Title
Neuroimaging Markers of Genetic Risk for Alzheimer's Disease in Cognitively Healthy Cohorts

Author
Harrison, Theresa Maria

Publication Date
2016

Peer reviewed|Thesis/dissertation

UNIVERSITY OF CALIFORNIA

Los Angeles

Neuroimaging Markers of Genetic Risk for Alzheimer's Disease in Cognitively Healthy Cohorts

A dissertation submitted in partial satisfaction of the requirements for the degree of Doctor of Philosophy in Neuroscience

by

Theresa Maria Harrison

2016

ABSTRACT OF THE DISSERTATION

Neuroimaging Genetic Risk for Alzheimer's
Disease in Cognitively Healthy Cohorts

by

Theresa Maria Harrison
Doctor of Philosophy in Neuroscience
University of California, Los Angeles, 2016
Professor Susan Y. Bookheimer, Chair

Drugs developed to slow, halt or reverse the progression of Alzheimer's Disease (AD) have failed to alter the course of the disease in clinical trials. One possible explanation is that drugs need to be administered earlier, before the onset of clinical symptoms. AD-related pathological processes that occur before clinical symptoms emerge define the preclinical phase of the disease. Neuroimaging biomarkers and genetics together present a powerful system for characterizing potential preclinical changes in the brain. The work presented in this volume is predicated on the need for a better understanding of genetic risk and neuroimaging biomarkers for AD in healthy adults. In Chapter 1, a thorough review of neuroimaging genetics in AD is presented. The studies described in Chapters 2 and 3 explore the relationship between functional connectivity and the apolioprotein E (*APOE*) risk allele, APOEε4. In the first study a pattern of context-dependent connectivity was uncovered that indicates APOEε4 carriers disengage key cortical regions from the hippocampus during a memory task. These findings support the growing consensus that functional connectivity changes may be among the earliest

preclinical markers of AD-related changes in the brain. The second study utilized resting state fMRI scans from 570 healthy college-age adults. Young carriers of APOEε4 showed decreased connectivity between key regions involved in AD and increased segregation of task-positive and task-negative regions. This work is a crucial reminder that genetic risk for AD has important implications across the lifespan and that gene-biomarker associations must be tracked over time to identify changes that might be signs of imminent clinical decline. In Chapter 4, the focus expands to include additional genetic risk factors for AD beyond *APOE*. This study is the first to show that a genetic risk score for AD is significantly associated with hippocampal thinning over two years in a cohort of older, cognitively healthy adults. Finally, Chapter 5 is a call for the further development of polygenic approaches to studying neuroimaging markers of genetic risk for AD. Together, this volume represents steps toward understanding how genetic risk for AD and neuroimaging can be used to identify individuals at greatest risk for decline.

The dissertation of Theresa Maria Harrison is approved.

Giovanni Coppola

Daniel Geschwind

Gary W. Small

Paul Thompson

Susan Y. Bookheimer, Committee Chair

University of California, Los Angeles

2016

I dedicate this work to my parents, Jeph and Josephine Harrison,

who have always been my greatest mentors.

TABLE OF CONTENTS

ABSTRACT ii

ACKNOWLEDGEMENTS viii

VITA xi

CHAPTER 1. Background: Neuroimaging Genetics of Alzheimer's Disease 1

Introduction 1
Pathological and Clinical Features of Alzheimer's Disease 4
Neuroimaging Highly Penetrant Genetic Causes of Alzheimer's Disease 7
Familial AD 10
Down Syndrome 17
Neuroimaging Genetic Risk for Alzheimer's Disease 20
APOE 20
TOMM40 33
TREM2 35
GWAS-Identified Alzheimer's Disease Risk Genes 37
BDNF 47
Neuroimaging-Identified Alzheimer's Disease Risk Genes 49
Family History of Alzheimer's Disease 53
Future Directions and Challenges 56
Relevance and Impact 59
Chapter 1 References 65

CHAPTER 2. Altered Memory-Related Functional Connectivity of the Anterior and Posterior Hippocampus in Older Adults at Increased Genetic Risk for Alzheimer's Disease 82

Abstract 83
Introduction 83
Materials and Methods 86
Results 95
Discussion 108
Supplementary Information 114
Chapter 2 References 120

CHAPTER 3. APOEε4 Effects on the Intrinsic Architecture of Neural Networks as Early Risk Biomarkers of Alzheimer's Disease 125

Abstract 125
Significance Statement 126
Introduction 127
Materials and Methods 129
Results 133
Discussion 143
Chapter 3 References 147

CHAPTER 4. An Alzheimer's Disease Genetic Risk Score Predicts Longitudinal Thinning of Hippocampal Complex Subregions in Healthy Older Adults 150

Abstract 150
Significance Statement 151
Introduction 152
Materials and Methods 154
Results 161
Discussion 170
Chapter 4 References 176

CHAPTER 5. Discussion: Neuroimaging genetic risk for Alzheimer's Disease in preclinical individuals 180

Abstract 180
Introduction 181
Neuroimaging Preclinical Alzheimer's Disease 181
Neuroimaging and AD Candidate Genes 182
Polygenic Risk Scores 187
Regression Approaches to Polygenic Risk 190
Advanced Association Models 192
Limitations 193
Implications for Clinical Trials 200
Chapter 5 References 203

CONCLUSION 211

Conclusion References 214

APPENDIX. A Model For Teaching Advanced Neuroscience Methods: A Student-Run Seminar to Increase Practical Understanding and Confidence 215

Abstract 215
Introduction 216
Materials and Methods 218
Results 223
Discussion 225
Appendix References 229

ACKNOWLEDGEMENTS

This work is the culmination of 4 years and 10 months of my earnest effort. The pursuit of a doctoral degree can sometimes feel like a very solitary and lonely endeavor. Thus, I am greatly indebted to those individuals who collaborated with me, supported me or otherwise made me feel like a friend and colleague during my time at UCLA. I extend my gratitude and thanks to each of you.

Mentor-mentee relationships take many forms. Susan and I have become friends and allies above all else. Susan's mentoring style allowed me to develop and grow into an independent researcher, which I hope will serve me well as I continue my academic career. She taught me the importance of collaboration and service within the academic model. Susan's skill in conveying scientific findings in the most powerful, persuasive and yet straightforward way is admirable and I hope to master it one day. Until then, I will be sending her my manuscript titles and abstracts for a touch of her unique style. Susan, thank you for taking me on as your graduate student and for supporting every idea I ever brought to your desk. I look forward to years of friendship and collaboration.

The Neuroscience Interdepartmental Graduate Program at UCLA attracts people from across the country and across the breadth of scientific inquiry. My peers in the 2011 cohort have inspired me again and again. I feel very lucky to have been part of this special group. I must specifically acknowledge two of them, Rachel Jonas and Esther Nie, for their incredible friendship and unwavering confidence in me. I have loved my time as a doctoral student, and it's always been Esther and Rachel who have made even the worst days seem like nothing more than an excuse for a coffee run or happy hour.

I had the great fortune of being born into a loving, stable family where academic excellence was the norm. My siblings' and my achievements were not celebrated so much as we were celebrated as individuals. My parents encouraged us to take ownership of our own life

trajectories and hardly ever questioned the steps we took. They trusted us, loved us and reminded us that they would always be there. For these, and countless other reasons, I am dedicating this body of work to my mother, Josephine, and my father, Jeph.

My greatest champion is my partner Jacob Aptekar. He takes true delight in my successes and has brushed aside every failure without a second thought. He greets me with a warm smile every single evening, even if his own day was challenging and stressful. Together we have built a life that seeks to balance hard work with plenty of time for family, friends, travel and each other. I feel confident that we will continue to seek that balance and support each other in every effort. As we leave our beloved Los Angeles to move on to new jobs, I know that we can start over and build a happy home because home is wherever we are together.

Portions of Chapter 1 are modified excerpts from Harrison, T.M., Burggren, A.C., Bookheimer, S.Y. (2015) Neuroimaging Genetics of Alzheimer's Disease in *Neuroimaging Genetics: Principles and Practices,* Kristin L Bigos, Ahmad R Hariri, Daniel R Weinberger (Eds.) Oxford University Press. Chapter 2 is a version of Harrison, T.M., Burggren, A.C., Small, G., Bookheimer, S.Y. (2015) Altered memory-related functional connectivity of the anterior and posterior hippocampus in older adults at increased genetic risk for Alzheimer's disease; *Human Brain Mapping.* Chapter 4 is a version of Harrison, T.M., Mahmood, Z., Lau, E.P., Karacozoff, A.M., Burggren, A.C., Small, G., Bookheimer, S.Y. (2016) An Alzheimer's Disease Genetic Risk Score Predicts Longitudinal Thinning of Hippocampal Complex Subregions in Healthy Older Adults; *eNeuro*. Portions of Chapter 5 are modified excerpts from Harrison, T.M. and Bookheimer, S.Y. (2015) Neuroimaging genetic risk for Alzheimer's Disease: From candidate genes to polygenic approaches. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging*. The appendix contains a version of Harrison, T.M., Ching C.R.K., Andrews, A.M. (2016) A Model for Teaching Advanced Neuroscience Methods: A Student-Run Seminar to Increase Practical Understanding and Confidence; *The Journal of Undergraduate Neuroscience*

Education. Much of this work was supported by the National Institute of Aging (grant number 1F31AG047041).

VITA

EDUCATION:

09/2011-12/2016*	University of California at Los Angeles, Los Angeles, CA
**expected*	Doctor of Philosophy in Neuroscience
08/2005-05/2009	Georgetown University, Washington, DC Bachelor of Science, Neurobiology Major, Psychology Minor

RESEARCH POSITIONS:

07/2012-12/2016	Center for Cognitive Neuroscience, UCLA, Los Angeles, CA, Principal Investigator: Dr. Susan Bookheimer – *Graduate Student Researcher*
10/2009-08/2011	Cognitive Neurology and Alzheimer's Disease Center, Northwestern University, Chicago, IL, Director: Dr. M. Marsel Mesulam, Principal Investigator: Dr. Emily Rogalski – *Research Programs Coordinator*
02/2007-06/2009	Kromer Laboratory, Georgetown University Medical School, Washington, DC, Principal Investigator: Dr. Lawrence Kromer - *Research Assistant*

MANUSCRIPTS:

1. Rogalski, E., Cobia, D., **Harrison, T.M.**, Wieneke, C., Thompson, C.K., Weintraub, S., Mesulam, M-M. (2011) Anatomy of language impairments in primary progressive aphasia. *The Journal of Neuroscience* 31(9): 3344-50
2. Rogalski, E., Rademaker, A., **Harrison, T.M.**, Helenowski, I., Johnson, N., Bigio, E., Mishra, M., Weintraub, S., Mesulam, M-M. (2011) APOE E4 in a susceptibility factor in amnestic but not aphasic dementias. *Alzheimer's Disease and Associated Disorders* 25(2): 159-63
3. Rogalski, E., Cobia, D., **Harrison, T.M.**, Wieneke, C., Weintraub, S., Mesulam, M-M. (2011) Progression of language decline and cortical atrophy in subtypes of primary progressive aphasia. *Neurology* 76(21): 1804-1810
4. **Harrison, T.M.**, Weintraub, S., Mesulam, M-M., Rogalski, E. (2012) Superior memory and higher cortical volumes in unusually successful advanced aging. *The Journal of the International Neuropsychological Society* 18:1-5
5. Olofsson, J.K., Rogalski, E., **Harrison, T.M.,** Mesulam, M-M., Gottfried, J.A. (2013) A cortical pathway to olfactory naming: evidence from primary progressive aphasia. *Brain* 136(4):1245-59
6. **Harrison, T.M.**, Burggren, A.C., Small, G., Bookheimer, S.Y. (2016) Altered memory-related functional connectivity of the anterior and posterior hippocampus in older adults at increased genetic risk for Alzheimer's disease. *Human Brain Mapping* 37(1):366-80 Epub 2015 Oct. 27
7. **Harrison, T.M.** and Bookheimer, S.Y. (2016) Neuroimaging genetic risk for Alzheimer's Disease: From candidate genes to polygenic approaches. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging* 1(1):14-23
8. **Harrison, T.M.**, Mahmood, Z., Lau, E.P., Karacozoff, A.M., Burggren, A.C., Small, G., Bookheimer, S.Y. (2016) An Alzheimer's Disease Genetic Risk Score Predicts Longitudinal Thinning of Hippocampal Complex Subregions in Healthy Older Adults. *eNeuro*
9. **Harrison, T.M.**, Ching, C.R., Andrews, A.M. (2016) A model for teaching advanced neuroscientific methods: A student-run seminar to increase practical understanding and confidence. *The Journal of Undergraduate Neuroscience Education.*
10. **Harrison, T.M.**, Brown, J.A., Knodt, A., Drabant Conley, E., Bookheimer, S.Y., Hariri, A.R. (*Under Review*) The Effects of APOEε4 on the Intrinsic Architecture of Neural Networks in Young Adults. *Molecular Psychiatry.*
11. Burggren, A.C., Mahmood, Z., **Harrison, T.M.**, Karacozoff, A., Small, G.W., Bookheimer, S.Y. (*Under Review*) Reduced hippocampal thickness in APOEε3 subjects with longer *TOMM40* poly-T lengths compared to subjects with shorter lengths. *Alzheimer's and Dementia*.

12. **Harrison, T.M.**, McLaren, D.G., Moody, T.D., Feusner, J.D., Bookheimer, S.Y. (*Under Review*) Generalized Psychophysiological Interaction (PPI) Analysis of Memory Related Connectivity in Individuals at Genetic Risk for Alzheimer's Disease. *JoVE.*
13. **Harrison, T.M.**, Bookheimer S.Y. (*In Prep*) Neuroimaging Correlates of APOEε4 Across the Human Lifespan: Implications for Biomarker Development in Alzheimer's Disease. *Neuroimage: Clinical*

BOOK CHAPTERS:

1. **Harrison, T.M.**, Burggren, A., Bookheimer, S.Y. (2015) Neuroimaging Genetics of Alzheimer's Disease. In *Neuroimaging Genetics,* Kristin L Bigos, Ahmad R Hariri, Daniel R Weinberger (Eds.) Oxford University Press

ABSTRACTS: *(selected from 18)*

Harrison, T.M., Geula, C., Shi, J., Samimi, M., Weintraub, S., Mesulam, M-M., Rogalski, E. Neuroanatomic and pathologic features of cognitive SuperAging. The Society for Neuroscience 2011

Harrison, T.M.* Ching,C.R.*, Einstein, M.C., Bonnano, S.L., Andrews, A.M., Levine, M. A model for teaching advanced neuroscience methods to graduate students: A student-run seminar to increase practical understanding and confidence. The Society for Neuroscience 2013 *co-first authors

Harrison, T.M. Bautista, A., Burggren, A.C., Coppola, G., Small, G., Bookheimer, S. *CLU* risk variant is associated with cortical thinning of the subiculum in a longitudinal study of cognitively healthy older adults. The Society for Neuroscience 2013

Harrison, T.M., Burgrren, A.C., Small, G., Bookheimer, S.Y. Memory-related functional connectivity differences in older adults at increased genetic risk for Alzheimer disease. Organization for Human Brain Mapping 2014

Harrison, T.M., Lau, E.P., Mahmood, Z., Burggren, A.C., Small, G.W., Bookheimer, S.Y. Weighted and Unweighted Genetic Risk Scores are Associated with Longitudinal Cortical Thinning of Hippocampal Complex Subregions. Alzheimer's Association International Conference 2015 & The Alzheimer's Imaging Consortium 2015

Harrison, T.M., Lau, E.P., Mahmood, Z., Burggren, A.C., Small, G.W., Bookheimer, S.Y. Genetic risk scores for Alzheimer's Disease are associated with thinning of hippocampal complex subregions. The Society for Neuroscience 2015

Harrison, T.M., Mahmood, Z., Lau, E.P., Burggren, A.C., Small, G.W., Bookheimer, S.Y. A risk score for Alzheimer disease including *APOE*, *CLU*, *PICALM* and family history is associated with longitudinal thinning of the hippocampus. Alzheimer's Disease International 2016

Harrison, T.M., Brown, J.A., Knodt, A., Drabant Conley, E., Bookheimer, S.Y., Hariri, A.R. Resting State Network Differences Related to APOEε4 in a Large Cohort of Young Adults. Organization for Human Brain Mapping 2016

FELLOWSHIPS AND AWARDS:

- Ruth L. Kirschstein National Research Service Award (NRSA): 1F31AG047041
- The Samuel Eiduson Student Lecture Award, UCLA, 2016
- Achievement Awards for College Scientists (ARCS) Scholar, 10/2012-11/2016
- Predoctoral Fellowship, Neurobehavioral Genetics, 08/2012-07/2014: 5T32MH073526
- UCLA Advanced Neuroimaging Program, Summer 2012: 5T90DA022768

Travel Awards:

- Organization for Human Brain Mapping Travel Award 2016
- Neuroscience Interdepartmental Graduate Program Travel Award for SfN 2015
- Alzheimer's Association International Conference 2015 Travel Fellowship
- Brain Research Institute/Semel Institute Graduate Travel Award for SfN 2013

CHAPTER 1

Background: Neuroimaging Genetics of Alzheimer's Disease

Introduction

When the human genome project was completed in 2003 many believed it marked the beginning of an era of genomic medicine. The genetic basis of highly heritable neurological disorders like Alzheimer's disease (AD), autism and schizophrenia would be discovered and applied to the development of new therapies. Some went one step further, predicting that personalized genomic medicine would soon follow the elucidation of disease-specific genetic fingerprints. These hopeful predictions have yet to come to fruition and, in their stead, an appreciation for the complexity of polygenic brain disorders has been steadily growing. Current estimates indicate that both autism and schizophrenia are associated with hundreds of genes and even more single nucleotide polymorphisms (SNPs) (1; 2). Similarly, the genetics of AD have proven to be a complex problem despite the early promise of the apolipoprotein E (*APOE*) gene. More than 20 years ago, Corder and colleagues discovered that a single copy of the E4 allele of *APOE* increased an individual's risk of getting AD 4-5-fold, and two copies increased AD risk up to 15-fold (3). Another way to measure the risk conferred by *APOE* is to examine what proportion of AD heritability can be accounted for by *APOE*. Twin studies reveal that the heritability of AD is 60-80% and *APOE* accounts for about 50% of the variation in heritability (4–6). Current understanding of human genetics allows one to appreciate that *APOE* has a relatively huge effect on AD risk. Still, roughly half of the variation in heritability is presumably due to other genetic risk factors, of which there are now greater than 20 validated candidates (7).

The increasing number of risk loci associated with AD and other neurological disorders is the result of a recent surge in the efficiency of genomic technologies combined with data sharing efforts that have allowed researchers to identify genomic loci associated with disease,

even with very low effect sizes. Low effect size associations require extremely large cohorts in order to provide sufficient power for detection. Recently, a consortium of AD researchers created a large dataset of ~70,000 subjects known as the International Genomics of Alzheimer's Project (IGAP) (8). Analyses performed on this large dataset were able to reveal 11 new AD risk loci, in addition to confirming previously identified loci (7). Based on this success and the success of other consortiums, there continues to be interest in ever-larger cohorts. However, as sample sizes continue to rise 'significant' effect sizes get smaller and smaller and there may be a risk of being statistically over-powered. Too much statistical power could lead to the identification of spurious risk loci that are not actually associated with the disease phenotype. The process of parsing out true and spurious associations will certainly be a topic of research in the coming years. Today, the major challenge is understanding the clinical and pathological roles played by each of the AD risk genes and their products. Neuroimaging has emerged as one popular method for characterizing genetic AD risk factors in humans. Progress on this front will be reviewed in subsequent sections.

Neuroimaging methods, including magnetic resonance imaging (MRI) and positron emission tomography (PET) imaging, are often used to study AD in humans. Recent advances in MRI hardware and pulse sequence development have enabled characterization of brain structure and function at improved spatial and temporal resolutions with higher signal to noise ratio and better tissue-type contrast. Structural MRI (sMRI) is now able to reliably delineate subregions of the hippocampus while functional MRI (fMRI) has reached spatial resolution high enough to resolve functional units in visual cortex (9; 10). Improved tissue contrast in sMRI allows for consistent delineation of white and gray matter, making cortical thickness and volumes estimates more robust (11). fMRI sequences are utilizing fast, multiband acquisition techniques that minimize signal dropout without sacrificing signal-to-noise ratios (12). In addition, diffusion weighted imaging (DWI) acquisition and analysis are constantly evolving and improving. Gradient strengths and the number of directions in which pulses are applied are

increasing as equipment improves, allowing for better estimation of water diffusion at every voxel (13). Spatial resolution continues to increase with recent studies boasting sub-millimeter in-plane resolution (14). In PET imaging, the development of ^{18}F radiotracers, such as florbetapir, has made it easier for more sites to acquire amyloid deposition data (a measure of AD pathology in the brain) than before when only the more volatile ^{11}C tracers were available (15). In addition, there is now preliminary data on tau-specific tracers including one ^{18}F tracer called T807 which, when analyzed alongside Aβ-specific tracers, will help to elucidate the temporal dynamics of Aβ and tau deposition in AD pathogenesis (16). Together, these advances in neuroimaging will allow for more sensitive and accurate biomarker detection and longitudinal monitoring, both of which are important to help identify individuals who are at increased risk for AD.

This chapter focuses on AD, the genetic basis of the disease and how genetic markers have been studied in combination with neuroimaging methods to help elucidate the effects of genetic risk for AD on the structure and function of the brain. After a brief review of the clinical and pathological features of AD, we begin by discussing the rare autosomal dominant forms of AD that result from a mutation in one of three genes: *APP*, *PSEN1* or *PSEN2*. Next, we cover how neuroimaging has helped to shed light on the still-murky relationship between AD and Down syndrome, a condition in which individuals have a third copy of chromosome 21 and, therefore, an extra copy of *APP*. The next portion of the chapter focuses on less penetrant, statistical genetic risk factors. Sections are organized to reflect the strength of the risk conferred by each locus, beginning with the discovery of the APOEε4 risk allele in the early 1990s, followed by the recent discoveries of strong AD associations with *TOMM40* and *TREM2* variants, and finally the identification of AD associated loci through genome wide association studies (GWAS). We will also cover the growing literature linking the neurotrophic factor *BDNF* with AD. Next, genetic risk factors identified through studies of AD-related neuroimaging biomarkers and endophenotypes will be discussed. In each section, relevant work in structural

and functional imaging will be reviewed. In addition, challenges in the field of neuroimaging genetics of AD will be discussed, alongside possible future directions for the field. Finally, the relevance and impact of neuroimaging genetics research in the fight against AD will be examined.

Pathological and Clinical Features of Alzheimer's Disease

More than 100 years ago, at a meeting of psychiatrists in Germany, Alois Alzheimer presented a case study of a woman who had suffered from progressive memory loss and other cognitive and psychiatric symptoms (17; 18). The most enduring aspect of his presentation was his description of abnormal deposits that he discovered after silver staining the patient's brain tissue. These deposits, called plaques and tangles based on their morphology under the microscope, remain the defining neuropathological features of AD today.

At the molecular level there are two main proteins that accumulate abnormally in AD, beta-amyloid (Aβ) and tau. Soluble Aβ oligomers collect to form extracellular neuritic plaques while hyperphosphorylated tau proteins form intracellular inclusions called neurofibrillary tangles. The gene that transcribes Aβ, as well as two genes that transcribe enzymes involved with regulating Aβ isoforms, are each the site of many mutations that give rise to dominantly inherited, familial AD. In addition, the link between Down syndrome and AD, which will be described in a subsequent section, can be traced back to Aβ. The connection is based on the fact that Aβ is transcribed from a gene on chromosome 21, the chromosome that is in triplicate in Down syndrome. It is believed that the third copy of this gene results in Aβ overexpression, which may be the cause of the near 100% incidence of AD in adults with Down syndrome. All of this evidence helped lead to the belief that Aβ is the primary pathology in AD and to the "amyloid cascade hypothesis" (19; 20). This hypothesis states, generally, that the ineffective clearance of Aβ leads to the deposition of plaques and that this is the first in a cascade of molecular events that eventually cause neuronal death and, in some cases, vascular damage. However, it has

been shown that Aβ pathology does not correlate with clinical symptoms of AD while tau pathology does (21). The formation of neurofibrillary tangles results from the polymerization of hyperphosphorylated tau. Under normal circumstances, tau is a major component of neuronal cytoskeleton but when tau becomes hyperphosphorylated it tends to accumulate into neurofibrillary tangles. These tangles are associated with the marked loss of synapses in AD, which is putatively caused, at least in part, by a breakdown in cytoskeletal maintenance (22). These findings have led to increased attention on tau and a convincing counterargument to the amyloid cascade hypothesis. Now it appears that other proteins may also play a role in AD, which may be further evidence that Aβ is not a trigger, but just one of several pathologies in AD. Very recent work has shown that two proteins known to play a role in other neurodegenerative diseases are, in fact, also associated with AD. For example, TDP-43 inclusions, a major feature of frontotemporal lobar degeneration (FTLD), have been shown to occur at a higher rate in those with AD compared to healthy controls (23). In addition, in mice it has been shown that decreased progranulin, another protein associated with FTLD, encourages Aβ deposition (24). Finally, the amyloid cascade hypothesis is also called into question based on data from PET imaging studies. PET scans acquired with Aβ specific tracers have revealed that individuals can be positive for Aβ in the brain and have no clinical symptoms. This state can persist for years. So while it is undeniable that Aβ pathology is *necessary* for a diagnosis of AD, it remains unclear whether it is *sufficient* to initiate the 'cascade' of events that leads to full-blown AD or whether it is just one of many factors that together cause AD. The emerging neuropathological picture of AD is complex. Hopefully, the development of additional, specific PET tracers will resolve some of the complexity by increasing our understanding of the temporal and spatial dynamics of each type of proteinaceous inclusion associated with AD.

Clinically, dementia is defined as the loss of cognitive ability that interferes with activities of daily living. Dementia can be caused by many conditions. Differential diagnosis based on clinical symptoms, neuroimaging biomarkers and other criteria is necessary in order to identify

the cause of a dementia syndrome. AD is the most frequent cause of dementia, accounting for 60-80% of cases (25; 26). The next most common cause is vascular dementia which accounts for about 10% of cases (26). It should be noted, however, that in about half of AD cases there is also concomitant vascular pathology discovered at autopsy (27). The most common presentation for AD involves a slow and steady decrease in episodic memory function as well as dysfunction in at least one other cognitive domain. In 2011, the diagnostic criteria for AD got a much-needed update. The National Institute on Aging worked alongside the Alzheimer's Association to form a working group of experts who reviewed the then-current criteria first published in 1984 (28). The 1984 guidelines were primarily based on clinical presentation and focused on memory impairment. This meant that in order to receive a diagnosis of AD, clinical symptoms needed to already be present and interfering with daily activities (26). However, research over the past 20 years has shown beyond a doubt that AD pathogenesis is a process that begins long before the emergence of cognitive impairment. This early, pre-symptomatic stage is estimated to last as long as 15-20 years in some patients (29). In order to better account for the long and slow progression of AD during the preclinical phase, the new criteria describe three stages of AD: preclinical AD, mild cognitive impairment (MCI) due to AD, and dementia due to AD (30). Another change to the criteria incorporates biomarker data in the process of diagnosis (31). Possible biomarkers for AD include levels of Aβ and tau analytes in cerebrospinal fluid (CSF), deposition of Aβ in the brain detected with PET imaging and hippocampal atrophy measured by MRI. It has been shown that decreased levels of Aβ in the CSF, increased levels of phosophorylated tau in the CSF, Aβ positivity as measured with PET tracers (cutoff ratios used to define positive or negative designations are still an active area of research) and hippocampal thickness or volume loss are all indications of possible AD. More work is needed before biomarker testing can be used to conclusively diagnose AD. The continued collection and development of these biomarker measurements are especially important to efforts to identify individuals who are in the early stages of AD, before clinical

symptoms emerge. These are the individuals who would benefit most from any available interventions or therapies. In addition, biomarkers provide critical benchmarks for monitoring the success of experimental treatments.

A key concept in AD pathophysiology is that clinical symptoms emerge only after there has been a long period of degeneration resulting in substantial neuronal loss (32). Indeed, this is true of all age-related neurodegenerative disorders. The superstructure of neuronal circuitry is complex and the result of both genetic and environmental effects compounded over one's lifetime. Thus, the prospect of regeneration after extensive cell death seems more like science fiction than a reasonable therapeutic goal. Therefore, investigators are increasingly focusing on the phase of AD progression that occurs before clinical symptoms can be detected. This phase, called preclinical or pre-symptomatic AD, is marked by progressive neuronal loss in the brain, especially the hippocampus, and by Aβ accumulation. It has been posited that some AD treatment trials have failed to show a positive effect because the drugs were given too late in the course of the disease (30). Preclinical AD is a hypothetical state (subjects may die before progressing to clinical AD) during which intervention aimed at halting disease progression may be most effective. Since its proposed addition to the AD diagnostic framework in 2011, many studies have attempted to focus on preclinical AD, which is still unfortunately difficult to define in the absence of longitudinal data. There are, however, exceptions to this definition problem. For example, the preclinical AD phase can be reliably identified in subjects who carry genetic mutations that cause dominantly inherited forms of AD. Therefore, families with these mutations are extremely valuable to the AD research community.

Neuroimaging Highly Penetrant Genetic Causes of Alzheimer's Disease

In the introduction or background sections of most papers written about AD the authors will list statistics detailing the incidence and prevalence of the disease. If the authors are interested in genetics, they may also cite studies that describe the heritability of AD and lifetime

risk given certain genetic risk factors, including family history of the disease. In these cases, the authors are invariably referring to sporadic, late-onset AD, which is, by far, the most common form of the disease. There are, however, people who develop AD as a result of an identifiable, underlying genetic cause. These genetic causes fall into two categories: familial AD and Down syndrome. The following sections will cover these highly penetrant forms of AD and how neuroimaging genetics findings in these unique cohorts can inform the study of AD in general. For a summary of the literature reviewed in this portion of the chapter see Table 1.1.

Table 1.1 Genetic Causes of and Risk Genes for AD: Neuroimaging Modalities in the Literature and Representative References. A ✔ mark indicates that there is published work exploring the relationship of a given genetic mutation or risk factor and a given neuroimaging modality. Citations of studies referred to in the text are given.

Genetic Anomaly	Gene	First Associated with AD	sMRI	DWI	t-fMRI	rs-fMRI	PET
Point Mutation	*APP*	1991 (33)	✔ (40–42,44, 45,48–51)	✔ (53)	✔ (56; 58)	✔ (60; 61)	✔ (42; 62)
	PSEN1	1992 (34)	✔ 40–42,44,45, 47–52	✔ (52; 53)	✔ (55; 56; 58; 59)	✔ (55; 60; 61)	✔ (42; 62; 63)
	PSEN2	1995 (35)	✔ (42; 45)			✔ (60; 61)	✔ (42)
Duplication	*APP*	2006 (64)					
Trisomy 21	*APP*	1948 (67)	✔ (69–74)	✔ (75)			✔ (77–79)
Poly-T repeat	*TOMM40*	2010 (154)	✔(161; 162)	✔(163)		✔(265)	
SNPs	*APOE*	1993 (3)	✔(89–98; 125–130; 139–145)	✔ (99–101, 120,126 ,141)	✔ (57,102–104, 106,107, 127,128)	✔(108–112; 133; 136)	✔(113–119,133, 142–146)
	TREM2	2013 (165; 166)	✔(176; 177)				
	CLU	1990 (182) (2009 (168; 179))	✔(186; 187)	✔(188)	✔(132; 190)	✔(191)	
	PICALM	2009 (179)	✔(186; 195; 196)			✔(191)	✔(197)
	CR1	2009 (164)	✔(195; 200)				
	BIN1	2010 (179)	✔(195)				✔(197)
	ABCA7	2011 (180)					✔(204)
	EphA1	2011 (180; 192)					✔(204)
	CD33	2011 (176,188)					✔(209)

Familial AD

Early-onset AD is the clinical manifestation of the disease before the age of 65. In the vast majority of cases, early-onset AD is caused by a rare, autosomal dominant form of the disease, characterized by a mutation in one of three genes. Together, patients who have one of these mutations are diagnosed with what is called familial AD (FAD). The three genes with known mutations that cause FAD are amyloid precursor protein (*APP*), presenilin 1 (*PSEN1*) and presenilin 2 (*PSEN2*) (33–35). More than 50 specific deleterious mutations in *APP* have been discovered but these only account for less than 10% of early-onset AD cases. In contrast, more than 175 specific deleterious mutations in *PSEN1* have been identified and these account for up to 70% of FAD cases. Less than 5% of cases are caused by *PSEN2* mutations, of which just over a dozen have been identified (36). Average age of onset varies across the three genes, with *PSEN1* mutations leading to earlier onsets (~42 years old) followed by APP (~52 years old) and *PSEN2* (~57 years old). Since the discovery of these highly penetrant AD genes over 20 years ago, studies of their function, both in normal and mutated conditions, have provided strong support to the so-called 'amyloid cascade hypothesis' (20). *APP*, *PSEN1* and *PSEN2* functionally converge on the production of Aβ, the peptide that aggregates to form extracellular amyloid plaques, one of the two primary neuropathological features of AD. Specifically, APP encodes the protein precursor of the pathogenic Aβ oligomer. *PSEN1* and *PSEN2* encode peptides that are components of secretase complexes, enzymes that modify proteins at specific cleavage sites. The protein presenilin 1 is a proteolytic subunit of a complex called gamma-secretase, which is perhaps best known for its role in cleaving the amyloid precursor protein, the protein product of the *APP* gene. Mutations in *PSEN1* result in an overproduction of the pathogenic Aβ peptide. Mutations in *PSEN2* cause a very similar effect, altering presenilin 2 such that gamma-secretase activity is disrupted leading to over production of pathogenic Aβ. Interestingly, recent work has indicated that there may also be mutations in these genes that decrease risk for AD. In a recent study led by Kari Stefansson and colleagues,

a unique Swedish population was found to harbor a protective mutation in *APP* that actually decreased risk for AD in carriers (37). The exact mechanism of this protection remains unknown but, presumably, the mutation causes a decrease either in amyloid precursor protein levels or, more downstream, results in a decrease of the amyloidogenic oligomers.

A clinical feature of FAD that is useful in research is that specific mutations are associated with a relatively precise age of onset of disease. Because of this, researchers can stage the preclinical phase of a mutation carrier based on the age of onset of a parent or family member who carried the same mutation (38). Thus, biomarker data from carriers of different mutations can be pooled according to preclinical stage, represented by years-to-expected-onset. Pooling data across specific rare mutation types is essential in order to assemble large cohorts for research. The Dominantly Inherited Alzheimer Network (DIAN) is a worldwide network of FAD research centers based out of Washington University in St. Louis, which has spearheaded much of the relatively large cohort neuroimaging research in FAD mutation carriers (39) .

sMRI-based measurements indicate that the rate of change of hippocampal volume is higher in FAD mutation carriers than in age-matched non-carriers (40; 41). Hippocampal thinning or shrinkage is a feature of AD but it is a tricky biomarker candidate because hippocampal volume loss is also associated with normal aging. It is now believed that it is the rate of that loss that is important, with slow changes indicating normal aging and a faster trajectory indicating AD. A significant difference between FAD mutation carriers and non-carriers in the rate of change of hippocampal volume loss is evident ~2-5 years before the expected onset of disease (40–42). Measuring rate of change, though, requires longitudinal data, which is not ideal for diagnostic use in a clinical setting. Cross-sectionally, it has been shown that FAD mutation carriers have decreased hippocampal volume bilaterally compared to non-mutation-carrier controls up to 15 years before the expected onset of disease (42). This is potentially a

very early biomarker, but difficult to assess on an individual basis since size and shape and, thus, volume of hippocampi vary in healthy populations as well as disease populations (43).

In addition to hippocampal changes, atrophy in the cortex in FAD mutation carriers has been studied using sMRI. One study, completed on preclinical FAD mutation carriers and non-carriers in a Swedish cohort, found that the mutation carriers had decreased gray matter volume in the left precuneus, superior temporal gyrus and fusiform gyrus (44). Another large study showed that there was a significant difference in gray matter volumes between mildly symptomatic (Clinical Dementia Rating scale =0.5) carriers and healthy non-carriers in the thalamus and putamen, as well as in cortical regions, including the temporal lobe, precuneus and the cingulate gyrus (45). The authors observed the same differences, with a greater magnitude, in moderate-to-severely symptomatic carriers. Another group of investigators, led by Bradford Dickerson, used Freesurfer, a computational neuroanatomy software suite, to test for differences between preclinical *PSEN1* mutation carriers and non-carriers in so-called AD-signature regions of cortex. These regions were based on previous studies comparing the cortical thickness of sporadic, late-onset AD patients and controls (46) (Figure 1.1A). In other words, the AD-signature regions represent cortical regions that are particularly vulnerable to atrophy in AD. In the *PSEN1* mutation carriers, they found that AD-signature regions as a whole were thinner when compared to non-carriers (47). Upon further analysis, the authors found that differences in the angular gyrus, superior parietal lobule and precuneus were driving this effect (Figure 1.1B). This is in agreement with other evidence that the precuneus is one of the earliest cortical regions to begin to atrophy in FAD mutation carriers (48). Another study that used tensor-based morphometry found significant differences in cortical regions only when demented FAD subjects were compared to non-carriers (49). However, the lack of differences between pre-symptomatic carriers and non-carriers might be related to low sample size, as other studies of relatively small cohorts also were unable to detect differences (50).

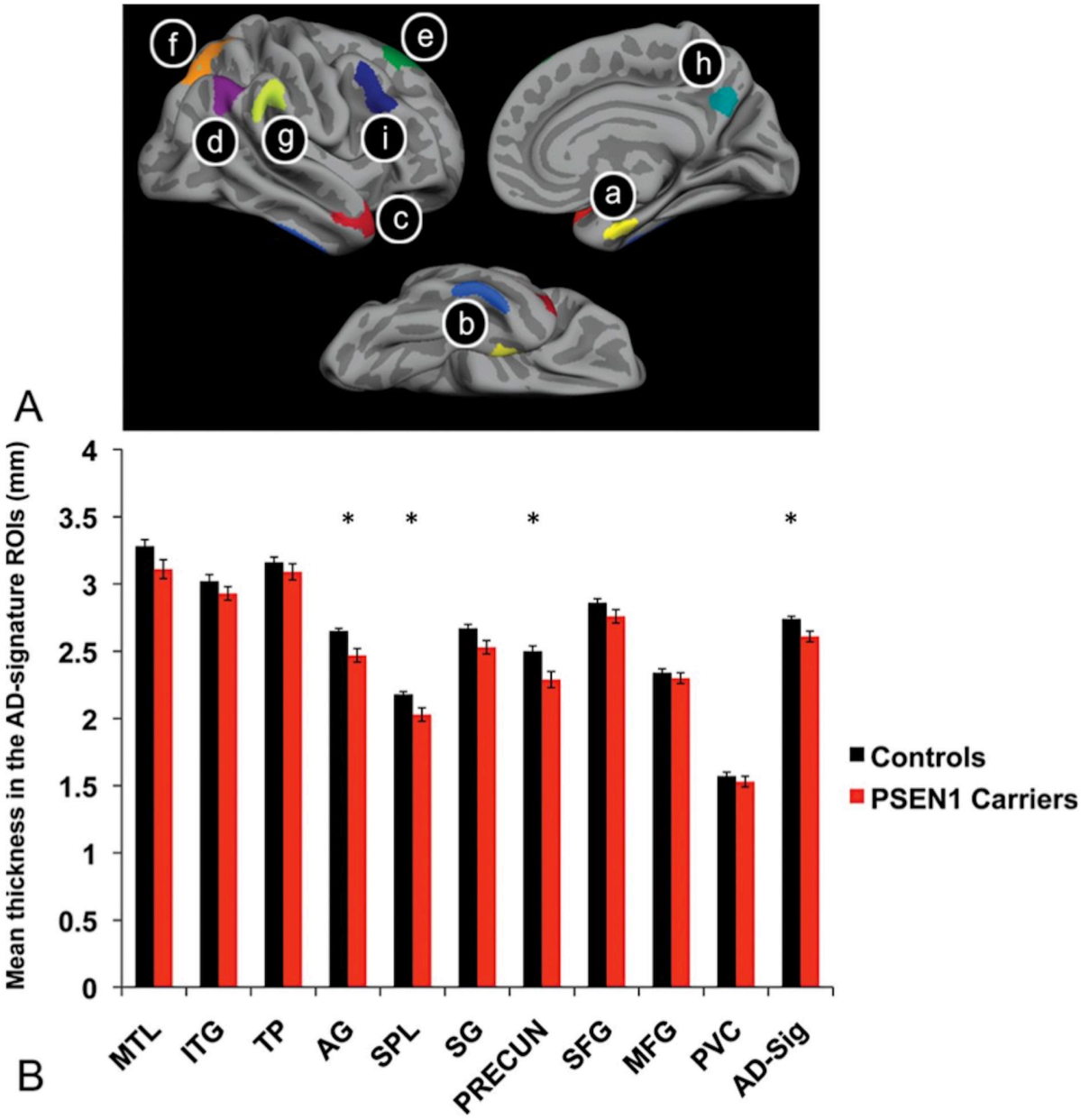


Figure 1.1: Comparison of presenilin 1 (PSEN1) FAD mutation carriers and non-carriers in cortical thickness from a priori ROIs that compose the 'Alzheimer's-signature' regions. Figure reproduced from Quiroz YT, et al. (2013), Journal of Neurology, Neurosurgery and Psychiatry. A. AD-signature regions: (a) Medial temporal lobe (MTL), (b) inferior temporal gyrus (ITG), (c) temporal pole (TP), (d) angular gyrus (AG), (e) superior frontal gyrus (SFG), (f) superior parietal lobule (SPL), (g) supramarginal gyrus (SG), (h) precuneus (Precun), (i) medial frontal gyrus (MFG), primary visual cortex (PVC). B. Bar graphs show mean cortical thickness within each ROI in the *PSEN1* mutation carriers and non-carriers, averaged across hemispheres ($p<0.005$). AD-signature (AD-sig) regions combined cortical thickness is also compared. Error bars show 1 SE of the mean. Reprinted with permission from BMJ Publishing Group Ltd.

Interestingly, there is some evidence to indicate that specific FAD mutations, especially in different genes, may have distinct effects on atrophy patterns and rates across the cortex and in the hippocampus (51). Other studies have found no differences between mutation gene types when tested explicitly (42). Still, one must consider the validity of combining individuals from families with different mutations in a single cohort based on preclinical stage. This approach certainly increases sample sizes and statistical power, which is likely a worthwhile trade-off of combining multiple genetic mutation types in carrier groups.

There has been relatively little work published examining white matter structure in FAD mutation carriers. One study showed that in carriers white matter volume is decreased in areas including the fornix and the cingulum, two important fiber bundles connecting the hippocampus and limbic areas (45). Another study examining white matter using tensor modeling found that symptomatic mutation carriers showed increased diffusivity (especially radial diffusivity) and reduced fractional anisotropy (FA) in the fornix, cingulum and the corpus callosum (52). The reduced FA phenotype in preclinical FAD mutation carriers has also been reported elsewhere (53). FA is an index that ranges from 0 to 1 that indicates, in a given voxel, the preference of water molecules to diffuse along the principal axis of diffusion. Reduced FA in a given region may indicate a breakdown of white matter, often referred to as decreased white matter integrity. Reduced FA is often, but not always, accompanied by increased diffusivity, an inversely related measure of how freely water molecules diffuse in a given voxel. FA and diffusivity are common metrics calculated based on tensor modeling of DWI data, and will be referred to in subsequent sections.

It has been posited that functional changes in the brain measured by fMRI might be a candidate for an early AD biomarker (54). Task-based and resting fMRI have both been used to examine possible differences between FAD mutation carriers and non-carriers. Several task-based studies found that FAD mutation carriers show reduced BOLD activity compared to non-carriers in regions normally associated with the task (55; 56). Specifically, in one study, there

was reduced activity in the hippocampus, inferior parietal cortex, precuneus, and posterior middle temporal gyrus during the retrieval phase of a memory task (56). The authors also looked at behavior, noting that in no scenario did higher activity in mutation carriers correlate with better performance. This is important because, as will be discussed in subsequent sections, the fMRI literature in genetic risk for sporadic, late-onset AD is contradictory, which leads to contradictory interpretations of results. One possible interpretation of higher activity in high genetic risk groups is that it is a compensatory mechanism and a sign of early disease (57). However, if there is no correlation between higher activity and behavioral performance, this interpretation is difficult to support with evidence. Another study in preclinical FAD mutation carriers found that activity in carriers increased as a function of preclinical stage (in other words, there was an inverse relationship between activity and years to expected onset) in the middle temporal gyri and fusiform (58). The authors suggest that this increasing activity phenotype, which was not observed in non-carriers, could be related to early AD processes. They did not test for an association of the increased activity with behavior. Another study that controlled for behavior during the functional task (as a variable of non-interest) found increased activation in the right anterior hippocampus during encoding in a group of *PSEN1* mutation carriers versus non-carriers (59).

Resting state fMRI, or task-free fMRI, has only recently gained traction in the FAD literature. Based on spatial coherency, resting state fMRI data can be used to identify specific functional networks in the brain. As expected from work in sporadic, late-onset AD, the default mode network (DMN) is the principal network that is disrupted in FAD. A study led by Reisa Sperling and colleagues used resting state data from 83 FAD mutation carriers and found that overall DMN functional connectivity decreased in mutation carriers as their Clinical Dementia Rating score increased (higher scores indicate greater severity of dementia symptoms) (60). This relationship was especially pronounced in the precuneus, posterior cingulate and the parietal cortices. These same regions also showed significantly decreased DMN functional

connectivity in mutation carriers when compared directly to non-carriers. These results are supported by other studies that suggest DMN dysfunction is a feature of preclinical FAD (55; 61).

