases: validated small scale interactions, protein complexes; and annotated pathways from BIND, KEGG and MIPS [47].

2.6 Pathway enrichment analysis

We aimed to determine which KEGG pathways were enriched for genes that comprised the significant SNP-SNP interactions. Using the DAVID software (http://david.abcc.ncifcrf.gov/), the list of genes from significant SNP-SNP interactions were compared to the HumanRef-8_V2_0_R4_11223162_A Illumina background marker set containing more than 5000 genes from KEGG. For each KEGG pathway, quantitative enrichment scores were calculated to quantify the likelihood that the pathway contained the observed number interacting genes compared to what would be expected by chance. Significance values were adjusted using Benjamini-Hochberg correction for multiple comparisons [39].

3. Results

3.1 Regional atrophy

A one-way ANOVA computed in SPSS (v 19.0; http://www-01.ibm.com/software/analytics/spss/) revealed that 12-month atrophy rates were significantly different among diagnostic groups for HIP and EC in both hemispheres and in the expected direction. Subjects with LOAD had the most atrophy, control subjects had the least and MCI subjects had intermediate values. Results are illustrated in Figure 3.

3.2 Interaction analysis

We identified 109 SNP-SNP interactions (mapped to 78 genes) and 125 SNP-SNP interactions (mapped to 102 genes) that were significantly associated with right HIP and right EC atrophy, respectively. No interactions were significant for the left hemisphere in these regions. Complete results are listed in Tables 2 and 3. Reported p-values were Bonferroni-corrected for multiple comparisons for all interaction tests ($N=1.51\times10^{8}$) performed.

3.3 Pathway enrichment analysis

Enrichment analysis identified three KEGG pathways enriched for significant SNP-SNP interactions associated with right HIP atrophy. These included, but were not limited to, calcium signaling, axon guidance and ErbB signaling pathways. Similarly, we found genes from significant SNP-SNP interactions with EC atrophy were overrepresented in several (N=14) biological pathways, including but not limited to, calcium signaling, axon guidance, Alzheimer's disease, long-term depression and potentiation, and neuroactive ligand-receptor interaction. The complete (all meeting a $p<0.05$ unadjusted alpha level) pathway enrichment results are presented in Tables 4 and 5 for HIP and EC, respectively.

3.4 Annotation and visualization

Mapping of SNPs to genes using Genotator software yielded 1287 genes previously associated with AD. The corresponding gene-gene pairs (with the previously associated AD genes in bold) that are here implicated in EC atrophy included: ***ENPP1***-*FHIT* (purine metabolism), ***ENPP1***-*PDE7B* (purine metabolism), ***CYP4F3***-*CYP2J2* (lipid metabolism), *ABCB5*-***ABCA1*** (ATP-binding cassette transporter system), *ITPR2*-***CHRNA7*** (calcium signaling), ***ADRA1A***-*GRIK1* (neuroactive ligand-receptor interaction), *GABBR2*-***GRIN2B*** (neuroactive ligand-receptor interaction), ***EFNA5***-*DPYSL2* (axon guidance), *ROBO1*-***FYN*** (axon guidance), *ROBO1*-***SEMA3A*** (axon guidance), *PRKCA*-***RELN*** (focal adhesion), ***IGF1R***-*TLN2* (focal adhesion), ***IGF1R***-*VAV2* (focal adhesion), ***IGF1R***-*PPP2CB* (long-term depression), ***ADAM10***-*IKBKB* (epithelial cell signaling in Helicobacter pylori infection), *CHSY3*-***GALNT2*** (glycan structure biosynthesis), ***CX3CL1***-*IL12RB2* (cytokine-cytokine receptor degradation), ***DOCK1***-*ACTN2* (focal adhesion & regulation of actin cytoskeleton), ***DOCK1***-*TIAM2* (Regulation of actin cytoskeleton), *GRM5*-***PRKACB*** (calcium signaling), ***IL1RAP***-*DFFB* (apoptosis), ***PPP3CA***-*DFFB* (apoptosis).

