Neurobiology of Aging 34 (2013) 1518.e9–1518.e18

Contents lists available at SciVerse ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

Genetic interactions associated with 12-month atrophy in hippocampus and entorhinal cortex in Alzheimer's Disease Neuroimaging Initiative

Shashwath A. Meda [a], Mary Ellen I. Koran [a,b], Jennifer R. Pryweller [c], Jennifer N. Vega [d], Tricia A. Thornton-Wells [a,*], for the Alzheimer's Disease Neuroimaging Initiative

[a] Center for Human Genetics and Research, Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN, USA
[b] Medical Scientist Training Program, School of Medicine, Vanderbilt University, Nashville, TN, USA
[c] Neuroscience PhD Program, Vanderbilt University, Nashville, TN, USA
[d] Neuroimaging of Neurodevelopmental Disorders Graduate Program, Vanderbilt University, Nashville, TN, USA

ARTICLE INFO

Article history:
Received 1 May 2012
Received in revised form 14 September 2012
Accepted 27 September 2012
Available online 27 October 2012

Keywords:
Alzheimer's disease
Biological pathway
Brain atrophy
Epistasis
Gene-gene interaction
GWIA
GWAS
KEGG
MRI
Neuroimaging
Pathway-based analysis

ABSTRACT

Missing heritability in late onset Alzheimer disease 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 magnetic resonance imaging data available from the Alzheimer's Disease Neuroimaging Initiative, 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 magnetic resonance imaging scans. Approximately 560,000 single nucleotide polymorphisms (SNPs) were available genome-wide. We tested all pairwise SNP-SNP interactions (approximately 151 million) within 212 Kyoto Encyclopedia of Genes and Genomes pathways for association with 12-month regional atrophy rates using linear regression, with sex, *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.

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. Neuropathologic hallmarks of AD include extracellular accumulation of amyloid-β 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 1 genetic risk factor of large effect for late-onset AD (LOAD)—apolipoprotein-E (*APOE*)—with 2 copies of the ε4 allele conferring approximately 6- to 30-fold risk for the disease (Akiyama et al., 1993). More recent genome-wide association studies (GWAS) have identified and replicated 9 additional AD susceptibility genes, including *BIN1*, *CLU*, *ABCA7*, *CR1*, *PICALM*, *MS4A6A*, *CD33*, *MS4A4E*, and *CD2AP* (Belbin et al., 2011; Carrasquillo et al., 2011; Harold et al., 2009; Hollingworth et al., 2011; Naj et al., 2011; Shi et al., 2012). However, all of these have low effect sizes (odds ratios of 0.87–1.23) and cumulatively account for approximately 35% of population-attributable risk (Naj et al., 2011).

A large portion of the "missing heritability" can be attributed to 2 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 that research has not yet explained more of the heritability in LOAD is that most genetic association studies compute statistics at the single marker level and fail to address the underlying biologic 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

* Corresponding author at: Center for Human Genetics and Research, 519 Light Hall, Nashville, TN 37232, USA. Tel.: +1 615 343 0396; fax: +1 615 343 8619.
E-mail address: t.thornton-wells@vanderbilt.edu (T.A. Thornton-Wells).

0197-4580/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neurobiolaging.2012.09.020

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 (Bence et al., 2001). 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 amyloid-β peptides in cerebrospinal fluid, selective measures of brain atrophy using magnetic resonance imaging (MRI), and imaging of glucose hypometabolism and amyloid using positron emission tomography (Dubois et al., 2007). However, out of the above-mentioned biomarkers, MRI-derived measures are the least invasive, thereby conferring a major advantage over other methods. 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 (Dickerson et al., 2011; Dubois et al., 2007; Holland and Dale, 2011; Salat et al., 2011). During early stages of the disease, in subjects with mild cognitive impairment (MCI) and early AD, atrophy is seen primarily in the entorhinal cortex (EC) and hippocampus (HIP) and can reliably predict MCI conversion to AD (Devanand et al., 2007; Pennanen et al., 2004).

Recent genetic studies in LOAD have employed quantitative MRI phenotypes (Potkin et al., 2009; Shen et al., 2010), and others have implemented pathway-based analysis (Akiyama et al., 1993; Hong et al., 2010). However, to our knowledge, no study has combined the 2 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 control subjects (Potkin et al., 2009). 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 (Shen et al., 2010). This study identified multiple single nucleotide polymorphisms (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 previous biological knowledge is 1 such approach offering an alternative way to explore GWAS of complex diseases such as LOAD (Herold et al., 2009). Using a computational bioinformatics approach, Liu et al. (2010) investigated spatial pathway clusters in different AD brain regions and the cross talk 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 used INTERSNP software (http://intersnp.meb.uni-bonn.de) (Herold et al., 2009) to evaluate how genetic interactions tested within known biological pathways influence 12-month atrophy rates in HIP and EC, which, as stated above, are brain regions known to be affected most significantly, consistently, and earliest in LOAD (Mizutani and Kasahara, 1997; Ridha et al., 2006).

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, the National Institute of Biomedical Imaging and Bioengineering, the Food and Drug Administration, private pharmaceutical companies, and nonprofit organizations, as a $60 million, 5-year public-private partnership. The primary goal of ADNI has been to test whether serial MRI, positron emission tomography, other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of MCI and early 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, Veterans Affairs Medical Center and University of California, San Francisco. ADNI is the result of efforts of many coinvestigators from a broad range of academic institutions and private corporations, and subjects have been recruited from more than 50 sites across the United States and Canada. The initial goal of ADNI was to recruit 800 adults, ages 55–90, to participate in the research, approximately 200 cognitively normal older individuals to be followed for 3 years, 400 people with MCI to be followed for 3 years, and 200 people with early AD to be followed for 2 years. For up-to-date information, see www.adni-info.org.

We applied for and were granted permission to use data from the 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. After applying quality control procedures (detailed below), the study sample included 156 control, 281 MCI, and 140 LOAD Caucasian subjects. Demographic and imaging characteristics of the sample are listed in Table 1.

2.2. Genotyping and quality control

SNP genotyping for 620,901 target SNPs covering the entire genome was completed on all subjects using the Illumina

Table 1
Descriptive statistics for ADNI dataset

	Control ($n = 156$)	MCI ($n = 281$)	AD ($n = 140$)	Statistic	
	Mean (SD)			F	p
Age (y)	75.60 (6.60)	74.80 (6.80)	75.50 (7.00)	0.84	NS
Education (y)	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 (%)			χ^2	p value
APOE e4 status					
0 Copies	117 (0.75)	121 (0.43)	50 (0.36)	62.00	1.30E-12
1 Copy	35 (0.22)	122 (0.43)	61 (0.44)		
2 Copies	4 (0.03)	38 (0.14)	29 (0.21)		
Sex					
Male	89 (0.57)	178 (0.63)	77 (0.55)	3.30	NS
Female	67 (0.43)	103 (0.37)	63 (0.45)		

Key: AD, Alzheimer's disease; ADNI, Alzheimer's Disease Neuroimaging Initiative; MCI, mild cognitive impairment; NS, not significant.