Perhaps some of the most important work being performed with FAD mutation carriers is the characterization of metabolic changes in the brain and amyloid deposition during preclinical AD using PET tracers. The precuneus, which is a region known to be affected by Aβ deposition and cortical thinning relatively early in AD, shows decreased glucose metabolism in FAD mutation carriers up to 10 years before the expected onset of symptoms (42). Metabolism is measured by using fluorodeoxyglucose (FDG)-PET, which is a radioactive glucose analog that gets taken up by tissues actively using glucose for energy. Lower uptake of the FDG tracer indicates reduced metabolic function, a relatively well-characterized feature of preclinical AD. Aβ imaging using Pittsburgh compound B (PiB)-PET reveals differences between FAD mutation carriers and non-carriers in the precuneus up to 15 years before expected onset of disease (42). In addition, imaging studies using PiB-PET identified one of the major pathological differences between FAD and sporadic, late-onset AD. Specifically, FAD mutation carriers have much higher levels of Aβ in the striatum than patients with sporadic, late-onset AD, even in the preclinical phase (62).

As discussed previously, FAD provides a unique opportunity to learn about AD progression during the pre-symptomatic phase. The genetic mutations that cause FAD and many of the imaging findings discussed in this section seem to support an amyloid-centric view of AD pathogenesis. However, as discussed briefly above, there are problems with the amyloid cascade hypothesis, which points to amyloid as the catalytic pathology in AD. Namely, it appears that being positive for Aβ in the brain is a necessary component of AD, but it is not sufficient to cause the disease. Research shows that 20-40% of cognitively healthy elderly adults are positive for Aβ and can remain healthy in that state for years (32). Of course, these subjects do not have a FAD mutation, but work linking FAD to the much more common

sporadic, late-onset AD is promising (47). However, while there are certainly many similarities between FAD and sporadic, late-onset AD, it is still open for debate whether or not the findings in FAD carriers will be directly applicable to developing treatments for sporadic, late-onset AD patients. One notable difference between these two types of AD that was elucidated via neuroimaging studies is that FAD mutation carriers have early Aβ deposition and volume loss in deep brain structures including the thalamus, caudate and putamen (49; 52; 63). The pattern of Aβ deposition and atrophy in sporadic, late-onset AD does not include significant involvement of these structures.

To close this section on autosomal dominant AD, let us consider another unique genetic event that can cause FAD. In addition to mutations in the *APP*, *PSEN1* and *PSEN2* genes, a duplication of *APP*, first identified in a Dutch sample, also leads to highly penetrant AD (64). While there is no neuroimaging work to review on these subjects, this is an appropriate segue into the next section that will focus on another genetic mechanism of presumed *APP* overexpression, Down syndrome.

Down Syndrome

Over 50 different mutations in *APP* are known to cause familial, autosomal dominant AD. *APP* is also implicated as a causative gene in the development of AD in individuals with Down syndrome (DS). DS results from an extra copy of chromosome 21. In ways not full understood, trisomy 21 causes intellectual disability and increases the risk for many medical conditions, including congenital heart defects, hearing and vision impairment, and endocrine dysfunction. Individuals with DS have an average life expectancy of 55 years and suffer from age-related cognitive decline after the age of 40 (65; 66). The *APP* gene is located on the long arm of chromosome 21 at position 21.3. Compared to healthy individuals with two copies of chromosome 21, there is a dose-dependent increase in the amount of Aβ produced in the brains of individuals with DS. The connection between DS and AD was first described in English

in the late 1940s (67; 68). The original paper describes the cognitive decline of three older DS patients. Post-mortem neuropathological examination of these three patients and countless others in the intervening decades have consistently revealed the presence of amyloid plaques and neurofibrillary tangles in the brains of individuals with DS over the age of 30 (66). Thus, there is assumed to be a connection between the increased expression of *APP* and the invariable and early appearance of amyloid pathology in the brain. The fact that tau pathology in the form of intracellular neurofibrillary tangles is also present in the brains of middle-aged persons with DS may support the theory that amyloid aggregation is the trigger in a cascade of physiological changes that lead to clinical AD.

The relatively limited neuroimaging work in adults with DS has primarily been focused on characterizing brain structure using sMRI. Many studies are designed to compare nondemented DS subjects to demented DS subjects. Because differences in these studies are likely attributable to the advanced disease state of the demented subjects we choose to not review those studies here. Instead we focus on reports that compare nondemented elderly DS subjects to younger DS subjects. Genetics is not the major factor in group differences, but these studies may shed light on features of preclinical AD. Because there are other developmental effects of DS, directly comparing a DS group to a healthy, control group makes it difficult to parse apart developmental differences versus changes due to preclinical AD. That said, the hippocampus and entorhinal cortex, key structures affected early in AD, are reported as reduced in volume in nondemented adults with DS in several studies (69–71). Also, as expected, with advancing age there is a decrease in volume of medial temporal lobe structures in individuals with DS (70). Because nearly all DS patients will develop AD, it is assumed that this decrease in volume is representative of a disease process rather than normal aging. One study found that in the cerebral cortex age is correlated with atrophy in regions of frontal and parietal cortices as well as parahippocampal gyrus (72). Another, using cross-sectional data, found a steeper age-related decrease in the volume of frontal, parietal and temporal lobes when compared with age-

matched healthy controls (73). A third examined DS brain morphology compared to healthy controls and found decreases in volume in the cingulate gyrus, left medial frontal lobe and regions of the right temporal lobe (74). Aside from these three studies, there is a dearth of publications that employ modern volumetric analysis techniques, such as voxel based morphometry, tensor-based morphometry or cortical thickness measurements with Freesurfer, in aged DS cohorts. There are also *almost* no studies that use DWI as a method to interrogate the putative preclinical AD phase in DS. The exception is a very interesting recent study that used tensor modeling with DWI to calculate FA across the brain in healthy older DS subjects. Their findings included a positive correlation between decreasing scores on a global functioning measure and FA in specific frontal ROIs, which indicates that late-myelinating white matter tracts may be particularly vulnerable in older individuals with DS (75).

Functional imaging in DS is limited to a handful of PET studies. To our knowledge, there are no fMRI studies in older or aging DS subjects. As mentioned previously, individuals with DS usually are positive for Aβ in the brain at, or soon after, age 30. Thus, the focus of much PET imaging work in DS has been to quantify this deposition *in vivo*. The first Aβ-specific tracer, PiB, was first published in 2004 (76). In 2011, a study to test the utility and safety of PiB in DS subjects was completed (77). The major findings of this study were that the tracer was successful in measuring Aβ plaque load and that age and a clinical diagnosis of AD were positive predictors of amyloid positivity (77). Also in 2011, there was a exhaustive case study published in which a 55-year-old DS subject with AD received a PET scan with florbetapir, another amyloid tracer (78). At death, the subject's brain was donated and neuropathological analysis was completed. In general, the pattern of amyloid deposition matched the pattern found in sporadic, late-onset AD and was corroborated by the neuropathological findings. Furthermore, results from another study show that the reduction in glucose metabolism (as measured by FDG-PET) that has been observed years before the onset of sporadic, late-onset AD is recapitulated in nondemented, older DS subjects (79). Findings like these have helped to

motivate the study of older DS subjects because there appear to be many similarities between AD in DS and sporadic, late-onset AD.

Concomitant with the increasing interest in the preclinical phase of AD, there is recent, renewed interest in the connection between DS and AD. However, because DS results from an extra copy of an entire chromosome, there could be as yet undiscovered aging related genes on chromosome 21 that help to influence lifespan, aging and AD in individuals with DS, affecting the interpretability of results (66).

Neuroimaging Genetic Risk for Alzheimer's Disease

In the vast majority of cases, AD presents with no clear, underlying genetic cause. This sporadic version of the disease usually affects patients later in life, with an average age of onset roughly 20 or 30 years later than FAD or AD associated with DS, respectively (36; 66; 80). The following sections will detail the genetic risk loci that have been associated with sporadic, late-onset AD and neuroimaging findings related to these risk factors. For a summary of the literature reviewed in this portion of the chapter see Table 1.

APOE

The explosion of the neuroimaging genetics field is due largely to the recent rapid identification of novel risk factors for sporadic diseases. In AD, these risk factors can be genotyped in healthy human subjects, allowing genetic risk for AD to be studied in a highly generalizable way in the population at large, rather than small restricted groups of individuals with a highly penetrant genetic mutations or DS. This makes recruitment of large numbers of subjects much more feasible, increasing statistical power. After age, genetic risk factors such as *APOE* are the strongest predictors of sporadic, late-onset AD currently available (81). Because sporadic AD accounts for ~99% of the diagnosed cases of AD, a better understanding of this disease is essential to the development of prevention and treatment strategies.

Sporadic AD, hereafter referred to simply as AD, is unique among polygenic human neurological diseases because there is a well-validated, non-causative genetic risk factor, *APOE*, which accounts for a relatively large portion of the variation in heritability. Specifically, twin studies reveal that the heritability of AD may exceed 60-80% and *APOE* genotype accounts for about 50% of the variation in heritability (4–6). A single *APOE* ε4 (APOEε4) allele increases lifetime risk for AD 4-5 fold, and two copies of the allele confer at least a 10-fold increase (3; 81). *APOE* was identified as a susceptibility gene for AD over 20 years ago and has been studied extensively since (3; 82; 83) . The *APOE* gene is localized on chromosome 19 and has three common alleles (e2, e3, and e4) determined by polymorphisms at two SNP sites, rs429358 and rs7412. Combinations of these three alleles result in six possible genotypes in the general population. APOE is a lipid transport protein that is believed to play a fundamental role in cell maintenance and repair (84). It has also been implicated as a regulator of normal cell metabolism, as well as other functions (84). In the years since the discovery of the association between *APOE* genotype and AD, fMRI has progressed from a novel, infant technology to one of the most popular methods in human neuroimaging research. The strength of the disease risk conferred by *APOE*, as well as the co-maturation of the fields of AD genetics and fMRI acquisition and analysis led to the first study combining a genetic risk factor for a disease and neuroimaging (57). This study, which found putative compensatory increases in activity in ε carriers, as well as others published shortly thereafter helped to expand the horizons of neuroimaging genetics, a new subfield of neuroscience and the topic of this book.

To date, there have been hundreds of publications focusing on neuroimaging the genetic risk for AD conferred by *APOE*. Because it is impossible to cover every aspect of this dense literature, it is worth noting that there are excellent reviews available to complement the information included in this section (85–88). We will summarize the key elements of this body of work, focusing on new and emerging research. Due to the heterogeneity of cohorts across the

literature, we have divided our summary of imaging findings into three subsections: healthy older adult cohorts, young healthy cohorts and, finally, MCI and AD cohorts.

Healthy, Older Adult Cohorts

In healthy older adults, hippocampal volumes have been shown to be smaller in APOEε4 carriers compared to non-carriers (89; 90). Hippocampal atrophy rates are also higher in APOEε4 carriers (91; 92). There is evidence that hippocampal volumes vary in an allele dose-dependent manner, but most studies' recruitment efforts conclude before they can amass enough homozygous APOEε4 carriers to consider them separately (93). In addition to whole hippocampal volume, sMRI can be used to measure structural changes within specific areas of the hippocampus. High resolution, partial field of view sMRI allows for the segmentation of the hippocampal complex into specific subregions, such as the subiculum, the entorhinal cortex and the CA subfields. Using this approach, several labs have reported smaller or thinner subregions in healthy APOEε4 carriers. Specifically, healthy APOEε4 carriers have been found to have thinner entorhinal cortex and subiculum compared to non-carriers (94). Two additional studies, each using MR images acquired at 4T, found thinner CA3 and dentate gyrus subfields in APOEε4 carriers (95; 96). However, some studies that examined hippocampal volumetric differences between healthy APOEε4 carriers and non-carriers did not find significant differences, although these are certainly in the minority (97).

There are very few reports of differences in cerebral cortex volume or thickness in healthy older adults based on *APOE* genotype so a consensus is difficult to develop. This may be because neutral results are not published as often as results showing significant differences. One published study that examined cortical volumetric differences between healthy APOEε4 carriers and non-carriers found no significant differences (97). However, another study found that APOEε4 carriers had thicker cortex in bilateral frontal and temporal regions, but a steeper longitudinal atrophic trajectory across the cortex (98). This points, again, to an emerging theme

that individuals with at least one copy of the APOEε4 allele experience an acceleration of the volume loss seen in normal aging.

A caveat of volumetric, structural findings in the *APOE* literature is that atrophy or volume loss is often seen as an indication of disease processes, while increased volumes or decreased atrophy rates are not. Thus, it is likely that intuitive results, for example where APOEε4 carriers have lower or smaller volumetric measurements, are favored in the published literature. A lack of such a biasing intuition in fMRI may partially explain why the results in the *APOE*-fMRI literature are more contradictory, as will be discussed in the following text.

Because APOE is a lipoprotein that transports endogenous lipids, there is interest in better understanding its relationship with myelin, which needs lipids for maintenance and repair. In neuroimaging, investigators can use DWI to examine the potential relationship between APOE and myelination, using 'white matter integrity' measured by FA as a proxy for myelin health. White matter integrity in the medial temporal lobe, but not entorhinal thickness, has been shown to be associated with improved performance on a verbal memory task (99). There is also evidence for a general decrease in FA in APOEε4 carriers (100). Diffusion tensor imaging (DTI) allows mathematical concepts from the field of graph theory to be applied to structural brain imaging data. In a study by Brown and colleagues, graph theory was used to measure global integration and local interconnectivity in healthy, older subjects. APOEε4 carriers had an age-related decrease in local interconnectivity that may indicate different aging trajectories in APOEε4 carriers and non-carriers (101). The application of graph theory to sMRI data as well as resting state fMRI data may help to elucidate the local and global network properties that change during early AD, but more research is needed in this area before such measures can be considered as potential biomarkers or endophenotypes of AD.

A quick review of the task-based fMRI-*APOE* literature reveals a frustratingly complex picture. Some studies have reported increased, putatively compensatory, activity in APOEε4 carriers (57; 102). Others have reported decreased activity, putatively caused by a loss of

function due to disease processes (103; 104). Part of the complexity stems from the heterogeneity of task designs (87). Differences can be stark. For example, it may be hard to compare results from a sematic memory task and a visuospatial memory task (57; 103). Other potentially confounding factors in task design can be more subtle. A task described as a “paired associates” memory task can actually vary widely on several factors including, but not limited to, method of presentation of stimuli (audio, visual, or both), types of stimuli (images, words, etc) and instructions (‘pay attention’ versus ‘remember these pairs’) (87). There are also many studies in the literature in which investigators used non-episodic-memory based tasks, complicating interpretation because there is evidence that APOEε4 exerts a specific effect on episodic memory systems (105). In contrast to the whole-brain approach of the studies cited here, the results from studies that examined BOLD activity in the hippocampus as an ROI are more cohesive. One study, which acquired data using a high-resolution fMRI sequence, found decreased activity in APOEε4 carriers in the CA2, CA3 and dentate gyrus subregions of the hippocampus (106). Another found decreased hippocampal activity during encoding in APOEε4 carriers (107).

Results from resting state fMRI work in healthy older APOEε4 carriers presents a more unified picture. There appears to be a convergence on the DMN and connectivity therein, by which APOEε4 carriers and non-carriers differ. In a very recent study, connectivity between the posterior cingulate cortex and the hippocampus, two major nodes of the DMN, was found to be diminished in APOEε4 carriers (108). Another study, focusing on female APOEε4 carriers, reported significantly reduced DMN connectivity compared to female non-carriers (109). Finally, decreased DMN connectivity and increased connectivity of another, opposing cognitive network, the salience network, have been described (110; 111). One theory explaining the DMN dysfunction reported in APOEε4 carriers states that the genetic vulnerability for AD may cause a loss of appropriate hippocampal decoupling from cortical DMN regions during activity, like when completing a task (112). This theory is supported by the discovery of a negative correlation

between hippocampus-DMN synchronization and performance on a memory test (112). More work is needed to explicitly test this theory.

PET imaging has helped elucidate the relationship between *APOE* and Aβ. Today we understand that, while the relationship is still far from fully understood, the protein products of *APOE* play a role in Aβ clearance, with APOEε4 performing this task less well than the ε3 or ε2 alleles (84). This idea is supported by PET imaging studies in which the relationship between Aβ (measured with PiB or florbetapir) and APOEε4 carrier status is examined. The majority of these studies report that healthy, older APOEε4 carriers have increased amyloid load compared to non-carriers (113–116) . There are also metabolic differences between healthy APOEε4 carriers and non-carriers. A very large study with 806 cognitively normal, PiB negative, subjects recently showed that glucose metabolism in APOEε4 carriers is lower than non-carriers in the posterior cingulate, precuneus, lateral parietal and inferior temporal regions (117) (Figure 1.2A). The magnitude of this difference was small but commensurate with differences observed between cognitively normal and MCI APOEε4 carriers (Figure 1.2B). There was also an overall negative correlation between FDG uptake and age across the whole cohort, with the posterior cingulate and precuneus exhibiting a particular vulnerability to both age and APOEε4 carrier status (117). This work is supported by previous studies that also reported hypometabolism in AD vulnerable regions in healthy APOEε4 carriers (118). However, a recent study of 600 cognitively normal older subjects found no FDG-PET metabolism differences in APOEε4 carriers and non-carrriers (119). This discrepancy may be based on the inclusion of PiB positive subjects in the latter report, who were stratified based on tracer uptake. Perhaps when subjects are binned by amyloid burden, the power to detect APOEε4 related differences in metabolism is diminished.

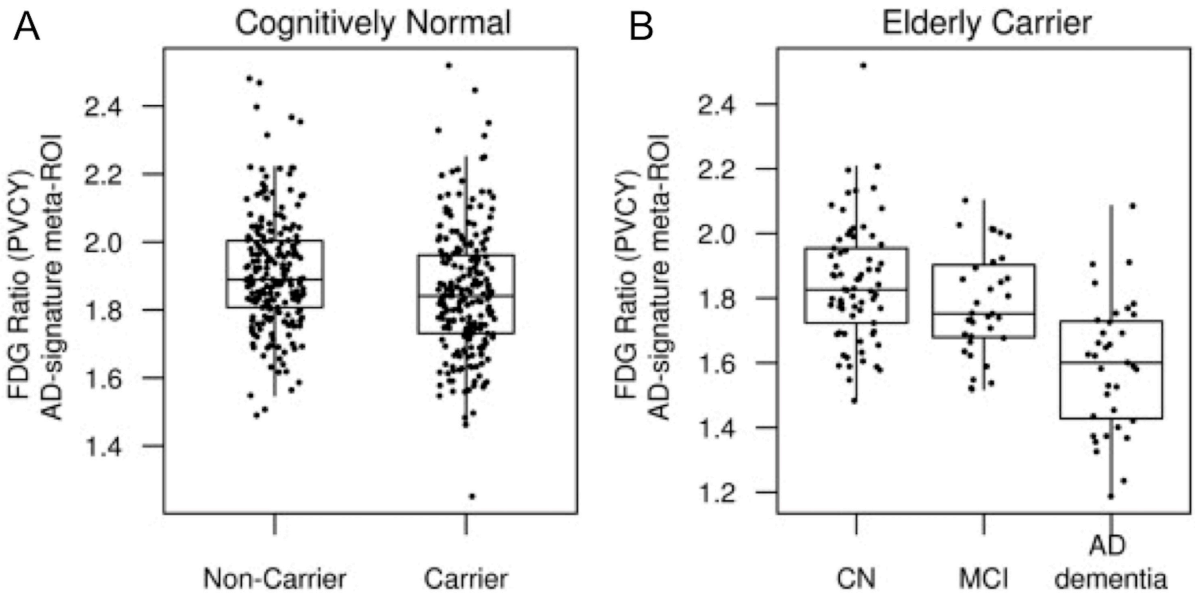


Figure 1.2: Boxplots of the FDG to reference region ratio (partial volume corrected; PVCY) in Alzheimer's-signature meta-ROI. Figure adapted from Knopman DS, et al. (2014), Neurobiology of Aging. A. FDG binding in CN APOEε4 carriers and non-carriers of all ages. B. FDG binding across elderly (age ≥70 years) cognitively normal (CN), MCI, and AD cohorts. Metabolism decreases as clinical disease severity, as represented by clinical diagnosis, increases. The magnitude of the differences between cognitively normal APOEε4 carriers and non-carriers is similar to the difference between CN and MCI groups, which were matched for APOEε4 carrier status in addition to age and sex. Reprinted with permission from Elsevier.

Magnetic resonance spectroscopy (MRS) is a technique that can be used to measure the relative concentrations of different hydrogen containing metabolites, each with a different peak resonance that can be plotted and quantified. Recent work using MRS in the posterior cingulate, a region particularly vulnerable to AD, has revealed that both GABA and glutamine/glutamate metabolites are reduced in individuals with MCI (120). However, the authors did not detect an association between the metabolites they measured and *APOE*ε4 status or amyloid deposition, which limits their usefulness as AD-specific biomarkers. In contrast, another recent study that also focused on the posterior cingulate examined choline/creatine and myoinositol/creatine ratios and found that they were significantly higher in older adult carriers of *APOE*ε4 compared to non-carriers (121). This finding supports earlier work in this field that found that myoinositol/creatine ratio is associated with neurodegenerative

disease, as opposed to normal age related cognitive decline (122). Examining creatine levels alone, another study observed significantly lower creatine in *APOE*ε4 carriers compared to non-carriers (123). There is also evidence that healthy older individuals with smaller hippocampal volume have a lower N-acetylaspartate/myoinositol ratio, which has been associated with AD, compared to their peers with larger hippocampal volume (124). Taken together, these results indicate that some metabolite measures and ratios may be useful biomarkers in individuals already at increased risk for AD.

Young, Healthy Cohorts

There is a burgeoning literature focusing on the effects of the APOEε4 allele in younger people, from middle-aged adults to young adults to infants. While there are few uncontested results, it is evident that APOEε4 carrier status affects brain structure and function well before old age. One thorough study of the effect of APOEε4 measured by various imaging modalities in young adults only found differences in fMRI activity, despite also acquiring and analyzing DWI for tensor modeling and sMRI for VBM in the same subjects (125). Other studies have also found no differences in hippocampal volume (126; 127). However, there is some evidence that hippocampal volume differs between APOEε4 carriers and non-carriers. In a small study of 44 subjects the authors found decreased hippocampal volume in the group of 22 APOEε4 carriers compared to non-carriers (128). Small sample size may be one reason that this finding does not fall in line with the others that interrogated hippocampal volume. In the cerebral cortex, reduced gray matter volume in AD-signature regions, including the lateral parietal, temporal and cingulate cortices, has been detected in young adult APOEε4 carriers (129). Another study found no differences in gray matter volume in young APOEε4 carriers and non-carriers (130). The contradictions in these structural findings will hopefully be resolved as larger datasets of young adults are being genotyped for larger numbers of SNPs (perhaps even undergoing whole genome sequencing). The expanding genetic data available may include AD risk factors that

were previously unlikely to be included in large data collection efforts focused on young adults. In contrast to sMRI, DWI appears to be a relatively sensitive imaging modality for uncovering differences between young APOEε4 carriers and non-carriers. A study of 203 subjects found a diffuse and widespread increase in mean diffusivity (MD) in APOEε4 carriers (131). Another found a general reduction in FA along with increased MD in carriers (100). More work is needed to establish alterations in DTI metrics as a potential biomarker of early *APOE*-mediated neural differences in young adults.

Contradictory results in fMRI experiments comparing APOEε4 carriers to non-carriers are not limited to older adult cohorts. Functional studies in young adults have reported decreased task-related activity in APOEε4 carriers that may indicate a blunted recruitment of the neural machinery necessary to complete the task efficiently (132). However, there is also evidence that hippocampal activation during memory tasks is higher in young APOEε4 carriers (133). Greater activation could be indicative of a compensatory mechanism in order to maintain performance. This theory does not appear to be supported by the *APOE* literature in young adults. In fact, differences in activity and cognitive performance suggest that APOEε4 carriers have better attention and memory function than non-carriers (134; 135). The latter findings and others have led to a moderately popular theory of antagonistic pleiotropy, still only tenuously supported, in which the APOEε4 allele confers some beneficial advantage in young people, only to then predispose older people to AD. One reason that this theory has gained some traction is that is may help explain why, despite the negative effects of the APOEε4 allele, it remains a relatively common variant, with 20-25% of the population carrying at least one copy (3). The argument is that an allele with deleterious effects would not be so common unless there were early life benefits. The counterargument to this evolutionary reasoning is that the human lifespan has only been long enough to experience the negative effects of the APOEε4 allele for a relatively brief epoch of our history as species. Furthermore, even in APOEε4 carriers, AD

usually manifests at the end of or after the reproductive phase of life, which would minimize selection pressure against APOEε4 carriers.

Using resting state fMRI, there is evidence of altered DMN function in young adults with the APOEε4 allele (133). This mirrors what has been discovered in older healthy adults and, as will be discussed below, in MCI and AD. There is also evidence that alterations in resting state networks mediated by the APOEε4 allele may not be tightly linked to risk for AD. In a study by Trachtenberg and colleagues, resting state networks that differed between APOEε4 carriers and APOEε3 homozygotes (including bilateral hippocampal networks, the auditory network, the left frontal-parietal network and the lateral visual network) also differed between APOEε2 carriers and APOEε3 homozygotes (136) (Figure 1.3). The APOEε2 allele has been shown to be protective against AD (137). Therefore, the authors reason, these findings would indicate that the differences between APOEε4 carriers and non-carriers were not a reflection of increased AD risk or early AD-related changes, but rather point to a role for *APOE* in neurodevelopment. Certainly, this study provides a compelling rationale for including APOEε2 allele carriers as an additional experimental group in future studies that aim to elucidate early AD-related changes in the brain.

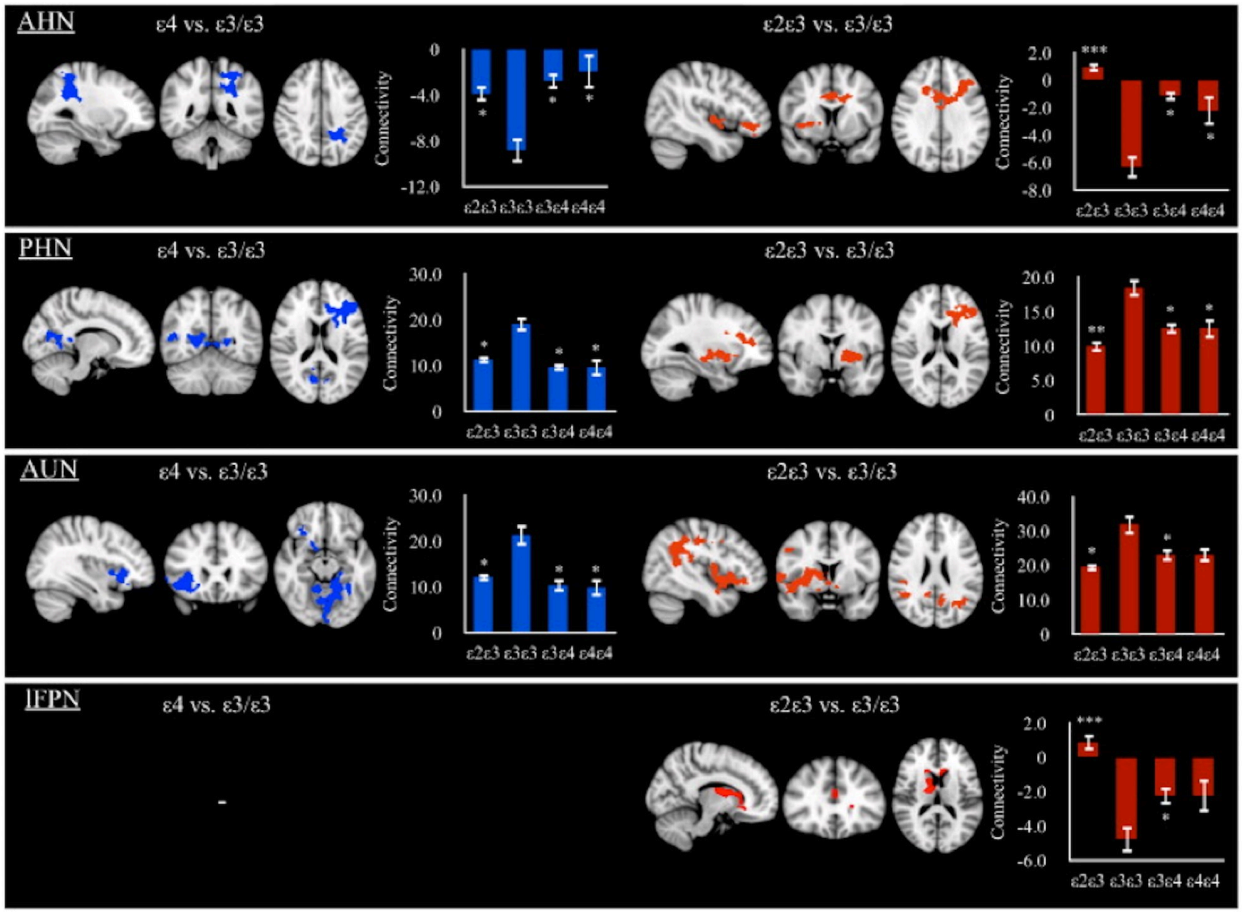


Figure 1.3: The effects of *APOE* genotype on connectivity of several resting state networks, including the anterior hippocampal network (AHN), the posterior hippocampal network (PHN), the auditory network (AUN), and the left frontal-parietal network (lFPN). Figure reproduced from Trachtenberg AJ, et al. (2012), NeuroImage. The left column shows the results of voxel-wise comparisons between APOEε4 carriers and APOEε3 homozygotes. The right column shows the results of voxel-wise comparisons between APOEε2/3 heterozygotes and APOEε3 homozygotes. Images are thresholded at z>2.3 and corrected for multiple comparisons using a corrected cluster significance threshold of p<0.05. Bar graphs show the ROI analyses on the significant regions from the voxel-wise comparisons. Error bars denote standard error of the mean. *Significantly different from APOE3/3 ($p < 0.05$); **significantly different from APOE3/3 and APOE3/4 ($p < 0.05$); ***significantly different from APOE3/3, APOE3/4, and APOEε4/4 ($p < 0.05$). Reprinted with permission from Elsevier.

As a final note on functional imaging of *APOE* genotype in young adults, a seminal FDG-PET study in 2004 found that in a small cohort of young adults, APOEε4 carriers were hypometabolic compared to non-carriers in the posterior cingulate cortex, parietal, temporal and frontal lobes (138). These regions are particularly vulnerable to AD and are the sites of marked hypometabolism early in the disease. Unfortunately, there are very few studies using PET imaging in young adults, so these results have not been properly reproduced.

We expect the number of studies that report on cohorts of adolescents, children and infants and the *APOE* gene will increase in the coming years. Today, there are several interesting published reports that are sure to inspire follow-up and further experiments. A study completed on 239 children and adolescents found that APOEε4 carriers had significantly thinner entorhinal cortex than non-carriers (139). Moving to even younger subjects, a recent study led by Eric Reiman scanned 162 infants from 2 to 25 months old and found that APOEε4 carriers had lower gray matter volume in the precuneus, the cingulate, lateral temporal cortex and medial fusiform gyrus (140). Support for these findings can be found in a paper examining the effects of psychiatric risk genes in prenatal development. Specifically, the APOEε4 allele was related to decreased gray matter volume in bilateral hippocampus, parahippocampus, fusiform and temporal gyri (141). Together, these studies suggest that there is a role for APOEε4 in development. This begs the question, is APOEε4 risk for AD a developmental susceptibility or a direct interaction with diseases processes? It has been shown that APOEε4 risk is specific to amnestic dementia (105). Studies on extremely young subjects might help to uncover if there are developmental clues as to the mechanism of this specificity. To date, there are no studies examining the functional consequences, measured with fMRI or PET, of *APOE* risk in infants. fMRI studies are likely to be completed soon as safety concerns for infants in fMRI experiments are minimal and motion correction techniques are constantly improving. PET studies in infants are extremely unlikely due to the required radiation exposure via the use of the radioactive tracer.

MCI and AD Cohorts

Similar to findings in healthy, older adults, APOEε4 carriers with AD or MCI have higher rates of hippocampal atrophy compared to non-carriers with AD or MCI, respectively (142; 143). Related to the increased rates of atrophy, AD and MCI subjects who carry at least one copy of APOEε4 have reduced hippocampal volume or more severe hippocampal thinning (143; 144).

Earlier, it was mentioned that clinical symptoms of AD generally follow marked atrophy and synaptic and neuronal loss, so it is not surprising that volumetric loss has occurred in these symptomatic cohorts. However, these studies are highlighting that the APOEε4 allele mediates more severe disease phenotypes, even in clinically affected patients. In studies of the cerebral cortex, APOEε4 carriers with MCI who then progress to AD show decreased gray matter volume in the temporal and parietal lobes (as well as decreased hippocampal volume) while APOEε4 non-carriers showed no gray matter volume changes over the same time elapsed (145). Taking together these sMRI findings and the many studies reporting that APOEε4 is associated with an earlier age of onset of AD, it is clear that APOEε4 is associated with a more rapid disease progression (80).

Alterations in DTI metrics based on APOEε4 carrier status appear to be a feature of young, healthy subjects and the preclinical phase of AD but not of symptomatic cohorts. A study that calculated FA and MD in two groups, AD patients and healthy controls, found that MD was significantly greater in APOEε4 carriers compared to non-carriers in the healthy control group but not in the group of AD subjects (146). In fact, there were no differences in DTI metrics mediated by *APOE* genotype in the AD group.

At the time of preparation of this chapter there were no published studies that employed either task-based or resting state fMRI to study differences in *symptomatic* populations *based on* APOE *genotype*. There is, however, a well-established literature in which PET tracers are used to assess differences in MCI and AD cohorts based on APOEε4 carrier status. With FDG-PET, AD patients who are APOEε4 carriers present with more dramatic metabolic reductions in the regions that are normally hypometabolic in AD, including the lateral parietal lobe, the posterior cingulate, precuneus and the temporal lobes (147). The spatial extent of the hypometabolic regions is also greater in APOEε4 carriers (148). PiB-PET studies that test for an association between Aβ deposition and APOEε4 carrier status in AD patients find that APOEε4 carriers have higher tracer uptake across diffuse regions of the cortex (149; 150).

Another PiB-PET study, this one in MCI patients, found that of the 61% of MCI subjects that were positive for Aβ, 80% of them were APOEε4 carriers (151). This indicates that APOEε4 carrier status is associated with an increased risk for amyloid positivity in subjects with MCI. Taking this one step further, it would indicate that individuals with MCI who are also carriers of the APOEε4 allele are more likely to convert to AD than non-carriers because amyloid positivity is a good predictor of progression (152).

As an interesting aside, let us consider *APOE* from another, extremely rare, perspective. Recently, it was discovered that a man with a severe form of dysbetalipoproteinemia was completely missing the *APOE* gene. A case study was published detailing his neurological status based on cognitive testing, MR imaging and CSF analytes (153). He was found to have no neurological deficits or structural abnormalities of the nervous system. The story of this remarkable patient has led to a resurgence of attention on *APOE* as a potential therapeutic target. If the absence of *APOE* does not negatively affect neurological function, perhaps the APOEε4 allele product can be silenced thus eliminating APOEε4-mediated AD risk. Of course, this therapy would need to be targeted to the CNS as a lack of APOE throughout the body results in excessively high cholesterol as well as other clinical problems.

TOMM40

In 2010, a non-coding region on chromosome 19 located just upstream from *APOE* was identified as a strong genetic risk locus for AD (154). This stretch of DNA, called *TOMM40* for translocase of outer mitochrondrial membrane 40, varies with respect to the length of a poly-T polymorphism. Longer length poly-T variants were found to be associated with increased risk for AD as well as a lower age of onset (154). The authors of these initial findings contended that the discovery was important because Tom40, the protein encoded by this region, is crucial to healthy mitochondrial function. The Tom40 protein forms a channel in the outer mitochondrial membrane that is used to import proteins (155). Since these initial findings, there has been

much disagreement in the field as to whether or not *TOMM40* is specifically associated with AD. Some believe that *TOMM40* is in such close linkage disequilibrium with *APOE* that any signal at the *TOMM40* locus in an AD association study is driven by the very strong *APOE* signal (156). These investigators postulate that the *TOMM40* polymorphism is "behaving as a surrogate for the well-established AD risk allele, *APOE* ε4". Subsequent to this, three groups attempted to replicate Roses and colleagues' original findings. One group found that longer lengths of the *TOMM40* poly-T repeat did in fact increase risk for AD, but only in the absence of APOEε4 (157). Another found no correlation between *TOMM40* poly-T repeat length and age of onset of AD (158). Finally, a third group found the *TOMM40* poly-T polymorphism association did not replicate, but reported another *TOMM40* polymorphism associated with increasing risk of AD in APOEε3 homozygotes, but in the opposite of the expected direction (i.e., increasing length was associated with a lower risk of AD) (159).

Given the various directions of the findings, the true implications of the *TOMM40* polymorphism remain to be determined. One group attempted to elucidate the complicated regulation of the relatively small haplotype block that encompasses *APOE* and *TOMM40*. The authors investigated the effect of putative *cis*-regulatory haplotypes on *in vitro* expression driven by *TOMM40* and *APOE* promoters, and their results suggest that genetic variation at the *TOMM40* locus may indeed be associated with late-onset AD, independently of *APOE* (160).

More recently, neuroimaging evidence that *TOMM40* is not merely a marker of *APOE* genotype has come to light (161). Specifically, *TOMM40* was found to have an additive and separable effect on the association between hippocampal volume and memory performance on a free recall task (161). Another study of healthy older adults who were not carriers of APOEε4 found a dose-dependent effect of high-risk *TOMM40* alleles correlated to decreasing performance on retrieval in a verbal memory task (162). The authors also reported a dose-dependent, high-risk allele correlation with decreasing gray matter volume in the ventral posterior cingulate cortex and medial ventral precuneus, both regions that are implicated early

in AD pathophysiology. Finally, in a cohort of generally healthy older adults assessed with DWI, the authors found significant and independent effects of both the APOEε4 and *TOMM40* 'short' alleles on specific tracts, independent of age, gender, vascular disease and childhood intelligence (163). For *TOMM40*, these tracts were the left uncinate fasciculus, left rostral cingulum, and left ventral cingulum. It remains unclear why specific tracts show significant deleterious effects of genetic variation at the *APOE* or *TOMM40* loci, but one hypothesis is that these late-myelinating tracts are particularly susceptible to injury or pathology (164). Clearly, a better understanding of the regulatory mechanisms affecting *TOMM40* and *APOE* will be necessary to tease apart their relationships to AD risk. In addition, continuing to measure genotype-driven pathological differences in the brain will help resolve whether the two genes have an additive effect on disease-related changes. Perhaps by investigating the rare variants within each of the genes that are not in linkage disequilibrium but that do associate with AD, it could be determined whether the genes exert their effects independently. Only a next generation sequencing method would support the large-scale effort required to implement such an approach.

TREM2

Triggering receptor expressed on myeloid cells 2 (*TREM2*) is a gene that has very recently been implicated in AD. Two independent studies were published in 2013 that linked a SNP (rs75932628) located within *TREM2* to AD (165; 166). The first studies quantifying the risk conferred by this *TREM2* variant have indicated that it could be as strong or stronger than the APOEε4 allele, which is, however, much more common. In one study, the allelic odds ratio for the *TREM2* variant was over 11 (167). For comparison, risk loci identified in genome-wide association studies for AD have odds ratios up to 1.5, while the *APOE* loci is often near or above 3 (see next section) (168). The strength of the association to AD in these initial reports has given rise to a burst of interest in the neurobiological underpinnings of the relationship.

The substitution of a C to a T base-pair results in the substitution of a histidine for arginine in the TREM2 protein and has been associated with low cell-surface expression of the protein and an increased risk for AD (166). TREM2 is an immune receptor responsible for regulating microglial cytokine production and phagocytosis of neuronal elements, neuritic debris, and bacteria (167). This protein is expressed in microglia and low cell-surface expression of TREM2 in transgenic mouse models of AD is associated with reduced phagocytic functions (169; 170). Recent evidence supports the notion that TREM2 is capable of phagocytosing Aβ, and mutations in TREM2 may lead to reduced clearance of protein aggregates in the brain (169). Additionally, upregulation of TREM2 has been shown to alleviate neuropathological symptoms of AD in a transgenic mouse model (171). Interestingly, *TREM2* variants have also been associated with other neurodegenerative diseases such as Parkinson's disease, frontotemporal dementia, and amyotrophic lateral sclerosis (172–174).

Despite its association with numerous neurodegenerative diseases, no systematic description of the clinical and neuropsychological features of the *TREM2* variant has been determined, in part due to the rarity of the mutation (175). The infrequency of the variant has limited the number of neuroimaging investigations dedicated to investigating the clinical presentation and patterns of gray and white matter morphology specific to *TREM2* variants. One cross-sectional study used voxel-based morphometric analysis to investigate regional patterns of gray and white matter loss associated with the at-risk variant (176). The authors found gray matter volume loss was largely restricted to frontobasal regions including orbitofrontal cortex and anterior cingulate cortex. In another tensor-based morphometry study, Rajagopalan and colleagues reported 1.4-3.3% annual rates of increased volume loss of the medial temporal lobe in at-risk *TREM2* subjects (177). However, no comprehensive whole-brain sMRI study of gray and white matter differences in *TREM2* variant carriers exists as of yet. Functional studies, especially PET imaging work exploring the relationship between *TREM2* and Aβ deposition, are likely forthcoming.

GWAS-Identified Alzheimer's Disease Risk Genes

Beginning in 2008, several large-scale genome-wide association studies (GWASs) examining genetic association with AD were published (168; 178–180). These studies confirmed previously identified risk factors (*APOE*, *CLU*) and identified new putative genetic risk factors for AD (*PICALM*, *CR1*, *BIN1* and others). Several of the GWAS-identified loci have been examined in subsequent studies of quantitative measures of cognitive decline and biomarkers for Alzheimer's disease. These phenotypes can include metrics of cognitive performance, functional and structural imaging biomarkers, PET tracer uptake, and CSF analytes. One purpose of genotype-driven phenotype studies is to better understand the role of GWAS-identified genes and their protein product(s) in AD pathogenesis. Neuroimaging data are acquired at a resolution much lower than the scale on which proteins act, so these data can provide only limited insight into pathogenic processes at the molecular or cellular level. Instead, what neuroimaging can do very well is help to assess the clinical utility of AD-associated genetic variants. In other words, in a living patient, can low effect size genetic risk factors be combined, along with biomarkers, to improve predictions about conversion to MCI and, subsequently, to AD? If so, this clinical utilization of GWAS-identified AD risk genes could allow clinical trial enrollment to be a more rigorous and specific process, with the ultimate goal of including only those individuals who are most likely to respond to the treatment, increasing statistical power to show an effect.

In 2013, the International Genomics of Alzheimer's Project (IGAP) consortium published their first GWAS effort, the largest ever on AD (7). Using a uniquely large cohort of 74,046 subjects amassed from four smaller data consortia the authors were able to detect 11 new AD risk loci, in addition to confirming previously identified loci (Figure 1.4). Using reference haplotype data from the 1000 Genomes Project for imputation and a predetermined genome-wide significance level of $p < 5 \times 10^{-8}$, the stage 1 analysis resulted in 15 genomic regions that

showed an association to AD. These regions included 10 previously identified AD genetic risk factors, including *APOE*, and 5 newly implicated loci. The 9 previously identified loci were *CR1*, *BIN1*, *CD2AP*, *EPHA1*, *CLU*, *MS4A6A*, *PICALM*, *ABCA7* and *CD33*. All available neuroimaging genetics findings for these loci will be reviewed in the following text. The six new loci were *HLA-DRB5-HLA-DRB1*, *PTK2B*, *SORL1*, *SLC24A4*, *RIN3* and *DSG2*. After the stage 2 replication analyses there were seven additional loci that reached statistical significance for association: *INPP5D*, *MEF2C*, *NME8*, *ZCWPW1*, *CELF1*, *FERMT2* and *CASS4*. Notably, in the stage 2 replication analyses there were two loci from stage 1 that did not reach statistical significance: *CD33* (a previously identified risk locus) and *DSG2* (a newly identified locus). This left a total of nine fully-replicated, previously identified risk loci, including *APOE*, as well as 11 newly identified potential risk loci (Figure 1.4).