Gene-gene pairs (with previously associated AD genes in bold) that are here implicated in HIP atrophy included: *ABCC4*-***ABCG1*** (ATP-binding cassette transporter system), ***PTGER3***-*CAMK2A* (calcium signaling), *RYR1*-***ADRA1A*** (calcium signaling), ***FSHR***-*GABRB2* (neuroactive ligand-receptor interaction), *GABRB2*-***AGTR1*** (neuroactive ligand-receptor interaction), ***TCF7L2***-*CAMK2A* (Wnt signalling), ***SMAD3***-***PRKACB*** (Wnt signalling), ***UNC5B***-*SLIT3* (axon guidance), *EPHB1*-***ABLIM1*** (axon guidance), *SRGAP1*-***NRP1*** (axon guidance), ***CTNNA3***-*IQGAP1* (adherens junction), *GUCY2F*-***IGF1R*** (long-term depression) & *ACCN1*-***CACNA1A*** (taste transduction - calcium signaling).

4. Discussion

The current study presents a GWIA of brain atrophy measures from 577 subjects from the ADNI cohort. To our knowledge this is the first study looking at genetic epistasis with neurodegenerative QT markers in a substantially large sample. We decided to focus on neurodegenerative MRI markers of HIP and EC as they are measures unequivocally affected in MCI and LOAD [12, 25, 26]. Our focused analytic approach yielded several interesting candidate gene-gene interactions for LOAD that could be readily interpreted in the light of known molecular pathways.

Prior evidence suggests that neuronal atrophy in AD might be initially characterized by decreased synaptic density following injury caused by the accumulation of neuropathological events such as astrogliosis and microglial cell proliferation, in addition to the observed amyloid and tau pathology [6, 14, 28]. Primary processes such as apoptosis, cell cycle impairment, ubiquitin system dysfunction, and failure of axonal and dendritic transport are key pathophysiological mechanisms thought to underlie neurodegeneration in AD [7, 8, 33]. The current study provides specific evidence of genetic interactions within

these and other diverse neurodegenerative functional pathways contributing to neuronal loss in HIP and EC.

In line with our hypothesis, we identified multiple genetic interactions associated with 12-month atrophy of HIP and EC. Several genes found in this study have already been implicated in AD, thus lending validation and confidence to the analytic procedure and results. However, perhaps more importantly, this study also identified a number of genes that had not yet been associated with AD in conventional GWAS studies investigating single-SNP effects. Thus, this study exposes several potential candidate genes that should be explored in future replication samples.

Apart from the above interesting findings, our study had several methodological and technical advantages over other imaging-genetics studies. 1) To our knowledge this is the first study to explore how SNP-SNP interactions influence atrophy of brain regions, measured using longitudinal MRI scan information, known to be compromised early in AD. 2) Focusing on interactions within known biological pathways allowed us to a) limit the number of multiple comparisons and b) make more informed interpretations in the context of known molecular systems. 3) The inclusion of continuous disease markers as quantitative traits confers higher statistical power than using conventional clinical status by modeling the underlying clinical heterogeneity. 4) Our sample included both MCI and clinically defined AD, thus providing more variation of the disease in the dataset. 5) Our sample size was relatively large and in most cases was larger than other imaging-genetics studies. 6) Our sample was derived from a well-controlled national study cohort. 7) Longitudinal atrophy measures were derived from a newly developed non-biased, non-linear registration technique shown to have higher effect sizes in detecting LOAD pathology compared to other similar imaging techniques.

Overall, EC atrophy was associated with interactions across a larger number of pathways compared to HIP atrophy. Our results were largely consistent with the highlighted pathways (apoptosis, cytokine receptor interaction, Wnt signaling, ErbB signaling, etc.) from a recent report investigating crosstalk between AD related pathways using gene expression data from brain regions including EC and HIP[29]. However, our study used previously associated quantitative intermediate phenotypes and, therefore, had greater power to detect interaction effects. We found significant interactions in pathways not identified by Liu et al., such as focal adhesion, regulation of actin and neuroactive ligand receptor interaction, all of which have been shown to be important processes contributing to LOAD pathology [46].