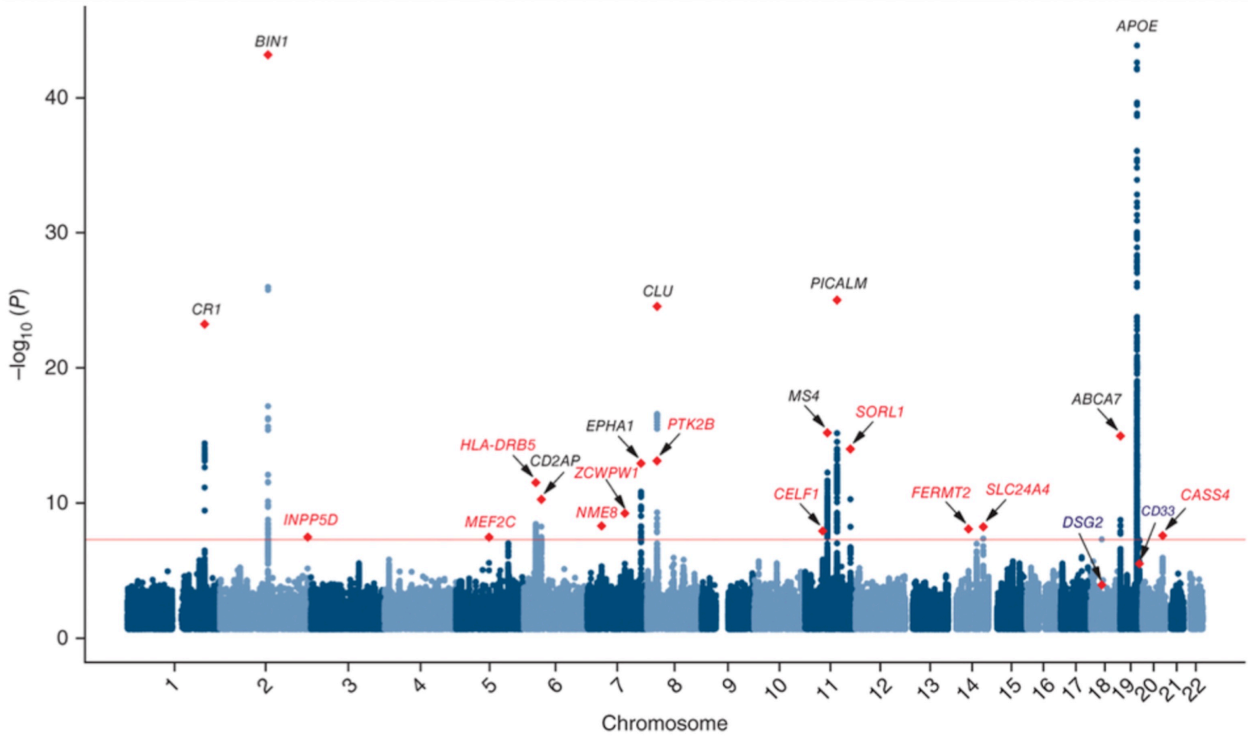


Figure 1.4: Manhattan plot depicting genome-wide associations with Alzheimer's Disease (17,008 cases and 37,154 controls). Figure reproduced from Lambert JC, et al. (2013), Nature Genetics. Genes that have been identified in previous GWASs are in shown in black and newly associated genes are shown in red. Red diamonds indicate loci with the smallest P values in the overall analysis. Reprinted with permission from Nature Publishing Group.

While this landmark study further complicated the genetic landscape of AD, it has helped to provide a more complete picture of the underlying genetics that lead to AD. These findings are very exciting to investigators taking an imaging genetics approach because this larger set of genetic markers will help to elucidate the role of the field in the future of AD research. Genetic risk markers identified in early GWAS studies are featured in a growing number of imaging genetics publications. Unfortunately, the new 11 loci described by Lambert and colleagues have not yet been queried using a neuroimaging genetics approach. Surely, these studies are underway. In several years, it is likely that these newly identified risk loci will also be represented in the imaging genetics literature. The remainder of this section will cover the published neuroimaging genetics findings for replicated GWAS-identified risk factors, including a brief background on each gene.

CLU

The gene clusterin (*CLU*) is ubiquitously expressed and its protein product has been implicated in a plethora of cellular functions, which seem to converge on *CLU's* role as a chaperone protein (181). Before the advent of the GWAS approach, *CLU* was already implicated in AD. Its potential relationship to the disease was first described by May and colleagues in 1990 when they found that *CLU* expression was significantly increased in the hippocampi of AD patients compared to controls (182). However, the coincident implication of *CLU* in two independent GWASs published in 2009 reignited the interest in *CLU* and its role in AD (168; 179). Furthermore, since those two initial reports, the association of a single SNP within the *CLU* gene has been replicated several times, making it a GWAS replication success story (183–185). One caveat that is especially important to molecular biologists is that the associated SNP (rs11136000) is intronic and therefore is not expected to have an effect on protein function (179). The search for the exonic, coding variants that are the true causative polymorphisms that underlie the association is ongoing.

Compared to other GWAS-identified genetic risk factors, *CLU* has been afforded the most attention in the neuroimaging genetics world. This may be because there was a preexisting literature linking *CLU* and AD and, thus, investigators felt that the *CLU* association was "real" and represented a signal strong enough to be picked up in neuroimaging approaches. The relatively large number of *CLU* studies may also be due to the highly reproduced nature of the genome-wide association with a single locus, again indicating a strong, "real" association.

There is no evidence that *CLU* genotype is associated with volumetric measures of medial temporal lobe structures. In healthy young adults, *CLU* was specifically shown *not* to be associated with hippocampal or entorhinal cortex volume (186). However, in a study of young healthy adults, the *CLU* risk allele was associated with poorer working memory performance and, further, this relationship was mediated by the gray matter volume of a region of the parietal lobe (187). The authors also tested for a relationship between *APOE*, working memory and gray matter morphology but found no significant association (187). Using DTI, the *CLU* risk variant has been associated with lower FA in several white matter regions, including the fornix, the splenium of the corpus callosum and the cingulum, which are all tracts that contribute directly to the structural connectivity of the medial temporal lobe (188). Decreases in FA have emerged in the *APOE* literature as a possible early indication of disease-susceptibility. More work is needed to ascertain whether or not there is an additive effect of risk genes on FA, but preliminary efforts are promising (189).

In contrast to findings in gray matter volume described earlier, an fMRI experiment that tested for an effect of *CLU* and/or *APOE* on brain activity during an executive attention task found that the effect of the genes was additive (132). Specifically, the authors found that as genetic risk across the two genes increased (represented by the number of risk alleles) the activity associated with executive attention decreased in the medial temporal lobe, as well as other regions (132). On its own, the *CLU* AD-risk variant mediated connectivity differences in

another fMRI study (190). Healthy carriers of the *CLU* risk variant showed decreased coupling of the hippocampus and prefrontal cortex during memory retrieval tasks (recall and recognition) (190) (Figure 1.5). Finally, the functional connectivity of the hippocampus and the relationship of this measure to the *CLU* polymorphism was recently reported in a study of resting state fMRI data. Compared to carriers of the protective allele, subjects who were homozygous for the *CLU* risk allele had the same general pattern of positive and negative functional connectivity but the magnitude of the connectivity was stronger in both the positive and negative directions (191). Taken together, these studies indicate that the BOLD signal as measured by fMRI may be modulated by *CLU* genotype. More studies are needed to confirm the association and define the dynamics of the modulation.

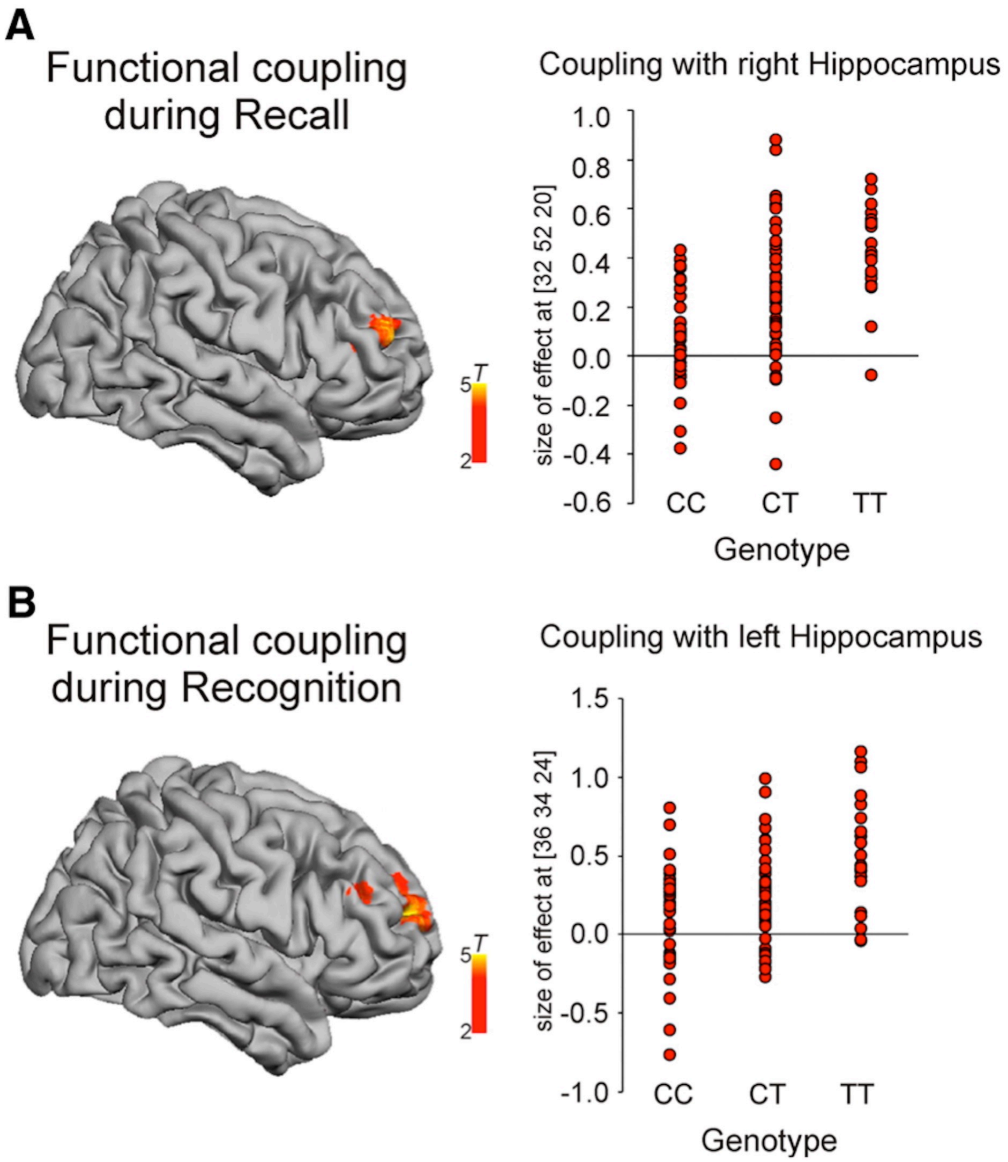


Figure 1.5: Functional coupling of the hippocampus and frontal cortex in three *CLU* genotype groups. Figure reproduced from Erk S, et al. (2011), The Journal of Neuroscience. A. Carriers of the risk allele (C at rs11136000) show decreased, allele dose-dependent coupling of the right DLPFC and the right hippocampus during recall ($Z = 5.06$; $p < 0.05$, family-wise error corrected for multiple testing across the whole brain). Each red dot represents the effect size for one subject and reflects connectivity between right DLPFC and the right hippocampal seed region. B. Carriers of risk allele exhibit significantly decreased allele dosage-dependent coupling of the right DLPFC with the left hippocampus during recognition ($Z = 4.73$; $p < 0.05$, family-wise error corrected for multiple testing across the whole brain). Each red dot represents the effect size for one subject and reflects connectivity between right DLPFC and left hippocampal seed region. Reprinted with permission from The Society for Neuroscience.

PICALM

The gene encoding phosphatidylinositol binding clathrin assembly protein (*PICALM*) was identified as an AD risk factor in 2009 (179). The implicated region (rs3851179) was located upstream from *PICALM*, but subsequent studies have not only replicated this finding but also identified AD-risk SNPs within the *PICALM* gene itself (192). *PICALM*, like *CLU*, has widespread expression in the brain. It is involved in many cellular processes, especially the trafficking of proteins and lipids via clathrin mediated endocytosis (193). Lately, this process, essential to synaptic transmission, has received increased attention in the study of AD, in part because of the strong association of *PICALM* to AD uncovered in GWASs (194). In terms of reproducibility in GWASs, *PICALM* ranks third after the *APOE*/*TOMM40* locus and *CLU* (7; 183; 184; 192). Perhaps because of this highly reproduced association, *PICALM* is fairly well-represented in the neuroimaging genetics of AD literature.

A study on older adults who ranged from cognitively healthy to diagnosed with AD reported a significant association between *PICALM* (rs3851179) and hippocampal volume such that carriers of the *PICALM* risk variant had lower hippocampal volume (195). The authors also described a similar relationship between *PICALM* risk and entorhinal cortex thickness. This finding has been replicated in another study that found that the *PICALM* risk allele is associated with a thinner entorhinal cortex (196). However, in young adults, *PICALM* was not associated with either hippocampal or entorhinal cortex volume (186).

The functional connectivity of the hippocampus and the relationship of this measure to *PICALM* was recently reported in a resting state fMRI experiment. Compared to subjects who were homozygous for the protective allele, risk allele carriers showed weaker negative functional connectivity of the hippocampus to many regions (191). This finding is preliminary and needs to be replicated. Lastly, a study of amyloid deposition as measured by florbetapir-PET found an epistatic effect involving *PICALM* and *BIN1*, another AD risk gene (197). This study is described later in this chapter (see *BIN1*).

CR1

Unlike *CLU* and *PICALM*, expression of complement component (3b/4b) receptor 1 (*CR1*) is likely to be low in the brain (198). The *CR1* protein's function is complex and varies by cell type, but it is generally involved in the regulation of the complement cascade, a major component of the innate immune system that helps to amplify the response of the immune system to potential targets. The CR1 protein is involved in transporting opsonized immune complexes through the circulatory system for removal (181). Neuroinflammation has been associated with AD for many years, but has often been dismissed as a consequence, not a cause, of the disease (199). This view is beginning to change and inflammatory processes are being studied as potential pathogenic processes in AD (194). One reason that interest in neuroinflammation and AD has been renewed is the identification of an association between a polymorphism in *CR1* and AD in a 2009 GWAS study (168).

The *CR1* risk variant has been shown to be associated with thinner entorhinal cortex in healthy older adults (195). Interestingly, there is also evidence that *CR1* is associated with lower entorhinal cortex volume in *young* healthy adults, a finding that was confirmed in two independent cohorts (200). Additional research is needed to assess whether or not this relationship between *CR1* and a potential endophenotype of AD is reproducible in larger samples.

BIN1

Bridge integrator 1 (*BIN1*) was conclusively reported as a risk gene for AD in 2010, after borderline associations were reported in one of the large 2009 GWASs (179; 183). Like *PICALM*, *BIN1* is associated with the intracellular trafficking of lipids and proteins. *BIN1* encodes a protein that has at least ten isoforms (181). Each isoform has specific domains that influence

the function of the protein. The isoform of BIN1 that is specific to the brain contains the clathrin-associated protein-binding (CLAP) domain, which plays a role in clathrin mediated endocytosis (201). Clathrin mediated endocytosis is an essential process in synaptic vesicle recycling, which is a crucial component of efficient synaptic transmission. It is interesting that both *PICALM* and *BIN1* are implicated as molecular components of this general cellular process, and it suggests that variability in synaptic transmission efficiency may contribute to AD pathology, especially during the early phase characterized by synaptic loss and neuronal death (194).

The convergence of *PICALM* and *BIN1* function led one group to test for epistatic genetic effects between the risk loci for each gene identified in GWASs. Hohman and colleagues analyzed florbetapir-PET scans from older adults to test for a possible interaction effect of BIN1 and PICALM on amyloid deposition (197). The authors found that there was indeed an interaction and that this interaction was reproducible in a second dataset. The *BIN1* risk variant was related to higher levels of amyloid burden, but only in individuals who were carriers of the *PICALM* protective variant. This study is a simple illustration of the weakness of candidate gene studies because both *BIN1* and *PICALM* are not related to amyloid deposition when tested on their own.

There is, however, evidence that *BIN1* genotype is directly associated with neuroimaging biomarkers of AD. Based on the preliminary, non-significant evidence from the 2009 GWASs that BIN1 was associated with AD, Biffi and colleagues tested for an association of the *BIN1* risk variant and a number of neuroimaging phenotypes. The authors found that the *BIN1* risk variant is associated with thinner temporal pole and entorhinal cortex in healthy older adults (195). The same year that paper was published, a *BIN1* locus reached genome-wide significance in a new AD GWAS (183).

ABCA7

ATP-binding cassette, subfamily A, member 7 (*ABCA7*) is one gene in a group of highly conserved transmembrane transporters that participate in active transport of various substrates across membranes, both cellular and organelle membranes (202). ABCA transporters have been linked to cholesterol and lipid homeostasis, and appear to work directly with APOE by transporting lipids out of the cell to APOE for clearance (203). This coordination with APOE may hint at the mechanism of the association between ABCA7 and AD. Still, *ABCA7* was first directly linked to AD through a GWAS in 2011 (180).

The *ABCA7* locus has been implicated in only one very recent neuroimaging study in which the authors were interested in the relationship between cholesterol levels and amyloid deposition (204). Hughes and colleagues described an over 2-fold increased risk of amyloid positivity in carriers of the *ABCA7* (rs3752246) risk variant.

EphA1

EphA1 was originally named after the cell line it was discovered in, erythropoietin-producing human hepatocellular carcinoma (181). EphA1 is a member of a superfamily of proteins called the receptor tyrosine kinases and is expressed widely in multiple tissues including the brain (205; 206). The Eph-ephrin family of receptors and ligands are all membrane bound proteins that are involved in cell adhesion and cell-cell contact mediated signaling, like in axonal guidance during development (206). The link between *EphA1* and AD was first described in two GWASs in 2011 (180; 192). There are few clues as to the neurobiological processes that link this gene to AD. It is known that EphA receptors, as a class, are highly expressed in the hippocampus, but the expression and function of specifically EphA1 in the hippocampus is not well understood (207).

Neuroimaging genetics results describing *EphA1* are limited to a single study. Like they did for the *ABCA7* genetic locus, Hughes and colleagues found that the *EphA1* is associated

with the likelihood of being amyloid positive, as measured by PiB-PET. In contrast to the findings for *ABCA7*, the authors described decreasing risk of amyloid positivity for each C allele of *EphA1* (rs11767557) (204).

CD33

Sialic acid binding immunoglobulin-like lectin-3 (*CD33*) is a membrane-bound receptor expressed on immune cells (208). It plays an important role in the differentiation of immature immune cells and the signaling of mature immune cells in the innate and adaptive immune system (208). Despite strong evidence from several GWASs that *CD33* is associated with AD, this association was not fully replicated in the second stage of the IGAP consortium GWAS (7; 180; 192). This may cast some doubt on the strength and reproducibility of this gene's association with AD. Perhaps the association is specific to certain regions and genetic backgrounds. In any case, there is some preliminary work using neuroimaging to measure neural substrates of *CD33* risk.

A single study examined the relationship between *CD33* genotype and a neuroimaging phenotype. Bradshaw and colleagues found that the risk variant of *CD33* was associated with greater, diffuse amyloid deposition as measured with PiB-PET imaging (209).

BDNF

Brain-derived neurotrophic factor (BDNF) is a growth factor that is widely expressed in the brain, including nearly all cortical areas. Through signaling with its main receptor tropomyosin-related kinase receptor type B (TRKB), BDNF is involved in regulating and supporting essential physiological functions in the adult brain. The literature supporting this assertion is vast and varied. For example, BDNF has been shown to modulate synaptic plasticity and dendritic spine dynamics and morphology (210). BDNF also supports long-term potentiation in the hippocampus, a process that is essential for learning and memory (211).

Furthermore, it has been suggested that BDNF plays an important role in energy homeostasis, providing a link between peripheral glucose metabolism and the brain that might be responsible for mediating the cognitive sequelae of physical exercise and a lack thereof (212). On account of the role of BDNF in these crucial processes, therapeutic uses of synthetic BDNF are actively being explored in a variety of neurological and psychiatric disorders (213). For example, in several animal models of AD, ranging from mice to non-human primates, treatment with BDNF has been shown to recover lost synapses, promote normal cell signaling and alleviate learning and memory deficits (214). Thus, despite the fact that *BDNF* has not been directly associated with AD incidence, there is certainly evidence that BDNF may mediate neuroprotective processes that may slow, or even reverse, some aging and AD-related neuronal changes (215).

There is a common polymorphism within the gene that encodes BDNF that results in a methionine substitution for a valine at codon 66 of the protein (Val66Met). The BDNF Met variant is expressed at normal levels, but the secretion of the protein from neurons is decreased (216). A possible relationship between this polymorphism and neuroimaging endophenotypes of AD has been explored in a small number of studies. In a report by Lim and colleagues, healthy Met carriers who had high Aβ, as measured by PiB-PET, had higher rates of hippocampal volume loss over 3 years than Val/Val homozygotes (217). In addition, the Met carriers with high Aβ showed more dramatic decline in cognition, including measures of executive functioning and episodic memory. There were no differences between healthy Met carriers and Val/Val homozygotes in the low Aβ group. These findings indicate that Met carrier status may help to predict decline in individuals who may be in the preclinical phase of AD (217). Another study found that Val66Met, as well as other SNPs within the *BDNF* gene, were associated with hippocampal and cortical atrophy over two years in a mixed cohort of healthy adults and patients with MCI and AD (218). The authors concluded that though *BDNF* was not associated with AD diagnosis, it is a factor in AD-related neurodegeneration measured by neuroimaging (218). In a study of healthy adults from age 19 to 82, Val66Met interacted with age to predict

cortical thinning in the entorhinal cortex and adjacent temporal areas (219). The authors of this study also found an Val66Met-age interaction that predicted decreased FA in temporal white matter tracts, indicating a loss of white matter integrity in these regions (219). These studies, and others, indicate that BDNF variants may modulate the severity of AD-related changes to cortical and hippocampal morphology (220). There is also some evidence that the Val66Met variant is related to glucose metabolism measured by FDG-PET. One study found decreased metabolism in healthy older Met carriers in the right parahippocampal gyrus and the superior temporal gyrus (221). The authors also report increased metabolism in healthy Met carriers in frontal regions. This pattern of differences was also observed in patients with MCI. Further studies examining *BDNF* variants and FDG-PET imaging are needed to replicate these results, as well as to help elucidate whether proposed connections between BDNF and peripheral metabolism extend to central glucose metabolism (212). Finally, a recent study by Adamczuk and colleagues showed an interesting relationship between APOEε4 and Val66Met, such that APOEε4 carriers who were also Met carriers had higher Aβ load than APOEε4 carriers who were Val/Val homozygotes (222). However, in APOEε4 non-carriers, there was no association between Aβ load and the Val66Met variant. These findings point to a potential interaction between APOEε4 and *BDNF* Met variant. Better characterization of this relationship, as well as the other findings described in this section, will be essential to developing potential *BDNF*-based therapies for use in the AD.

Neuroimaging-Identified Alzheimer's Disease Risk Genes

In addition to *APOE*, *TOMM40*, *TREM2* and GWAS-identified AD-risk loci, there are genes that have been identified as potential risk factors for AD through the use of human neuroimaging. This represents a reversal of the types of studies we have covered thus far in this chapter, in which genetic associations are discovered via epidemiological studies, molecular biology experiments, linkage analyses or GWAS of a disease state, and then the effects of

these genetic variants are studied in living humans using neuroimaging. In contrast, the following studies use neuroimaging and creative experimental design in order to search for genetic associations with disease biomarkers, such as hippocampal atrophy. These studies are examples of some of the most exciting work materializing from the field of neuroimaging genetics because they are an example of true co-operation of human genetics methods and neuroimaging biomarkers/endophenotypes.

In a creative experiment by Nho and colleagues, a small set of subjects from the Alzheimer's Disease Neuroimaging Initiative (ADNI) who had experienced either extreme or very little hippocampal atrophy over two years were selected and their exomes were sequenced (223). Often, the loci identified in genetic association studies are not coding variants, but exome sequencing ensures that any associations one finds will be a potentially functional variant. The authors isolated 57 SNP variants that were found in all rapid atrophy subjects and in none of the slow atrophy subjects. Next, they used these SNPs and performed a quantitative trait analysis on a separate, larger cohort of subjects homozygous for the APOEε3 allele. Two genes, *PARP1* and *CARD10*, were associated with the rate of hippocampal atrophy in the larger validation group. While further research is required to assess the reproducibility and clinical utility of these results, this cross-discipline design utilizes large cohort enrollment efforts by selecting for extreme cases, advancing human genetics methods for exome sequencing and neuroimaging for measurement of a potential biomarker. We believe that experiments like this will become more common as neuroimaging genetics methods are further integrated into mainstream biomedical research.

A study by Shen and colleagues, also used ADNI data in order to perform many GWASs using a different neuroimaging measure as the target phenotype in each case (224). The authors used voxel-based morphometry to define cortical gray matter volume, as well as the volume of 43 ROIs in each hemisphere. Freesufer was also used to calculate cortical thickness and volume measures for ROIs across the cortical mantle. After an iterative GWAS was

performed for each of these potential endophenotypes, it was not surprising that *APOE* and *TOMM40* were associated with several ROIs, including bilateral hippocampus and amygdala, volume of right cerebral cortex and cortical thickness of left cerebral cortex. Several additional genes were implicated, including *EphA4*, *TP63* and *NXPH1*. Further analyses focused on the locus proximal to *NXPH1* showed that subjects homozygous for the putative risk allele had significantly reduced bilateral hippocampal gray matter density (224).

Another area of interest in neuroimaging genetics involves testing for the association of functional pathways with neuroimaging measures (225; 226). In other words, based on previous understanding of protein interactions and signaling, genes that encode proteins in a given biological pathway can be assessed for an aggregate association with a phenotype. Putative gene pathways are available in a variety of databases including the Molecular Signatures Database (227). In a study by Silver and colleagues, the authors present a new statistical method for testing biological pathway associations called sparse reduced-rank regression (225). Using this method and a voxel-wise measurement of structural change intended to maximize the difference in trajectories between normal controls and AD patients, the authors found that insulin signaling, vascular smooth muscle contraction and focal adhesion pathways were associated with AD-related changes. They then took their analyses a step further and tried to identify single SNPs that might be driving the association of these pathways and found nearly 10 candidates. The authors also found that APOE, TOMM40 and CR1 were associated with their voxel-wise endophenotype, suggesting that it captures disease-related structural changes in AD (225).

Thus far, genetic risk factors for AD have predominantly been identified in large cohorts of white American or European individuals, with relatively little effort made to replicate findings in different ethnoracial groups. In a 2012 study by Melville and colleagues, a specific aim of their research was to identify SNPs that associate with neuroimaging phenotypes in both Caucasian and African American cohorts (228). The neuroimaging endophenotypes they chose to query

were total cerebral volume, hippocampal volume and the volume of white matter hyperintensities. SNP associations were evaluated in a 2-stage reproducible analysis, a common study design in GWASs. The unique feature of this study was that the stage 1 cohorts were Caucasian cohorts and the stage 2 cohort was composed of African American individuals. Thus, they reported only genes that were associated with a given phenotype in these two different ethnoracial groups. The authors found that loci within APOE, as well as F5/SELP, LHFP and GCFC2, were associated with hippocampal volume. In addition, they reported that two different SNPs both in the PICALM gene were associated with hippocampal volume in the Caucasian and African American cohorts (228). This finding in PICALM is very interesting because it indicates that specific risk variants may differ between ethnic or racial groups, even within the same risk gene.

Recently, a new, potential dementia associated gene, SPON1, was shown to be associated with the density of structural connections between the left posterior cingulate gyrus and the left superior parietal lobe, such that carrying two copies of the minor allele was associated with increased structural connectivity (229). Before this relationship was uncovered, the authors used their cohort of young adult twins to assess the heritability of a structural connectome created using tractography and a common cortical parcellation. This was an important step because it indicated that portions of the structural connectome were heritable enough to perform genome-wide association scanning. After SPON1 was implicated as a genetic factor mediating structural connectivity between two nodes, the authors tested the relationship between the putatively protective SPON1 variant and the morphology of the brain, finding associations with larger posterior cingulate cortex volume and smaller ventricular size. Furthermore, the minor allele was associated with milder dementia as measured by the Clinical Dementia Rating scale (229).

The publications discussed in this section are examples of neuroimaging genetics studies that aim to identify genetic risk loci associated with neuroimaging biomarkers or

endophenotypes. This requires authors to perform interdisciplinary work that spans the fields of human genetics and neuroimaging. It is likely that neuroimaging genetics will move further into this interdisciplinary space in the future, with fewer 'candidate gene' studies and more experiments like those reviewed here. In addition, efforts to develop new methods to statistically test the association of many genetic risk factors as a single polygenic risk score or metric are sure to be a major focus of the neuroimaging genetics field moving forward. Unfortunately, the sample sizes of these kinds of studies are lagging behind those that have been achieved in AD GWASs. Although there is evidence that GWASs of neuroimaging endophenotypes are more statistically efficient than GWASs based on behavior, sample sizes still need to grow in order to ensure generalizability and reproducibility (230). The smaller sample sizes are due in part to difficulties in combining neuroimaging datasets caused by differences in acquisition parameters, processing and inclusion/exclusion criteria that may produce a confounding affect in a given neuroimaging measure. However, data sharing efforts that focus on resolving these issues with standardized protocols are gaining ground. One effort that is specifically focused on neuroimaging genetics is the Enhancing NeuroImaging Genetics through Meta-Analysis (ENIGMA) project (231). The aim of the ENIGMA project is to increase the ability of neuroimaging genetics researchers to share their own data, as well as access others' data in order to increase statistical power in their own studies.

Family History of Alzheimer's Disease

Thus far, we have discussed neuroimaging genetics approaches that focus on either a known genetic cause of AD, a known genetic risk factor for AD or identifying new genetic associations. Another approach in neuroimaging genetics of AD is to study the effect of a family history of the disease. A family history of AD confers a strong predisposition to the disease, doubling one's chances of developing AD (232). A positive family history of AD may indicate that a subject carries genetic risk factors for AD perhaps even above and beyond the known

susceptibility loci that were covered in previous sections. It can be thought of as a composite genetic risk factor that may reflect susceptibility conferred by both known and unknown risk genes (88). The risk for AD captured by family history cannot be explained by *APOE* alone. Several studies have found additive effects of family history and *APOE* (88). This supports the idea that family history is a composite measure and cannot be explained or supplanted by even the strong genetic association of *APOE* to AD.

A positive family history of AD is associated with higher rates of thinning in the hippocampus, especially in the entorhinal cortex and subiculum, in cognitively normal, older subjects (233). Family history has also been linked to hippocampal volume in middle-aged adults, specifically in the left hippocampus (234). Furthermore, cognitively normal, older subjects with a family history of AD show more severe *whole-brain* gray matter volume loss than subjects with a negative family history (235). Interestingly, gray matter volume loss was significant in the precuneus, parahippocampal gyrus and the hippocampus when subjects with only a maternal history of AD were compared to the negative family history group or to the paternal family history group (235). This indicates that phenotypic differences ascribed to subjects with a family history of AD might actually be driven by subjects with a maternal history of AD. Evidence in the literature supports the theory that a maternal history of AD results in more severe changes in structural endophenotypes than a paternal history of AD (236; 237). There is also evidence that a maternal history of AD is related to Aβ load. PiB tracer uptake in people with a maternal history of AD reveals significantly more Aβ in parietal cortex, the precuneus, posterior cingulate and sensorimotor cortex when compared to subjects with a paternal history of AD (238; 239). The mechanism of this relationship between maternal family history of AD and risk for the disease is not known. However, it has been posited that maternal-lineage inheritance of mitochondrial DNA may play an important role (240). In fact, there is preliminary evidence that adult children whose mothers have AD show mitochondrial

dysfunction and reduced cytochrome oxidase activity compared to subjects with an affected father and to controls with a negative family history (240).

In a recent study by Mosconi and colleagues, subjects with a family history of AD were further subdivided into groups of individuals who had a maternal history of AD, a paternal history of AD *or both* a maternal and paternal history of AD (241). These three groups were then each compared to a reference group of age and sex-matched subjects who had a no family history of AD. The authors found that the subjects who had a history of AD in both their maternal and paternal lineages showed more severe alterations in all of the neuroimaging phenotypes they measured. Specifically, these subjects had higher retention of the PiB tracer, indicative of higher Aβ load, as well as lower FDG uptake, indicative of hypometabolism. In addition, subjects with a history of AD on both sides of their family had more severe gray matter volume reductions across the cortex (241). When the authors examined the subjects with only a maternal history of AD, they found intermediate phenotypes, followed by subjects with only a paternal history. Their findings are in line with others that reported more severe changes in neuroimaging endophenotype measures in people with a maternal family history of AD. The discovery that there is an additional additive effect of a maternal and paternal history of AD is a novel finding that will certainly be important in designing studies of family history of AD in the future.

As we have discussed, DMN connectivity is altered in FAD and in APOEε4 carriers of varying ages. There is also evidence that DMN connectivity is modulated by family history of AD (242; 243). One study found that subjects with a family history of AD (they did not specify maternal or paternal lineage) was associated with reduced connectivity between the posterior cingulate and the medial temporal lobe (243). Another paper describes a direct comparison of the ability of task-based fMRI and resting state fMRI to differentiate between two groups stratified by AD risk: subjects with a family history of AD and at least one copy of APOEε4 versus subjects with no family history and no APOEε4 alleles (242). Comparing DMN average connectivity between the two groups was the best differentiator, accounting for 62% of the

variance due to risk group membership compared to only 25% accounted for when comparing task-related fMRI activations (242). The consistency of the DMN disruption findings across different cohorts representing preclinical AD or risk for AD indicates that DMN functional connectivity may be a uniquely viable fMRI-based endophenotype for AD.

Future Directions and Challenges

The challenges associated with the neuroimaging genetics field apply to neuroimaging genetics of AD with one major exception. As described earlier, *APOE* accounts for a large amount of the genetic variance of AD, more than any single genetic locus in another human polygenic disorder. Because of this, there is a particularly large body of work using *APOE* in 'candidate gene' type neuroimaging experiments. The sheer volume of these studies, although not all in agreement, is indicative of the unique position of *APOE* in the field of human genetics. In theory, because *APOE* is soaking up a good portion of heritability variance in AD, it is possible that polygenic risk modeling will be easier in AD than in other common polygenic diseases. This makes AD an attractive disease for neuroimaging genetics researchers. Indeed, attempts to model multiple genetic risk factors in neuroimaging studies of AD have showed promise, predicting conversion from MCI to AD as well as cortical thickness changes in AD-vulnerable regions (244; 245).

The causal variants that give rise to the APOEε4 allele, which then confers increased risk for AD, are known polymorphisms at rs429358 and rs7412. Variants at these sites alter the structure and function of the APOE protein (246). In fact, so-called APOEε4 "structure correctors" which make APOEε4 behave like the more common APOE3 are currently being developed as a possible treatment for AD (247). In contrast, many of the GWAS-identified AD risk loci (*CLU*, *BIN1*, *ABCA7*, *EphA1*) are located in intronic (*CLU*, *ABCA7*) or intragenic (*BIN1*, *EphA1*) regions with no evidence that variants affect protein structure or function. In general, an intragenic region may play some regulatory function, but in the cases of *EphA1* and *BIN1* there

is little evidence of conservation of these intragenic regions, therefore making a regulatory role in genetic expression unlikely (181). Thus, the search for the causal variants is still ongoing for these genes and, also, for genes implicated in other common disease by GWASs (248). Ostensibly, the causal variant for one of these genetic risk loci will be a polymorphism in high linkage disequilibrium with the GWAS locus that affects the function of the gene's protein product in some way. It is possible that the polymorphisms driving the signal of these GWAS associations are rare variants occurring in less than 5% of people (minor allele frequency <0.05) (248). If this is the case, large sample sizes in GWASs will increase our ability to detect rare polymorphisms associated with disease. Still, it remains to be seen if the underlying genetics of a common disease like AD will be best described as the coincidence of several strong-effect rare variants or of many low-effect common variants. In either case, until the casual variants of GWAS-identified risk loci are identified, the utility of these risk factors in targeted efforts like drug development is minimal.

From the perspective of neuroimaging, there are advantages and disadvantages to the rare-variant or common-variant theory of AD genetics. Obviously, because rare variants occur in so few individuals it would be difficult to amass a large enough cohort of carriers to produce statistically significant results. However, the field is moving fast toward larger and larger datasets through data sharing efforts and multi-center study designs. Access to ever-expanding reservoirs of data may mean that reasonably sized samples of individuals with specific rare variants may be plausible. The great advantage of studying rare variants with neuroimaging is that the effect size of these rare variants is likely to be much larger than common variants, likely making differences between carrier groups easier to detect, even at smaller sample sizes. In contrast, methods for modeling multiple genetic risk factors in a single experiment are actively being developed and may help to exploit the synergistic predictive power of many low-effect-size common variants.

As a final note on GWAS-identified genetic risk factors for AD, it is important to recognize that the 20 loci discussed in this chapter were identified using large cohorts of Caucasian European or American subjects. There are many reasons the genetic loci implicated by these studies might fail to replicate in a cohort of subjects from a different ethnic background, including population specific variants, differing patterns of linkage disequilibrium or even a heterogeneous genetic basis of AD in different ethnic groups. As an illustrative example, many small GWAS studies have tried to replicate the association of CLU with AD in non-Caucasian cohorts. The results of these studies tell us that there appears to be an association between CLU and AD in Chinese cohorts, but not in cohorts of non-white Americans or Europeans (184; 249; 250). Clearly, this is a limitation of the published large GWASs in AD and a greater effort must be made to amass comparably large samples of different ethnoracial groups for study. It is possible that this effort may result in the identification of certain genes that are associated with AD regardless of genetic background, and that these genes could then be the focus of increased research resources due to their greater generalizability. In addition, further exploration of the genetic basis of AD in people of African and Hispanic descent may help elucidate epidemiological differences observed in these ethnic groups, including higher incidence and earlier onset of AD (251).

Another challenge in the field is the predominant use of cross-sectional experimental designs in trying to elucidate the pathophysiological trajectory of AD. Given the importance of early detection in neurodegenerative diseases as well as the published associations of various AD risk genes with differences in brain structure and function in young people (even children and infants), it is clear that longitudinal mapping of disease progression is essential in the fight against AD. A better understanding of how the disease manifests in individuals, each with his or her own unique genetic and environmental risk profile, would help clinicians detect preclinical AD. As described earlier, preclinical AD, or the phase of AD before changes in behavior and symptoms emerge, is believed to provide the best opportunity for treatment, especially with a

progression-halting drug. Such a drug is not available yet, but accurate definition of preclinical AD will be crucial to the success of any candidates. So how do we design experiments to study AD risk and preclinical AD? Overwhelmingly, inferences about the trajectory of AD are made from cross-sectional studies in which data are collected from each subject only once and all the subjects are randomly distributed across the age range under investigation. This approach is problematic because cross-sectional studies are excellent at confounding between-subject and within-subject variation (252). In other words, in a cross-sectional study, one loses the ability to separate differences mediated by normal variation in a given subject from variation across two different subjects, or cohorts of subjects. Thus, drawing longitudinal conclusions based on cross-sectional evidence, even based on many cross-sectional studies, is precarious and should be done cautiously (253). Longitudinal designs are better for making inferences about disease trajectory but they are difficult in practice. Still, multi-cohort longitudinal designs are feasible in today's pro-collaboration atmosphere because many sites can collect longitudinal data on a relatively small number of subjects and then, assuming that proper standardization and oversight are in place, these subjects can be combined to create a much larger cohort. Indeed, ADNI is a good example of this type of effort in neuroimaging genetics of AD. We believe that future efforts to define the pathophysiological trajectory of AD through neuroimaging genetics should follow this example of a multi-cohort longitudinal design.

Relevance and Impact

Alzheimer's disease affects more than 13% of individuals aged 65 years and older, a subset of the population that is rapidly growing across the world. According to a recent report by the United States Census Department, by the benchmark year of 2050 there will be nearly 84 million people aged 65 years and older, with at least 13 million individuals suffering from AD (254; 255). The number is likely to be higher as the risk for AD grows proportionately with age and the fraction of the above 65 set who are the oldest old (say, over 95 years, when over 40%

of individuals have AD) is continuously growing as people live longer and longer lives (25). Although the focus of this chapter is on neuroimaging genetics of AD, age is the most predictive risk factor for AD so demographic trends in aging are an important factor to weigh in understanding the future need for AD research.

AD is a debilitating disease that, especially when combined with other age-related health struggles, can require years of part or full-time care for a single patient. The economic impact of this looming need for elder care providers is difficult to fully grasp. There is, of course, the high cost of professional care, either in the home or in an institution, which is prohibitive for many older Americans. There is also the economic burden that families will take on to care for aging relatives. A large proportion of elder care will be provided wage-free by adult children who, in order to be available to ailing parents, may be forced to leave jobs or dip into their own savings.

The predictions for the future of AD in the United States, and indeed across the world, paint a dark picture in which advancing medical care, public health interventions and programs and improved healthcare literacy will lead to more and more people reaching extreme old age with nothing to protect them from the reality of high AD incidence.

Imagine a scenario where, in the future, an individual could undergo a battery of non-invasive tests including cognitive testing, a blood draw for genetic profiling and MR and/or PET imaging in order to generate a report or panel detailing the likelihood that he or she will go on to develop AD. What if that report could estimate with a high certainty the age of onset? Of course, these revelations in the absence of effective treatment would lead to a situation similar to the current state of Huntington's disease (HD) diagnosis. Many at risk for HD choose to learn what their genetic fate is but many do not (256). The latter group sees no benefit to knowing their fate in advance, especially if that fate is to die of a terrible degenerative disease. Genetic counseling is a crucial aspect of these difficult decisions. But even with genetic counseling and support, learning that you will one day develop an unpreventable and untreatable disease can hardly be argued as universally empowering knowledge.

However, the ability to make such predictions and then adjust the predictions when they fall short is an important part of clinical trial design. In other words, the better we are at estimating, on an individual basis, AD risk or AD clinical stage the better we will be at enrolling a homogenous group in treatment trials (see Figure 1.6 for schematic). Phase 3 AD treatment trials in humans have almost exclusively failed, even after very promising data in model organisms and in earlier trial phases (257; 258). A potential reason for this high failure rate is the highly heterogenous nature of the subjects being enrolled in these trials. One problem is neuropathological variation. For example, of the people with AD who come to autopsy, up to 75% of those patients also have vascular pathology severe enough to have contributed to their dementia syndrome (259). Before neuropathological processing, it is extremely difficult to differentiate so-called “pure AD” from mixed AD and vascular disease. Furthermore, a clinical diagnosis of AD corresponds to a neuropathological diagnosis of AD (pure or mixed pathology) about 80% of the time (260). These leaves 20% of clinically diagnosed AD subjects who in fact had another disease entirely, like frontotemporal lobar degeneration (FTLD) or corticobasal degeneration (CBD). It is not unreasonable to assume that subjects with each of these diseases, from pure AD and mixed AD pathology to FTLD and CBD, will respond differently (if at all) to treatments that target a single molecular species, like Aβ oligomers or plaques. Therefore, a concerted effort must be made to minimize incorrect clinical-pathological diagnoses in subjects enrolled in clinical trials. Unfortunately, the only way to make a pathological diagnosis is by examining the brain tissue at autopsy. Luckily, PET imaging allows clinicians and researchers to shed some light on what is inside the black box. Using PET imaging of Aβ and tau as a prescreening technique in clinical trials, while expensive, may increase our ability to amass a pathologically homogeneous cohort. Indeed, neuropathological prescreening using PET imaging has just been implemented for the first time as part of the Anti-Amyloid Treatment in Asymptomatic AD (A4) trial which is requiring a positive Aβ florbetapir-PET scan for enrollment into the treatment arm of the trial (261).

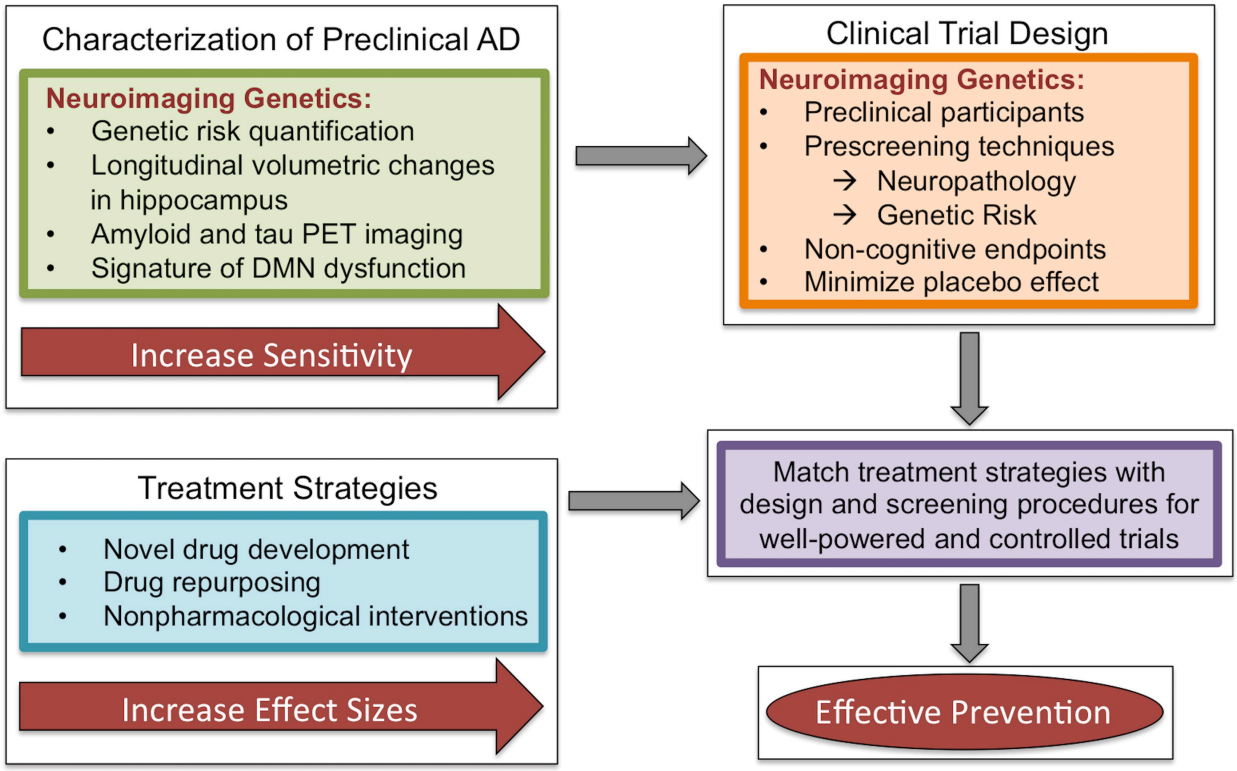


Figure 1.6: Neuroimaging Genetics in the Path to Alzheimer's Disease Prevention. Neuroimaging genetics research characterizing the preclinical phase of AD is aimed at improving our understanding of the pathophysiology of AD, specifically the prodrome that precedes memory loss (and other cognitive symptoms). Neuroimaging genetics will empower clinical trials by informing enrollment procedures, increasing the ability to enroll participants who have preclinical AD, thus increasing AD incidence in the cohort. When potential treatments come to clinical trial, neuroimaging genetics approaches will play a critical role in prescreening procedures and end point (outcome) definition.

In addition to heterogeneity of neuropathology in clinical trial subjects, we must also consider the heterogeneity of the underlying genetics in each individual subject (Figure 1.6). It is not a particularly new idea that genetic variation can predispose individuals to respond well or less well to pharmacological treatments (262). One well-validated example of this is the connection between genetic variation and the efficacy of antidepressants, a class of drugs targeted to the brain. Specifically, variants in the FKBP5 gene have been linked to better response to antidepressants, especially when given in combination (263). FKBP5 is a co-chaperone of the glucocorticoid receptor, which is involved in hypothalamic-pituitary-adrenal axis activation, a pathogenic state for depression (263). Given these findings, it is reasonable to

assume that there may be variation in drug response in trial participants with different genetic risk factors for AD. In order to control for as many of these genetic variables as possible, clinical trials should consider implementing genetic prescreening measures that select for participants that have certain genetic risk factors for AD. An interesting study by Kohannim and colleagues tested the theory that a genetic prescreening protocol would decrease the sample size necessary to detect a treatment effect (264). In other words, they were interested in understanding how homogenizing the genetic risk profile of trial participants in favor of higher risk would affect the statistical power of a hypothetical trial. Specifically, the authors ranked 394 cognitively healthy and MCI ADNI subjects in order of decreasing genetic risk score, calculated based on multiplying risk alleles for APOE, CLU, CR1 and PICALM by the logarithm of the odds ratios reported for each gene in GWASs. They found that by selecting only the top 15% of subjects in order of highest genetic risk, the required sample size to show differences in temporal lobe atrophy decreased from 142 to 69 (264). This provides excellent preliminary evidence that genetic pre-screening would increase statistical power in trials. Binning participants by genetic risk may very well be the next frontier in AD clinical trial design.

Let us return to the hypothetical report detailing one's AD risk and prognosis. The measures that will comprise that report are minimally invasive cognitive testing, genetic sequencing, CSF analysis and neuroimaging. These are the tools we have available when studying living humans. The field of neuroimaging genetics of AD is performing research that explicitly combines two of these data types, and often incorporates the others. This research has and will continue to lead to the insights needed in order to prescreen participants for clinical trials, increasing the ability of those trials to detect the effect of a useful drug. Another important role for neuroimaging genetics in the fight against AD is the development of hard (non-cognitive) endpoints to assess treatment efficacy in clinical trials (Figure 1.6). Most AD trials to date have used soft endpoints like paper-and-pencil memory measures or a composite dementia severity

scores (257; 258). These soft endpoints are particularly vulnerable to confounding effects, such as the placebo effect and the within-subject variance (the ‘good day, bad day’ phenomenon).

The proximal goal of an individualized AD risk report based on genetics, neuroimaging biomarkers and other measures is the pre-selection of clinical trial participants (Figure 1.6). The distal goal is to provide detailed prognoses in the clinic, combined with effective treatment. Neuroimaging genetics research in AD will play an essential role in achieving these goals.

Chapter 1 References:

1. SFARI Gene Database Webpage (n.d.).: . Retrieved from https://gene.sfari.org/autdb/HG_Home.do.
2. Ripke S, Neale BM, Corvin A, Walters JTR, Farh K-H, Holmans PA, *et al.* (2014): Biological insights from 108 schizophrenia-associated genetic loci. *Nature* 511: Nature Publishing Group, a division of Macmillan Publishers Limited. All Rights Reserved.421–427.
3. Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, *et al.* (1993): Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* 261: 921–3.
4. Bergem AL, Engedal K, Kringlen E (1997): The role of heredity in late-onset Alzheimer disease and vascular dementia. A twin study. *Arch Gen Psychiatry* 54: 264–70.
5. Gatz M, Reynolds CA, Fratiglioni L, Johansson B, Mortimer JA, Berg S, *et al.* (2006): Role of genes and environments for explaining Alzheimer disease. *Arch Gen Psychiatry* 63: 168–74.
6. Waring SC, Rosenberg RN (2008): Genome-wide association studies in Alzheimer disease. *Arch Neurol* 65: 329–34.
7. Lambert JC, Ibrahim-Verbaas CA, Harold D, Naj AC, Sims R, Bellenguez C, *et al.* (2013): Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. *Nat Genet* 45: Nature Publishing Group, a division of Macmillan Publishers Limited. All Rights Reserved.1452–8.
8. IGAP Website (n.d.).: . Retrieved from http://www.wikigenes.org/e/art/e/258.html.
9. Wisse LEM, Biessels GJ, Heringa SM, Kuijf HJ, Koek DHL, Luijten PR, Geerlings MI (2014): Hippocampal subfield volumes at 7T in early Alzheimer's disease and normal aging. *Neurobiol Aging* 35: 2039–45.
10. Yacoub E, Harel N, Ugurbil K (2008): High-field fMRI unveils orientation columns in humans. *Proc Natl Acad Sci U S A* 105: 10607–12.
11. Fujimoto K, Polimeni JR, van der Kouwe AJW, Reuter M, Kober T, Benner T, *et al.* (2014): Quantitative comparison of cortical surface reconstructions from MP2RAGE and multi-echo MPRAGE data at 3 and 7 T. *Neuroimage* 90: 60–73.
12. Boyacioğlu R, Schulz J, Müller NCJ, Koopmans PJ, Barth M, Norris DG (2014): Whole brain, high resolution multiband spin-echo EPI fMRI at 7 T: a comparison with gradient-echo EPI using a color-word Stroop task. *Neuroimage* 97: 142–50.
13. Alexander AL, Lee JE, Lazar M, Field AS (2007): Diffusion tensor imaging of the brain. *Neurotherapeutics* 4: 316–29.
14. Shi X, Ma X, Wu W, Huang F, Yuan C, Guo H (2014): Parallel imaging and compressed sensing combined framework for accelerating high-resolution diffusion tensor imaging using inter-image correlation. *Magn Reson Med*. doi: 10.1002/mrm.25290.
15. Wong DF, Rosenberg PB, Zhou Y, Kumar A, Raymont V, Ravert HT, *et al.* (2010): In vivo imaging of amyloid deposition in Alzheimer disease using the radioligand 18F-AV-45 (florbetapir [corrected] F 18). *J Nucl Med* 51: 913–20.
16. Xia C-F, Arteaga J, Chen G, Gangadharmath U, Gomez LF, Kasi D, *et al.* (2013): [(18)F]T807, a novel tau positron emission tomography imaging agent for Alzheimer's disease. *Alzheimers Dement* 9: Elsevier666–76.

17. Alzheimer A, Stelzmann RA, Schnitzlein HN, Murtagh FR (1995): An English translation of Alzheimer's 1907 paper, "Uber eine eigenartige Erkankung der Hirnrinde". *Clin Anat* 8: 429–31.

18. Goedert M, Spillantini MG (2006): A century of Alzheimer's disease. *Science* 314: 777–81.

19. Hardy JA, Higgins GA (n.d.).: Alzheimer's disease: The amyloid cascade hypothesis.

20. Hardy J, Selkoe DJ (2002): The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* 297: 353–6.

21. Bennett DA, Schneider JA, Wilson RS, Bienias JL, Arnold SE (2004): Neurofibrillary tangles mediate the association of amyloid load with clinical Alzheimer disease and level of cognitive function. *Arch Neurol* 61: American Medical Association378–84.

22. Pooler AM, Noble W, Hanger DP (2014): A role for tau at the synapse in Alzheimer's disease pathogenesis. *Neuropharmacology* 76 Pt A: 1–8.

23. Keage HAD, Hunter S, Matthews FE, Ince PG, Hodges J, Hokkanen SRK, *et al.* (2014): TDP-43 pathology in the population: prevalence and associations with dementia and age. *J Alzheimers Dis* 42: 641–50.

24. Minami SS, Min S-W, Krabbe G, Wang C, Zhou Y, Asgarov R, *et al.* (2014): Progranulin protects against amyloid β deposition and toxicity in Alzheimer's disease mouse models. *Nat Med* advance on: Nature Publishing Group, a division of Macmillan Publishers Limited. All Rights Reserved. doi: 10.1038/nm.3672.

25. Fratiglioni L, De Ronchi D, Ag??ero Torres H (1999): Worldwide Prevalence and Incidence of Dementia. *Drugs Aging* 15: 365–375.

26. Mendez MF, Cummings JL (2003): *Dementia: A Clinical Approach*. . Butterworth-Heinemann. Retrieved September 29, 2014, from http://books.google.com/books?id=LzM4v_OruZ8C&pgis=1.

27. Fernando MS, Ince PG (2004): Vascular pathologies and cognition in a population-based cohort of elderly people. *J Neurol Sci* 226: 13–7.

28. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM (1984): Clinical diagnosis of Alzheimer's disease: Report of the NINCDS-ADRDA Work Group* under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology* 34: 939–939.

29. Morris JC (2005): Early-stage and preclinical Alzheimer disease. *Alzheimer Dis Assoc Disord* 19: 163–5.

30. Sperling RA, Aisen PS, Beckett LA, Bennett DA, Craft S, Fagan AM, *et al.* (2011): Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* 7: 280–92.

31. McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR, Kawas CH, *et al.* (2011): The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* 7: 263–9.

32. Jack CR, Knopman DS, Jagust WJ, Shaw LM, Aisen PS, Weiner MW, *et al.* (2010): Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. *Lancet Neurol* 9: 119–28.

33. Goate A, Chartier-Harlin MC, Mullan M, Brown J, Crawford F, Fidani L, *et al.* (1991):

Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature* 349: 704–6.

34. Schellenberg G, Bird T, Wijsman E, Orr H, Anderson L, Nemens E, *et al.* (1992): Genetic linkage evidence for a familial Alzheimer's disease locus on chromosome 14. *Science (80-)* 258: 668–671.
35. Rogaev EI, Sherrington R, Rogaeva EA, Levesque G, Ikeda M, Liang Y, *et al.* (1995): Familial Alzheimer's disease in kindreds with missense mutations in a gene on chromosome 1 related to the Alzheimer's disease type 3 gene. *Nature* 376: 775–8.
36. Cruts M, Theuns J, Van Broeckhoven C (2012): Locus-specific mutation databases for neurodegenerative brain diseases. *Hum Mutat* 33: 1340–4.
37. Jonsson T, Atwal JK, Steinberg S, Snaedal J, Jonsson P V, Bjornsson S, *et al.* (2012): A mutation in APP protects against Alzheimer's disease and age-related cognitive decline. *Nature* 488: Nature Publishing Group, a division of Macmillan Publishers Limited. All Rights Reserved.96–9.
38. Lopera F (1997): Clinical Features of Early-Onset Alzheimer Disease in a Large Kindred With an E280A Presenilin-1 Mutation. *JAMA J Am Med Assoc* 277: American Medical Association793.
39. DIAN webpage (n.d.).: . Retrieved from http://dian-info.org/default.htm.
40. Ridha BH, Barnes J, Bartlett JW, Godbolt A, Pepple T, Rossor MN, Fox NC (2006): Tracking atrophy progression in familial Alzheimer's disease: a serial MRI study. *Lancet Neurol* 5: 828–34.
41. Schott JM, Fox NC, Frost C, Scahill RI, Janssen JC, Chan D, *et al.* (2003): Assessing the onset of structural change in familial Alzheimer's disease. *Ann Neurol* 53: 181–8.
42. Bateman RJ, Xiong C, Benzinger TLS, Fagan AM, Goate A, Fox NC, *et al.* (2012): Clinical and biomarker changes in dominantly inherited Alzheimer's disease. *N Engl J Med* 367: 795–804.
43. Ringman JM, Pope W, Salamon N (2010): Insensitivity of visual assessment of hippocampal atrophy in familial Alzheimer's disease. *J Neurol* 257: 839–42.
44. Thordardottir S, Ståhlbom AK, Ferreira D, Almkvist O, Westman E, Zetterberg H, *et al.* (2014): Preclinical Cerebrospinal Fluid and Volumetric Magnetic Resonance Imaging Biomarkers in Swedish Familial Alzheimer's Disease. *J Alzheimers Dis*. doi: 10.3233/JAD-140339.
45. Cash DM, Ridgway GR, Liang Y, Ryan NS, Kinnunen KM, Yeatman T, *et al.* (2013): The pattern of atrophy in familial Alzheimer disease: volumetric MRI results from the DIAN study. *Neurology* 81: 1425–33.
46. Dickerson BC, Bakkour A, Salat DH, Feczko E, Pacheco J, Greve DN, *et al.* (2009): The cortical signature of Alzheimer's disease: regionally specific cortical thinning relates to symptom severity in very mild to mild AD dementia and is detectable in asymptomatic amyloid-positive individuals. *Cereb Cortex* 19: 497–510.
47. Quiroz YT, Stern CE, Reiman EM, Brickhouse M, Ruiz A, Sperling RA, *et al.* (2013): Cortical atrophy in presymptomatic Alzheimer's disease presenilin 1 mutation carriers. *J Neurol Neurosurg Psychiatry* 84: 556–61.
48. Knight WD, Kim LG, Douiri A, Frost C, Rossor MN, Fox NC (2011): Acceleration of cortical thinning in familial Alzheimer's disease. *Neurobiol Aging* 32: 1765–73.

49. Lee GJ, Lu PH, Medina LD, Rodriguez-Agudelo Y, Melchor S, Coppola G, *et al.* (2013): Regional brain volume differences in symptomatic and presymptomatic carriers of familial Alzheimer's disease mutations. *J Neurol Neurosurg Psychiatry* 84: 154–62.

50. Apostolova LG, Hwang KS, Medina LD, Green AE, Braskie MN, Dutton RA, *et al.* (2011): Cortical and hippocampal atrophy in patients with autosomal dominant familial Alzheimer's disease. *Dement Geriatr Cogn Disord* 32: 118–25.

51. Scahill RI, Ridgway GR, Bartlett JW, Barnes J, Ryan NS, Mead S, *et al.* (2013): Genetic influences on atrophy patterns in familial Alzheimer's disease: a comparison of APP and PSEN1 mutations. *J Alzheimers Dis* 35: 199–212.

52. Ryan NS, Keihaninejad S, Shakespeare TJ, Lehmann M, Crutch SJ, Malone IB, *et al.* (2013): Magnetic resonance imaging evidence for presymptomatic change in thalamus and caudate in familial Alzheimer's disease. *Brain* 136: 1399–414.

53. Ringman JM, O'Neill J, Geschwind D, Medina L, Apostolova LG, Rodriguez Y, *et al.* (2007): Diffusion tensor imaging in preclinical and presymptomatic carriers of familial Alzheimer's disease mutations. *Brain* 130: 1767–76.

54. Sperling R (2011): Potential of functional MRI as a biomarker in early Alzheimer's disease. *Neurobiol Aging* 32 Suppl 1: S37–43.

55. Sala-Llonch R, Fortea J, Bartrés-Faz D, Bosch B, Lladó A, Peña-Gómez C, *et al.* (2013): Evolving brain functional abnormalities in PSEN1 mutation carriers: a resting and visual encoding fMRI study. *J Alzheimers Dis* 36: 165–75.

56. Braskie MN, Medina LD, Rodriguez-Agudelo Y, Geschwind DH, Macias-Islas MA, Thompson PM, *et al.* (2013): Memory performance and fMRI signal in presymptomatic familial Alzheimer's disease. *Hum Brain Mapp* 34: 3308–19.

57. Bookheimer SY, Strojwas MH, Cohen MS, Saunders AM, Pericak-Vance MA, Mazziotta JC, Small GW (2000): Patterns of brain activation in people at risk for Alzheimer's disease. *N Engl J Med* 343: 450–6.

58. Braskie MN, Medina LD, Rodriguez-Agudelo Y, Geschwind DH, Macias-Islas MA, Cummings JL, *et al.* (2012): Increased fMRI signal with age in familial Alzheimer's disease mutation carriers. *Neurobiol Aging* 33: 424.e11–21.

59. Quiroz YT, Budson AE, Celone K, Ruiz A, Newmark R, Castrillón G, *et al.* (2010): Hippocampal hyperactivation in presymptomatic familial Alzheimer's disease. *Ann Neurol* 68: 865–75.

60. Chhatwal JP, Schultz AP, Johnson K, Benzinger TLS, Jack C, Ances BM, *et al.* (2013): Impaired default network functional connectivity in autosomal dominant Alzheimer disease. *Neurology* 81: 736–44.

61. Thomas JB, Brier MR, Bateman RJ, Snyder AZ, Benzinger TL, Xiong C, *et al.* (2014): Functional Connectivity in Autosomal Dominant and Late-Onset Alzheimer Disease. *JAMA Neurol* 71: American Medical Association1111–1122.

62. Villemagne VL, Ataka S, Mizuno T, Brooks WS, Wada Y, Kondo M, *et al.* (2009): High striatal amyloid beta-peptide deposition across different autosomal Alzheimer disease mutation types. *Arch Neurol* 66: 1537–44.

63. Koivunen J, Verkkoniemi A, Aalto S, Paetau A, Ahonen J-P, Viitanen M, *et al.* (2008): PET amyloid ligand [11C]PIB uptake shows predominantly striatal increase in variant Alzheimer's disease. *Brain* 131: 1845–53.

64. Sleegers K, Brouwers N, Gijselinck I, Theuns J, Goossens D, Wauters J, *et al.* (2006): APP

duplication is sufficient to cause early onset Alzheimer's dementia with cerebral amyloid angiopathy. *Brain* 129: 2977–83.

65. Alexander GE, Saunders AM, Szczepanik J, Strassburger TL, Pietrini P, Dani A, *et al.* (1997): Relation of age and apolipoprotein E to cognitive function in Down syndrome adults. *Neuroreport* 8: 1835–40.

66. Zigman W, Silverman W, Wisniewski HM (1996): Aging and Alzheimer's disease in Down syndrome: Clinical and pathological changes. *Ment Retard Dev Disabil Res Rev* 2: 73–79.

67. JERVIS GA (1948): Early senile dementia in mongoloid idiocy. *Am J Psychiatry* 105: 102–6.

68. Lott IT, Head E (2001): Down syndrome and Alzheimer's disease: a link between development and aging. *Ment Retard Dev Disabil Res Rev* 7: 172–8.

69. Krasuski JS, Alexander GE, Horwitz B, Rapoport SI, Schapiro MB (2002): Relation of medial temporal lobe volumes to age and memory function in nondemented adults with Down's syndrome: implications for the prodromal phase of Alzheimer's disease. *Am J Psychiatry* 159: 74–81.

70. Teipel SJ (2003): Relation of Corpus Callosum and Hippocampal Size to Age in Nondemented Adults With Down's Syndrome. *Am J Psychiatry* 160: American Psychiatric Association1870–1878.

71. Kesslak JP, Nagata SF, Lott I, Nalcioglu O (1994): Magnetic resonance imaging analysis of age-related changes in the brains of individuals with Down's syndrome. *Neurology* 44: 1039–45.

72. Teipel SJ, Alexander GE, Schapiro MB, Möller H-J, Rapoport SI, Hampel H (2004): Age-related cortical grey matter reductions in non-demented Down's syndrome adults determined by MRI with voxel-based morphometry. *Brain* 127: 811–24.

73. Beacher F, Daly E, Simmons A, Prasher V, Morris R, Robinson C, *et al.* (2010): Brain anatomy and ageing in non-demented adults with Down's syndrome: an *in vivo* MRI study. *Psychol Med* 40: Cambridge University Press611–619.

74. White NS, Alkire MT, Haier RJ (2003): A voxel-based morphometric study of nondemented adults with Down Syndrome. *Neuroimage* 20: 393–403.

75. Powell D, Caban-Holt A, Jicha G, Robertson W, Davis R, Gold BT, *et al.* (2014): Frontal white matter integrity in adults with Down syndrome with and without dementia. *Neurobiol Aging* 35: 1562–9.

76. Klunk WE, Engler H, Nordberg A, Wang Y, Blomqvist G, Holt DP, *et al.* (2004): Imaging brain amyloid in Alzheimer's disease with Pittsburgh Compound-B. *Ann Neurol* 55: 306–19.

77. Landt J, D'Abrera JC, Holland AJ, Aigbirhio FI, Fryer TD, Canales R, *et al.* (2011): Using positron emission tomography and Carbon 11-labeled Pittsburgh Compound B to image Brain Fibrillar β-amyloid in adults with down syndrome: safety, acceptability, and feasibility. *Arch Neurol* 68: American Medical Association890–6.

78. Sabbagh MN, Fleisher A, Chen K, Rogers J, Berk C, Reiman E, *et al.* (2011): Positron emission tomography and neuropathologic estimates of fibrillar amyloid-β in a patient with Down syndrome and Alzheimer disease. *Arch Neurol* 68: American Medical Association1461–6.

79. Pietrini P, Dani A, Furey ML, Alexander GE, Freo U, Grady CL, *et al.* (1997): Low glucose metabolism during brain stimulation in older Down's syndrome subjects at risk for Alzheimer's disease prior to dementia. *Am J Psychiatry* 154: 1063–9.

80. Thambisetty M, An Y, Tanaka T (2013): Alzheimer's disease risk genes and the age-at-onset phenotype. *Neurobiol Aging* 34: 2696.e1–5.

81. Bertram L, Tanzi RE (2012): The genetics of Alzheimer's disease. *Prog Mol Biol Transl Sci* 107: 79–100.

82. Strittmatter WJ, Weisgraber KH, Huang DY, Dong LM, Salvesen GS, Pericak-Vance M, *et al.* (1993): Binding of human apolipoprotein E to synthetic amyloid beta peptide: isoform-specific effects and implications for late-onset Alzheimer disease. *Proc Natl Acad Sci U S A* 90: 8098–102.

83. Schmechel DE, Saunders AM, Strittmatter WJ, Crain BJ, Hulette CM, Joo SH, *et al.* (1993): Increased amyloid beta-peptide deposition in cerebral cortex as a consequence of apolipoprotein E genotype in late-onset Alzheimer disease. *Proc Natl Acad Sci U S A* 90: 9649–53.

84. Leduc V, Domenger D, De Beaumont L, Lalonde D, Bélanger-Jasmin S, Poirier J (2011): Function and comorbidities of apolipoprotein e in Alzheimer's disease. *Int J Alzheimers Dis* 2011: 974361.

85. Cherbuin N, Leach LS, Christensen H, Anstey KJ (2007): Neuroimaging and APOE genotype: a systematic qualitative review. *Dement Geriatr Cogn Disord* 24: Karger Publishers348–62.

86. Bookheimer S, Burggren A (2009): APOE-4 genotype and neurophysiological vulnerability to Alzheimer's and cognitive aging. *Annu Rev Clin Psychol* 5: 343–62.

87. Trachtenberg AJ, Filippini N, Mackay CE (2012): The effects of APOE-ε4 on the BOLD response. *Neurobiol Aging* 33: 323–34.

88. Donix M, Small GW, Bookheimer SY (2012): Family history and APOE-4 genetic risk in Alzheimer's disease. *Neuropsychol Rev* 22: 298–309.

89. Lu PH, Thompson PM, Leow A, Lee GJ, Lee A, Yanovsky I, *et al.* (2011): Apolipoprotein E genotype is associated with temporal and hippocampal atrophy rates in healthy elderly adults: a tensor-based morphometry study. *J Alzheimers Dis* 23: 433–42.

90. Taylor JL, Scanlon BK, Farrell M, Hernandez B, Adamson MM, Ashford JW, *et al.* (2014): APOE-epsilon4 and aging of medial temporal lobe gray matter in healthy adults older than 50 years. *Neurobiol Aging* 35: 2479–85.

91. Jak AJ, Houston WS, Nagel BJ, Corey-Bloom J, Bondi MW (2007): Differential cross-sectional and longitudinal impact of APOE genotype on hippocampal volumes in nondemented older adults. *Dement Geriatr Cogn Disord* 23: 382–9.

92. Donix M, Burggren AC, Suthana NA, Siddarth P, Ekstrom AD, Krupa AK, *et al.* (2010): Longitudinal changes in medial temporal cortical thickness in normal subjects with the APOE-4 polymorphism. *Neuroimage* 53: 37–43.

93. Hostage CA, Roy Choudhury K, Doraiswamy PM, Petrella JR (2013): Dissecting the gene dose-effects of the APOE ε4 and ε2 alleles on hippocampal volumes in aging and Alzheimer's disease. *PLoS One* 8: e54483.

94. Burggren AC, Zeineh MM, Ekstrom AD, Braskie MN, Thompson PM, Small GW, Bookheimer SY (2008): Reduced cortical thickness in hippocampal subregions among cognitively normal apolipoprotein E e4 carriers. *Neuroimage* 41: 1177–83.

95. Mueller SG, Schuff N, Raptentsetsang S, Elman J, Weiner MW (2008): Selective effect of Apo e4 on CA3 and dentate in normal aging and Alzheimer's disease using high resolution MRI at 4 T. *Neuroimage* 42: 42–8.

96. Mueller SG, Weiner MW (2009): Selective effect of age, Apo e4, and Alzheimer's disease on hippocampal subfields. *Hippocampus* 19: 558–64.

97. Cherbuin N, Anstey KJ, Sachdev PS, Maller JJ, Meslin C, Mack HA, *et al.* (2008): Total and regional gray matter volume is not related to APOE*E4 status in a community sample of middle-aged individuals. *J Gerontol A Biol Sci Med Sci* 63: 501–4.

98. Espeseth T, Westlye LT, Fjell AM, Walhovd KB, Rootwelt H, Reinvang I (2008): Accelerated age-related cortical thinning in healthy carriers of apolipoprotein E epsilon 4. *Neurobiol Aging* 29: 329–40.

99. Westlye ET, Hodneland E, Haász J, Espeseth T, Lundervold A, Lundervold AJ (2012): Episodic memory of APOE ε4 carriers is correlated with fractional anisotropy, but not cortical thickness, in the medial temporal lobe. *Neuroimage* 63: 507–16.

100. Heise V, Filippini N, Ebmeier KP, Mackay CE (2011): The APOE ε4 allele modulates brain white matter integrity in healthy adults. *Mol Psychiatry* 16: Macmillan Publishers Limited908–16.

101. Brown JA, Terashima KH, Burggren AC, Ercoli LM, Miller KJ, Small GW, Bookheimer SY (2011): Brain network local interconnectivity loss in aging APOE-4 allele carriers. *Proc Natl Acad Sci U S A* 108: 20760–5.

102. Bondi MW, Houston WS, Eyler LT, Brown GG (2005): fMRI evidence of compensatory mechanisms in older adults at genetic risk for Alzheimer disease. *Neurology* 64: 501–8.

103. Borghesani PR, Johnson LC, Shelton AL, Peskind ER, Aylward EH, Schellenberg GD, Cherrier MM (2008): Altered medial temporal lobe responses during visuospatial encoding in healthy APOE*4 carriers. *Neurobiol Aging* 29: 981–91.

104. Xu G, McLaren DG, Ries ML, Fitzgerald ME, Bendlin BB, Rowley HA, *et al.* (2009): The influence of parental history of Alzheimer's disease and apolipoprotein E epsilon4 on the BOLD signal during recognition memory. *Brain* 132: 383–91.

105. Rogalski EJ, Rademaker A, Harrison TM, Helenowski I, Johnson N, Bigio E, *et al.* (n.d.).: ApoE E4 is a susceptibility factor in amnestic but not aphasic dementias. *Alzheimer Dis Assoc Disord* 25: 159–63.

106. Suthana NA, Krupa A, Donix M, Burggren A, Ekstrom AD, Jones M, *et al.* (2010): Reduced hippocampal CA2, CA3, and dentate gyrus activity in asymptomatic people at genetic risk for Alzheimer's disease. *Neuroimage* 53: 1077–84.

107. Adamson MM, Hutchinson JB, Shelton AL, Wagner AD, Taylor JL (2011): Reduced hippocampal activity during encoding in cognitively normal adults carrying the APOE ε4 allele. *Neuropsychologia* 49: 2448–55.

108. Heise V, Filippini N, Trachtenberg AJ, Suri S, Ebmeier KP, Mackay CE (2014): Apolipoprotein E genotype, gender and age modulate connectivity of the hippocampus in healthy adults. *Neuroimage* 98: 23–30.

109. Damoiseaux JS, Seeley WW, Zhou J, Shirer WR, Coppola G, Karydas A, *et al.* (2012): Gender modulates the APOE ε4 effect in healthy older adults: convergent evidence from functional brain connectivity and spinal fluid tau levels. *J Neurosci* 32: 8254–62.

110. Machulda MM, Jones DT, Vemuri P, McDade E, Avula R, Przybelski S, *et al.* (2011): Effect of APOE ε4 status on intrinsic network connectivity in cognitively normal elderly subjects. *Arch Neurol* 68: 1131–6.

111. Sheline YI, Morris JC, Snyder AZ, Price JL, Yan Z, D'Angelo G, *et al.* (2010): APOE4 allele disrupts resting state fMRI connectivity in the absence of amyloid plaques or decreased

CSF Aβ42. *J Neurosci* 30: 17035–40.

112. Westlye ET, Lundervold A, Rootwelt H, Lundervold AJ, Westlye LT (2011): Increased hippocampal default mode synchronization during rest in middle-aged and elderly APOE ε4 carriers: relationships with memory performance. *J Neurosci* 31: 7775–83.
113. Fleisher AS, Chen K, Liu X, Roontiva A, Thiyyagura P, Ayutyanont N, *et al.* (2011): Using positron emission tomography and florbetapir F18 to image cortical amyloid in patients with mild cognitive impairment or dementia due to Alzheimer disease. *Arch Neurol* 68: 1404–11.
114. Morris JC, Roe CM, Xiong C, Fagan AM, Goate AM, Holtzman DM, Mintun MA (2010): APOE predicts amyloid-beta but not tau Alzheimer pathology in cognitively normal aging. *Ann Neurol* 67: 122–31.
115. Fleisher AS, Chen K, Liu X, Ayutyanont N, Roontiva A, Thiyyagura P, *et al.* (2013): Apolipoprotein E ε4 and age effects on florbetapir positron emission tomography in healthy aging and Alzheimer disease. *Neurobiol Aging* 34: 1–12.
116. Scheinin NM, Wikman K, Jula A, Perola M, Vahlberg T, Rokka J, *et al.* (2014): Cortical ^{11}C-PIB uptake is associated with age, APOE genotype, and gender in "healthy aging". *J Alzheimers Dis* 41: 193–202.
117. Knopman DS, Jack CR, Wiste HJ, Lundt ES, Weigand SD, Vemuri P, *et al.* (2014): 18F-fluorodeoxyglucose positron emission tomography, aging, and apolipoprotein E genotype in cognitively normal persons. *Neurobiol Aging* 35: 2096–106.
118. Reiman EM, Caselli RJ, Yun LS, Chen K, Bandy D, Minoshima S, *et al.* (1996): Preclinical evidence of Alzheimer's disease in persons homozygous for the epsilon 4 allele for apolipoprotein E. *N Engl J Med* 334: 752–8.
119. Lowe VJ, Weigand SD, Senjem ML, Vemuri P, Jordan L, Kantarci K, *et al.* (2014): Association of hypometabolism and amyloid levels in aging, normal subjects. *Neurology*. doi: 10.1212/WNL.0000000000000467.
120. Riese F, Gietl A, Zölch N, Henning A, O'Gorman R, Kälin AM, *et al.* (2015): Posterior cingulate γ-aminobutyric acid and glutamate/glutamine are reduced in amnestic mild cognitive impairment and are unrelated to amyloid deposition and apolipoprotein E genotype. *Neurobiol Aging* 36: 53–9.
121. Gomar JJ, Gordon ML, Dickinson D, Kingsley PB, Uluğ AM, Keehlisen L, *et al.* (2014): APOE genotype modulates proton magnetic resonance spectroscopy metabolites in the aging brain. *Biol Psychiatry* 75: 686–92.
122. Kantarci K, Smith GE, Ivnik RJ, Petersen RC, Boeve BF, Knopman DS, *et al.* (2002): 1H magnetic resonance spectroscopy, cognitive function, and apolipoprotein E genotype in normal aging, mild cognitive impairment and Alzheimer's disease. *J Int Neuropsychol Soc* 8: 934–42.
123. Laakso MP, Hiltunen Y, Könönen M, Kivipelto M, Koivisto A, Hallikainen M, Soininen H (2003): Decreased brain creatine levels in elderly apolipoprotein E epsilon 4 carriers. *J Neural Transm* 110: 267–75.
124. Spencer DC, Zitzelberger T, Spielman D, Kaye J (2003): MRS in relation to hippocampal volume in the oldest old. *Neurology* 60: 1194–6.
125. Matura S, Prvulovic D, Jurcoane A, Hartmann D, Miller J, Scheibe M, *et al.* (2014): Differential effects of the ApoE4 genotype on brain structure and function. *Neuroimage* 89: 81–91.
126. Richter-Schmidinger T, Alexopoulos P, Horn M, Maus S, Reichel M, Rhein C, *et al.* (2011):

Influence of brain-derived neurotrophic-factor and apolipoprotein E genetic variants on hippocampal volume and memory performance in healthy young adults. *J Neural Transm* 118: 249–57.

127. Khan W, Giampietro V, Ginestet C, Dell'Acqua F, Bouls D, Newhouse S, *et al.* (2014): No differences in hippocampal volume between carriers and non-carriers of the ApoE ε4 and ε2 alleles in young healthy adolescents. *J Alzheimers Dis* 40: 37–43.
128. O'Dwyer L, Lamberton F, Matura S, Tanner C, Scheibe M, Miller J, *et al.* (2012): Reduced hippocampal volume in healthy young ApoE4 carriers: an MRI study. *PLoS One* 7: e48895.
129. Alexander GE, Bergfield KL, Chen K, Reiman EM, Hanson KD, Lin L, *et al.* (2012): Gray matter network associated with risk for Alzheimer's disease in young to middle-aged adults. *Neurobiol Aging* 33: 2723–32.
130. Samuraki M, Matsunari I, Chen W-P, Shima K, Yanase D, Takeda N, *et al.* (2012): Glucose metabolism and gray-matter concentration in apolipoprotein E ε4 positive normal subjects. *Neurobiol Aging* 33: 2321–3.
131. Westlye LT, Reinvang I, Rootwelt H, Espeseth T (2012): Effects of APOE on brain white matter microstructure in healthy adults. *Neurology* 79: 1961–9.
132. Green AE, Gray JR, Deyoung CG, Mhyre TR, Padilla R, Dibattista AM, William Rebeck G (2014): A combined effect of two Alzheimer's risk genes on medial temporal activity during executive attention in young adults. *Neuropsychologia* 56: 1–8.
133. Filippini N, MacIntosh BJ, Hough MG, Goodwin GM, Frisoni GB, Smith SM, *et al.* (2009): Distinct patterns of brain activity in young carriers of the APOE-epsilon4 allele. *Proc Natl Acad Sci U S A* 106: 7209–14.
134. Rusted JM, Evans SL, King SL, Dowell N, Tabet N, Tofts PS (2013): APOE e4 polymorphism in young adults is associated with improved attention and indexed by distinct neural signatures. *Neuroimage* 65: 364–73.
135. Mondadori CRA, de Quervain DJ-F, Buchmann A, Mustovic H, Wollmer MA, Schmidt CF, *et al.* (2007): Better memory and neural efficiency in young apolipoprotein E epsilon4 carriers. *Cereb Cortex* 17: 1934–47.
136. Trachtenberg AJ, Filippini N, Ebmeier KP, Smith SM, Karpe F, Mackay CE (2012): The effects of APOE on the functional architecture of the resting brain. *Neuroimage* 59: 565–72.
137. Corder EH, Saunders AM, Risch NJ, Strittmatter WJ, Schmechel DE, Gaskell PC, *et al.* (1994): Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer disease. *Nat Genet* 7: 180–4.
138. Reiman EM, Chen K, Alexander GE, Caselli RJ, Bandy D, Osborne D, *et al.* (2004): Functional brain abnormalities in young adults at genetic risk for late-onset Alzheimer's dementia. *Proc Natl Acad Sci U S A* 101: 284–9.
139. Shaw P, Lerch JP, Pruessner JC, Taylor KN, Rose AB, Greenstein D, *et al.* (2007): Cortical morphology in children and adolescents with different apolipoprotein E gene polymorphisms: an observational study. *Lancet Neurol* 6: 494–500.
140. Dean DC, Jerskey BA, Chen K, Protas H, Thiyyagura P, Roontiva A, *et al.* (2014): Brain differences in infants at differential genetic risk for late-onset Alzheimer disease: a cross-sectional imaging study. *JAMA Neurol* 71: American Medical Association11–22.
141. Knickmeyer RC, Wang J, Zhu H, Geng X, Woolson S, Hamer RM, *et al.* (2014): Common variants in psychiatric risk genes predict brain structure at birth. *Cereb Cortex* 24: 1230–46.

142. Manning EN, Barnes J, Cash DM, Bartlett JW, Leung KK, Ourselin S, Fox NC (2014): APOE ε4 is associated with disproportionate progressive hippocampal atrophy in AD. *PLoS One* 9: e97608.

143. Pievani M, Galluzzi S, Thompson PM, Rasser PE, Bonetti M, Frisoni GB (2011): APOE4 is associated with greater atrophy of the hippocampal formation in Alzheimer's disease. *Neuroimage* 55: 909–19.

144. Liu Y, Paajanen T, Westman E, Wahlund L-O, Simmons A, Tunnard C, *et al.* (2010): Effect of APOE ε4 allele on cortical thicknesses and volumes: the AddNeuroMed study. *J Alzheimers Dis* 21: 947–66.

145. Spampinato MV, Rumboldt Z, Hosker RJ, Mintzer JE (2011): Apolipoprotein E and gray matter volume loss in patients with mild cognitive impairment and Alzheimer disease. *Radiology* 258: 843–52.

146. Kljajevic V, Meyer P, Holzmann C, Dyrba M, Kasper E, Bokde ALW, *et al.* (2014): The ε4 genotype of apolipoprotein E and white matter integrity in Alzheimer's disease. *Alzheimers Dement* 10: 401–4.

147. Mosconi L, Nacmias B, Sorbi S, De Cristofaro MTR, Fayazz M, Tedde A, *et al.* (2004): Brain metabolic decreases related to the dose of the ApoE e4 allele in Alzheimer's disease. *J Neurol Neurosurg Psychiatry* 75: 370–6.

148. Drzezga A, Grimmer T, Riemenschneider M, Lautenschlager N, Siebner H, Alexopoulus P, *et al.* (2005): Prediction of individual clinical outcome in MCI by means of genetic assessment and (18)F-FDG PET. *J Nucl Med* 46: 1625–32.

149. Lehmann M, Ghosh PM, Madison C, Karydas A, Coppola G, O'Neil JP, *et al.* (2014): Greater medial temporal hypometabolism and lower cortical amyloid burden in ApoE4-positive AD patients. *J Neurol Neurosurg Psychiatry* 85: 266–73.

150. Ossenkoppele R, van der Flier WM, Zwan MD, Adriaanse SF, Boellaard R, Windhorst AD, *et al.* (2013): Differential effect of APOE genotype on amyloid load and glucose metabolism in AD dementia. *Neurology* 80: 359–65.

151. Pike KE, Savage G, Villemagne VL, Ng S, Moss SA, Maruff P, *et al.* (2007): Beta-amyloid imaging and memory in non-demented individuals: evidence for preclinical Alzheimer's disease. *Brain* 130: 2837–44.

152. Hatashita S, Yamasaki H (2013): Diagnosed mild cognitive impairment due to Alzheimer's disease with PET biomarkers of beta amyloid and neuronal dysfunction. *PLoS One* 8: e66877.

153. Mak ACY, Pullinger CR, Tang LF, Wong JS, Deo RC, Schwarz J-M, *et al.* (2014): Effects of the Absence of Apolipoprotein E on Lipoproteins, Neurocognitive Function, and Retinal Function. *JAMA Neurol*. doi: 10.1001/jamaneurol.2014.2011.

154. Roses AD, Lutz MW, Amrine-Madsen H, Saunders AM, Crenshaw DG, Sundseth SS, *et al.* (2010): A TOMM40 variable-length polymorphism predicts the age of late-onset Alzheimer's disease. *Pharmacogenomics J* 10: 375–84.

155. Hong M-G, Alexeyenko A, Lambert J-C, Amouyel P, Prince JA (2010): Genome-wide pathway analysis implicates intracellular transmembrane protein transport in Alzheimer disease. *J Hum Genet* 55: The Japan Society of Human Genetics707–9.

156. Carrasquillo MM, Morgan K (2012): Commentary on "Functional analysis of APOE locus genetic variation implicates regional enhancers in the regulation of both TOMM40 and APOE". *J Hum Genet* 57: The Japan Society of Human Genetics3–4.

157. Maruszak A, Pepłońska B, Safranow K, Chodakowska-Żebrowska M, Barcikowska M, Zekanowski C (2012): TOMM40 rs10524523 polymorphism's role in late-onset Alzheimer's disease and in longevity. *J Alzheimers Dis* 28: 309–22.

158. Chu SH, Roeder K, Ferrell RE, Devlin B, DeMichele-Sweet MAA, Kamboh MI, *et al.* (2011): TOMM40 poly-T repeat lengths, age of onset and psychosis risk in Alzheimer disease. *Neurobiol Aging* 32: 2328.e1–9.

159. Cruchaga C, Nowotny P, Kauwe JSK, Ridge PG, Mayo K, Bertelsen S, *et al.* (2011): Association and expression analyses with single-nucleotide polymorphisms in TOMM40 in Alzheimer disease. *Arch Neurol* 68: 1013–9.

160. Bekris LM, Lutz F, Yu C-E (2012): Functional analysis of APOE locus genetic variation implicates regional enhancers in the regulation of both TOMM40 and APOE. *J Hum Genet* 57: The Japan Society of Human Genetics18–25.

161. Ferencz B, Laukka EJ, Lövdén M, Kalpouzos G, Keller L, Graff C, *et al.* (2013): The influence of APOE and TOMM40 polymorphisms on hippocampal volume and episodic memory in old age. *Front Hum Neurosci* 7: 198.

162. Johnson SC, La Rue A, Hermann BP, Xu G, Koscik RL, Jonaitis EM, *et al.* (2011): The effect of TOMM40 poly-T length on gray matter volume and cognition in middle-aged persons with APOE ε3/ε3 genotype. *Alzheimers Dement* 7: 456–65.

163. Lyall DM, Harris SE, Bastin ME, Muñoz Maniega S, Murray C, Lutz MW, *et al.* (2014): Alzheimer's disease susceptibility genes APOE and TOMM40, and brain white matter integrity in the Lothian Birth Cohort 1936. *Neurobiol Aging* 35: 1513.e25–33.

164. Benitez A, Fieremans E, Jensen JH, Falangola MF, Tabesh A, Ferris SH, Helpern JA (2014): White matter tract integrity metrics reflect the vulnerability of late-myelinating tracts in Alzheimer's disease. *NeuroImage Clin* 4: 64–71.

165. Jonsson T, Stefansson H, Steinberg S, Jonsdottir I, Jonsson P V, Snaedal J, *et al.* (2013): Variant of TREM2 associated with the risk of Alzheimer's disease. *N Engl J Med* 368: 107–16.

166. Guerreiro R, Wojtas A, Bras J, Carrasquillo M, Rogaeva E, Majounie E, *et al.* (2013): TREM2 variants in Alzheimer's disease. *N Engl J Med* 368: 117–27.

167. Finelli D, Rollinson S, Harris J, Jones M, Richardson A, Gerhard A, *et al.* (2014): TREM2 analysis and increased risk of Alzheimer's disease. *Neurobiol Aging*. doi: 10.1016/j.neurobiolaging.2014.08.001.

168. Lambert J-C, Heath S, Even G, Campion D, Sleegers K, Hiltunen M, *et al.* (2009): Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. *Nat Genet* 41: 1094–9.

169. Melchior B, Garcia AE, Hsiung B-K, Lo KM, Doose JM, Thrash JC, *et al.* (2010): Dual induction of TREM2 and tolerance-related transcript, Tmem176b, in amyloid transgenic mice: implications for vaccine-based therapies for Alzheimer's disease. *ASN Neuro* 2: e00037.