Several of the pathways identified in the present study have been previously implicated in AD. Gene enrichment analysis indicated that associations with HIP atrophy in AD were restricted primarily to three molecular networks, 1) Calcium signaling, 2) axon guidance and 3) ErbB signaling. It is interesting to note that some pathways were common to both HIP and EC atrophy (calcium signaling and axon guidance), although the underlying gene-gene pairs were different for the two regions of interest. However the specific genes involved in significant interactions (within a common pathway) were different between the two regions of interest. Dysregulation of intracellular calcium signaling has been implicated as a central feature in the pathogenesis of AD. It has been shown that neurodegeneration and cell death induced by amyloid beta is indirectly mediated by changes in calcium homeostasis [28, 30]. Our study lends direct support to these mechanisms by finding significant genetic interactions in the Ca2+ signaling pathway associated with HIP and EC atrophy.

Genes in the axon guidance molecular pathway play a key role in the formation of neuronal networks. In particular, semaphorins, which are axon-guiding proteins have been shown to play a key role in the pathophysiology of AD [18]. A recent study showed that

semaphorin3A accumulates excessively in HIP in patients with AD[27]. The present study identified several variations of the semaphorin gene (including semaphorin 3A) that significantly interact with other axon guiding genes in relation to atrophy of HIP and EC. In particular, we found SNPs in semaphorin 3A to significantly interact with SNPs in the ROBO1 gene, which is a candidate gene for neurodevelopmental disorders such as dyslexia [19].

ErbB signaling pathway was found to be unique to HIP atrophy and was not involved with EC degeneration. In addition to the previously mentioned molecular processes, recent evidence also suggests interference of adult hippocampal neurogenesis contributes to neurodegeneration in AD [14]. Neurogenesis occurs in adults throughout life and is a complex multistep process involving cell proliferation, migration, differentiation and maturation, including growth and synaptogenesis. Interestingly, genes in the ErbB signaling pathway regulate diverse biologic mechanisms, including all of those listed above, by facilitating binding of extracellular growth factor ligands to intracellular signaling pathways. Specifically, we found interaction of genes Neureglin3/4 and ErbB4 in this pathway to be responsible for HIP atrophy, consistent with evidence showing that neuregulin-1 and ErbB4 distribution in the hippocampus is altered in AD [10]. Further immunoreactivity of Neureglin and ErbB4 is known to be associated with neuritic plaques in human and mice brain models of AD [10]. Our study also identified interactions within the MAGI2 gene to be associated with HIP atrophy in MCI and AD. MAGI2 has been speculated to play a critical role in the ubiquitin system, the failure or misregulation of which could lead to increased accumulation of misfolded proteins, cell cycle abnormalities and even apoptosis [4]. MAGI2 was identified in a previous QT study on the ADNI cohort as being associated with hippocampal atrophy in AD [36]. The key differences between that study and ours were: 1) they used a different brain volumetric estimation method (voxel based morphometry) that operated only on cross-sectional data from a single time point, and 2) they only included controls and AD (not MCI) subjects.

The current study also found genes involved in both long-term potentiation (LTP) and depression (LTD) to be associated with EC atrophy. These processes refer to activity-dependent (increased or decreased) efficacy of neuronal synapses lasting hours or longer. Both LTP and LTD are thought to be involved in learning and memory and are believed to underlie elimination of synapses in neurodegenerative diseases[11]. The role of EC in episodic memory is paramount, and it serves as a communication point between HIP and the neocortex to enable and complete the memory formation and retrieval loop. A recent study showed that the white matter projections between HIP and EC are capable of sustaining long-term synaptic changes in the form of both LTP and LTD [13]. The current study provides evidence that genes related to LTP/LTD (*GRIN2A*, *GRIN2B*, etc.) influence EC atrophy through interactions with genes in other pathways, such as *FPR3* and *GPR156* in the neuroactive ligand-receptor interaction gene set. Interestingly, all of the above genes have been implicated directly in the pathogenesis of AD or molecular processes involved in causing AD like disorders to varying degrees [5, 43]. It was also extremely interesting to note that even though our analysis was not limited to AD-related genes, our results showed the Alzheimer disease pathway was enriched for significant SNP-SNP interactions associated with 12-month EC atrophy. We could speculate that since we know atrophy occurs earlier in the entorhinal cortex, compared to the hippocampus, more of the variability in EC atrophy could be explained by a genetic factor predisposing to disease onset.