170. Lue L-F, Schmitz CT, Sorrano G, Sue LI, Beach TG, Walker DG (2014): TREM2 protein expression changes correlate with Alzheimer's disease neurodegenerative pathologies in postmortem temporal cortices. *Brain Pathol*. doi: 10.1111/bpa.12190.

171. Jiang T, Tan L, Zhu X-C, Zhang Q-Q, Cao L, Tan M-S, *et al.* (2014): Upregulation of TREM2 Ameliorates Neuropathology and Rescues Spatial Cognitive Impairment in a Transgenic Mouse Model of Alzheimer's Disease. *Neuropsychopharmacology*. doi:

10.1038/npp.2014.164.

172. Rayaprolu S, Mullen B, Baker M, Lynch T, Finger E, Seeley WW, *et al.* (2013): TREM2 in neurodegeneration: evidence for association of the p.R47H variant with frontotemporal dementia and Parkinson's disease. *Mol Neurodegener* 8: 19.

173. Ruiz A, Dols-Icardo O, Bullido MJ, Pastor P, Rodríguez-Rodríguez E, López de Munain A, *et al.* (2014): Assessing the role of the TREM2 p.R47H variant as a risk factor for Alzheimer's disease and frontotemporal dementia. *Neurobiol Aging* 35: 444.e1–4.

174. Cady J, Koval ED, Benitez BA, Zaidman C, Jockel-Balsarotti J, Allred P, *et al.* (2014): TREM2 variant p.R47H as a risk factor for sporadic amyotrophic lateral sclerosis. *JAMA Neurol* 71: 449–53.

175. Reitz C, Mayeux R (2013): TREM2 and neurodegenerative disease. *N Engl J Med* 369: 1564–5.

176. Luis EO, Ortega-Cubero S, Lamet I, Razquin C, Cruchaga C, Benitez BA, *et al.* (2014): Frontobasal gray matter loss is associated with the TREM2 p.R47H variant. *Neurobiol Aging*. doi: 10.1016/j.neurobiolaging.2014.06.007.

177. Rajagopalan P, Hibar DP, Thompson PM (2013): TREM2 and neurodegenerative disease. *N Engl J Med* 369: 1565–7.

178. Bertram L, Lange C, Mullin K, Parkinson M, Hsiao M, Hogan MF, *et al.* (2008): Genome-wide association analysis reveals putative Alzheimer's disease susceptibility loci in addition to APOE. *Am J Hum Genet* 83: 623–32.

179. Harold D, Abraham R, Hollingworth P, Sims R, Gerrish A, Hamshere ML, *et al.* (2009): Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. *Nat Genet* 41: 1088–93.

180. Hollingworth P, Harold D, Sims R, Gerrish A, Lambert J-C, Carrasquillo MM, *et al.* (2011): Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease. *Nat Genet* 43: 429–35.

181. Morgan K, Carrasquillo MM (2013): *Genetic Variants in Alzheimer's Disease*. . New York: Springer Science+Business Media.

182. May PC, Lampert-Etchells M, Johnson SA, Poirier J, Masters JN, Finch CE (1990): Dynamics of gene expression for a hippocampal glycoprotein elevated in Alzheimer's disease and in response to experimental lesions in rat. *Neuron* 5: 831–9.

183. Seshadri S, Fitzpatrick AL, Ikram MA, DeStefano AL, Gudnason V, Boada M, *et al.* (2010): Genome-wide analysis of genetic loci associated with Alzheimer disease. *JAMA* 303: 1832–40.

184. Jun G, Naj AC, Beecham GW, Wang L-S, Buros J, Gallins PJ, *et al.* (2010): Meta-analysis confirms CR1, CLU, and PICALM as alzheimer disease risk loci and reveals interactions with APOE genotypes. *Arch Neurol* 67: 1473–84.

185. Corneveaux JJ, Myers AJ, Allen AN, Pruzin JJ, Ramirez M, Engel A, *et al.* (2010): Association of CR1, CLU and PICALM with Alzheimer's disease in a cohort of clinically characterized and neuropathologically verified individuals. *Hum Mol Genet* 19: 3295–301.

186. Bralten J, Franke B, Arias-Vásquez A, Heister A, Brunner HG, Fernández G, Rijpkema M (2011): CR1 genotype is associated with entorhinal cortex volume in young healthy adults. *Neurobiol Aging* 32: 2106.e7–11.

187. Stevens BW, DiBattista AM, William Rebeck G, Green AE (2014): A gene-brain-cognition

pathway for the effect of an Alzheimer's risk gene on working memory in young adults. *Neuropsychologia* 61: 143–9.

188. Braskie MN, Jahanshad N, Stein JL, Barysheva M, McMahon KL, de Zubicaray GI, *et al.* (2011): Common Alzheimer's disease risk variant within the CLU gene affects white matter microstructure in young adults. *J Neurosci* 31: 6764–70.
189. Kohannim O, Jahanshad N, Braskie MN, Stein JL, Chiang M-C, Reese AH, *et al.* (2012): Predicting white matter integrity from multiple common genetic variants. *Neuropsychopharmacology* 37: 2012–9.
190. Erk S, Meyer-Lindenberg A, Opitz von Boberfeld C, Esslinger C, Schnell K, Kirsch P, *et al.* (2011): Hippocampal function in healthy carriers of the CLU Alzheimer's disease risk variant. *J Neurosci* 31: 18180–4.
191. Zhang P, Qin W, Wang D, Liu B, Zhang Y, Jiang T, Yu C (2014): Impacts of PICALM and CLU variants associated with Alzheimer's disease on the functional connectivity of the hippocampus in healthy young adults. *Brain Struct Funct*. doi: 10.1007/s00429-014-0738-4.
192. Naj AC, Jun G, Beecham GW, Wang L-S, Vardarajan BN, Buros J, *et al.* (2011): Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease. *Nat Genet* 43: 436–41.
193. Tebar F, Bohlander SK, Sorkin A (1999): Clathrin assembly lymphoid myeloid leukemia (CALM) protein: localization in endocytic-coated pits, interactions with clathrin, and the impact of overexpression on clathrin-mediated traffic. *Mol Biol Cell* 10: 2687–702.
194. Morgan K (2011): The three new pathways leading to Alzheimer's disease. *Neuropathol Appl Neurobiol* 37: 353–7.
195. Biffi A, Anderson CD, Desikan RS, Sabuncu M, Cortellini L, Schmansky N, *et al.* (2010): Genetic variation and neuroimaging measures in Alzheimer disease. *Arch Neurol* 67: 677–85.
196. Furney SJ, Simmons A, Breen G, Pedroso I, Lunnon K, Proitsi P, *et al.* (2011): Genome-wide association with MRI atrophy measures as a quantitative trait locus for Alzheimer's disease. *Mol Psychiatry* 16: 1130–8.
197. Hohman TJ, Koran ME, Thornton-Wells T (2013): Epistatic genetic effects among Alzheimer's candidate genes. *PLoS One* 8: e80839.
198. Singhrao SK, Neal JW, Rushmere NK, Morgan BP, Gasque P (1999): Differential expression of individual complement regulators in the brain and choroid plexus. *Lab Invest* 79: 1247–59.
199. Hensley K (2010): Neuroinflammation in Alzheimer's disease: mechanisms, pathologic consequences, and potential for therapeutic manipulation. *J Alzheimers Dis* 21: 1–14.
200. Bralten J, Franke B, Arias-Vásquez A, Heister A, Brunner HG, Fernández G, Rijpkema M (2011): CR1 genotype is associated with entorhinal cortex volume in young healthy adults. *Neurobiol Aging* 32: 2106.e7–11.
201. Pant S, Sharma M, Patel K, Caplan S, Carr CM, Grant BD (2009): AMPH-1/Amphiphysin/Bin1 functions with RME-1/Ehd1 in endocytic recycling. *Nat Cell Biol* 11: 1399–410.
202. Vasiliou V, Vasiliou K, Nebert DW (2009): Human ATP-binding cassette (ABC) transporter family. *Hum Genomics* 3: 281–90.
203. Hirsch-Reinshagen V, Zhou S, Burgess BL, Bernier L, McIsaac SA, Chan JY, *et al.* (2004):

Deficiency of ABCA1 impairs apolipoprotein E metabolism in brain. *J Biol Chem* 279: 41197–207.

204. Hughes TM, Lopez OL, Evans RW, Kamboh MI, Williamson JD, Klunk WE, *et al.* (2014): Markers of cholesterol transport are associated with amyloid deposition in the brain. *Neurobiol Aging* 35: 802–7.
205. Hirai H, Maru Y, Hagiwara K, Nishida J, Takaku F (1987): A novel putative tyrosine kinase receptor encoded by the eph gene. *Science* 238: 1717–20.
206. Chen Y, Fu AKY, Ip NY (2012): Eph receptors at synapses: implications in neurodegenerative diseases. *Cell Signal* 24: 606–11.
207. Nakamura-Hirota T, Kadoyama K, Takano M, Otani M, Matsuyama S (2012): The expression changes of EphA3 receptor during synaptic plasticity in mouse hippocampus through activation of nicotinic acetylcholine receptor. *Neuroreport* 23: 746–51.
208. Crocker PR, Paulson JC, Varki A (2007): Siglecs and their roles in the immune system. *Nat Rev Immunol* 7: 255–66.
209. Bradshaw EM, Chibnik LB, Keenan BT, Ottoboni L, Raj T, Tang A, *et al.* (2013): CD33 Alzheimer's disease locus: altered monocyte function and amyloid biology. *Nat Neurosci* 16: 848–50.
210. Tanaka J-I, Horiike Y, Matsuzaki M, Miyazaki T, Ellis-Davies GCR, Kasai H (2008): Protein synthesis and neurotrophin-dependent structural plasticity of single dendritic spines. *Science* 319: 1683–7.
211. Figurov A, Pozzo-Miller LD, Olafsson P, Wang T, Lu B (1996): Regulation of synaptic responses to high-frequency stimulation and LTP by neurotrophins in the hippocampus. *Nature* 381: 706–9.
212. Marosi K, Mattson MP (2014): BDNF mediates adaptive brain and body responses to energetic challenges. *Trends Endocrinol Metab* 25: 89–98.
213. Nagahara AH, Tuszynski MH (2011): Potential therapeutic uses of BDNF in neurological and psychiatric disorders. *Nat Rev Drug Discov* 10: Nature Publishing Group209–19.
214. Nagahara AH, Merrill DA, Coppola G, Tsukada S, Schroeder BE, Shaked GM, *et al.* (2009): Neuroprotective effects of brain-derived neurotrophic factor in rodent and primate models of Alzheimer's disease. *Nat Med* 15: Nature Publishing Group331–7.
215. von Bohlen und Halbach O (2010): Involvement of BDNF in age-dependent alterations in the hippocampus. *Front Aging Neurosci* 2: . doi: 10.3389/fnagi.2010.00036.
216. Chen Z-Y, Bath K, McEwen B, Hempstead B, Lee F (2008): Impact of genetic variant BDNF (Val66Met) on brain structure and function. *Novartis Found Symp* 289: 180–8; discussion 188–95.
217. Lim YY, Villemagne VL, Laws SM, Ames D, Pietrzak RH, Ellis KA, *et al.* (2013): BDNF Val66Met, Aβ amyloid, and cognitive decline in preclinical Alzheimer's disease. *Neurobiol Aging* 34: 2457–64.
218. Honea RA, Cruchaga C, Perea RD, Saykin AJ, Burns JM, Weinberger DR, Goate AM (2013): Characterizing the role of brain derived neurotrophic factor genetic variation in Alzheimer's disease neurodegeneration. *PLoS One* 8: e76001.
219. Voineskos AN, Lerch JP, Felsky D, Shaikh S, Rajji TK, Miranda D, *et al.* (2011): The brain-derived neurotrophic factor Val66Met polymorphism and prediction of neural risk for Alzheimer disease. *Arch Gen Psychiatry* 68: 198–206.

220. Hashimoto R, Hirata Y, Asada T, Yamashita F, Nemoto K, Mori T, *et al.* (2009): Effect of the brain-derived neurotrophic factor and the apolipoprotein E polymorphisms on disease progression in preclinical Alzheimer's disease. *Genes Brain Behav* 8: 43–52.

221. Xu C, Wang Z, Fan M, Liu B, Song M, Zhen X, Jiang T (2010): Effects of BDNF Val66Met polymorphism on brain metabolism in Alzheimer's disease. *Neuroreport* 21: 802–7.

222. Adamczuk K, De Weer A-S, Nelissen N, Chen K, Sleegers K, Bettens K, *et al.* (2013): Polymorphism of brain derived neurotrophic factor influences β amyloid load in cognitively intact apolipoprotein E ε4 carriers. *NeuroImage Clin* 2: 512–20.

223. Nho K, Corneveaux JJ, Kim S, Lin H, Risacher SL, Shen L, *et al.* (2013): Whole-exome sequencing and imaging genetics identify functional variants for rate of change in hippocampal volume in mild cognitive impairment. *Mol Psychiatry* 18: 781–7.

224. Shen L, Kim S, Risacher SL, Nho K, Swaminathan S, West JD, *et al.* (2010): Whole genome association study of brain-wide imaging phenotypes for identifying quantitative trait loci in MCI and AD: A study of the ADNI cohort. *Neuroimage* 53: 1051–63.

225. Silver M, Janousova E, Hua X, Thompson PM, Montana G (2012): Identification of gene pathways implicated in Alzheimer's disease using longitudinal imaging phenotypes with sparse regression. *Neuroimage* 63: 1681–94.

226. Meda SA, Narayanan B, Liu J, Perrone-Bizzozero NI, Stevens MC, Calhoun VD, *et al.* (2012): A large scale multivariate parallel ICA method reveals novel imaging-genetic relationships for Alzheimer's disease in the ADNI cohort. *Neuroimage* 60: 1608–21.

227. Molecular Signatures Database (n.d.).: . Retrieved from http://www.broadinstitute.org/gsea/msigdb/index.jsp.

228. Melville SA, Buros J, Parrado AR, Vardarajan B, Logue MW, Shen L, *et al.* (2012): Multiple loci influencing hippocampal degeneration identified by genome scan. *Ann Neurol* 72: 65–75.

229. Jahanshad N, Rajagopalan P, Hua X, Hibar DP, Nir TM, Toga AW, *et al.* (2013): Genome-wide scan of healthy human connectome discovers SPON1 gene variant influencing dementia severity. *Proc Natl Acad Sci U S A* 110: 4768–73.

230. Rose EJ, Donohoe G (2013): Brain vs behavior: an effect size comparison of neuroimaging and cognitive studies of genetic risk for schizophrenia. *Schizophr Bull* 39: 518–26.

231. Thompson PM, Stein JL, Medland SE, Hibar DP, Vasquez AA, Renteria ME, *et al.* (2014): The ENIGMA Consortium: large-scale collaborative analyses of neuroimaging and genetic data. *Brain Imaging Behav* 8: 153–82.

232. The Canadian Study of Health and Aging (1994): The Canadian Study of Health and Aging*: Risk factors for Alzheimer's disease in Canada. *Neurology* 44: 2073–2073.

233. Donix M, Burggren AC, Suthana NA, Siddarth P, Ekstrom AD, Krupa AK, *et al.* (2010): Family history of Alzheimer's disease and hippocampal structure in healthy people. *Am J Psychiatry* 167: 1399–406.

234. Okonkwo OC, Xu G, Dowling NM, Bendlin BB, Larue A, Hermann BP, *et al.* (2012): Family history of Alzheimer disease predicts hippocampal atrophy in healthy middle-aged adults. *Neurology* 78: 1769–76.

235. Honea RA, Swerdlow RH, Vidoni ED, Burns JM (2011): Progressive regional atrophy in normal adults with a maternal history of Alzheimer disease. *Neurology* 76: 822–9.

236. Berti V, Mosconi L, Glodzik L, Li Y, Murray J, De Santi S, *et al.* (2011): Structural brain

changes in normal individuals with a maternal history of Alzheimer's. *Neurobiol Aging* 32: 2325.e17–26.

237. Honea RA, Swerdlow RH, Vidoni ED, Goodwin J, Burns JM (2010): Reduced gray matter volume in normal adults with a maternal family history of Alzheimer disease. *Neurology* 74: 113–20.
238. Honea RA, Vidoni ED, Swerdlow RH, Burns JM (2012): Maternal family history is associated with Alzheimer's disease biomarkers. *J Alzheimers Dis* 31: 659–68.
239. Mosconi L, Rinne JO, Tsui WH, Murray J, Li Y, Glodzik L, *et al.* (2013): Amyloid and metabolic positron emission tomography imaging of cognitively normal adults with Alzheimer's parents. *Neurobiol Aging* 34: 22–34.
240. Mosconi L, de Leon M, Murray J, E L, Lu J, Javier E, *et al.* (2011): Reduced mitochondria cytochrome oxidase activity in adult children of mothers with Alzheimer's disease. *J Alzheimers Dis* 27: 483–90.
241. Mosconi L, Murray J, Tsui WH, Li Y, Spector N, Goldowsky A, *et al.* (2014): Brain imaging of cognitively normal individuals with 2 parents affected by late-onset AD. *Neurology* 82: 752–60.
242. Fleisher AS, Sherzai A, Taylor C, Langbaum JBS, Chen K, Buxton RB (2009): Resting-state BOLD networks versus task-associated functional MRI for distinguishing Alzheimer's disease risk groups. *Neuroimage* 47: 1678–90.
243. Wang L, Roe CM, Snyder AZ, Brier MR, Thomas JB, Xiong C, *et al.* (2012): Alzheimer disease family history impacts resting state functional connectivity. *Ann Neurol* 72: 571–7.
244. Rodríguez-Rodríguez E, Sánchez-Juan P, Vázquez-Higuera JL, Mateo I, Pozueta A, Berciano J, *et al.* (2013): Genetic risk score predicting accelerated progression from mild cognitive impairment to Alzheimer's disease. *J Neural Transm* 120: 807–12.
245. Sabuncu MR, Buckner RL, Smoller JW, Lee PH, Fischl B, Sperling RA (2012): The association between a polygenic Alzheimer score and cortical thickness in clinically normal subjects. *Cereb Cortex* 22: 2653–61.
246. Mahley RW, Weisgraber KH, Huang Y (2009): Apolipoprotein E: structure determines function, from atherosclerosis to Alzheimer's disease to AIDS. *J Lipid Res* 50 Suppl: S183–8.
247. Chen H-K, Liu Z, Meyer-Franke A, Brodbeck J, Miranda RD, McGuire JG, *et al.* (2012): Small molecule structure correctors abolish detrimental effects of apolipoprotein E4 in cultured neurons. *J Biol Chem* 287: 5253–66.
248. Cirulli ET, Goldstein DB (2010): Uncovering the roles of rare variants in common disease through whole-genome sequencing. *Nat Rev Genet* 11: Nature Publishing Group415–25.
249. Yu J-T, Li L, Zhu Q-X, Zhang Q, Zhang W, Wu Z-C, *et al.* (2010): Implication of CLU gene polymorphisms in Chinese patients with Alzheimer's disease. *Clin Chim Acta* 411: 1516–9.
250. Chen LH, Kao PYP, Fan YH, Ho DTY, Chan CSY, Yik PY, *et al.* (2012): Polymorphisms of CR1, CLU and PICALM confer susceptibility of Alzheimer's disease in a southern Chinese population. *Neurobiol Aging* 33: 210.e1–7.
251. Gurland BJ, Wilder DE, Lantigua R, Stern Y, Chen J, Killeffer EH, Mayeux R (1999): Rates of dementia in three ethnoracial groups. *Int J Geriatr Psychiatry* 14: 481–93.
252. Thompson WK, Hallmayer J, O'Hara R (2011): Design considerations for characterizing psychiatric trajectories across the lifespan: application to effects of APOE-ε4 on cerebral

cortical thickness in Alzheimer's disease. *Am J Psychiatry* 168: 894–903.

253. Kraemer HC, Yesavage JA, Taylor JL, Kupfer D (2000): How can we learn about developmental processes from cross-sectional studies, or can we? *Am J Psychiatry* 157: 163–71.
254. Hebert LE, Scherr PA, Bienias JL, Bennett DA, Evans DA (2003): Alzheimer disease in the US population: prevalence estimates using the 2000 census. *Arch Neurol* 60: 1119–22.
255. Census Projections by Age Group (n.d.).: . Retrieved from http://www.census.gov/population/projections/data/national/2012/summarytables.html.
256. Dufrasne S, Roy M, Galvez M, Rosenblatt DS (2011): Experience over fifteen years with a protocol for predictive testing for Huntington disease. *Mol Genet Metab* 102: 494–504.
257. Salloway S, Sperling R, Fox NC, Blennow K, Klunk W, Raskind M, *et al.* (2014): Two phase 3 trials of bapineuzumab in mild-to-moderate Alzheimer's disease. *N Engl J Med* 370: 322–33.
258. Doody RS, Thomas RG, Farlow M, Iwatsubo T, Vellas B, Joffe S, *et al.* (2014): Phase 3 trials of solanezumab for mild-to-moderate Alzheimer's disease. *N Engl J Med* 370: 311–21.
259. Toledo JB, Arnold SE, Raible K, Brettschneider J, Xie SX, Grossman M, *et al.* (2013): Contribution of cerebrovascular disease in autopsy confirmed neurodegenerative disease cases in the National Alzheimer's Coordinating Centre. *Brain* 136: 2697–706.
260. Struyfs H, Molinuevo JL, Martin J-J, De Deyn PP, Engelborghs S (2014): Validation of the AD-CSF-index in autopsy-confirmed Alzheimer's disease patients and healthy controls. *J Alzheimers Dis* 41: 903–9.
261. Sperling RA, Rentz DM, Johnson KA, Karlawish J, Donohue M, Salmon DP, Aisen P (2014): The A4 study: stopping AD before symptoms begin? *Sci Transl Med* 6: 228fs13.
262. Roden DM, George AL (2002): The genetic basis of variability in drug responses. *Nat Rev Drug Discov* 1: 37–44.
263. Kirchheiner J, Lorch R, Lebedeva E, Seeringer A, Roots I, Sasse J, Brockmöller J (2008): Genetic variants in FKBP5 affecting response to antidepressant drug treatment. *Pharmacogenomics* 9: 841–6.
264. Kohannim O, Hua X, Rajagopalan P, Hibar DP, Jahanshad N, Grill JD, *et al.* (2013): Multilocus genetic profiling to empower drug trials and predict brain atrophy. *NeuroImage Clin* 2: 827–35.
265. Liu X, Bai F, Yue C, Shi Y, Yu H, Luo B, Zhang Z (2014): The association between TOMM40 gene polymorphism and spontaneous brain activity in amnestic mild cognitive impairment. *J Neurol* 261: 1499–507.
266. Fleisher AS, Chen K, Liu X, Ayutyanont N, Roontiva A, Thiyyagura P, *et al.* (2012): Apolipoprotein E ε4 and age effects on florbetapir positron emission tomography in healthy aging and Alzheimer disease. *Neurobiol Aging*. doi: 10.1016/j.neurobiolaging.2012.04.017.

CHAPTER 2

Altered Memory-Related Functional Connectivity of the Anterior and Posterior Hippocampus in Older Adults at Increased Genetic Risk for Alzheimer's Disease

Abstract

The hippocampal complex is affected early in Alzheimer's disease (AD). Increasingly, altered functional connectivity of the hippocampus is recognized as an important feature of preclinical AD. Carriers of the APOEε4 allele are at an increased risk for AD, which could lead to altered hippocampal connectivity even in healthy older adults. To test this hypothesis, we used a paired-associates memory task to examine differences in task-dependent functional connectivity of the anterior and posterior hippocampus in non-demented APOEε4 carriers (n=34, 18F) and non-carriers (n=46, 31F). We examined anterior and posterior portions of the hippocampus separately to test the theory that APOEε4-mediated differences would be more pronounced in the anterior region, which is affected earlier in the AD course. This study is the first to use a psychophysiological interaction approach to query the context-dependent connectivity of subregions of the hippocampus during a memory task in adults at increased genetic risk for AD. During encoding, APOEε4 carriers had lower functional connectivity change compared to baseline between the anterior hippocampus and right precuneus, anterior insula and cingulate cortex. During retrieval, bilateral supramarginal gyrus and right precuneus showed lower functional connectivity change with anterior hippocampus in carriers. Also during retrieval, carriers showed lower connectivity change in the posterior hippocampus with auditory cortex. In each case, APOEε4 carriers showed strong negative connectivity changes compared to non-carriers where positive connectivity change was measured. These differences may represent prodromal functional changes mediated in part by APOEε4 and are consistent with the anterior-to-posterior theory of AD progression in the hippocampus.

Introduction

Alzheimer's disease (AD) is the most common cause of dementia and currently affects more than five million Americans. The illness is unique among polygenic human diseases because there is a single genetic risk factor, *APOE*, which accounts for a relatively large portion of the variation in heritability, yet is not a causative gene. Specifically, twin studies reveal that the heritability of AD may exceed 60-80% (1; 2). *APOE* was identified as a susceptibility gene for AD over 20 years ago and has been studied extensively since (3–5). *APOE* allele status accounts for about 50% of the variation in heritability estimates (6). A single copy of the ε4 allele of *APOE* (APOEε4) increases lifetime risk for AD fourfold, and 2 copies of the allele confer a 10-fold increase (7). Here, we examined the effect of APOEε4 on the functional connectivity of the anterior and posterior hippocampus during encoding and retrieval. This design allowed us to interrogate group differences while also testing the theory that APOEε4-mediated differences in an asymptomatic cohort would be more severe in the anterior hippocampus, the region of the structure where AD pathology first occurs (8).

One popular method for studying the effects of *APOE* allele status in humans is task-based functional magnetic resonance imaging (fMRI). Task-based fMRI allows investigators to localize significant increases in the blood-oxygen-level dependent (BOLD) signal associated with particular cognitive processes. Because the APOEε4 allele is a strong risk factor for AD, there is particular interest in how the neural substrates of memory function are modulated by *APOE*. Since 2000 investigators have attempted to characterize the neural signature of the risk conferred by the APOEε4 allele, but results have been contradictory (for a review see Trachtenberg, Filippini, and Mackay 2012). Roughly half of memory task-based fMRI studies describe significant increases in activity (BOLD signal) in carriers of the APOEε4 allele compared to non-carriers, while the other half report the opposite effect. This may be due to the heterogeneity of the tasks used in these studies (9). In addition, differences in other non-*APOE*

genetic risk factors (including family history) may affect results, especially in small cohorts (Burggren et al. 2002).

In contrast to task-based fMRI, resting state fMRI (rs-fMRI) measures fluctuations in BOLD signal while the subject is at rest, as opposed to performing a specific cognitive task (11). rs-fMRI studies have revealed complex differences in functional connectivity mediated by *APOE* allele status in healthy older adults (12–15). These network-based alterations have been suggested as a potential early endophenotype for AD (16). This, as well as the inconsistent findings in task-based fMRI, has led to the idea that functional connectivity alterations capture more of the complex interaction between *APOE* and brain function than task-induced activations. As task-based fMRI analysis methods continue to be improved and refined, we have an opportunity to resolve the conflicts in the *APOE*-fMRI literature. One way to tease out the complex relationship between APOEε4 allele and memory function is to measure the context-dependent functional connectivity of an anatomical region (seed) and a specific task phase using a psychophysiological interaction (PPI) model (17). This approach allows investigators to examine functional connectivity in the context of specific cognitive processes. In addition, PPI modeling requires differences between groups to be limited to the connectivity relationships between an *a priori* seed and regions where activity is mediated or modified by that seed in certain behavioral contexts, such as memory encoding or retrieval. Thus, differences between groups are differences in functional connectivity of the seed during the particular phase of the task that is being modeled. Here, we employ a method of modeling PPIs that has been shown to increase the sensitivity and specificity of findings (18).

Focusing on subregions of the hippocampus during an associative memory task allows us to sensitively interrogate the effect of APOEε4 allele on connectivity alterations in functionally distinct regions of the hippocampus during specific task phases. One reason we chose to examine the anterior portion and the posterior portion of the hippocampus separately is because of the known functional and anatomical segregation of the hippocampus along the longitudinal

axis (19–22). In general, anterior regions of the hippocampal complex, including the entorhinal cortex, are the main input regions and are involved in encoding new memories while posterior regions are output regions involved in memory retrieval and consolidation (23–25). At the cellular level, the entorhinal cortex is the first area to be affected by AD pathology so we might expect that there would be early functional changes in anterior hippocampus before posterior regions (8; 26; 27). In fact, structural imaging has revealed that entorhinal cortex is significantly thinner in healthy, older APOEε4 carriers than non-carriers (28). Therefore, we were interested in interrogating the two active phases of the memory task, encoding and retrieval, and the phase-dependent functional connectivity of the anterior and posterior portions of the hippocampus in order to better understand memory-induced connectivity of functional subregions of the hippocampus.

This study is the first to examine differences in context-dependent functional connectivity of subregions of the hippocampus during the performance of a complex memory task in healthy adults. Our participants were non-demented older adults who generally have a high incidence of family history of AD and a high carriage rate of AD risk variants such as APOEε4. This allows us to examine differences in task-related hippocampal functional connectivity changes between well-matched groups of APOEε4 carriers and non-carriers. We specifically compare the hippocampal connectivity that is related to either encoding or retrieval processes in APOEε4 carriers and non-carriers. Recent work at the molecular level has suggested that AD pathology moves in a trans-synaptic fashion (29; 30). One of the earliest sites of neurofibrillary tangle deposition is the entorhinal cortex, adjacent to the anterior hippocampus (8; 31). Thus, our study design was based on a pair of nested hypotheses: first, that carriers in of the APOEε4 allele would show decreased context-dependent functional connectivity of the hippocampus with cortical regions during a memory task and second, that these differences would be more pronounced when interrogating the anterior subregion of the hippocampus. Our findings provide evidence from functional imaging in humans that supports the hypothesis that anterior regions

of the hippocampus are more susceptible to differences in function based on APOEε4. We believe these findings highlight a susceptibility in APOEε4 carriers to AD-related hippocampal functional changes (32). Our focus on genetic risk for AD is motivated by the need to better understand how risk factors like APOEε4 affect brain function *before* the onset of symptoms. The effects of genetic risk for AD on functional endophenotypes for AD may help to define preclinical AD patients who are candidates for preventative therapies.

Materials and Methods

Participants

Participants were recruited by the UCLA Longevity Center as part of an ongoing initiative to study aging, AD genetic risk and dementia. Recruitment efforts included posting flyers in older adult communities and adult day care centers, the local Alzheimer's Association chapter, memory groups, and other groups catering to older adults with age-related memory concerns. This strategy enabled the recruitment of approximately 40-50% of participants carrying at least one copy of the APOEε4 allele, as opposed to the 20-25% that would be expected from a purely random recruitment (33; 34). In the present study, all participants were healthy and cognitively intact at the time of imaging acquisition. Participants are defined as non-demented in our study if they are cognitively intact based on the results of the Mini Mental State Exam (MMSE; for gross cognition, threshold≥26) and standard criteria for AAMI (Age Associated Memory Impairment); that is, participants were excluded if they had scores more than two standard deviations below normal on two or more of the memory tests described below. Finally, participants with clinical anxiety, depression or any neuropsychiatric or neurological illness were excluded. This study was performed in compliance with the UCLA Institutional Review Board (IRB) protocols and approved by the UCLA Human Subjects Protection Committee. All participants gave written informed consent in order to enroll in this study.

Neuropsychological Assessment

Participants performed a 3-hour neuropsychological battery including tests of the following: General Intelligence (Subtests of the WAIS-III) (35), Fluency (Fruits and Vegetables) (36), Attention (Digits Forward and Backward) (35), Language (Boston Naming Test) (37), Verbal Memory (Buschke-Fuld Selective Reminding Task) (38), WMS-III Logical Memory and Verbal Paired Associates learning (35) and Visual Memory (Rey-Osterrieth Figure test) (39). Participants also completed the following: Family history questionnaire (40), memory complaints self-report questionnaire (41), Hamilton Depression and Anxiety Inventory (Hamilton 1959; Hamilton 1960), Neuropsychiatric Inventory (44) and the MMSE (45).

Genotyping

A blood sample was drawn from each participant by a trained phlebotomist at the UCLA Clinical and Translational Research Laboratory. Leukocytes from 10ml of the sample were frozen and stored at -80°C. 200ug genomic DNA was isolated from the remaining 10ml and screened using a PCR-based mutation detection assay and a microsatellite marker based genotyping. *APOE* SNP (rs429358 and rs7412) genotyping was carried out by Real Time PCR on an Applied Biosystems 7900HT Real Time PCR machine. In addition to a standard curve amplification protocol, an allelic discrimination step was added to facilitate the contrast between the two alleles and their respective reporter dyes. These dyes are incorporated into a Taqman SNP Genotyping Assay with identification numbers C___3084793_20 and C___904973_10 for rs429358 and rs7412, respectively (Applied Biosystems, Foster City, CA). The experiment was performed in duplicate to confirm results. SDS software (version 2.3, Applied Biosystems) was used to analyze the SNP genotyping data. This program calculates the affinity of the sample to one of the two reporter dyes that, in turn, represents one allele over the other. The results of these tests are strictly confidential and are never made available to the research participant.

Imaging Acquisition

MRI scanning was conducted using a Siemens 3T Trio magnet located at the UCLA Center for Cognitive Neuroscience in the Semel Institute. Whole-brain, structural MRI was collected using a 3D T1-weighted Magnetization Prepared Rapid Gradient Echo (MPRAGE) volumetric scan sequence with axial slicing, TR=1900ms, TE=2.26ms, FOV=250mm x 218mm, flip angle=9°, matrix=256x215, 176 slices, slice thickness=1mm, zero-filled to a matrix of 256x224 resulting in a voxel size=1x0.976x0.976 mm^3. To facilitate registration of functional images, co-planar, T2-weighted structural images were also acquired in axial slices with TR=5000ms, TE=34ms, FOV=200mmx200mm, flip angle=90°, matrix=128x128, 28 slices, slice thickness=3mm, interslice gap=1mm and voxel size=1.6x1.6x4mm. Whole-brain, functional MRI scans were acquired using a sequence with the following parameters: interleaved axial slices, TR=2500ms, TE=21ms, FOV=200mmx200mm, flip angle=75°, matrix=64x64, 33 slices, slice thickness=3mm, interslice gap=0.75mm, voxel size=3.125x3.125x3.75mm. This acquisition sequence was designed to minimize signal drop-out caused by susceptibility artifact in the medial temporal lobes, an area of particular interest in older participants and in the analyses described here. The functional imaging data acquired during the course of this study have not been analyzed in other publications. Participants were also scanned using a high-resolution hippocampal structural sequence that was not analyzed as a part of this study. Some participants' structural imaging data have been used in previous publications (46–51). Previous work from our group on the effect of the APOEε4 allele on brain function using whole-brain fMRI was completed with a separate, older dataset. The current dataset was collected from Spring 2006 to Fall 2012.

Memory Task

During the functional scan participants completed a paired-associates memory task that has been previously shown to be sensitive to subtle memory impairment in disease and normal

aging and to differentiate across APOEε4 carriers and non-carriers (33; 52–54). Participants were presented with seven pairs of unrelated words that had to be learned and then recalled (Figure 2.1). The task includes six blocks each of alternating encoding and retrieval phases (30 seconds each) separated by a baseline condition (20 seconds). During encoding, seven unrelated word pairs (e.g., clock/green, jazz/beast) were presented sequentially and participants were asked to learn the word pairs. Words were presented as simultaneous auditory and visual stimuli. Following each encoding block participants completed a baseline control task in which they were instructed to fixate on a symbol in the center of the screen ("+" or "o") and press a button every time the symbol changed (55). Next, participants completed a retrieval block in which they saw and heard the first word of each pair and were asked to silently recall the second word of the pair. Because the retrieval phase of the task requires a spontaneous recall response, all participants completed an alternate form of the task outside the scanner where we assessed performance using the WMS-III Verbal Paired Associates. This generates a valid proxy of in-scanner performance, which is preferable to using a recognition-based response that would fundamentally change the nature of the memory task; prior work in our lab has verified the comparability of performance in and outside the scanner using this approach (33).

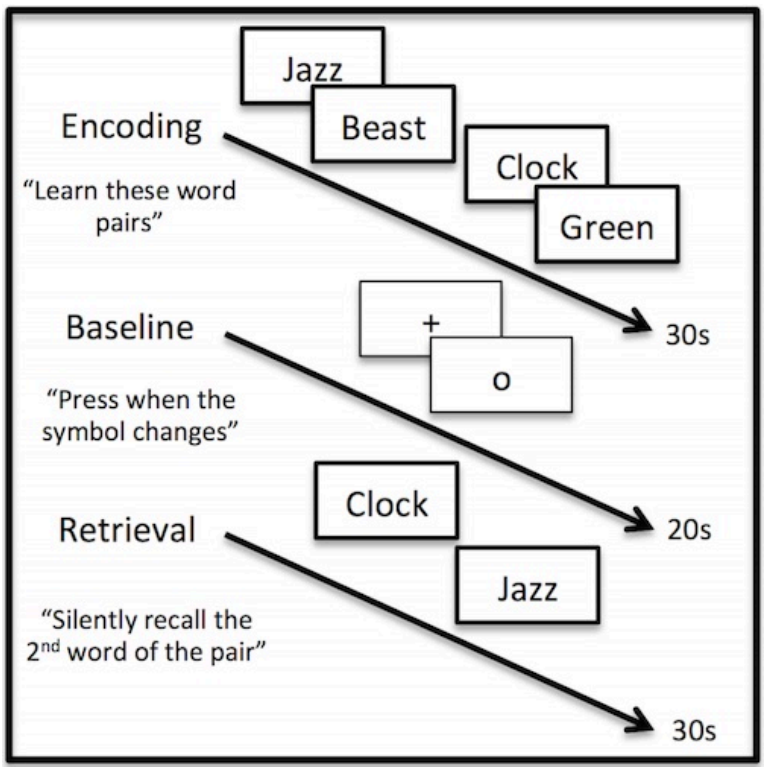


Figure 2.1: Unrelated Words, Paired-Associates Memory Task Design. This is a block design task that includes six blocks each of alternating encoding and retrieval phases separated by a baseline condition. During encoding (30 second block), seven unrelated word pairs (e.g., jazz/beast, clock/green) are presented sequentially using both audio and visual stimuli and participants are asked to learn the word pairs. Next, during the baseline block (20 second block), participants are instructed to fixate on a symbol in the center of the screen ("+" or "o") and press a button every time the symbol changes. Finally, during the retrieval phase (30 second block) participants see and hear the first word of each pair and are asked to silently recall the second word of the pair. s = seconds.

Statistical and Imaging Analyses

Neuropsychological Performance

To test whether the APOEε4 carrier and non-carrier groups differed in cognitive ability, scores on each neuropsychological test were compared using two-sample, two-tailed *t* tests. Fisher's exact tests were used to test for group differences in the categorical variables of sex and family history of AD. These tests were completed using tools from R Project for Statistical Computing (http://www.r-project.org).

Hippocampal Seeds

A mask of the left hippocampus in each participant's high resolution structural space was created using FSL's FIRST and a hippocampal model based on 336 subjects as a prior (56). We focused our analysis on the left hippocampus because of the preferential engagement of left-lateralized hippocampal complex areas during verbal memory tasks (57). Masks were checked manually for accuracy, eroded and binarized. Next, for each participant's unique hippocampal mask, the anterior and posterior thirds of the structure were identified using custom code in MATLAB (version R2012a) (Figure 2.2). Specifically, the length of the volumetric hippocampal mask in the anterior-posterior plane was determined and then used to generate coordinates demarking the anterior and posterior thirds of this plane for each participant. Next, using FSL tools, we generated anterior and posterior hippocampal mask images based on these coordinates. Finally, we transformed the anterior and posterior hippocampal masks into native functional space. Using the anterior and posterior thirds prevented signal blurring across the two hippocampal seeds after registration to functional space while still allowing us to include the majority of the hippocampus in our study. Also, the anterior third of the hippocampus is perfused by a different arterial supply (anterior choroidal) than the posterior two thirds (posterior cerebral) which may affect BOLD signal (58). We followed the example of previous studies that have also examined the anterior and posterior thirds of the hippocampus for these reasons (59; 60).

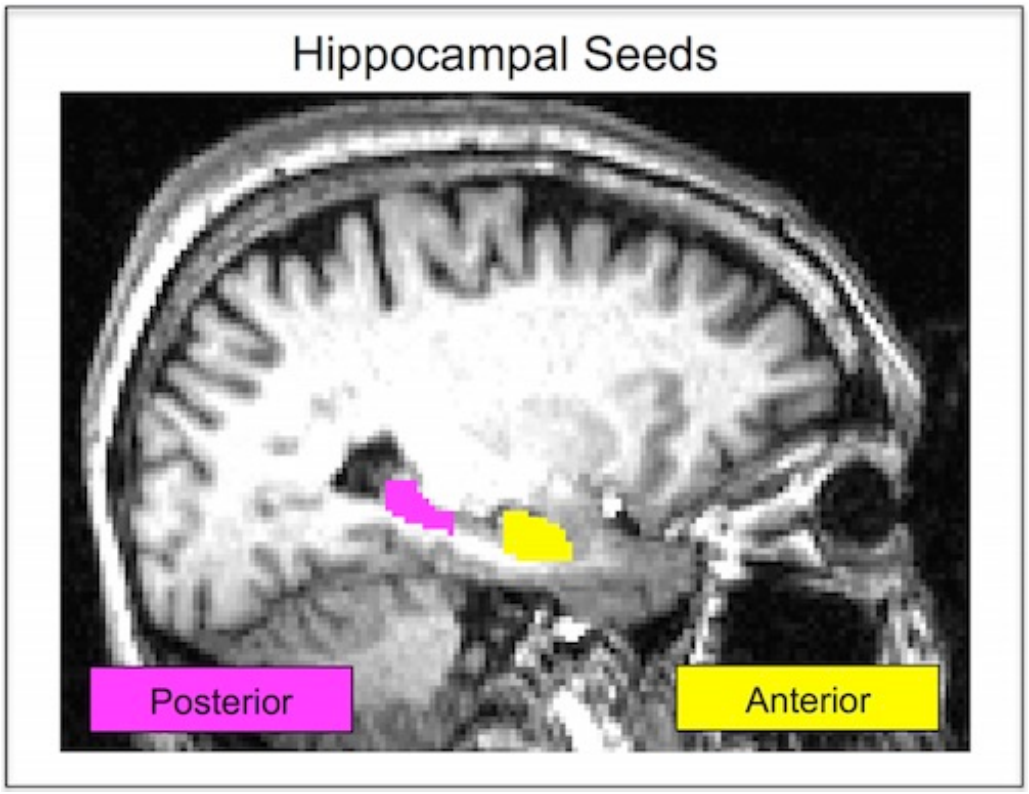


Figure 2.2: Hippocampal Seeds. In native space, a single participant's anterior hippocampus seed is shown in yellow. The posterior hippocampus seed for the same participant is shown in pink. Seeds are defined in each participant's unique structural image and then registered to their functional scan. Seeds are never in a standardized space which improves the accuracy of the hippocampal segmentation.

Structural Imaging

Differences in cortical integrity caused by atrophy can confound functional imaging studies in older subjects, especially when one group is at increased risk for a neurodegenerative disease like AD. To ensure that are were no differences in gray matter thickness between APOEε4 carriers and non-carriers in this study, whole-brain structural MRI scans were processed using Freesurfer (version 5.1.0 available at freesurfer.net). This computational neuroanatomy software suite uses tissue contrast to determine the boundary between gray and white matter as well as delineate the pial surface of the brain. A mesh of vertices is plotted across each of these boundaries or surfaces. The software calculates the distance between each pair of vertices to measure cortical thickness. The details of the FreeSurfer pipeline are described in previous publications (61). After completing the FreeSurfer automated pipeline,

each participant's scan was visually checked for accuracy. Minimal manual edits were completed when necessary by a single individual (TMH). Vertex-wise general linear models (GLMs) were used to compare cortical thickness across groups with a statistical threshold set at false discovery rate (FDR) of $p<0.05$. We also examined differences thresholded at $p<0.01$, uncorrected to check for regions trending toward differences.

Functional Imaging

First-Level Analysis: Preprocessing and Task Activation Model

Functional imaging preprocessing was completed using FSL (version 6.0: http://fsl.fmrib.ox.ac.uk). Preprocessing included skull-stripping and head motion correction (62) (63). A Gaussian kernel of FWHM 5mm was applied to the data for spatial smoothing. This kernel size is slightly below the 6mm kernel that is recommended based on the Nyquiest theorem. However, we chose to use a 5mm kernel due to concern about over-smoothing in the hippocampus, which is a structure with a small diameter and very intricate anatomy. Images were high-pass filtered at sigma=100s and prewhitened (64). The functional data was registered to co-planar T2 structural images with 6 degrees of freedom. The co-planar structural images were then registered to each participant's high-resolution structural image using boundary-based registration (65). Finally, each high-resolution structural scan was registered to the MNI152 standard using 12 parameter affine transformation. A linear transformation was used because this method produced more accurate alignment results than the more common non-linear approach. Within-subjects analysis was completed with a GLM including the two active phases of the functional task, 6 motion parameters as well as a regressor for each motion outlier volume, as determined by frame displacement (FD) calculations and standard outlier identification (75th percentile + 1.5 times the interquartile range; (66)). After these preprocessing steps were completed, the denoised average timeseries from both hippocampal seeds were extracted for each participant.