There are some notable limitations of our study that could be usefully addressed in future investigations. (1) The limited number of non-Caucasian samples in the ADNI cohort meant we did not have the power to analyze these samples separately, which limits the generalizability of our findings. Cohorts with larger sample sizes, or ones that oversampled

for different ancestral groups, might allow for replication and generalization of these findings. (2) The restriction of tested SNP-SNP interactions within known biological pathways prohibits discovery of significant interactions between genes whose structure or function is not well understood. (3) The restriction of analyses to HIP and EC precluded the discovery of associations with atrophy in other regions across of the brain. (4) The inclusion of other longitudinal time points (24 or 36 months) from the ADNI dataset might enable the creation of a temporal map of AD related atrophy, which could be mapped onto associations with biological pathways. (5) In a larger dataset, such as the one being accrued in ADNI-GO and ADNI-2, it would be interesting to focus primarily on subjects with normal cognition at baseline, since the earliest changes in brain are more likely to associate with a specific genetic risk model. (6) Due to linkage disequilibrium among SNPs, not all tests performed were independent. Thus, our use of the Benjamini-Hochberg correction for multiple comparisons was overly stringent, perhaps leading to an increased false negative rate. Future studies could increase their power by imploring computational methods of correction, such as permutation testing, or spectral decomposition [34]. (7) It is important to note that SNPs from the Illumina Human610-Quad BeadChip are common SNPs more likely to be tagging the location of an AD susceptibility locus than to be the functional SNPs responsible for influencing risk for AD. (8) We want to emphasize that the genetic interactions with brain atrophy rate discovered in this study are *statistical* associations, which need to be replicated in additional human genetic studies and, ideally, should be validated using functional studies in cell lines or model systems.

In conclusion, we provide an innovative analytic framework using intermediate quantitative phenotypes to confirm genes associated with known pathologic mechanisms and to discover new disease-relevant genes that interact but do not necessarily show significant main effects. Extending existing GWAS and hypothesis-driven analyses on the ADNI data set, the current analysis identifies epistatic interactions within plausible biological pathways associated with atrophy in primary LOAD related brain regions. Importantly, our study shows that atrophy in different brain regions might be mediated by different biogenetic mechanisms. Further, we also believe our analytic technique could inform and be readily applied to other complex neurological or psychiatric disorders where the neuropathology and genetic basis might be less well-defined than for LOAD. Future studies should attempt to replicate these results in independent datasets of similar size with neuroimaging and genetic data, as they become available. Additional molecular studies aimed at understanding the link between cellular processes such as calcium signaling, axon guidance, inflammation (actin regulation) and focal adhesion would provide critical functional evidence in support of our statistical association findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Miguel Herrera and Lan Jiang for helping with custom scripts to aid in performing our data analysis and organizing the results.

The study was funded in part by the following: Recruitment for Genetic Aging Research (1P30AG036445), Analysis Tool for Heritable and Environmental Network Associations (R01LM010040), and the Vanderbilt CTSA Grant 1 UL1 RR024975 from NCRR/NIH. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Data collection and sharing for this project was funded by the Alzheimer's Disease Neuroimaging Initiative (ADNI) (National Institutes of Health Grant U01 AG024904). ADNI is funded by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, and through generouscontributions from the

following: Abbott; Alzheimer's Association; Alzheimer's Drug Discovery Foundation; Amorfix Life Sciences Ltd.; AstraZeneca; Bayer HealthCare; BioClinica, Inc.; Biogen Idec Inc.; Bristol-Myers Squibb Company; Eisai Inc.; Elan Pharmaceuticals Inc.; Eli Lilly and Company; F. Hoffmann-La Roche Ltd and its affiliated company Genentech, Inc.; GE Healthcare; Innogenetics, N.V.; IXICO Ltd.; Janssen Alzheimer Immunotherapy Research & Development, LLC.; Johnson & Johnson Pharmaceutical Research & Development LLC.; Medpace, Inc.; Merck & Co., Inc.; Meso Scale Diagnostics, LLC.; Novartis Pharmaceuticals Corporation; Pfizer Inc.; Servier; Synarc Inc.; and Takeda Pharmaceutical Company. The Canadian Institutes of Health Research is providing funds to support ADNI clinical sites in Canada. Private sector contributions are facilitated by the Foundation for the National Institutes of Health (www.fnih.org). The grantee organization is the Northern California Institute for Research and Education, and the study is coordinated by the Alzheimer's Disease Cooperative Study at the University of California, San Diego. ADNI data are disseminated by the Laboratory for Neuro Imaging at the University of California, Los Angeles. This research was also supported by NIH grants P30 AG010129 and K01 AG030514.

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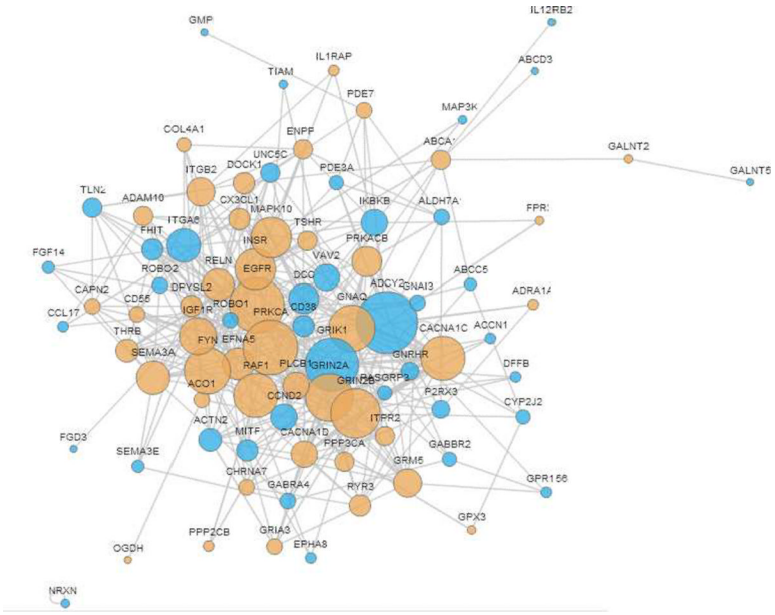

Figure 1.

Gene-Gene Interaction Network for Entorhinal Atrophy. Each circle is a gene that participated in a significant SNP-SNP interaction model. Circles colored orange are genes previously identified as a possible Alzheimer disease risk gene.

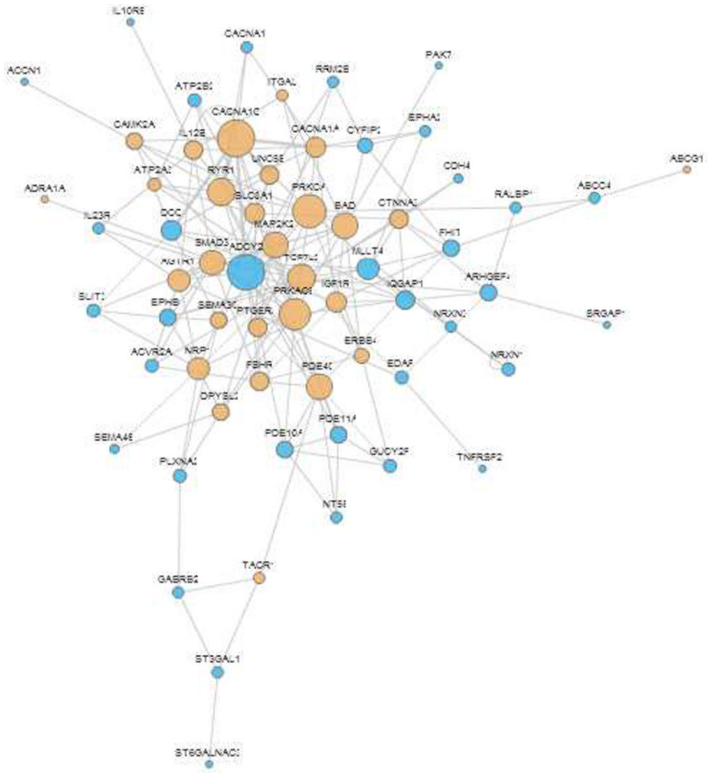

Figure 2.