Mid-Level Analysis: gPPI

A generalized psychophysiological interaction (gPPI) analysis strategy was used to interrogate functional coupling of the hippocampus with the rest of the brain during the active phases of the paired associates task. Separate gPPI analyses were run for the anterior and posterior hippocampus seeds. A GLM, which included regressors for the encoding and retrieval phases of the task, a regressor for the denoised, average timeseries of either the left anterior or posterior hippocampal seed and a PPI regressor for each phase of the task was used to analyze activation in individual participants. These models also included the motion parameters and motion outlier regressors from the first-level analyses. Standard PPI includes a single PPI regressor in each GLM. However, by more comprehensively modeling the entire task the gPPI method has been shown to more accurately fit the data, leading to improvements in sensitivity and specificity (18).

Second-Level Analysis: Group Comparisons

To compare the context-dependent functional connectivity of the two seeds of interest between APOEε4 carriers and non-carriers, individual contrast of parameter estimates maps for each of the two PPI regressors in each of the two PPI models were registered from native space to MNI space using the registration parameters from the first-level analyses. The PPI regressors were seed x encoding and seed x retrieval, the two PPI models were anterior seed and posterior seed, and the registration to MNI space used 2mm isotropic voxels. Thus, for each participant, 4 statistical maps were examined: anterior seed x encoding, anterior seed x retrieval, posterior seed x encoding and posterior seed x retrieval. Unpaired t-tests, with memory performance included as regressor, were run in SPM8 comparing APOEε4 carriers to non-carriers.

Significance thresholding for group analyses was carried out using tools available in the AFNI software suite. First, spatial smoothness was estimated on the residuals across the whole

cohort. Smoothness estimates were extremely similar for each gPPI model and did not differ based on the seed included. Thus, for simplicity, a single average smoothness estimate (FWHM (x,y,z)= 7.06, 7.11, 6.50) was used in Monte Carlo simulations to estimate cluster extent minimums at uncorrected voxel thresholds. After simulations, 3dClustSim creates a table with cluster extent estimates at different voxel-wise p-values and cluster-wise alpha values. Thus, rather than testing many voxel and cluster threshold combinations, 3dClustSim minimizes guesswork and allows the investigators' hypotheses about cluster size to guide significance testing. In the present study, results were thresholded to reveal clusters significant at alpha <0.05 with a voxelwise threshold of p<0.005. Using this method and these thresholds, the significant cluster size minimum was 108 contiguous voxels. Masks were created from all significant clusters in each analysis in order to extract summary statistics from each participant to illustrate the shape of the effect.

Results

Participants

For this study 93 non-demented adults aged 55 and older were recruited. Of the 93 participants, 9 were excluded because they carried at least one ε2 allele (2 ε2/ε2, 5 ε2/ε3, and 2 ε2/ε4). Another 4 participants were excluded because they were homozygous for the ε4 allele. The remaining cohort included 34 APOEε4 carriers (all ε3/ε4) and 46 non-carriers (all ε3/ε3). Across the two experimental groups, APOEε4 carriers and non-carriers, there were no significant differences in age, sex, education or family history of AD (Table 2.1). Two-sample, two-tailed t-tests revealed that the groups did not differ in cognitive ability except in two measures of verbal memory: Logical Memory Delay and Verbal Paired Associates Delay. These two measures were highly correlated across the entire sample (r=0.43, p<0.0001). To control for the differences between groups in verbal memory, performance on Verbal Paired Associates was included as a regressor in all higher-level functional analyses. We ran group comparisons

without controlling for verbal memory performance in order to determine how performance differences might influence the results (Figure 2.3). We also tested for correlations between memory performance and the four PPIs that we examined (Figure 2.4).

Table 2.1: Cohort Characteristics. APOEε4 carriers and non-carriers do not significantly differ in age, sex, family history of AD or education. Measures of intelligence and cognition did not differ between groups, except on two verbal memory tests. As a result, verbal memory performance was regressed out of imaging analyses. APOEε4 = apolipoprotein E ε4 MMSE = Mini Mental State Exam; WMS = Wechsler Memory Scale; LM = Logical Memory; VP = Verbal Paired Associates; CLTR = Consistent Long-Term Retrieval; WAIS = Wechsler Adult Intelligence Scale; * = p<0.05; ** = p<0.01

Characteristic/Test	APOEε4 Carriers (n=34)	Non-Carriers (n=46)	*P*-value
Age (yr)	68.1	66.7	0.470
Sex (M/F)	16 / 18	15 / 31	0.247
Family History (Yes/No)	26 / 8	30 / 16	0.330
Education (yr)	17.0	17.2	0.593
MMSE (0-30)	28.6	28.9	0.390
Boston Naming (0-60)	56.1	56.0	0.973
WMS LM Delay Total (0-50)	23.4	28.9	***0.007***
WMS VP Delay (0-10)	6.1	7.1	***0.024****
Buschke CLTR (0-144)	58.2	60.9	0.742
WAIS Digit Span	18.4	17.6	0.399
WAIS Digit Symbol	64.1	63.0	0.780
Fluency: Fruits and Vegs	18.4	19.6	0.294

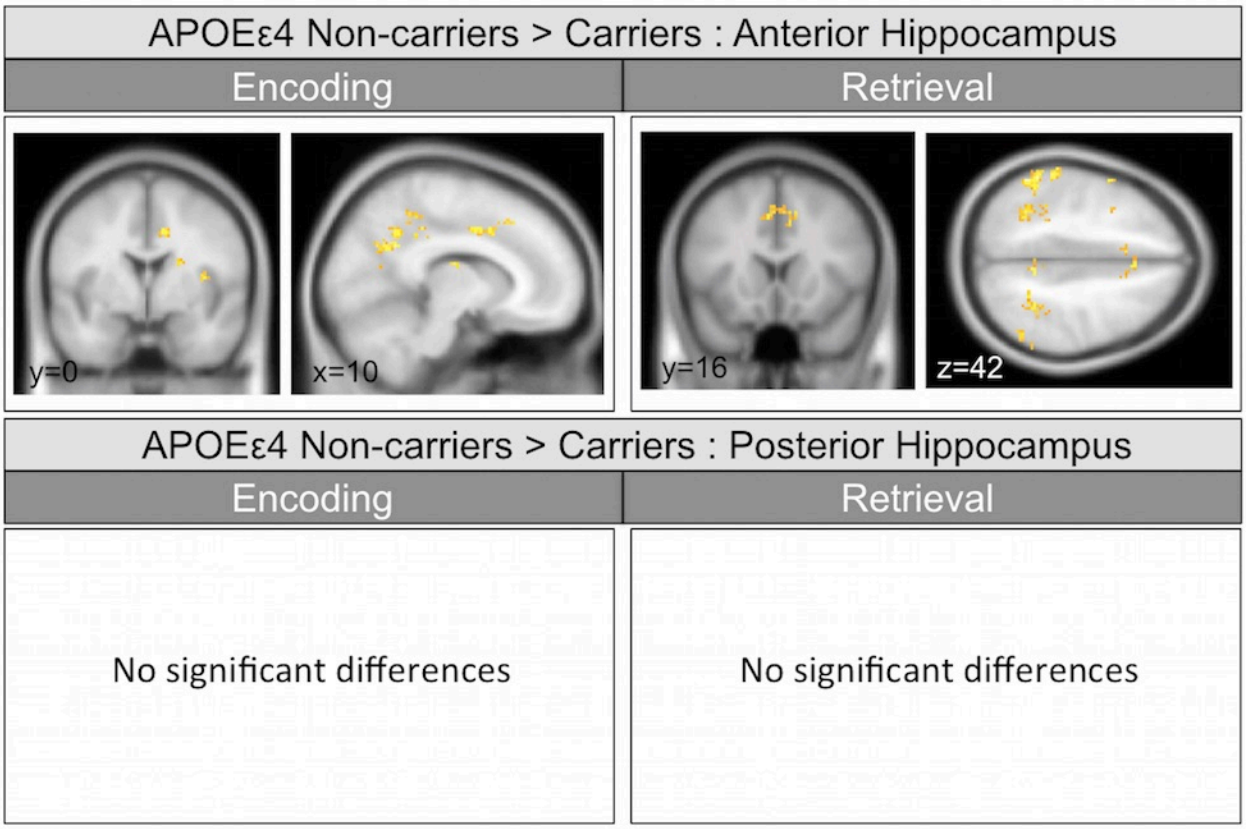


Figure 2.3: PPI analyses with no behavioral covariates. We ran gPPI group comparisons without controlling for verbal memory. All other aspects of this analysis were performed as described in the main text. Coronal and sagittal views of significant differences between APOEε4 carriers and non-carriers are shown (APOEε4 non-carriers > carriers). Relevant plane coordinates are provided. Differences between groups were similar in location and direction but greater in extext for the anterior hippocampus seed in both task conditions (upper panel). However, there were no significant results in either the encoding or retrieval phases using the posterior hippocampus seed (lower panel).

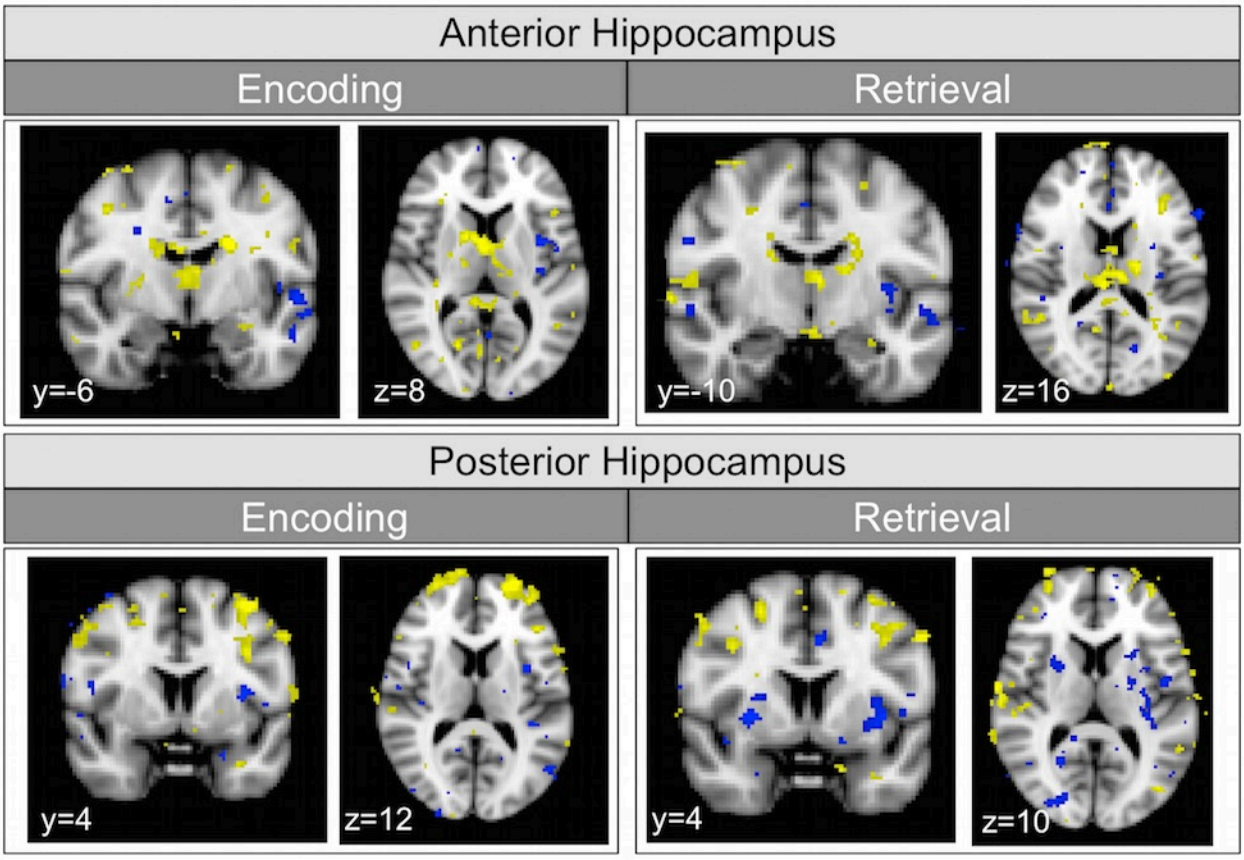


Figure 2.4: Main effect of memory performance on PPIs. Coronal and axial views of the whole group (APOEε4 carriers and non-carriers) correlation of memory performance and gPPI values. The upper panel shows results for the anterior hippocampus seed. Lower panel depicts results for posterior hippocampus seed. Maps are thresholded at $p<0.05$, uncorrected. Regions where there is a positive correlation are shown in yellow overlaid with regions where a negative correlation is found, shown in blue.

Hippocampal Seeds Volume

We calculated the volume of both the anterior and posterior hippocampal seeds in each participant. Two-sample t-tests revealed that there was no significant difference in seed volume between APOEε4 carriers and non-carriers for either the anterior (carriers average [SD] = 1946.6 mm^3 [311.0], non-carriers = 1949.8 mm^3 [302.6], p=0.96) or posterior hippocampus (carriers average [SD] = 1446.6 mm^3 [244.3], non-carriers = 1437.3 mm^3 [211.1], p=0.86).

Cortical Thickness

After visual inspection and manual intervention, one participant's FreeSurfer-processed structural scan did not meet our accuracy standards (female, 65-year-old APOEε4 non-carrier). This left 79 subjects with usable FreeSurfer data. Cortical thickness did not differ in any region of the cortex between the APOEε4 carrier and non-carrier groups at FDR of $p<0.05$ or at $p<0.01$ uncorrected. Additional models were evaluated that accounted for sex and that examined differences in age-cortical thickness correlations between APOEε4 carriers and non-carriers. There were no significant differences in cortical thickness is any region in these two models at either of the two statistical thresholds that were employed.

Head Motion

Differences in head motion between experimental groups may lead to spurious results (67). To ensure that the APOEε4 carriers and non-carriers in this study do not differ in head motion estimates, we calculated the average FD for each participant's functional scan. A two-sample t-test revealed that there was no significant difference in FD between APOEε4 carriers and non-carriers (carriers average [SD] = 0.21 mm [0.09], non-carrier = 0.20 mm [0.10], $p=0.45$).

Univariate Task Activation

There were no significant differences between APOEε4 carriers and non-carriers in task activation during encoding or retrieval. The within-group task activation maps show that the occipital lobe, auditory cortex, large regions of parietal lobe, frontal language areas, superior temporal gyrus and caudate (more pronounced during retrieval) show significant BOLD signal increases during encoding and retrieval in both experimental groups (Figure 2.5).

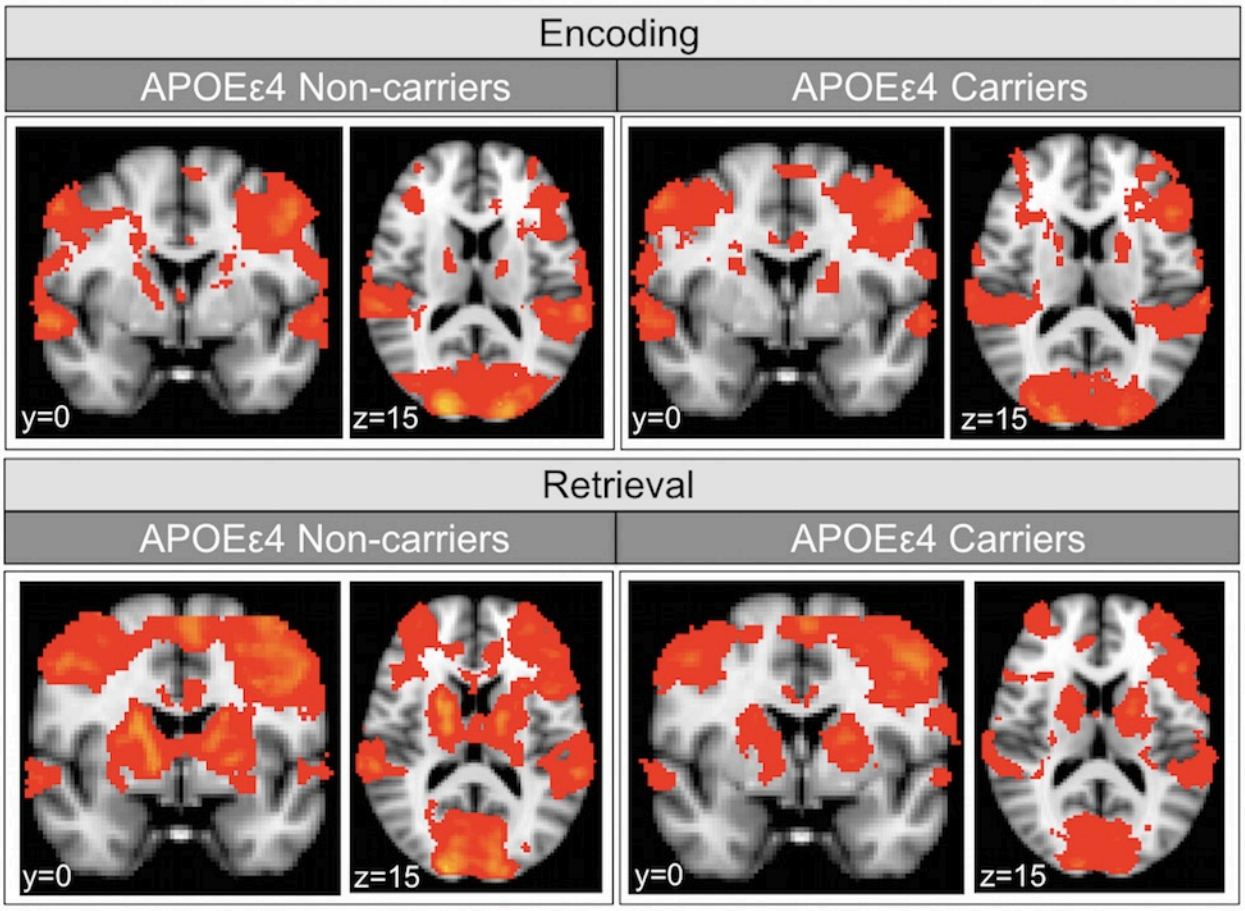


Figure 2.5: Univariate group maps for APOEε4 non-carriers and carriers showing significant activity during encoding and retrieval. Coronal and axial views of group average BOLD signal maps in APOEε4 non-carriers and carriers in encoding (upper panel) and retrieval (lower panel). Relevant plane coordinates are provided. Maps were thresholded at height z=3.0 and cluster corrected for extent at p<0.01. Regions in red denote regions where BOLD signal was, on average across the group, significantly higher during the active task phase compared to baseline.

Task-Dependent Connectivity (PPI): Anterior Seed

Using the anterior left hippocampus as a seed, significant differences between APOEε4 carriers and non-carriers were found for both encoding and retrieval phases of the task, such that APOEε4 non-carriers had more positive task-dependent connectivity change than carriers in several cortical regions (Figures 2.6 & 2.9). In contrast, there were no cortical regions in which connectivity change was significantly more positive for APOEε4 carriers compared to non-carriers in either task phase. Three clusters in the right hemisphere including the precuneus, the anterior insula and an area of anterior middle cingulate differed significantly

between APOEε4 carriers and non-carriers for the PPI of the encoding phase with the anterior hippocampus seed (Figure 2.6). Each of these clusters was examined as a region of interest (ROI) in order to better characterize group differences. The average parameter estimate from every participant was extracted from each ROI and then plotted by group (Figure 2.6). These plots show that the direction of the difference between APOEε4 carriers and non-carriers is consistent across clusters. Specifically, APOEε4 non-carriers on average have a greater-than-baseline relationship between BOLD activity and the PPI, while APOEε4 carriers have a lower-than-baseline relationship between BOLD activity and the PPI. This means that in APOEε4 non-carriers during encoding anterior hippocampus activity predicts higher activity in precuneus, anterior insula and a region of the cingulate, while in APOEε4 carriers anterior hippocampus activity during encoding predicts lower activity in these regions. One sample t-tests showed that within each group these activity-PPI relationships are significantly different from zero (Table 2.2). In other words, in the regions where significant differences between groups were found, the APOEε4 non-carriers show significant increases in activity while APOEε4 carriers show significant decreases in activity. The within-group functional connectivity maps show that there are no significant increases in functional connectivity of the hippocampal seeds in either APOEε4 carriers or non-carriers (Figure 2.7), but there are significant decreases in functional connectivity in APOEε4 carriers in each condition and in APOEε4 non-carriers only for posterior hippocampus during encoding (Figure 2.8). These maps, in contrast to the univariate activation maps which showed no differences, show a divergence between APOEε4 carriers and non-carriers in how hippocampal functional connectivity changes during a memory task. This divergence can be measured as a significant difference in the precuneus, anterior insula and the cingulate, as discussed above.

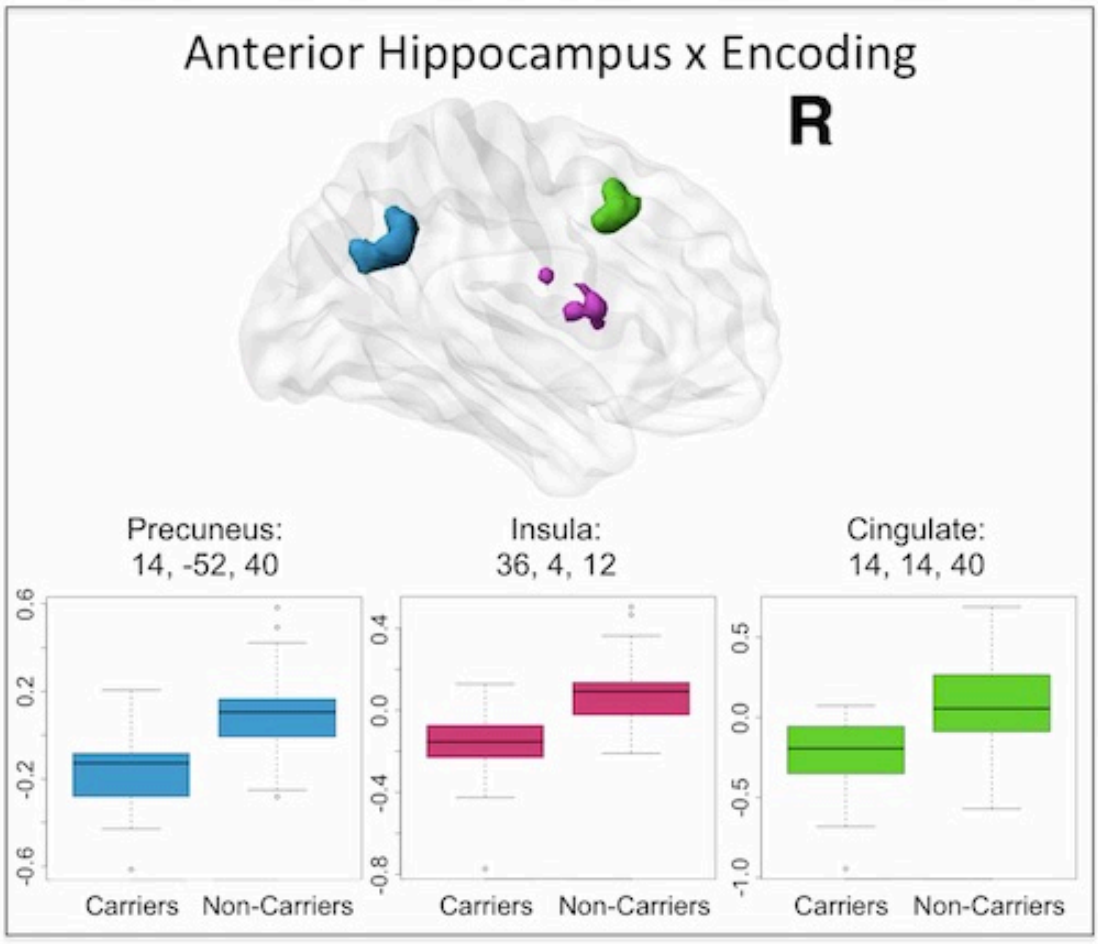


Figure 2.6: Anterior hippocampal seed connectivity differences in APOEε4 carriers and non-carriers during encoding. During encoding, significant differences in anterior hippocampus connectivity between APOEε4 carriers and non-carriers were found in right precuneus (blue), right anterior insula (pink) as well as right middle cingulate cortex (green). The peak coordinate for each cluster is reported in Montreal Neurological Institute (MNI) space, in x, y, z planes (mm). For illustration of the direction and magnitude of the difference between groups, contrasts of parameter estimates from each cluster are plotted by group in boxplots. The band within the box represents the median while the upper and lower edges of the box represent the first and third quartiles, respectively. The whiskers extend up to 1.5 times the interquartile range. Data points outside this range are plotted as outliers.

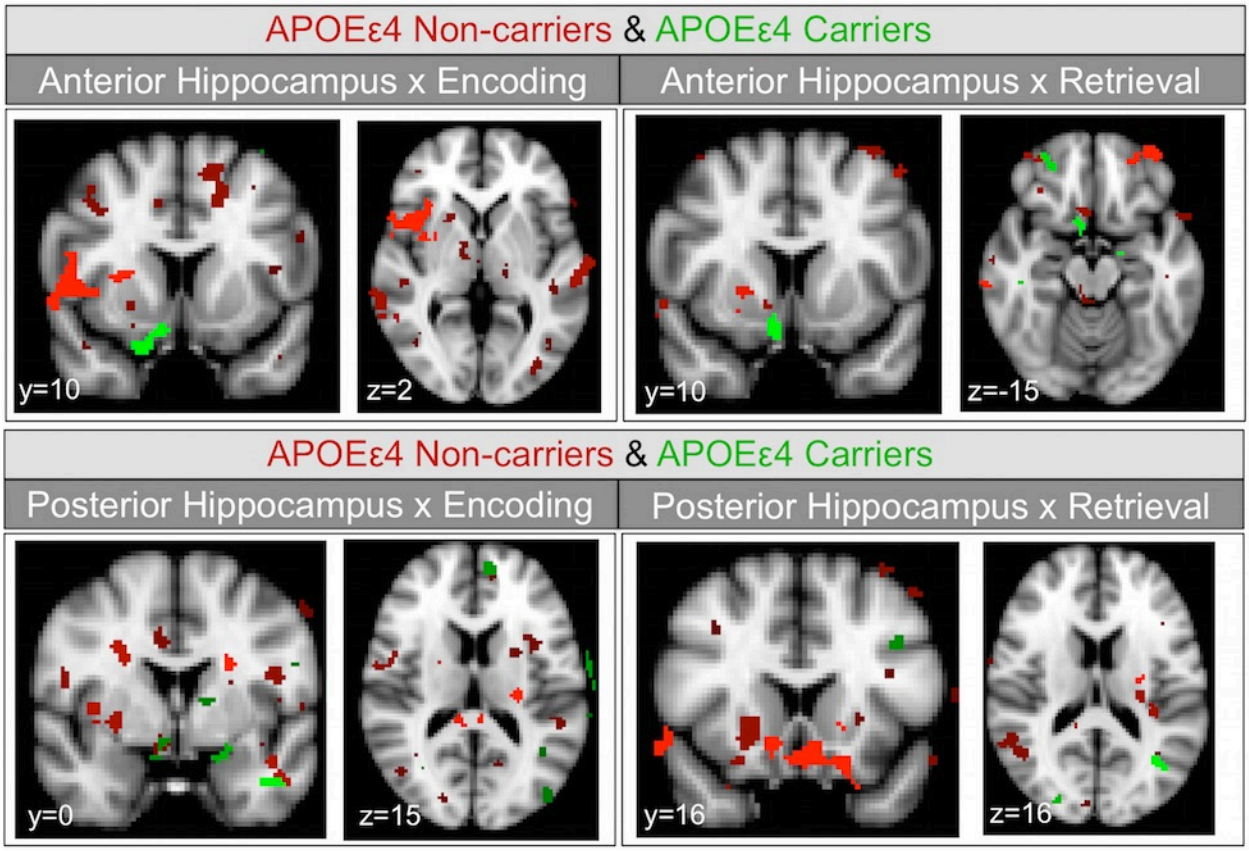


Figure 2.7: Hippocampal seeds task-dependent positive functional connectivity change maps. Coronal and axial views of group average task-dependent positive functional connectivity change of hippocampal subregions in APOEε4 non-carriers and carriers. Task-dependent connectivity of the anterior hippocampus seed is shown in the upper panel. The lower panel shows task-dependent connectivity maps of the posterior hippocampus. Maps were thresholded at $p<0.05$, uncorrected. Clusters of less than 10 voxels are not shown. Voxels meeting threshold in APOEε4 non-carriers (in red) and carriers (in green) are overlaid.

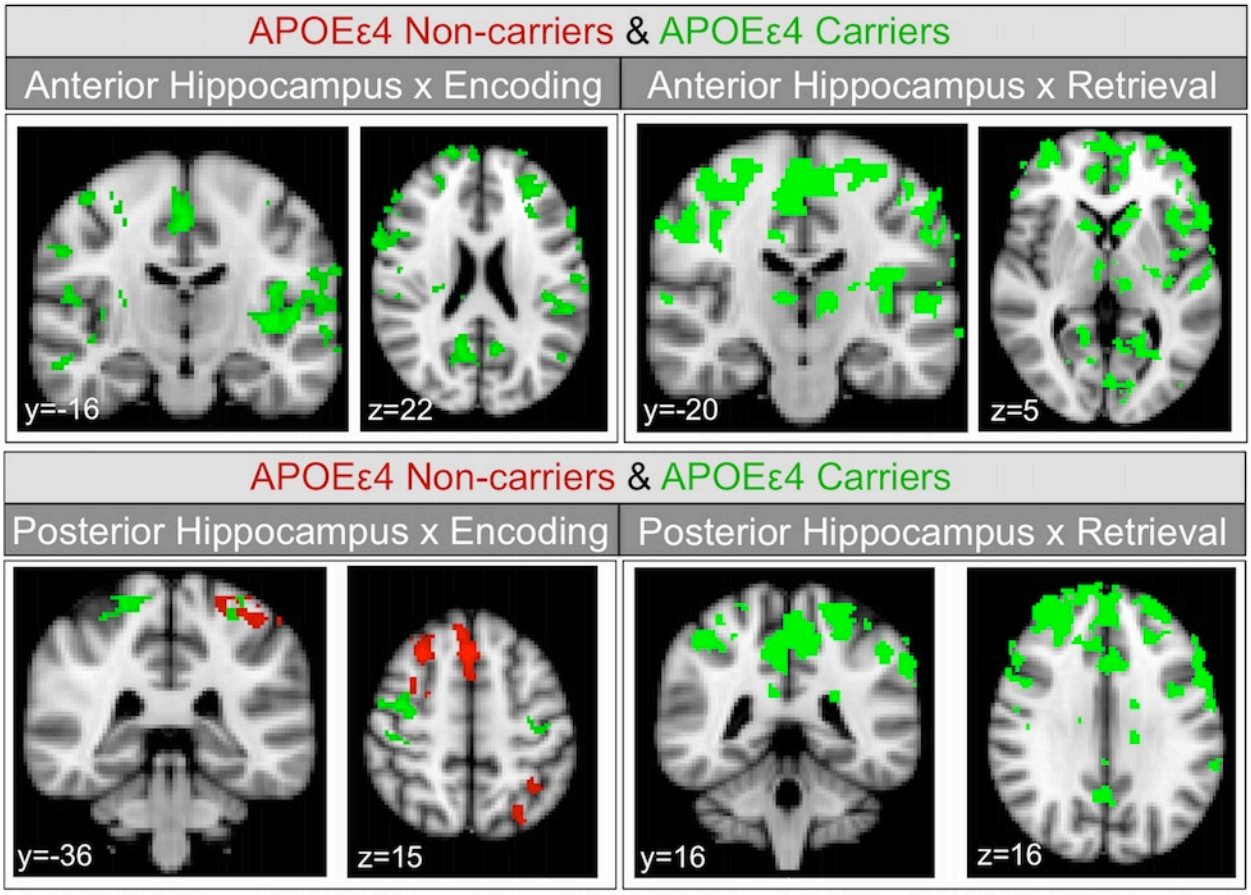


Figure 2.8: Hippocampal seeds task-dependent negative functional connectivity change maps. Coronal and axial views of group average task-dependent negative functional connectivity change of hippocampal subregions in APOEε4 non-carriers and carriers. Task-dependent connectivity of the anterior hippocampus seed is shown in the upper panel. The lower panel shows task-dependent connectivity maps of the posterior hippocampus. Maps were thresholded at z=2.3, cluster corrected at p<0.05. Voxels meeting threshold in APOEε4 non-carriers (in red) and carriers (in green) are overlaid.

The retrieval phase PPI with anterior hippocampus revealed significant group differences in three clusters located in bilateral supramarginal (with some angular gyrus in the right hemisphere) and right precuneus. ROI analyses of these clusters showed an effect of APOEε4 carrier status similar to the encoding phase PPI with anterior hippocampus. Specifically, in APOEε4 non-carriers activity in the anterior hippocampus positively predicts BOLD signal in bilateral supramarginal gyri and right precuneus while in APOEε4 carriers the anterior hippocampus shows lower-than-baseline functional connectivity to these regions during retrieval (Figure 2.9). Once again, one sample t-tests showed that within each group these BOLD signal-

PPI relationships are significantly different from zero indicating that the parameter estimates represent a significant change from baseline in these regions (Table 2.2).

Although there were no group differences in age, we did test the main effect of age on functional connectivity changes of the anterior hippocampus during encoding and retrieval. There were no regions where an effect of age was significant in either phase. We also tested for correlations between memory performance and task-related functional connectivity changes and found no significant results (Figure 2.4).

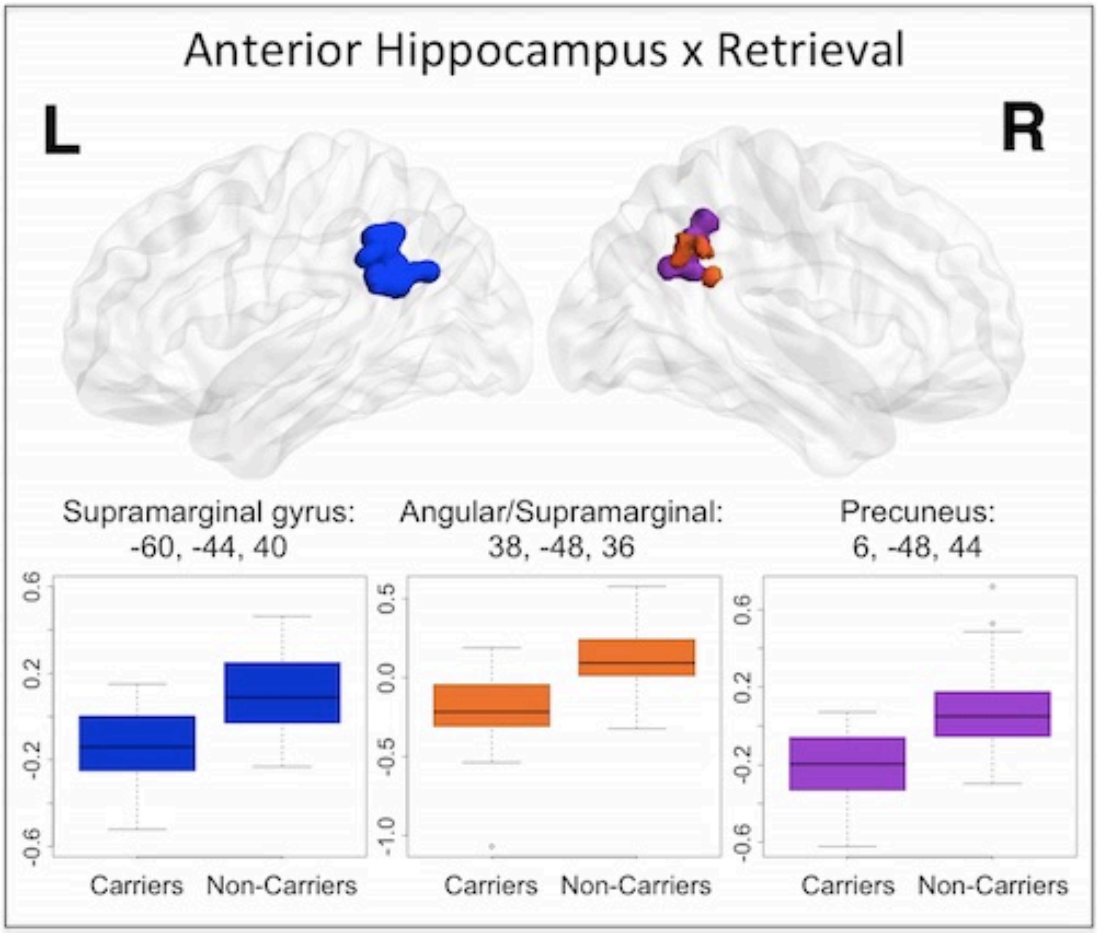


Figure 2.9: Anterior hippocampal seed connectivity differences in APOEε4 carriers and non-carriers during retrieval. During retrieval, significant differences between APOEε4 carriers and non-carriers were found in left supramarginal gyrus (dark blue), right supramarginal/angular junction (orange) as well as right precuneus (purple). The peak coordinate for each cluster is reported in MNI space, in x, y, z planes (mm). For illustration of the direction and magnitude of the difference between groups, contrasts of parameter estimates from each cluster are plotted by group. The band within the box represents the median while the upper and lower edges of the box represent the first and third quartiles, respectively. The whiskers extend up to 1.5 times the interquartile range. Data points outside this range are plotted as outliers.

Task-Dependent Connectivity (PPI): Posterior Seed

Using the posterior left hippocampus as a seed, significant group differences were found for only the retrieval phase of the unrelated words task. Similar to the results from the anterior hippocampus seed, differences were found such that APOEε4 non-carriers had significantly higher retrieval-dependent posterior hippocampal connectivity change to cortical areas compared to APOEε4 carriers. There were no cortical regions in which connectivity change was significantly more positive for APOEε4 carriers compared to non-carriers. The significant cluster, in left auditory cortex (transverse temporal gyri) and superior temporal gyrus, was examined as an ROI (Figure 2.10). As with the anterior hippocampus seed, APOEε4 non-carriers on average have a higher-than-baseline relationship between the PPI of the retrieval phase with the posterior hippocampus and BOLD activity in the ROI. In contrast, APOEε4 carriers have a lower-than-baseline relationship between the PPI of the retrieval phase with the posterior hippocampus and BOLD activity in the ROI. One sample t-tests showed that within each group these BOLD signal-PPI relationships are significantly different from zero (Table 2.2). Finally, there were no main effects of age or memory performance on functional connectivity changes of the posterior hippocampus during either the encoding or retrieval phase of the memory task.

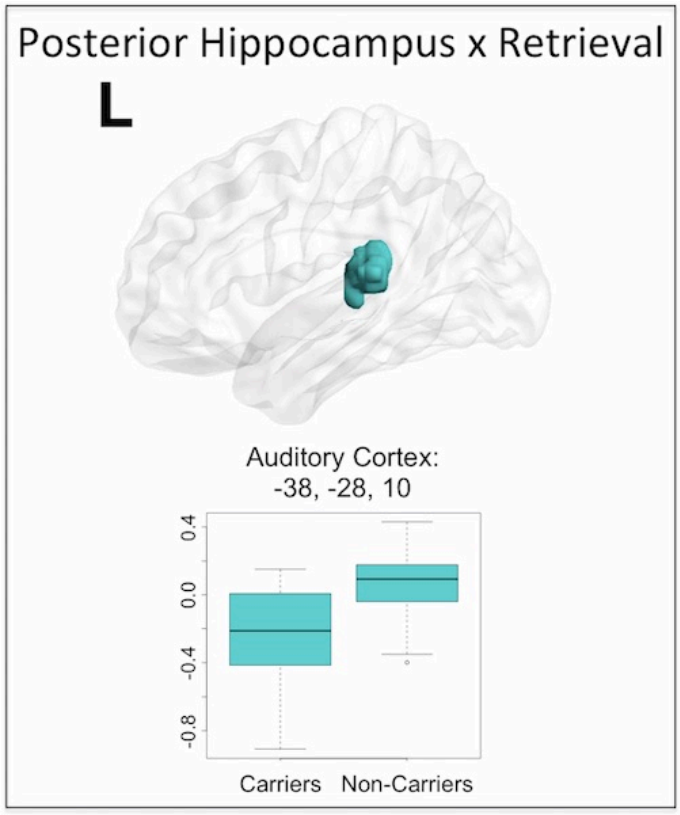


Figure 2.10: Posterior hippocampal seed connectivity differences in APOEε4 carriers and non-carriers during retrieval. During retrieval, significant differences in posterior hippocampus connectivity between APOEε4 carriers and non-carriers were found in a single cluster including left auditory cortex and some superior temporal gyrus (teal). The peak coordinate for the cluster is reported in Montreal Neurological Institute space, in x, y, z planes (mm). For illustration of the direction and magnitude of the difference between groups, contrasts of parameter estimates from each cluster are plotted by group in boxplots. The band within the box represents the median while the upper and lower edges of the box represent the first and third quartiles, respectively. The whiskers extend up to 1.5 times the interquartile range. Data points outside this range are plotted as outliers.

Table 2.2: ROI Analyses of Significant Clusters. One sample t-tests show that for each region where significant differences between groups were observed APOEε4 carriers' contrasts of parameter estimates were significantly less than 0 while non-carriers' contrasts of parameter estimates were significantly greater than 0. PPI = psychophysiological interaction; MNI = Montreal Neurological Institute; PE = parameter estimate * = p<0.05; ** = p<0.01; *** = p<0.001

PPI	Cluster Peak MNI Coordinates (mm)			APOEε4 Carriers		APOEε4 Non-Carriers	
	x	y	z	Average Contrast PE	One Sample T-test	Average Contrast PE	One Sample T-test
Anterior x Encoding	14	14	40	-0.237	*0.000****	0.079	*0.030**
	14	-52	40	-0.162	*0.000****	0.086	*0.001***
	36	4	12	-0.163	*0.000****	0.080	*0.001***
Anterior x Retrieval	38	-48	36	-0.201	*0.000****	0.124	*0.000****
	-60	-44	40	-0.158	*0.000****	0.128	*0.000****
	6	-48	44	-0.218	*0.000****	0.078	*0.008***
Posterior x Retrieval	-38	-28	10	-0.220	*0.000****	0.077	*0.004***

Discussion

This study identified differences in task-dependent functional connectivity between APOEε4 carriers and non-carriers during memory encoding and retrieval. During both encoding and retrieval of word pair associate learning we found significant differences in task-related functional connectivity of the hippocampus and several cortical regions. Group differences, regardless of task phase or hippocampal seed, were consistent in both direction and magnitude. Specifically, the relationship between the PPI regressor (the interaction of the task phase and activity in the hippocampal seed) and cortical activity was higher-than-baseline in APOEε4 non-carriers and lower-than-baseline in carriers. This consistency across task phase and hippocampal seed indicates that there is a characteristic difference between APOEε4 carriers and non-carriers in memory-related functional connectivity of the hippocampus and cortex. We found evidence of active disengagement in APOEε4 carriers of memory and language cortical regions that were positively modulated by the hippocampus in APOEε4 non-carriers during the memory task. These regions included right precuneus, right anterior insula, right middle cingulate cortex and bilateral supramarginal gyri. Our data suggest that a different functional

network could be mediating memory performance in APOEε4 carriers compared to non-carriers. Furthermore, *APOE* group differences in task-dependent functional connectivity change of the anterior hippocampus were present in both encoding and retrieval phases of the task. However, the posterior hippocampus functional connectivity change was only different between groups during the retrieval phase, indicating that the severity of APOEε4 carrier effects is greater in the anterior hippocampus.

rs-fMRI studies suggest that an early endophenotype of AD that is detectable even before the onset of clinical symptoms is dysfunction of the default mode network (DMN) (16; 68; 69). Activity within the DMN is relatively increased when the brain is not engaged in a specific cognitive task. The DMN has been linked to introspective processes and includes the hippocampus as one the nodes in the network (70). One of the key functions of the hippocampus is consolidation, which is a process that occurs when the brain is in a "resting state". This is likely to be one reason why hippocampal activity is correlated with the DMN, as measured with rs-fMRI. In healthy older APOEε4 carriers, decreased DMN connectivity has been described in several studies (12–15). One theory explaining this DMN dysfunction in APOEε4 carriers states that the genetic vulnerability for AD may cause a loss of appropriate hippocampal decoupling from cortical DMN regions during active states, like when completing a task (71). This theory is supported by a negative correlation between hippocampus-DMN synchronization and performance on a memory test that has been reported (71). It has also been shown that greater resting hippocampal connectivity is associated with cognitive decline in normal aging (72). Thus, it may be that impairment in switching hippocampal network engagement from *resting* functional connectivity state to *task-based* functional connectivity state recruiting memory-relevant regions underlies the apparent disengagement results described in the present study. Dynamic connectivity of hippocampal complex regions and DMN mediated by behavior has also been reported in other studies not specifically interested in *APOE* (73; 74).

The strong associations to memory, language and early AD-related changes of the regions identified as significantly different between groups in this study converge on the potential importance of these regions and the effect of APOEε4 on their function. Specifically, we found lower task-dependent connectivity change among APOEε4 carriers between the anterior hippocampus and right precuneus, anterior insula and a region of the cingulate during encoding. The precuneus is part of the DMN and, like other regions of this network, has high metabolic activity at rest (75). In addition, the precuneus is one of the first cortical regions to be affected by AD, showing decreased glucose metabolism and amyloid deposition in the earliest phases of the disease and in those at increased risk (76; 77). We also found a significant difference between APOEε4 carriers and non-carriers in the right precuneus when we examined change in functional connectivity of the anterior hippocampus during retrieval. Given these findings, it may be that APOEε4 carriers have a strong negative change in task-dependent connectivity in this region because of some early AD-related process or a baseline susceptibility in this region conferred by APOEε4. The anterior insula, another region where group differences were identified for the anterior hippocampus and encoding interaction, is a key region of the salience network (78). The anterior insula and its functional network have been previously associated with episodic memory decline in patients with mild cognitive impairment (79). Similarly, the cingulate has been implicated as a crucial region for normal memory function, especially the posterior portion (80). Lastly, in addition to right precuneus, during the retrieval phase, we found significant differences in task-dependent functional connectivity changes of the anterior hippocampus and bilateral parietal language areas, including supramarginal gyrus. These areas are responsible for aspects of language comprehension and repetition (81–83). These regions must work in concert with memory systems in order complete verbal memory tasks, like the paradigm used in this study.

The posterior hippocampus is important for episodic memory retrieval. We found no significant differences in APOEε4 carriers and non-carriers when we examined coupling of the

posterior hippocampus and whole cortex during *encoding*. This is not surprising given that encoding processes have been linked primarily in the anterior portions of the structure (25). However, there was a significant difference between groups when we examined change in functional connectivity of the posterior hippocampus during retrieval. Specifically, we found lower connectivity change of posterior hippocampus with left primary auditory cortex in APOEε4 carriers. This difference in primary auditory cortex, located along the transverse temporal gyri, may be related to the effort of recalling the second word of a word pair (words are simultaneously presented as both visual and auditory stimuli). We posit that this area may be involved in the active recalling of the spoken word pairs in order to select the appropriate word that paired with the retrieval stimulus. This finding, in contrast to those we reported using the anterior hippocampus seed, is unique as it involves a primary sensory cortical region, as opposed to higher order sensory integration regions. It is also important to note that the difference between groups in this region is not significant when verbal memory performance is not statistically controlled in the model (Figure 2.3). Thus, the difference between groups in this region may be related to accuracy and performance, but further studies are needed to formally test this hypothesis in a new cohort. Within our cohort, we found no significant association between memory performance and the PPI of either seed in either encoding or retrieval (Figure 2.4).

A possible limitation of this study is the lack of significant within-group increases in functional connectivity of the hippocampal seeds to cortical regions during encoding and retrieval (Figure 2.7). However, we do see significant decreases in functional connectivity of the hippocampal seeds within group, especially for APOEε4 carriers (Figure 2.8). Certainly, if these significant effects were in the positive direction interpretation of the results would be more straightforward. However, we believe these results show that there is a disconnection phenotype of the hippocampus from cortical regions during active memory function in APOEε4 carriers and that this finding is valuable in itself. We argue that this might be part of an overall

disruption of normal functional connectivity both in resting networks and in response to task demands.

The participants in this study are older adults and it is likely that some of them have begun the process of hippocampal atrophy and dysfunction that is associated with normal aging (Small et al. 2011). However, because none of the participants exhibited clinical features of cognitive dysfunction, we believe that they are an ideal group in which to examine the effects of the APOEε4 allele. Because of our unique recruitment strategy, our APOEε4 non-carrier group may be enriched for other genetic risk factors for AD, such as family history of AD, despite their lack of an APOEε4 allele. We consider this a strength because our results can be more confidently attributed to APOEε4 carrier status because of how closely matched our groups are on other factors, including family history of AD, which is usually higher in APOEε4 carriers than non-carriers. It is possible that some of our results may be related to amyloid deposition, especially in the APOEε4 carriers, but a large portion of our cohort is young enough (average age = 67.3) that severe amyloid deposition is not a primary concern. In future follow-up studies of these participants as they age, it will be critically important to acquire amyloid imaging. It is not known whether the results described here are evidence of a compensatory strategy in APOEε4 carriers that affects BOLD activity, nor is there sufficient information to determine whether the findings are related to baseline perfusion differences (84; 85).

The cortical regions where we identified differences between APOEε4 carriers and non-carriers are all putatively related to task-performance, which indicates our approach was strong and our findings are valid. It is also important to note that in this study no masking procedures were used to amplify the power of the PPI to detect differences between groups in specific areas. While a masking approach is sound and supported when there is a strong hypothesis about a specific cortical area, we chose to interrogate the whole brain in order to elucidate robust differences between groups without restriction.

Conclusion

There is an increasing emphasis on the development of neuroimaging endophenotypes for AD. The ultimate goal is to use neuroimaging biomarkers to detect *preclinical* AD on the individual level in order to ensure that preclinical patients receive available interventions or are invited to enroll in treatment trials. One way to identify potential neuroimaging endophenotypes is to examine groups of participants at increased genetic risk for AD. Our findings suggest that there are cortical regions in which APOEε4 carriers and non-carriers show consistent differences in task-based hippocampal connectivity. The consistency of these findings across memory task phases and hippocampal subregion seeds suggests that task-based hippocampal functional connectivity changes differ between APOEε4 carriers and non-carriers at the network level, as opposed to in specific, homogenous functional regions. This may be related to the well-validated dysfunction of the DMN in preclinical AD, as well as cohorts of healthy APOEε4 carriers (12–15; 86). The results described here are consistent with neuropathological evidence suggesting that anterior hippocampus is affected earlier in the course of AD pathophysiology and thus may be more susceptible to the earliest preclinical changes. Future studies linking task-based functional connectivity changes and rs-fMRI cognitive networks in healthy older APOEε4 carriers and non-carriers are necessary to better understand how alterations in network connectivity at 'rest' influence functional connectivity alterations during a memory task.

Supplementary Information

Univariate Analyses

We ran univariate analyses to create average group activation maps for APOEε4 carriers and non-carriers during encoding and retrieval (Figure 2.5). Despite previous findings from our group and others, there were no significant differences between groups in these analyses. We hypothesize that alterations to the task protocol (presenting the stimuli as both visual and auditory input when previously the stimuli were auditory only) may have diminished task difficulty and made replication of previous results not possible.

Hippocampal Seeds Task-Dependent Connectivity by Group

After the mid-level of functional imaging processing in which the gPPI models were run (described in *Methods*), we examined the task-dependent connectivity maps of each hippocampal seed during encoding and retrieval in both groups, APOEε4 carriers and non-carriers, separately. There were no regions that reached significance in the group maps reporting positive changes in functional connectivity. For illustration, we have included hippocampal seed task-dependent connectivity maps not corrected for multiple comparisons with $p<0.05$ and thresholded to only include clusters of 10 or more voxels (Figure 2.7). Qualitatively, it can be observed that there are more voxels that reach this uncorrected threshold in the APOEε4 non-carriers compared to carriers. We also examined within-group task-dependent negative functional connectivity changes and found that in each condition there were regions of significant decreases in functional connectivity in APOEε4 carriers (Figure 2.8). There are significant decreases in functional connectivity in APOEε4 non-carriers only of the posterior hippocampus during encoding. Based on our group comparison findings described in the main text, it is not surprising that there are no significant results in group average maps of positive changes in functional connectivity as we show that APOEε4 carriers and non-carriers have opposite relationships (lower-than-baseline in carriers, higher-than-baseline in non-

carriers) between the PPI of seed and task phase and the activity in the regions where significant group differences are detected.

Group Differences Without Controlling for Memory Performance

We ran gPPI group comparisons between APOEε4 carriers and non-carriers without controlling for verbal memory, a cognitive domain where performance differed significantly between groups. Differences between groups were similar in location and direction but greater in extent for the anterior hippocampus seed in both task conditions (Figure 2.3, upper panel). However, there were no significant results in either the encoding or retrieval phases using the posterior hippocampus seed (Figure 2.3, lower panel). Thus, the significant difference we detect between groups in functional connectivity of the posterior hippocampus to auditory cortex during retrieval is dependent on statistical control of group differences in memory performance.

Memory – PPI Correlations

In order to determine if there is a relationship between memory performance and the PPIs explored in this study, we tested for negative and positive correlations between performance on Verbal Paired Associates and PPI values. We observed no significant results for either positive or negative correlations between memory performance and PPIs. We examined these correlations for both positive and negative PPI contrasts. For illustrative purposes, we have included a supplemental figure depicting the memory-PPI correlation maps thresholded at $p<0.05$, uncorrected, using the positive PPI contrasts (Figure 2.4). The lack of significant findings in these analyses strengthens our focus on the APOEε4 allele as the factor mediating differences in memory task-dependent connectivity of the anterior and posterior hippocampus in this cohort of cognitively healthy older adults.

Task-Dependent Connectivity (PPI): Orbitofrontal ROI

It is important to consider the possibility that the group differences observed in the PPI models are consistent across the entire brain but only reached significance in some regions. To address this, we examined a region of interest (ROI) in left orbitofrontal cortex, an area we would not expect to be involved in a verbal memory task. The connectivity of the anterior hippocampus to this ROI during encoding was estimated as described in *Methods*. To create the ROI, a 6mm sphere was grown around the coordinates [-30, 36, -10] (in mm) and the average contrast of parameter estimates within the ROI was extracted for each subject (Figure 2.11). We plotted these values for each group separately and found that the average for both APOEε4 carriers and non-carriers was close to 0 (-0.04 for carriers and -0.009 for non-carriers) (Figure 2.11). One sample t-tests empirically showed that contrast of parameter estimates from APOEε4 carriers and non-carriers did not differ from 0 (carriers p=0.16; non-carriers p=0.78). This is an illustrative control that suggests our main findings are attributable to memory-dependent hippocampal connectivity differences between APOEε4 carriers and non-carriers.

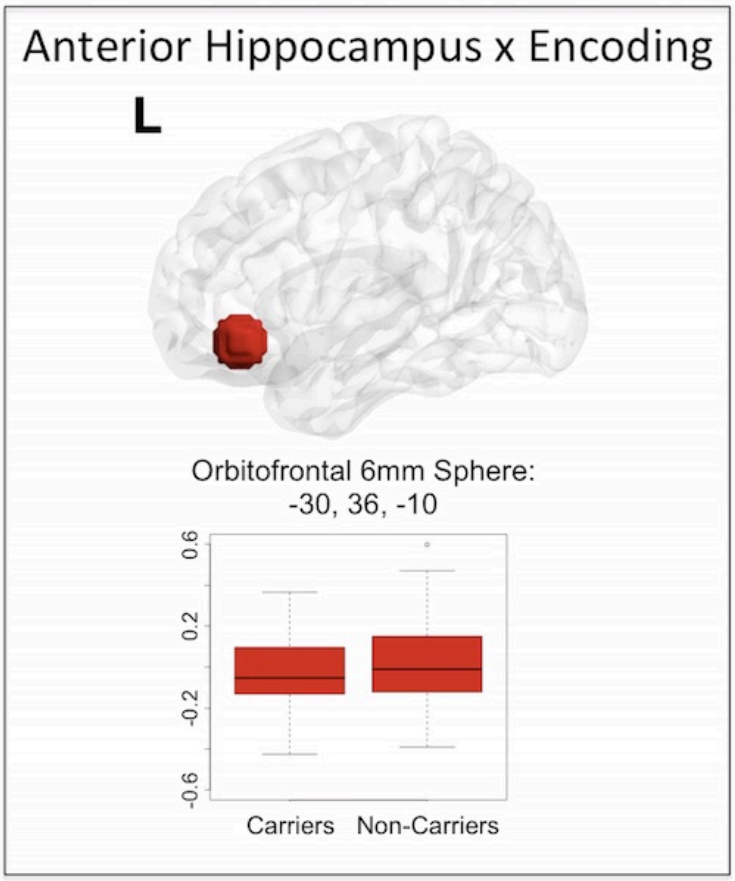


Figure 2.11: Comparison of APOEε4 carriers' and non-carriers' anterior hippocampus connectivity to an orbitofrontal ROI during encoding. During encoding, anterior hippocampus connectivity to a 6mm sphere located in orbitofrontal was compared between APOEε4 carriers and non-carriers. As expected, no significant differences were found. The center coordinate for the ROI is reported in Montreal Neurological Institute space, in x, y, z planes (mm). For each group, contrasts of parameter estimates from the ROI are plotted. The band within the box represents the median while the upper and lower edges of the box represent the first and third quartiles, respectively. The whiskers extend up to 1.5 times the interquartile range. Data points outside this range are plotted as outliers. Neither group has parameter estimates significantly different from zero.

Table 2.3: Complete Cluster Peak Information. The complete peak information for each of the significant clusters reported in the main text and illustrated in Figures 2.6, 2.9 & 2.10 is included here to aid in meta-analyses. We used a freely available SPM8 toolbox to extract these data (https://www.nitrc.org/projects/peak_nii). This toolbox is superior to SPM peak output because it reports all the peaks within a cluster instead of only three, which is the standard in SPM. We used standard parameters and parameters matched to our analyses (voxel threshold $p<0.005$, cluster minimum at 108 voxels). PPI = psychophysiological interaction; MNI = Montreal Neurological Institute.

PPI	Num. Voxels in Cluster	T-Statistic of Peak	MNI Coordinates (mm)		
			x	y	z
Anterior x Encoding	171	4.574	14	-52	40
		4.138	9	-57	30
		3.777	4	-66	29
		3.414	3	-55	36
	115	4.370	12	16	42
		3.940	22	17	49
		3.616	20	24	46
	180	4.052	35	1	9
		3.968	26	8	9
		3.796	22	-7	20
		3.780	20	6	20
		3.528	34	14	8
		3.497	18	-4	11
		2.846	16	12	8
Anterior x Retrieval	241	4.968	-56	-47	39
		4.289	-67	-44	34
		4.221	-59	-49	24
		3.744	-54	-62	28
		3.566	-48	-44	44
		3.317	-52	-52	32
		2.834	-52	-54	18
	141	4.618	5	-48	41
		4.429	7	-55	28
		3.952	11	-53	38
		3.859	-6	-54	34
		2.869	10	-44	52
	134	3.999	44	-47	34
		3.972	63	-42	24
		3.809	62	-48	33
		3.521	52	-54	37
		3.432	49	-59	30
Posterior x Retrieval	147	4.438	-40	-32	12
		3.810	-37	-31	21

		3.595	-34	-26	4
		3.582	-50	-32	12
		2.947	-54	-39	13
		2.905	-30	-32	12

Chapter 2 References

1. Bergem AL, Engedal K, Kringlen E (1997): The role of heredity in late-onset Alzheimer disease and vascular dementia. A twin study. *Arch Gen Psychiatry* 54: 264–70.
2. Gatz M, Reynolds CA, Fratiglioni L, Johansson B, Mortimer JA, Berg S, *et al.* (2006): Role of genes and environments for explaining Alzheimer disease. *Arch Gen Psychiatry* 63: 168–74.
3. Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, *et al.* (1993): Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* 261: 921–3.
4. Strittmatter WJ, Weisgraber KH, Huang DY, Dong LM, Salvesen GS, Pericak-Vance M, *et al.* (1993): Binding of human apolipoprotein E to synthetic amyloid beta peptide: isoform-specific effects and implications for late-onset Alzheimer disease. *Proc Natl Acad Sci U S A* 90: 8098–102.
5. Schmechel DE, Saunders AM, Strittmatter WJ, Crain BJ, Hulette CM, Joo SH, *et al.* (1993): Increased amyloid beta-peptide deposition in cerebral cortex as a consequence of apolipoprotein E genotype in late-onset Alzheimer disease. *Proc Natl Acad Sci U S A* 90: 9649–53.
6. Waring SC, Rosenberg RN (2008): Genome-wide association studies in Alzheimer disease. *Arch Neurol* 65: 329–34.
7. Bertram L, Tanzi RE (2012): The genetics of Alzheimer's disease. *Prog Mol Biol Transl Sci* 107: 79–100.
8. Braak H, Braak E, Bohl J (1993): Staging of Alzheimer-related cortical destruction. *Eur Neurol* 33: 403–8.
9. Trachtenberg AJ, Filippini N, Mackay CE (2012): The effects of APOE-ε4 on the BOLD response. *Neurobiol Aging* 33: 323–34.
10. Burggren AC, Small GW, Sabb FW, Bookheimer SY (2002): Specificity of brain activation patterns in people at genetic risk for Alzheimer disease. *Am J Geriatr Psychiatry* 10: 44–51.
11. Damoiseaux JS, Rombouts SARB, Barkhof F, Scheltens P, Stam CJ, Smith SM, Beckmann CF (2006): Consistent resting-state networks across healthy subjects. *Proc Natl Acad Sci U S A* 103: 13848–53.
12. Heise V, Filippini N, Trachtenberg AJ, Suri S, Ebmeier KP, Mackay CE (2014): Apolipoprotein E genotype, gender and age modulate connectivity of the hippocampus in healthy adults. *Neuroimage* 98: 23–30.
13. Damoiseaux JS, Seeley WW, Zhou J, Shirer WR, Coppola G, Karydas A, *et al.* (2012): Gender modulates the APOE ε4 effect in healthy older adults: convergent evidence from functional brain connectivity and spinal fluid tau levels. *J Neurosci* 32: 8254–62.
14. Machulda MM, Jones DT, Vemuri P, McDade E, Avula R, Przybelski S, *et al.* (2011): Effect of APOE ε4 status on intrinsic network connectivity in cognitively normal elderly subjects. *Arch Neurol* 68: 1131–6.
15. Sheline YI, Morris JC, Snyder AZ, Price JL, Yan Z, D'Angelo G, *et al.* (2010): APOE4 allele disrupts resting state fMRI connectivity in the absence of amyloid plaques or decreased CSF Aβ42. *J Neurosci* 30: 17035–40.
16. Sperling R (2011): Potential of functional MRI as a biomarker in early Alzheimer's disease.

Neurobiol Aging 32 Suppl 1: S37–43.

17. Friston KJ, Buechel C, Fink GR, Morris J, Rolls E, Dolan RJ (1997): Psychophysiological and modulatory interactions in neuroimaging. *Neuroimage* 6: 218–29.
18. McLaren DG, Ries ML, Xu G, Johnson SC (2012): A generalized form of context-dependent psychophysiological interactions (gPPI): a comparison to standard approaches. *Neuroimage* 61: 1277–86.
19. Strange BA, Fletcher PC, Henson RN, Friston KJ, Dolan RJ (1999): Segregating the functions of human hippocampus. *Proc Natl Acad Sci U S A* 96: 4034–9.
20. Strange B, Dolan R (1999): Functional segregation within the human hippocampus. *Mol Psychiatry* 4: 508–11.
21. Schacter DL, Wagner AD (1999): Medial temporal lobe activations in fMRI and PET studies of episodic encoding and retrieval. *Hippocampus* 9: 7–24.
22. Salami A, Eriksson J, Nyberg L (2012): Opposing effects of aging on large-scale brain systems for memory encoding and cognitive control. *J Neurosci* 32: 10749–57.
23. Eldridge LL, Engel SA, Zeineh MM, Bookheimer SY, Knowlton BJ (2005): A dissociation of encoding and retrieval processes in the human hippocampus. *J Neurosci* 25: 3280–6.
24. Zeineh MM, Engel SA, Thompson PM, Bookheimer SY (2003): Dynamics of the hippocampus during encoding and retrieval of face-name pairs. *Science* 299: 577–80.
25. Strange BA, Witter MP, Lein ES, Moser EI (2014): Functional organization of the hippocampal longitudinal axis. *Nat Rev Neurosci* 15: 655–669.
26. Thal DR, Rüb U, Orantes M, Braak H (2002): Phases of A beta-deposition in the human brain and its relevance for the development of AD. *Neurology* 58: 1791–800.
27. Small SA, Schobel SA, Buxton RB, Witter MP, Barnes CA (2011): A pathophysiological framework of hippocampal dysfunction in ageing and disease. *Nat Rev Neurosci* 12: 585–601.
28. Burggren AC, Zeineh MM, Ekstrom AD, Braskie MN, Thompson PM, Small GW, Bookheimer SY (2008): Reduced cortical thickness in hippocampal subregions among cognitively normal apolipoprotein E e4 carriers. *Neuroimage* 41: 1177–83.
29. Harris JA, Devidze N, Verret L, Ho K, Halabisky B, Thwin MT, *et al.* (2010): Transsynaptic progression of amyloid-β-induced neuronal dysfunction within the entorhinal-hippocampal network. *Neuron* 68: 428–41.
30. Liu L, Drouet V, Wu JW, Witter MP, Small SA, Clelland C, Duff K (2012): Trans-synaptic spread of tau pathology in vivo. *PLoS One* 7: Public Library of Sciencee31302.
31. Frankó E, Joly O (2013): Evaluating Alzheimer's disease progression using rate of regional hippocampal atrophy. *PLoS One* 8: e71354.
32. Reinvang I, Espeseth T, Westlye LT (2013): APOE-related biomarker profiles in non-pathological aging and early phases of Alzheimer's disease. *Neurosci Biobehav Rev* 37: 1322–35.
33. Bookheimer SY, Strojwas MH, Cohen MS, Saunders AM, Pericak-Vance MA, Mazziotta JC, Small GW (2000): Patterns of brain activation in people at risk for Alzheimer's disease. *N Engl J Med* 343: 450–6.
34. Small GW, Ercoli LM, Silverman DH, Huang SC, Komo S, Bookheimer SY, *et al.* (2000): Cerebral metabolic and cognitive decline in persons at genetic risk for Alzheimer's disease. *Proc Natl Acad Sci U S A* 97: 6037–42.

35. Wechsler D (1997): *Wecshler Adult Intelligence Scale, 3rd Edition*. . San Antonio, TX: Harcourt Assessement.

36. Cauthen NR (1978): Verbal fluency: normative data. *J Clin Psychol* 34: 126–9.

37. Goodglass HP, Kaplan EP (2001): Boston Naming Test, 3rd EditionLippincott Williams & Wilkins.

38. Buschke H, Fuld PA (1974): Evaluating storage, retention, and retrieval in disordered memory and learning. *Neurology* 24: 1019–25.

39. Osterrieth PA (1944): Le test de copie d'une figure complex: Contribution à l'étude de la perception et de la memoir. *Arch Psychol (Geneve)*286–356.

40. Breitner JC, Folstein MF (1984): Familial Alzheimer Dementia: a prevalent disorder with specific clinical features. *Psychol Med* 14: 63–80.

41. Gilewski MJ, Zelinski EM, Schaie KW (1990): The Memory Functioning Questionnaire for assessment of memory complaints in adulthood and old age. *Psychol Aging* 5: 482–90.

42. HAMILTON M (1959): The assessment of anxiety states by rating. *Br J Med Psychol* 32: 50–5.

43. HAMILTON M (1960): A rating scale for depression. *J Neurol Neurosurg Psychiatry* 23: 56–62.

44. Cummings JL, Mega M, Gray K, Rosenberg-Thompson S, Carusi DA, Gornbein J (1994): The Neuropsychiatric Inventory: comprehensive assessment of psychopathology in dementia. *Neurology* 44: 2308–14.

45. Folstein MF, Robins LN, Helzer JE (1983): The Mini-Mental State Examination. *Arch Gen Psychiatry* 40: 812.

46. Burggren A, Brown J (2013): Imaging markers of structural and functional brain changes that precede cognitive symptoms in risk for Alzheimer's disease. *Brain Imaging Behav*. doi: 10.1007/s11682-013-9278-4.

47. Burggren AC, Renner B, Jones M, Donix M, Suthana NA, Martin-Harris L, *et al.* (2011): Thickness in entorhinal and subicular cortex predicts episodic memory decline in mild cognitive impairment. *Int J Alzheimers Dis* 2011: 956053.

48. Donix M, Burggren AC, Scharf M, Marschner K, Suthana NA, Siddarth P, *et al.* (2013): APOE associated hemispheric asymmetry of entorhinal cortical thickness in aging and Alzheimer's disease. *Psychiatry Res* 214: 212–20.

49. Donix M, Burggren AC, Suthana NA, Siddarth P, Ekstrom AD, Krupa AK, *et al.* (2010): Longitudinal changes in medial temporal cortical thickness in normal subjects with the APOE-4 polymorphism. *Neuroimage* 53: 37–43.

50. Brown JA, Terashima KH, Burggren AC, Ercoli LM, Miller KJ, Small GW, Bookheimer SY (2011): Brain network local interconnectivity loss in aging APOE-4 allele carriers. *Proc Natl Acad Sci U S A* 108: 20760–5.

51. Donix M, Burggren AC, Suthana NA, Siddarth P, Ekstrom AD, Krupa AK, *et al.* (2010): Family history of Alzheimer's disease and hippocampal structure in healthy people. *Am J Psychiatry* 167: 1399–406.

52. Persson J, Kalpouzos G, Nilsson L-G, Ryberg M, Nyberg L (2011): Preserved hippocampus activation in normal aging as revealed by fMRI. *Hippocampus* 21: 753–66.

53. Sperling R, Greve D, Dale A, Killiany R, Holmes J, Rosas HD, *et al.* (2002): Functional MRI detection of pharmacologically induced memory impairment. *Proc Natl Acad Sci U S A* 99:

455–60.

54. Suthana NA, Krupa A, Donix M, Burggren A, Ekstrom AD, Jones M, *et al.* (2010): Reduced hippocampal CA2, CA3, and dentate gyrus activity in asymptomatic people at genetic risk for Alzheimer's disease. *Neuroimage* 53: 1077–84.

55. Stark CE, Squire LR (2001): When zero is not zero: the problem of ambiguous baseline conditions in fMRI. *Proc Natl Acad Sci U S A* 98: 12760–6.

56. Patenaude B, Smith SM, Kennedy DN, Jenkinson M (2011): A Bayesian model of shape and appearance for subcortical brain segmentation. *Neuroimage* 56: 907–22.

57. Ryan L, Cox C, Hayes SM, Nadel L (2008): Hippocampal activation during episodic and semantic memory retrieval: comparing category production and category cued recall. *Neuropsychologia* 46: 2109–21.

58. Duvernoy HM (2005): *The Human Hippocampus*. . Berlin/Heidelberg: Springer-Verlag. doi: 10.1007/b138576.

59. Greicius MD, Krasnow B, Boyett-Anderson JM, Eliez S, Schatzberg AF, Reiss AL, Menon V (2003): Regional analysis of hippocampal activation during memory encoding and retrieval: fMRI study. *Hippocampus* 13: 164–74.

60. Duarte IC, Ferreira C, Marques J, Castelo-Branco M (2014): Anterior/posterior competitive deactivation/activation dichotomy in the human hippocampus as revealed by a 3D navigation task. *PLoS One* 9: Public Library of Sciencee86213.

61. Fischl B, Dale AM (2000): Measuring the thickness of the human cerebral cortex from magnetic resonance images. *Proc Natl Acad Sci U S A* 97: 11050–5.

62. Smith SM (2002): Fast robust automated brain extraction. *Hum Brain Mapp* 17: 143–55.

63. Jenkinson M, Bannister P, Brady M, Smith S (2002): Improved optimization for the robust and accurate linear registration and motion correction of brain images. *Neuroimage* 17: 825–41.

64. Woolrich MW, Ripley BD, Brady M, Smith SM (2001): Temporal autocorrelation in univariate linear modeling of FMRI data. *Neuroimage* 14: 1370–86.

65. Greve DN, Fischl B (2009): Accurate and robust brain image alignment using boundary-based registration. *Neuroimage* 48: 63–72.

66. Power JD, Mitra A, Laumann TO, Snyder AZ, Schlaggar BL, Petersen SE (2014): Methods to detect, characterize, and remove motion artifact in resting state fMRI. *Neuroimage* 84: 320–41.

67. Power JD, Barnes KA, Snyder AZ, Schlaggar BL, Petersen SE (2012): Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion. *Neuroimage* 59: 2142–54.

68. Hafkemeijer A, Altmann-Schneider I, Oleksik AM, van de Wiel L, Middelkoop HAM, van Buchem MA, *et al.* (2013): Increased functional connectivity and brain atrophy in elderly with subjective memory complaints. *Brain Connect* 3: 353–62.

69. Fleisher AS, Sherzai A, Taylor C, Langbaum JBS, Chen K, Buxton RB (2009): Resting-state BOLD networks versus task-associated functional MRI for distinguishing Alzheimer's disease risk groups. *Neuroimage* 47: 1678–90.

70. Greicius MD, Krasnow B, Reiss AL, Menon V (2003): Functional connectivity in the resting brain: a network analysis of the default mode hypothesis. *Proc Natl Acad Sci U S A* 100: 253–8.

71. Westlye ET, Lundervold A, Rootwelt H, Lundervold AJ, Westlye LT (2011): Increased hippocampal default mode synchronization during rest in middle-aged and elderly APOE ε4 carriers: relationships with memory performance. *J Neurosci* 31: 7775–83.

72. Salami A, Pudas S, Nyberg L (2014): Elevated hippocampal resting-state connectivity underlies deficient neurocognitive function in aging. *Proc Natl Acad Sci* 111: 201410233.

73. McLaren DG, Sperling RA, Atri A (2014): Flexible modulation of network connectivity related to cognition in Alzheimer's disease. *Neuroimage* 100: 544–57.

74. Ward AM, Schultz AP, Huijbers W, Van Dijk KRA, Hedden T, Sperling RA (2014): The parahippocampal gyrus links the default-mode cortical network with the medial temporal lobe memory system. *Hum Brain Mapp* 35: 1061–73.

75. Raichle ME, MacLeod AM, Snyder AZ, Powers WJ, Gusnard DA, Shulman GL (2001): A default mode of brain function. *Proc Natl Acad Sci U S A* 98: 676–82.

76. Reiman EM, Caselli RJ, Yun LS, Chen K, Bandy D, Minoshima S, *et al.* (1996): Preclinical evidence of Alzheimer's disease in persons homozygous for the epsilon 4 allele for apolipoprotein E. *N Engl J Med* 334: 752–8.

77. Buckner RL, Snyder AZ, Shannon BJ, LaRossa G, Sachs R, Fotenos AF, *et al.* (2005): Molecular, structural, and functional characterization of Alzheimer's disease: evidence for a relationship between default activity, amyloid, and memory. *J Neurosci* 25: 7709–17.

78. Seeley WW, Menon V, Schatzberg AF, Keller J, Glover GH, Kenna H, *et al.* (2007): Dissociable intrinsic connectivity networks for salience processing and executive control. *J Neurosci* 27: 2349–56.

79. Xie C, Bai F, Yu H, Shi Y, Yuan Y, Chen G, *et al.* (2012): Abnormal insula functional network is associated with episodic memory decline in amnestic mild cognitive impairment. *Neuroimage* 63: 320–7.

80. Maddock R., Garrett A., Buonocore M. (2001): Remembering familiar people: the posterior cingulate cortex and autobiographical memory retrieval. *Neuroscience* 104: 667–676.

81. Paulesu E, Frith CD, Frackowiak RS (1993): The neural correlates of the verbal component of working memory. *Nature* 362: 342–5.

82. Rogalski E, Cobia D, Harrison TM, Wieneke C, Thompson CK, Weintraub S, Mesulam M-M (2011): Anatomy of language impairments in primary progressive aphasia. *J Neurosci* 31: 3344–50.

83. Damasio H, Damasio AR (1980): The anatomical basis of conduction aphasia. *Brain* 103: 337–50.

84. Wierenga CE, Stricker NH, McCauley A, Simmons A, Jak AJ, Chang Y-L, *et al.* (2010): Increased functional brain response during word retrieval in cognitively intact older adults at genetic risk for Alzheimer's disease. *Neuroimage* 51: 1222–33.

85. Fleisher AS, Podraza KM, Bangen KJ, Taylor C, Sherzai A, Sidhar K, *et al.* (2009): Cerebral perfusion and oxygenation differences in Alzheimer's disease risk. *Neurobiol Aging* 30: 1737–48.

86. Chhatwal JP, Schultz AP, Johnson K, Benzinger TLS, Jack C, Ances BM, *et al.* (2013): Impaired default network functional connectivity in autosomal dominant Alzheimer disease. *Neurology* 81: 736–44.

CHAPTER 3

APOEε4 Effects on the Intrinsic Architecture of Neural Networks as Early Risk Biomarkers of Alzheimer's Disease

Abstract

Emerging evidence suggests that disruption of intrinsic neural networks associated with genetic risk for Alzheimer's Disease (AD) may be present long before disease onset. Better characterization of such neural signatures of genetic risk for AD throughout the lifespan is critical to identifying predictive biomarkers to guide intervention. In the present study, resting state fMRI data for 570 healthy 18-22 year olds were used to generate intrinsic connectivity maps to examine the impact of genetic risk for AD on neural network architecture in early life, long before the age of possible AD onset. Graph theoretical analyses were employed to further examine network differences. Carriers of the AD risk allele APOEε4 had decreased intrinsic connectivity between multiple nodes within the default mode network. In the sensorimotor network, APOEε4 carriers also had relatively decreased connectivity between primary motor cortex and both presupplemental motor area and anterior insula. Increased connectivity in APOEε4 carriers was only observed between frontoinsular cortex of the salience network and dorsal posterior cingulate. More broadly, APOEε4 carriers had more negative connectivity between task-positive and task-negative networks as well as relatively greater segregation between networks. Importantly, there were no significant differences between APOEε4 carriers and non-carriers in age, years of education, or IQ. These complimentary analyses of intrinsic neural network architecture indicate that decreased connectivity and increased segregation between task-negative and task-positive networks in healthy young adult APOEε4 carriers may represent early brain biomarkers of increased AD risk in later life.

Significance Statement

Studies of early risk biomarkers for Alzheimer's Disease (AD) have been limited by low statistical power to detect small but informative effects. Here we leverage unprecedented statistical power to identify brain biomarkers of genetic risk for AD using neuroimaging and genetic data from 570 young adults. Increased risk conveyed by the APOEε4 allele was associated with decreased connectivity and increased segregation between task-positive and task-negative neural networks. The detection of these brain biomarkers early in life (mean age = 19.7 years) suggest that characterizing patterns of intrinsic neural network architecture in longitudinal research across the lifespan is crucial to tracking gene-biomarker associations and identifying changes in these associations that might be signs of imminent clinical decline.

Introduction

As the strongest genetic risk factor for Alzheimer's Disease (AD), apolipoprotein E (*APOE*) has been studied more than any genomic locus in both elderly and younger cohorts. In studies of cognitively healthy elderly cohorts, differences that are associated with the *APOE* risk allele ε4 (APOEε4) are often interpreted as preclinical changes that may indicate incipient AD. Importantly, differences in brain structure and function associated with APOEε4 have also been uncovered in younger people, from infants to middle-aged adults, well before the possible emergence of clinical symptoms(1–3). It is not known, however, if *APOE*-mediated differences in young adults and children are compensatory, beneficial (e.g., supporting a theory of antagonistic pleiotropy(4)) or behaviorally neutral susceptibilities to later disease. Thus, elucidating the effects of genetic risk factors like APOEε4 throughout the lifespan is necessary for interpreting imaging correlates of genetic risk for AD in older adults and for vetting of potential risk biomarkers.

There is increasing interest in utilizing resting state fMRI to capture changes in the intrinsic architecture of neural networks (i.e, connectivity) that may be an early biomarker of dysfunction in preclinical AD(5; 6). Intrinsic, resting state neural networks, including the default mode network (DMN) and the salience network, are comprised of specific regions where spontaneous low-frequency fluctuations in the blood-oxygen-level-dependent (BOLD) signal are highly correlated. Generally, the DMN is relatively more active when an individual is at rest, and less active while an individual is engaged in a task(7). For this reason, the DMN is sometimes referred to as the task-negative network. In contrast, task-positive networks are more active in response to specific task stimuli and demands than at rest. The salience network, for example, is active in response to emotionally and behaviorally relevant stimuli(8).

DMN function is disrupted in AD and in individuals with mild cognitive impairment(9). It has been hypothesized that there may be a relationship between AD and the DMN because the constituent regions including the posterior cingulate cortex, precuneus, lateral parietal cortex

and medial prefrontal cortex are selectively vulnerable to AD, showing early hypometabolism and cortical thinning as well as functional disruption(10–13). Areas defined as functional “hubs” overlap with the DMN and are particularly vulnerable to these changes(14). Studies examining intrinsic neural network architecture in healthy, older APOEε4 carriers have found decreased connectivity within nodes of the DMN in comparison with non-carriers(15; 16). In young people, several studies have also reported differences in DMN connectivity based on *APOE* genotype, but these studies are contradictory possibly because they were underpowered to reliably detect the likely small effects of genetic risk(17–19).

In the present study, we aimed to expand this literature in two ways: first, by examining not only DMN but also other robust and well-defined task-positive neural networks, including the salience and sensorimotor networks, and second, by increasing the participant sample size by an order of magnitude to afford necessary statistical power to detect reliable effects. Because large data collection efforts in AD have focused on older adults, published studies on younger individuals have relatively small sample sizes. In contrast, we utilized a large dataset of 18-22 year old university students for whom resting state fMRI and *APOE* data were available (n=570). Thus, our current study is the largest to-date focused on genetic risk for AD and intrinsic neural network architecture in healthy young adults. We hypothesized, based on the most compelling previous research in younger adults, that APOEε4 carriers would show relatively increased connectivity within the DMN(17). We also hypothesized APOEε4 carrier connectivity in the salience network would be relatively weaker, as the salience network and DMN are opposing networks and there is some evidence that alterations in one network may impact the connectivity of other, related networks(9). We expected that there would be no *APOE*-related connectivity differences in the sensorimotor network, due to the relatively lower order cognitive functions associated with this network. Lastly, we conducted graph theoretical analyses to further examine network differences as a function of *APOE* genotype.

Materials and Methods

Participants

Data were available for 632 participants recruited as part of the ongoing Duke Neurogenetics Study (DNS), between January 13, 2012 and July 10, 2014. Each participant completed written informed consent in compliance with the Duke University Institutional Review Board protocols before their participation. A standardized neuropsychological battery was administered to each participant. This battery included measures of intelligence (Wechsler Abbreviated Scale of Intelligence Full-Scale IQ [WASI FSIQ](20)), learning and memory (California Verbal Learning Test [CVLT](21)), processing speed (Trails B(22)) and working memory (digit span(23)).

Exclusion criteria for the DNS include: 1) medical diagnoses of cancer, stroke, head injury with loss of consciousness, untreated migraine headaches, diabetes requiring management with insulin, chronic liver or kidney disease or a history of psychosis; 2) use of psychotropic, glucocorticoid or hypolipidemic medication; 3) hypertension or other conditions affecting cerebral blood flow. Exclusion criteria did not include a past or present Axis I or select Axis II (borderline and antisocial personality) disorder as defined by the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM IV)(24) because the DNS aims to include a broad range of cognitive and psychological variability. Incidence of Axis I disorders did not differ between APOEε4 carriers and non-carriers ($p=0.729$). In the whole group, 128 individuals had either a past or current history of at least one DSM-IV diagnosis, including 75 with alcohol use disorders, 21 with non-alcohol substance use disorders, 32 with major depressive disorders, 25 with bipolar disorders, 8 with panic disorder (no agoraphobia), 9 with panic disorder including agoraphobia, 6 with social anxiety disorder, 7 with generalized anxiety disorder, 9 with obsessive compulsive disorder, 6 with eating disorders, and 0 with post traumatic stress disorder. Participants were asked to report a history of AD in first-degree

relatives. Unfortunately, this did not include grandparents and is therefore not a useful measure in a cohort aged 18-22 years old.

All comparisons between groups on demographic and cognitive factors were completed using tools from the R Project for Statistical Computing (http://www.r-project.org)

Genotyping

The DNS works in collaboration with 23andMe, Inc. (Mountain View, CA) in order to genotype participants at single nucleotide polymorphisms (SNPs) across the genome. Genomic DNA from each participant was isolated from buccal cells derived from Oragene DNA self-collection kits (DNA Genotek, Inc., Kanata, Canada) that were further customized for 23andMe. DNA extraction and subsequent genotyping were performed at the National Genetics Institute, a CLIA-certified clinical laboratory.

Optimal genomic coverage is obtained by using Illumina microarray chips along with additional custom SNPs(25; 26). Each participant was genotyped at *APOE* rs429358 and rs7412 and the results were extracted from the master database using PLINK(27). Participants' two *APOE* alleles were then determined (T/T=ε2, T/C=ε3, C/C=ε4).

Imaging

Participants were scanned on one of two identical research-dedicated GE MR750 3T scanners at the Duke-UNC Brain Imaging and Analysis Center. Before scan acquisition a semi-automated high-order shimming procedure was used to maximize global field inhomogeneity. For each participant, 2 back-to-back 4-minute 16-second resting state functional MRI scans were acquired in 34 interleaved axial slices with the following parameters: repetition time (TR)=2000ms, echo time (TE)=30ms, flip angle=60°, field of view (FOV)=240 mm, voxel size=3.75×3.75×4mm, interslice gap=0. For spatial registration of the functional resting state scans to a common coordinate space, 3-dimensional structural MRI scans were acquired in 34

axial slices co-planar with the functional scans: TR=7.7s, TE=3.0ms, flip angle=12°, voxel size=0.9x0.9x4mm, FOV=240mm, interslice gap=0.

Participants were instructed to remain awake, with their eyes open during each resting state scan. They were shown a blank gray screen and asked to think about nothing in particular. Variability in single-subject whole-brain functional volumes was determined using the Artifact Recognition Toolbox (http://www.nitrc.org/projects/artifact_detect). Individual whole-brain BOLD fMRI volumes meeting at least one of two criteria were flagged: 1) significant mean-volume signal intensity variation (i.e., within volume mean signal greater or less than 4 standard deviations of mean signal of all volumes in time series), and 2) individual volumes where scan-to-scan movement exceeded 2mm translation or 2° rotation in any direction. Participants with 5% or more flagged volumes were excluded from analysis. Standard resting state fMRI spatial preprocessing steps were completed in SPM8 (www.fil.ion.ucl.ac.uk/spm).

Intrinsic Network Connectivity: Seed-Based Analyses

Seed-based correlation maps of the DMN, salience, and sensorimotor networks were generated for each participant using the Conn toolbox(28). Individual head motion realignment parameters were included as confound regressors to remove the effects of residual head motion. Signal from three principal noise components associated with white matter and cerebrospinal fluid and one component associated with grey matter were also included. The seeds were 5mm radius spheres centered around a coordinate in posterior cingulate cortex (PCC) [0 -53 26] for DMN, right frontoinsular cortex [30 20 -12] for salience and right primary motor cortex [36 -25 57] for sensorimotor network (adapted from (29–31)). Mean timeseries were extracted from each seed and used to create whole brain correlation maps for each participant corresponding to the three anatomical seeds.

Intrinsic Network Connectivity: Functional Node Set and Graph Theoretical Analyses

Next, we conducted independent analyses using a previously published functional node set to define networks with 5mm radius spheres sampled across key regions(32). We focused on nodes included in cognitive networks, eliminating any primary sensory resting state networks. These included nodes composing the task-negative DMN and task-positive salience, cingulo-opercular task control (COT), fronto-parietal task control (FPT), ventral attention (vAtt), and dorsal attention (dAtt) networks. After identifying this subset of 135 nodes, we calculated mean intra- and inter-network connectivity values for task-positive and task-negative regions. For example, intra-network mean connectivity for the DMN is the average of the Pearson correlation coefficients for each pair of nodes within the network. Inter-network mean connectivity is the average of the Pearson correlation coefficients between each pair of nodes across two sets of nodes or networks.

Graph theory is a branch of mathematics that describes the properties of networks. We used specific graph theory metrics to compare global network properties between APOEε4 carriers and non-carriers. First, a full Pearson correlation coefficient was calculated pairwise across our 135 nodes and stored in a unique connectivity matrix for each participant. The top 30% strongest connections within each matrix were used to calculate graph theory metrics (33; 34). Measures of clustering, global efficiency and modularity were calculated(35). We examined measures of clustering, global efficiency and modularity specifically because these metrics could be interpreted as either supporting or rejecting our hypothesis that the strength of task-negative and task-positive opposition differed between APOEε4 carriers and non-carriers. Briefly, clustering coefficient indicates how densely interconnected neighboring regions are. Global efficiency is related to how well a given network supports the fast transmission of information(36). Finally, modularity is the degree to which a network can be delineated into non-overlapping groups of regions by maximizing within module connections and minimizing

between module connections(37). Greater modularity indicates that modules are more segregated from one another within the greater network.

Results

Participants

For the current study, primary analyses were conducted on data from 570 participants (318F, mean age=19.7 years +/- 1.22). Before primary analyses, data from twenty-three participants were excluded due to excessive motion artifact in their resting state functional imaging data, and six were excluded due to scanner malfunction and/or other artifact. Data from thirty-two additional participants were excluded due to low confidence in genotyping one or both of the single nucleotide polymorphism sites within *APOE* (rs429358 and rs7412) required to determine allele type (ε2, ε3 or ε4). For the remaining participants, there was a 25.2% rate of carriage of at least one APOEε4 allele, which is consistent with the expected population frequency of this AD risk allele (26.5%) (Table 3.1)(38). *APOE* met Hardy Weinberg equilibrium criteria in our sample with a non-significant difference between expected and observed genotypes (p=0.142). The frequency of APOEε4 differed across ancestry groups (Table 3.2). According to an F-test controlling for multiple comparisons, reported effects did not differ across ancestry groups so we present our results for the full sample (Table 3.3).

There were no differences between APOEε4 carrier and non-carrier groups in age, sex, education, incidence of DSM-IV disorder, full-scale IQ or cognitive performance on tests of verbal and working memory as well as processing speed (Table 3.4).