Gene-Gene Interaction Network for Hippocampal Atrophy. Each circle is a gene that participated in a significant SNP-SNP interaction model. Circles colored orange are genes previously identified as a possible Alzheimer disease risk gene.

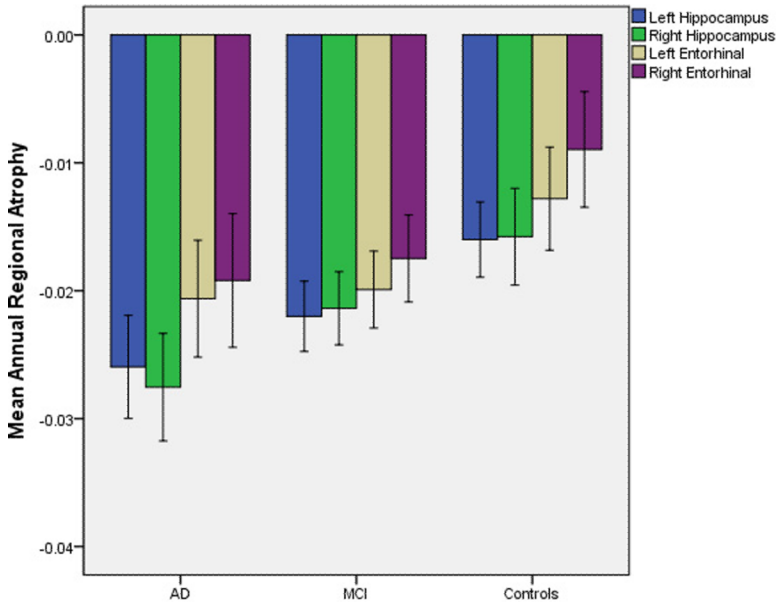

Figure 3.
Mean Annual Regional Atrophy by Group for left and right Entorhinal Cortex and Hippocampus

Table 1

Descriptive Statistics for ADNI Dataset

		Controls (N=156)		MCI (N=281)		AD (N=140)		Statistic	
		Mean	***SD***	***Mean***	***SD***	***Mean***	***SD***	***F***	***p value***
Age (Years)		75.60	6.60	74.80	6.80	75.50	7.00	0.84	NS
Education (Years)		16.20	2.70	15.70	2.80	15.30	3.00	3.90	0.034
Annual Entorhinal Atrophy (%)	***Left***	−1.3	0.02	−2	0.02	−2.1	0.02	4.69	0.01
	Right	−0.8	0.02	−1.7	0.02	−1.9	0.03	5.75	0.003
Annual Hippocampal Atrophy (%)	***Left***	−1.6	0.18	−2.2	0.02	−2.6	0.02	7.74	4.80E-04
	Right	−1.57	0.02	−2.1	0.02	−2.8	0.02	8.78	1.74E-04
		N	**%**	***N***	**%**	***N***	**%**	***Chi-Square***	***p value***
APOE e4 status	***0 copies*** ***1 copy*** ***2 copies***	117 35 4	0.75 0.22 0.03	121 122 38	0.43 0.43 0.14	50 61 29	0.36 0.44 0.21	62.00	1.30E-12
Sex	***Male*** ***Female***	89 67	0.57 0.43	178 103	0.63 0.37	77 63	0.55 0.45	3.30	NS

Table 2

Pathway Enrichment Analysis for Entorhinal Atrophy

Pathway	#Genes Signif in Study	# Background Genes	% Genes Signif in Study	Fold Enrichment	P-val unadjusted	Benjamini-Hochberg p-value
Calcium signaling pathway	18	176	17.48	5.25	2.69E-08	**2.99E-06**
Long-term potentiation	11	68	10.68	8.31	5.13E-07	**2.85E-05**
GnRH signaling pathway	12	98	11.65	6.29	2.16E-06	**8.01E-05**
Long-term depression	10	69	9.71	7.44	5.52E-06	**1.23E-04**
Axon guidance	13	129	12.62	5.18	5.40E-06	**1.50E-04**
Focal adhesion	15	201	14.56	3.83	2.51E-05	**3.97E-04**
Neuroactive ligand-receptor interaction	17	256	16.50	3.41	2.44E-05	**4.51E-04**
Gap junction	10	89	9.71	5.77	4.50E-05	**6.25E-04**
Vascular smooth muscle contraction	10	112	9.71	4.59	2.70E-04	**3.33E-03**
Alzheimer's disease	11	163	10.68	3.47	1.07E-03	**1.18E-02**
Melanogenesis	8	99	7.77	4.15	2.79E-03	**2.78E-02**
Adherens junction	7	77	6.80	4.67	3.46E-03	**3.15E-02**
MAPK signaling pathway	13	267	12.62	2.50	4.87E-03	**4.09E-02**
Progesterone-mediated oocyte maturation	7	86	6.80	4.18	5.97E-03	**4.64E-02**
Regulation of actin cytoskeleton	11	215	10.68	2.63	7.99E-03	5.76E-02
Chemokine signaling pathway	10	187	9.71	2.75	9.49E-03	6.40E-02
Type II diabetes mellitus	5	47	4.85	5.46	1.23E-02	7.75E-02
Pathways in cancer	13	328	12.62	2.04	2.28E-02	1.33E-01
Wnt signaling pathway	8	151	7.77	2.72	2.58E-02	1.35E-01
Apoptosis	6	87	5.83	3.54	2.56E-02	1.40E-01
Melanoma	5	71	4.85	3.62	4.73E-02	2.26E-01

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Table 3

Pathway Enrichment Analysis for Hippocampal Atrophy

Pathway	#Genes Signif in Study	# Background Genes	% Genes Signif in Study	Fold Enrichment	P-val unadjusted	Benjamini-Hochberg p-value
Calcium signaling pathway	16	176	2.00	5.90	4.40E-08	**3.70E-06**
Axon guidance	13	129	1.60	6.50	4.40E-07	**1.90E-05**
ErbB signaling pathway	7	87	0.90	5.20	2.00E-03	5.60E-02
Adherens junction	6	77	0.70	5.00	6.20E-03	1.20E-01
Vascular smooth muscle contraction	7	112	0.90	4.00	7.10E-03	1.10E-01
Purine metabolism	8	153	1.00	3.40	8.60E-03	1.20E-01
Non-small cell lung cancer	5	54	0.60	6.00	9.10E-03	1.00E-01
Glioma	5	63	0.60	5.10	1.50E-02	1.50E-01
GnRH signaling pathway	6	98	0.70	3.90	1.70E-02	1.50E-01
Melanogenesis	6	99	0.70	3.90	1.70E-02	1.40E-01
Long-term potentiation	5	68	0.60	4.70	2.00E-02	1.40E-01
Long-term depression	5	69	0.60	4.70	2.10E-02	1.40E-01
Pathways in cancer	11	328	1.40	2.20	2.70E-02	1.60E-01
Colorectal cancer	5	84	0.60	3.80	3.90E-02	2.20E-01
Endometrial cancer	4	52	0.50	5.00	4.50E-02	2.30E-01
Regulation of actin cytoskeleton	8	215	1.00	2.40	4.60E-02	2.20E-01
Prostate cancer	5	89	0.60	3.60	4.70E-02	2.10E-01
O-Glycan biosynthesis	3	30	0.40	6.40	7.70E-02	3.10E-01
Melanoma	4	71	0.50	3.60	9.50E-02	3.60E-01
Neuroactive ligand-receptor interaction	8	256	1.00	2.00	9.60E-02	3.50E-01
Pancreatic cancer	4	72	0.50	3.60	9.80E-02	3.40E-01