Table 3.1: *APOE* Genotype Frequencies

***APOE* Allelic Distribution**						
Genotype	ε2/ε2	ε2/ε3	ε2/ε4	ε3/ε3	ε3/ε4	ε4/ε4
No. Participants	1	54	14	372	114	15
Percent (%) of total	0.2	9.5	2.5	65.3	20.0	2.6

Table 3.2: Frequency of APOEε4 Within Self-Reported Ancestry Groups

Ancestry	No. Carriers / Total	% Carriers
White	76 / 257	29.6%
Black	25 / 63	39.7%
Asian	25 / 153	16.3%
Latino/a	5 / 35	14.2%
Multi/Other	12 / 62	20.0%

Table 3.3: Descriptive Statistics of Experimental Values Within Self-Reported Ancestry Groups. DMN = default mode network

		White (n=257)	Black (n=63)	Asian (n=153)	Latino/a (n=35)	Multi/Other (n=62)
DMN – DMN	**Mean**	0.159	0.149	0.146	0.142	0.148
	St. Dev	0.043	0.048	0.041	0.040	0.046
DMN – Task Positive	**Mean**	-0.061	-0.056	-0.045	-0.055	-0.051
	St. Dev	0.032	0.034	0.034	0.028	0.036
DMN – Task Control	**Mean**	-0.032	-0.031	-0.019	-0.031	-0.022
	St. Dev	0.039	0.040	0.039	0.039	0.042
Clustering Coefficient	**Mean**	0.200	0.193	0.195	0.199	0.193
	St. Dev	0.026	0.030	0.023	0.024	0.024
Modularity	**Mean**	0.479	0.477	0.459	0.475	0.465
	St. Dev	0.053	0.059	0.053	0.054	0.055

Table 3.4: Cohort Characteristics. Carriers and non-carriers of APOEε4 do not differ in age, sex, education, DSM Axis I disorders, IQ or cognition. DSM-IV = Diagnostic and Statistical Manual of Mental Disorders, 4th Edition; WASI FSIQ = Wechsler Abbreviated Scale of Intelligence Full-Scale Intelligence Quotient; CVLT = California Verbal Learning Test

Characteristic/Measure	Carriers (n=143)	Non-Carriers (n=427)	*p*-value
Age (yrs)	19.72 [1.19]	19.73 [1.24]	0.990
Sex (M/F)	63 / 80	189 / 238	0.923
Education (yrs)	13.51 [1.19]	13.55 [1.17]	0.732
DSM-IV Axis I Disorder (yes/no)	34 / 109	94 / 333	0.729
WASI FSIQ	122.02 [8.91]	121.03 [8.58]	0.240
CVLT Long Delay (0-16)	12.44 [2.61]	12.43 [2.49]	0.971
Digit Span Total (0-48)	32.32 [4.35]	32.38 [5.22]	0.897
Trails B Time (sec)	47.54 [14.45]	46.19 [13.75]	0.313

Intrinsic Network Connectivity: Seed-Based Analyses

We first used a seed-based functional connectivity approach to define three networks of interest: DMN, salience network and sensorimotor network. Seed-based correlation maps produced robust DMN, salience, and sensorimotor networks across participants (Figure 3.1). Comparison of the seed-based DMN maps revealed that APOEε4 carriers had relatively reduced intrinsic connectivity between posterior cingulate cortex (PCC) and bilateral medial temporal lobe (MTL) (cluster $p<0.05$, Monte Carlo corrected for each group comparison; Figure 3.2). Sensorimotor network maps showed carriers had relatively decreased connectivity between primary motor cortex and both pre-supplemental motor area and anterior insula. Salience network maps revealed that carriers had decreased connectivity between frontoinsular cortex and sensorimotor regions. Across all the seed-based analyses, relatively greater intrinsic connectivity in carriers was only observed in the salience network between frontoinsular cortex and dorsal PCC. Cluster peaks for all regions exhibiting differences are reported in Table 3.5.

The center of mass for each significant cluster was used to plot spherical nodes alongside seed regions to summarize the seed-based correlation map findings (Figure 3.3).

Table 3.5: Cluster Peak Coordinates from Seed-Based Correlation Map Analyses. MTL = medial temporal lobe; PCC = posterior cingulate cortex; SMA = supplementary motor area

Network	Contrast	Cluster Coordinates (Peaks)			Region
		x	y	z	
DMN	NC > C	-10	-10	-28	Left MTL
		36	-14	-22	Right MTL
Salience	NC > C	40	-18	54	Right Postcentral Gyrus
	C > NC	16	-44	26	Bilateral PCC
Sensorimotor	NC > C	8	12	58	Right Pre. SMA
		36	16	-10	Right Anterior Insula

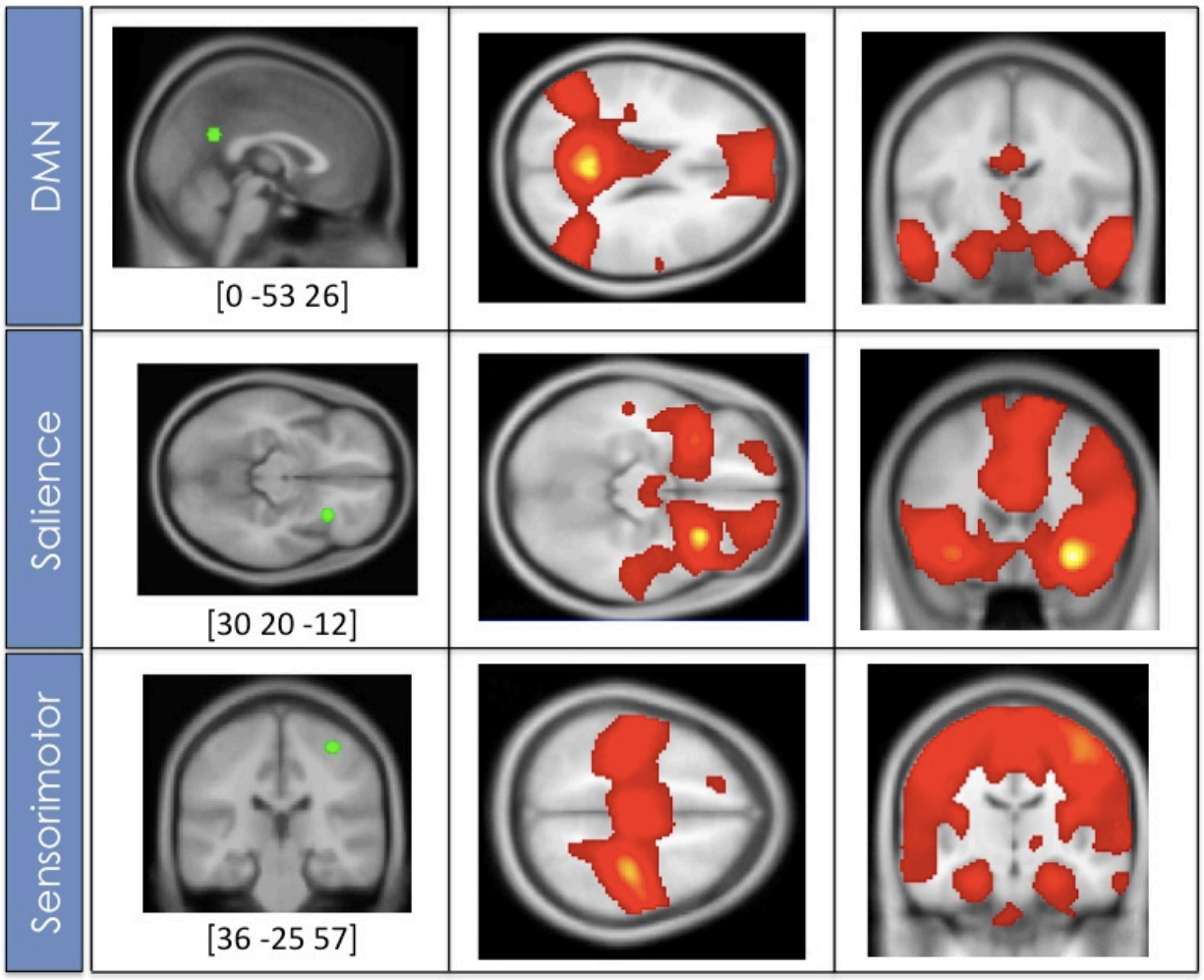


Figure 3.1: Seed-based resting state networks. Seed regions used to produce intrinsic functional connectivity maps are showed in the leftmost column (green). Axial and coronal views of the resulting default mode (DMN), salience and sensorimotor networks for the whole group (n=570) are provided. These maps were corrected for multiple comparisons at FDR p=0.05.

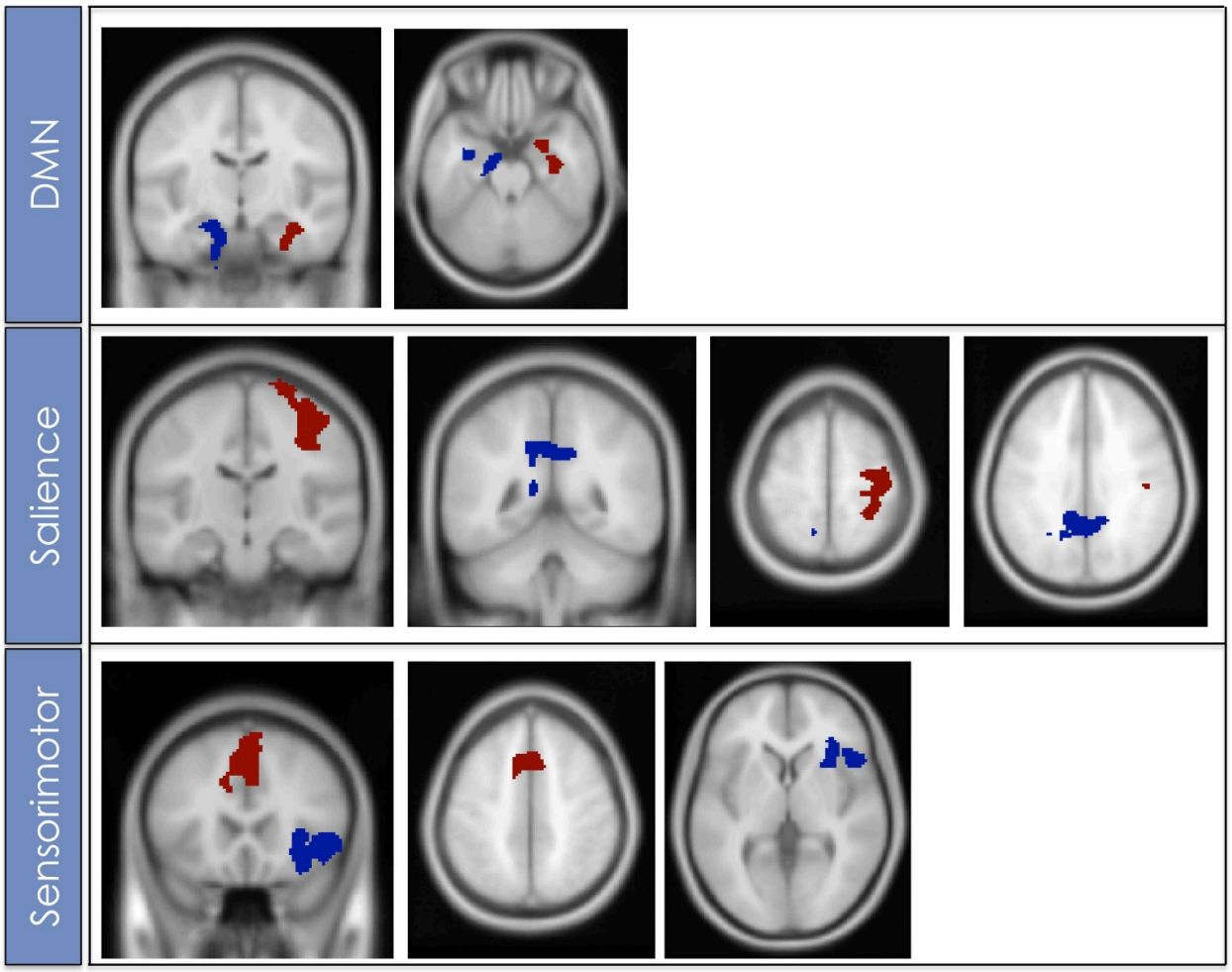


Figure 3.2: Significant functional connectivity differences between APOEε4 carriers and non-carriers in seed-based analyses. For each network, there were two significant clusters (one shown in red, one shown in blue). Connectivity is relative to the seed region and summarized in Table 3.5. The center of mass of each of these clusters is plotted in Figure 3.3.

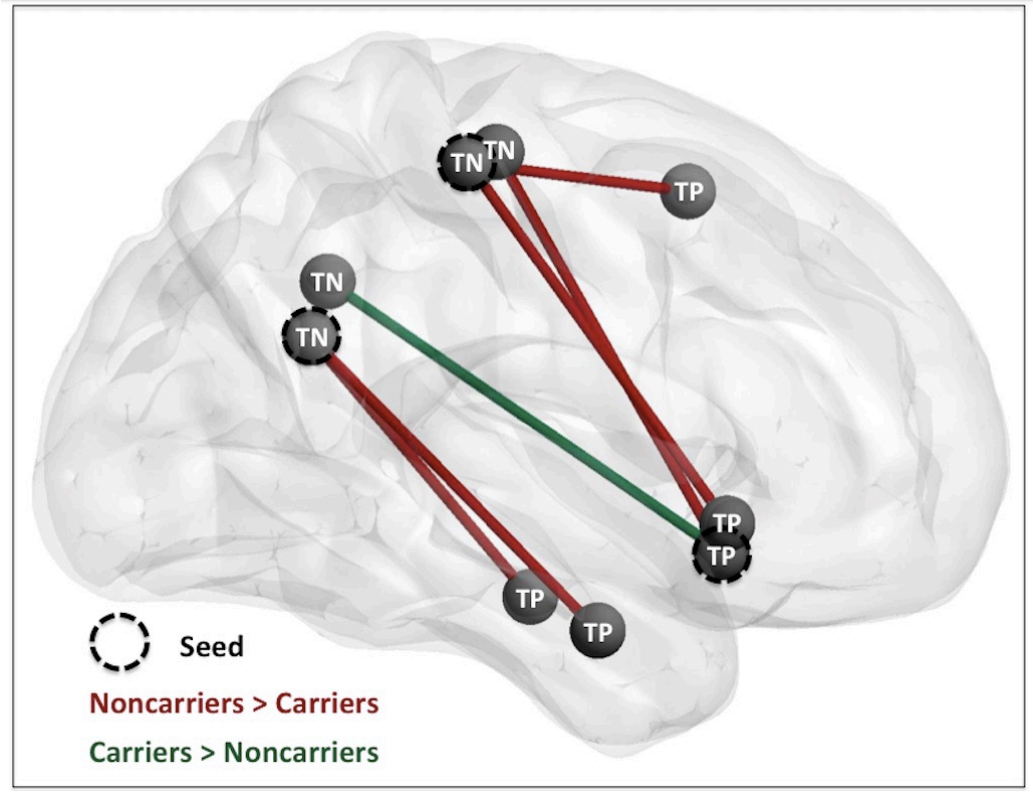


Figure 3.3: Summary model of results comparing functional connectivity of APOEε4 carriers and non-carriers in seed-based correlation map analyses. Significant group differences revealed lower functional connectivity in APOEε4 carriers compared to carriers (dark red) except in the case of one connection between frontoinsular cortex and PCC (dark green). Seeds used in the seed-based correlation maps analyses are demarked by black, dashed circles. The non-seed spheres were plotted based on the center of mass of the significant cluster. In general, carriers had relatively lower connectivity between task negative (TN) and task positive (TP) regions.

Intrinsic Network Connectivity: Functional Node Set and Graph Theoretical Analyses

To be certain that our findings were not biased by the seed-based correlation map method, we employed a second network analysis approach to corroborate our findings. Using a previously published functional node set, we selected 135 nodes sampled over the regions comprising DMN, salience network, dorsal and ventral attention (dAtt, vAtt) networks, cingulo-opercular task control (COT) network and frontoparietal task control (FPT) network (32). Spatial overlap was high between our seed-based resting state networks and the corresponding functional node set (Figure 3.4). Analyses of connectivity values within and between task-positive and task-negative networks revealed no differences in mean intra-network connectivity

of the task-negative DMN between APOEε4 carriers and non-carriers (Figure 3.5A). In contrast, there was a significant difference in mean connectivity between task-positive networks and task-negative DMN (Figure 3.5B). Specifically, carriers exhibited significantly greater segregation (connectivity was more negative) between task-positive networks (including salience, dAtt, vAtt, COT and FPT) and the task-negative DMN compared to non-carriers (p=0.005, Cohen's d=0.24). Further, there was significantly greater segregation between task control regions (COT and FPT) and the DMN in carriers (p=0.012, Cohen's d=0.21).

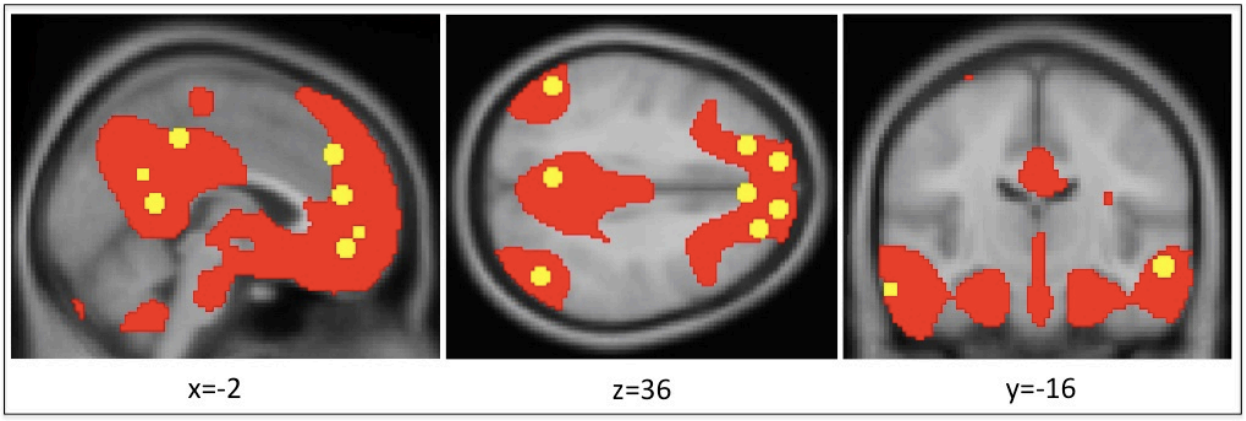


Figure 3.4: Demonstration of high overlap between DMN-assigned nodes from Power and colleagues (2011) and DMN from seed-based approach. 57 of 58 total functional nodes assigned to the DMN (yellow) overlapped with our seed-based DMN (red). Thus, the spatial overlap of DMN in the functional node set with our seed-based DMN was 98%.

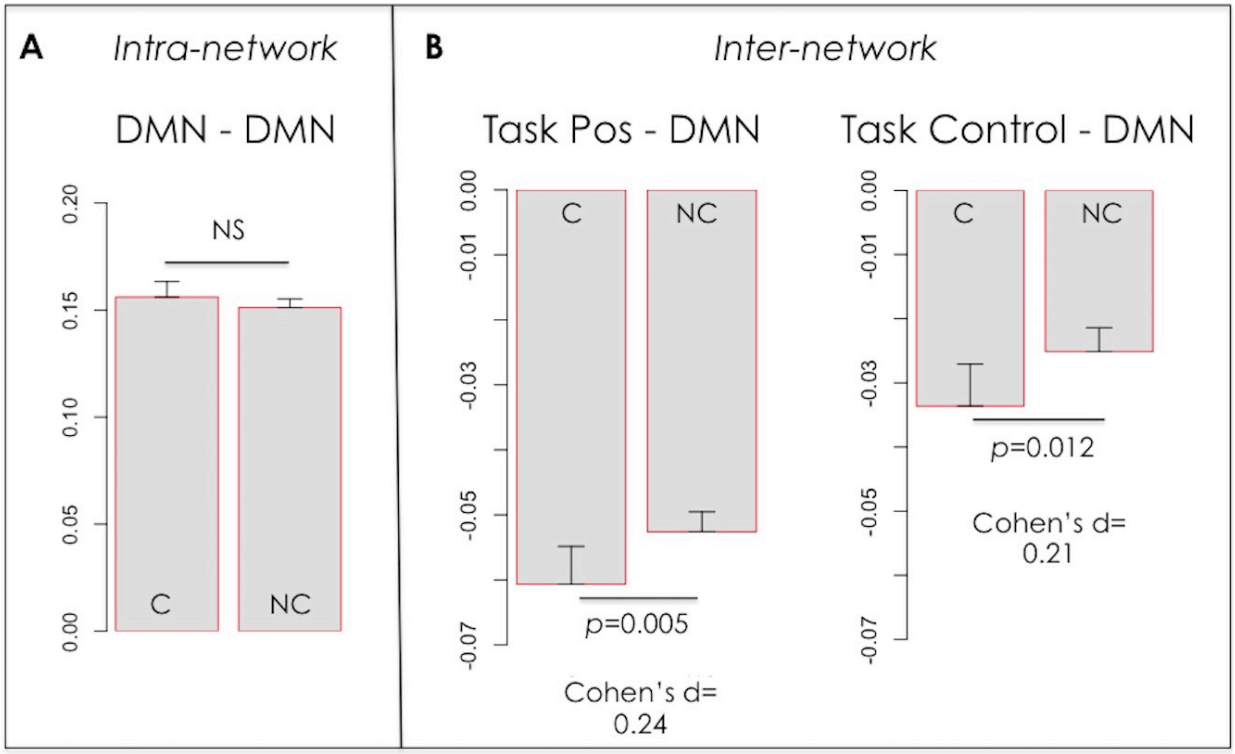


Figure 3.5: Comparison of APOEε4 carriers (C) and non-carriers (NC) mean inter-network connectivity measures shows that carriers have lower connectivity between the task-negative DMN and task-positive regions. A) No differences between groups were observed in intra-network connectivity in the DMN. B) Carriers had significantly more negative mean connectivity between task-positive (and a subset of task-control networks) and task-negative DMN. Error bars represent the 95% confidence interval.

Graph theory is a branch of mathematics that describes of the properties of networks. We selected specific graph theory metrics to compare global network properties between APOEε4 carriers and non-carriers. First, the full Pearson correlation coefficient was calculated pairwise across our 135 nodes and stored in a unique connectivity matrix for each participant. The top 30% strongest connections within each matrix were used to calculate graph theory metrics (33; 34). We examined measures of clustering, global efficiency and modularity because we hypothesized these metrics could be interpreted as either supporting or rejecting our hypothesis that the strength of task-negative and task-positive opposition differed between APOEε4 carriers and non-carriers. Graph theory analyses revealed that APOEε4 carriers had a relatively higher mean clustering coefficient (p=0.043) and higher modularity (p=0.026) which

supports the preceding findings that, overall, APOEε4 carriers have more segregated networks (Figure 3.6). There were no differences between APOEε4 carriers and non-carriers in global efficiency (p=0.12). Spring plots of modules revealed typical organization of modules in both APOEε4 carriers and non-carriers with the task-negative DMN segregated from task-positive networks (Figure 3.7).

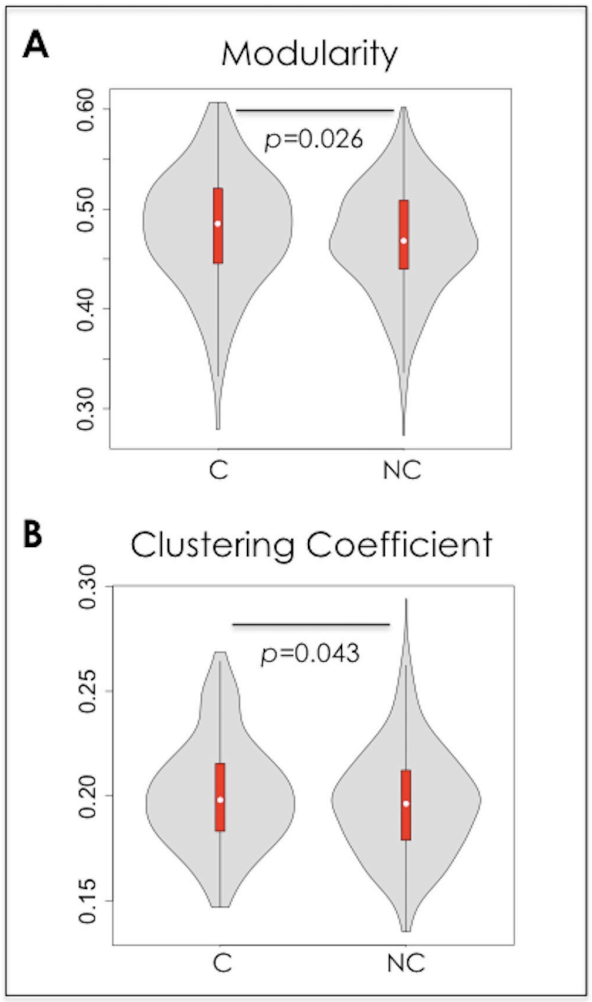


Figure 3.6: APOEε4 carriers (C) have significantly higher clustering coefficient and modularity compared to non-carriers (NC). A) Modularity was higher in carriers of the APOEε4 allele compared to non-carriers. B) Mean clustering coefficient across all 135 functional nodes was significantly higher in APOEε4 carriers. Violin plots show data probability density on either side of box plots where the white dot within the box (red) represents the median while the upper and lower edges of the box represent the first and third quartiles, respectively. The whiskers extend up to 1.5 times the interquartile range.

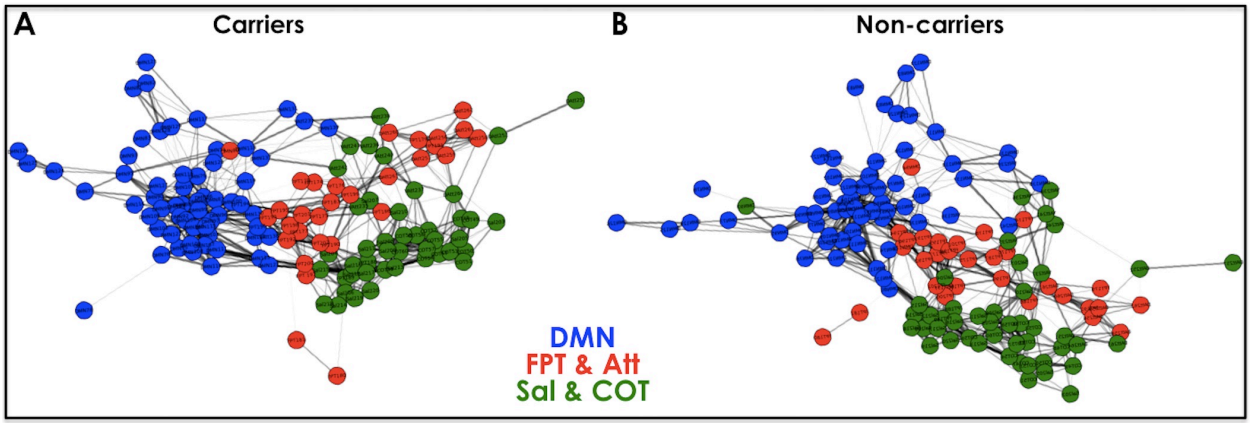


Figure 3.7: APOEε4 carriers and non-carriers do not differ in module assignments. As expected, spring plots based on modularity and colored by module assignment show DMN (blue) clustering separately from task-positive networks in both APOEε4 carriers and non-carriers. This conformation is typical in healthy brains.

Discussion

Our results provide critical evidence that the intrinsic architecture of neural networks is affected by APOEε4 carrier status even in young healthy adults. Specifically, we found across complimentary analysis methods that connectivity between task-negative and task-positive networks is relatively decreased in carriers of the APOEε4 allele. The two-pronged approach to defining networks and the concordance of findings across these methods bolsters our conclusion that the APOEε4 risk allele affects the strength of the opposition between task-positive and task-negative intrinsic neural networks. Broadly, our findings indicate that differences based on *APOE* genotype are not always related to AD, but may reflect lifelong differences in brain organization and associated function.

Our initial hypotheses predicting unique connectivity phenotypes in DMN, salience network and sensorimotor network for carriers of APOEε4 were disproven. Instead, our findings indicate a more global phenotype related to the connectivity of opposing task-negative and task-positive regions, regardless of specific network association. Additional analyses using a previously published node set support the idea that it is not a solely specific task-positive

network like salience network, but task-positive networks in general that show altered connectivity to task-negative DMN in APOEε4 carriers. The ability to detect this global effect may be due to the large sample in this study, close to 600 young adults, which is uniquely powered to investigate possible brain biomarkers of early genetic risk for AD.

Previous studies have reported differences in DMN connectivity in young adults at increased genetic risk for AD(17; 18; 39). Each of these studies had under 100 participants and focused on the DMN, reporting either relative hyper- or hypo-connectivity in APOEε4 carriers. Only one study has looked beyond the DMN to other intrinsic networks, reporting differences between APOEε4 carriers and non-carriers in a hippocampal network, auditory network and left fronto-parietal network, as defined by group independent component analysis (ICA)(18). In contrast to the data-driven ICA approach, we chose to limit our analyses to previously defined networks that can be reliably compared across different studies. This will make our procedure easier to implement with new datasets and will, therefore, facilitate necessary replication efforts.

Our findings are in line with previous work in older, cognitively intact adults. For example, a study that examined the effect of family history of AD on intrinsic connectivity in healthy older adults found significantly decreased connectivity between the PCC and the medial temporal lobe, key nodes within the DMN(40). In another study of healthy adults aged 30-72 years old, female APOEε4 carriers had lower intrinsic connectivity between hippocampus and PCC(41). Finally, a third study used a seed-based approach to examine connectivity of the DMN and found relatively decreased PCC-medial temporal lobe connectivity in APOEε4 carriers(16). Our large sample size gives us increased power to detect these subtle genetic risk effects on DMN intrinsic connectivity even in healthy young adult university students, and suggests that a weakened connection between the medial temporal lobes and PCC represents a lifelong susceptibility for cognitive decline and development of later AD.

Functional connectivity differences between groups detected using seed-based correlation can only be interpreted in reference to the seed region. To confirm that our findings

were not biased by the choice of our seeds, we used a previously published set of neural networks, which are applicable to any dataset, to examine mean inter-network connectivity(32). APOEε4 carriers showed decreased mean intrinsic connectivity between task-negative and task-positive networks. The effect size of this difference falls between a small (d=0.10) and a medium (d=0.30) effect with Cohen's d=0.24. While not specific to a particular functional connection, this finding supports the general pattern of decreased connectivity between task-negative and task-positive network nodes observed in the seed-based analyses. Spring plots that show module structure within the larger set of networks illustrate how closely APOEε4 carriers and non-carriers resemble each other in their healthy, typical neural architecture (Figure 3.7). However, subtle differences in clustering coefficient and modularity exist and support our findings indicating APOEε4 carriers have relatively greater segregation between opposing intrinsic connectivity networks. Previous studies have shown that segregation of networks decreases across the lifespan and that greater segregation is associated with higher IQ in adolescence(42; 43). While there was no significant IQ differences by *APOE* genotype in our cohort, future research will aim to uncover the consequences of relative changes in network segregation in general, as well as in the context of specific *APOE* genotypes.

It is important to note that associations between genetic loci and neuroimaging phenotypes can be difficult to interpret. In the context of AD research, there is a temptation to attribute differences between high and low genetic risk groups to the pathophysiological processes in AD. In healthy older adults, differences between high and low genetic risk groups are often discussed as possible preclinical, AD-related changes. Another interpretation of differences mediated by genetic risk for AD is that the increased genetic risk necessitates compensatory mechanisms to support normal, healthy behavior. If the cohort is younger, it might be argued that group differences are evidence of antagonistic pleiotropy, or the theory that a genetic variant that confers risk in late life may be advantageous earlier in life(4). The association also could be driven by neutral neurodevelopmental differences mediated by the

risk gene. In the present study, with participants ranging in age from 18-22, we are able to rule out preclinical, AD-related changes as underlying our results. Compensation is also very unlikely given the age of these participants and the absence of a clear behavioral or network-efficiency advantage in APOEε4 carriers. After that, however, we are not able to easily rule out antagonistic pleiotropy, neutral neurodevelopmental differences or a lifelong susceptibility to disease. Nevertheless, our current findings do suggest that an emphasis on assessing these patterns of intrinsic neural network architecture in future longitudinal research may be key to tracking gene-biomarker associations over time and pinpointing changes in these associations that might be signs of imminent clinical decline.

Chapter 3 References

1. Green AE, Gray JR, Deyoung CG, Mhyre TR, Padilla R, Dibattista AM, William Rebeck G (2014): A combined effect of two Alzheimer's risk genes on medial temporal activity during executive attention in young adults. *Neuropsychologia* 56: 1–8.
2. Dean DC, Jerskey BA, Chen K, Protas H, Thiyyagura P, Roontiva A, *et al.* (2014): Brain differences in infants at differential genetic risk for late-onset Alzheimer disease: a cross-sectional imaging study. *JAMA Neurol* 71: 11–22.
3. Patel KT, Stevens MC, Pearlson GD, Winkler AM, Hawkins KA, Skudlarski P, Bauer LO (2013): Default mode network activity and white matter integrity in healthy middle-aged ApoE4 carriers. *Brain Imaging Behav* 7: 60–7.
4. Tuminello ER, Han SD (2011): The apolipoprotein e antagonistic pleiotropy hypothesis: review and recommendations. *Int J Alzheimers Dis* 2011: 726197.
5. Sperling R (2011): Potential of functional MRI as a biomarker in early Alzheimer's disease. *Neurobiol Aging* 32 Suppl 1: S37–43.
6. Sheline YI, Raichle ME (2013): Resting state functional connectivity in preclinical Alzheimer's disease. *Biol Psychiatry* 74: 340–7.
7. Fox MD, Snyder AZ, Vincent JL, Corbetta M, Van Essen DC, Raichle ME (2005): The human brain is intrinsically organized into dynamic, anticorrelated functional networks. *Proc Natl Acad Sci U S A* 102: 9673–8.
8. Seeley WW, Menon V, Schatzberg AF, Keller J, Glover GH, Kenna H, *et al.* (2007): Dissociable intrinsic connectivity networks for salience processing and executive control. *J Neurosci* 27: 2349–56.
9. Jones DT, Knopman DS, Gunter JL, Graff-Radford J, Vemuri P, Boeve BF, *et al.* (2015): Cascading network failure across the Alzheimer's disease spectrum. *Brain* 139: 547–62.
10. Del Sole A, Clerici F, Chiti A, Lecchi M, Mariani C, Maggiore L, *et al.* (2008): Individual cerebral metabolic deficits in Alzheimer's disease and amnestic mild cognitive impairment: an FDG PET study. *Eur J Nucl Med Mol Imaging* 35: 1357–66.
11. Greicius MD, Srivastava G, Reiss AL, Menon V (2004): Default-mode network activity distinguishes Alzheimer's disease from healthy aging: evidence from functional MRI. *Proc Natl Acad Sci U S A* 101: 4637–42.
12. Dickerson BC, Bakkour A, Salat DH, Feczko E, Pacheco J, Greve DN, *et al.* (2009): The cortical signature of Alzheimer's disease: regionally specific cortical thinning relates to symptom severity in very mild to mild AD dementia and is detectable in asymptomatic amyloid-positive individuals. *Cereb Cortex* 19: 497–510.
13. Buckner RL, Snyder AZ, Shannon BJ, LaRossa G, Sachs R, Fotenos AF, *et al.* (2005): Molecular, structural, and functional characterization of Alzheimer's disease: evidence for a relationship between default activity, amyloid, and memory. *J Neurosci* 25: 7709–17.
14. Buckner RL, Sepulcre J, Talukdar T, Krienen FM, Liu H, Hedden T, *et al.* (2009): Cortical hubs revealed by intrinsic functional connectivity: mapping, assessment of stability, and relation to Alzheimer's disease. *J Neurosci* 29: 1860–73.
15. Sheline YI, Morris JC, Snyder AZ, Price JL, Yan Z, D'Angelo G, *et al.* (2010): APOE4 allele disrupts resting state fMRI connectivity in the absence of amyloid plaques or decreased CSF Aβ42. *J Neurosci* 30: 17035–40.

16. Machulda MM, Jones DT, Vemuri P, McDade E, Avula R, Przybelski S, *et al.* (2011): Effect of APOE ε4 status on intrinsic network connectivity in cognitively normal elderly subjects. *Arch Neurol* 68: 1131–6.

17. Filippini N, MacIntosh BJ, Hough MG, Goodwin GM, Frisoni GB, Smith SM, *et al.* (2009): Distinct patterns of brain activity in young carriers of the APOE-epsilon4 allele. *Proc Natl Acad Sci U S A* 106: 7209–14.

18. Trachtenberg AJ, Filippini N, Ebmeier KP, Smith SM, Karpe F, Mackay CE (2012): The effects of APOE on the functional architecture of the resting brain. *Neuroimage* 59: 565–72.

19. Trachtenberg AJ, Filippini N, Mackay CE (2012): The effects of APOE-ε4 on the BOLD response. *Neurobiol Aging* 33: 323–34.

20. Wechsler D (1999): *Wechsler Abbreviated Scale of Intelligence*. . San Antonio, TX: The Psychological Corporation.

21. Delis DC, Kramer JH, Kaplan E, Ober BA (2000): California Verbal Learning Test. 2nd ed.San Antonio, TX: The Psychological Corporation.

22. Reitan RM, Wolfson D (1985): The Halstead-Reitan Neuropsychological Test Battery: Therapy and Clinical InterpretationTucson, AZ: Neuropsychological Press.

23. Wechsler D (1997): *Wecshler Adult Intelligence Scale, 3rd Edition*. . San Antonio, TX: Harcourt Assessement.

24. American Psychiatric Association (1994): Diagnostic and Statistical Manual of Mental Disorders: DSM IV (4th ed)Washington, DC: American Psychiatric Association.

25. Eriksson N, Macpherson JM, Tung JY, Hon LS, Naughton B, Saxonov S, *et al.* (2010): Web-based, participant-driven studies yield novel genetic associations for common traits. *PLoS Genet* 6: Public Library of Sciencee1000993.

26. Tung JY, Do CB, Hinds DA, Kiefer AK, Macpherson JM, Chowdry AB, *et al.* (2011): Efficient replication of over 180 genetic associations with self-reported medical data. *PLoS One* 6: Public Library of Sciencee23473.

27. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, Bender D, *et al.* (2007): PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 81: 559–75.

28. Whitfield-Gabrieli S, Nieto-Castanon A (2012): Conn: a functional connectivity toolbox for correlated and anticorrelated brain networks. *Brain Connect* 2: Mary Ann Liebert, Inc. 140 Huguenot Street, 3rd Floor New Rochelle, NY 10801 USA125–41.

29. Zhou J, Gennatas ED, Kramer JH, Miller BL, Seeley WW (2012): Predicting Regional Neurodegeneration from the Healthy Brain Functional Connectome. *Neuron* 73: 1216–1227.

30. Fransson P, Marrelec G (2008): The precuneus/posterior cingulate cortex plays a pivotal role in the default mode network: Evidence from a partial correlation network analysis. *Neuroimage* 42: 1178–1184.

31. Van Dijk KRA, Hedden T, Venkataraman A, Evans KC, Lazar SW, Buckner RL (2010): Intrinsic functional connectivity as a tool for human connectomics: theory, properties, and optimization. *J Neurophysiol* 103: 297–321.

32. Power JD, Cohen AL, Nelson SM, Wig GS, Barnes KA, Church JA, *et al.* (2011): Functional network organization of the human brain. *Neuron* 72: 665–78.

33. Braun U, Plichta MM, Esslinger C, Sauer C, Haddad L, Grimm O, *et al.* (2012): Test-retest

reliability of resting-state connectivity network characteristics using fMRI and graph theoretical measures. *Neuroimage* 59: 1404–12.

34. Guo CC, Kurth F, Zhou J, Mayer EA, Eickhoff SB, Kramer JH, Seeley WW (2012): One-year test-retest reliability of intrinsic connectivity network fMRI in older adults. *Neuroimage* 61: 1471–83.

35. Rubinov M, Sporns O (2010): Complex network measures of brain connectivity: uses and interpretations. *Neuroimage* 52: 1059–69.

36. Latora V, Marchiori M (2001): Efficient Behavior of Small-World Networks. *Phys Rev Lett* 87: American Physical Society198701.

37. Girvan M, Newman MEJ (2002): Community structure in social and biological networks. *Proc Natl Acad Sci U S A* 99: 7821–6.

38. Bertram L, McQueen MB, Mullin K, Blacker D, Tanzi RE (2007): Systematic meta-analyses of Alzheimer disease genetic association studies: the AlzGene database. *Nat Genet* 39: 17–23.

39. Su YY, Liang X, Schoepf UJ, Varga-Szemes A, West HC, Qi R, *et al.* (2015): APOE Polymorphism Affects Brain Default Mode Network in Healthy Young Adults: A STROBE Article. *Medicine (Baltimore)* 94: e1734.

40. Wang L, Roe CM, Snyder AZ, Brier MR, Thomas JB, Xiong C, *et al.* (2012): Alzheimer disease family history impacts resting state functional connectivity. *Ann Neurol* 72: 571–7.

41. Heise V, Filippini N, Trachtenberg AJ, Suri S, Ebmeier KP, Mackay CE (2014): Apolipoprotein E genotype, gender and age modulate connectivity of the hippocampus in healthy adults. *Neuroimage* 98: 23–30.

42. Sherman LE, Rudie JD, Pfeifer JH, Masten CL, McNealy K, Dapretto M (2014): Development of the default mode and central executive networks across early adolescence: a longitudinal study. *Dev Cogn Neurosci* 10: 148–59.

43. Chan MY, Park DC, Savalia NK, Petersen SE, Wig GS (2014): Decreased segregation of brain systems across the healthy adult lifespan. *Proc Natl Acad Sci U S A* 111: E4997–5006.

CHAPTER 4

An Alzheimer's Disease Genetic Risk Score Predicts Longitudinal Thinning of Hippocampal Complex Subregions in Healthy Older Adults

Abstract

Variants at twenty-one genetic loci have been associated with an increased risk for Alzheimer's disease (AD). An important unresolved question is whether multiple genetic risk factors can be combined to increase power to detect changes in neuroimaging biomarkers for AD. We acquired high-resolution structural images of the hippocampus in 66 healthy, older human subjects. For 45 of these subjects, longitudinal two-year follow-up data were also available. We calculated an additive AD genetic risk score for each participant and contrasted this with a weighted risk score approach. Each score included *APOE*, *CLU*, *PICALM* and family history of AD. Both unweighted (URS) and weighted (WRS) risk scores correlated strongly to percent change in thickness across the whole hippocampal complex (URS r=-0.40, p=0.003; WRS r=-0.25, p=0.048), driven by a strong relationship to entorhinal cortex thinning (URS r=-0.35, p=0.009; WRS r=-0.35, p=0.009). By contrast, at baseline the risk scores showed no relationship to thickness in any hippocampal complex subregion. These results provide compelling evidence that polygenic AD risk scores may be especially sensitive to structural change over time in regions affected early in AD, like the hippocampus and adjacent entorhinal cortex. This work also supports the paradigm of studying genetic risk for disease in healthy volunteers. Together, these findings will inform clinical trial design by supporting the idea that genetic prescreening in healthy controls can be useful to maximize the ability to detect an effect on a longitudinal neuroimaging endpoint, like hippocampal complex cortical thickness.

Significance Statement

This is the first study to show a relationship between a genetic risk score (GRS) for Alzheimer's disease (AD) and hippocampal thinning in healthy adults. We found that a GRS composed of AD risk factors that have been shown to relate to hippocampal structure or function in humans predicted thinning of the hippocampal complex. Our ability to interpret these findings is bolstered by the association of genetic risk with longitudinal atrophy as opposed to cross-sectional morphology, which might be driven by neurodevelopmental differences. This work has implications for clinical trials focused on preclinical subjects such that screening by polygenic risk might increase the ability to detect an effect of a drug in a trial where hippocampal integrity is an endpoint.

Introduction

The development of preclinical biomarkers for sporadic, late-onset Alzheimer's disease (AD) is critical for clinical trial design and ultimately disease prevention. Neuronal loss in the hippocampus occurs early in the course of AD. This neuronal loss leads to morphological changes over time resulting in severe atrophy of the entire hippocampus in advanced AD. The hippocampus, however, begins to shrink long before the emergence of clinical symptoms. Research on families who carry genetic mutations for dominantly inherited AD has revealed that hippocampal volume loss is detectable up to 15 years before the expected onset of symptoms (1). Studies have shown that a key difference between normal age-related hippocampal thinning and pathological thinning related to AD may be the rate of thinning over time (2; 3). Longitudinal data is, therefore, extremely important in predicting trajectories of normal and pathological aging.

Genetic risk for AD is also related to hippocampal thinning. Carriage of the APOEε4 allele accelerates age-related hippocampal shrinkage in older healthy adults which may make individuals more susceptible to AD (4; 5). While *APOE* is the strongest genetic risk factor for AD, at least 20 other genes have been identified as associated with the disease (6). Among these non-*APOE* AD risk genes, clusterin (*CLU*) and phosphatidylinositol binding clathrin assembly protein (*PICALM*) have been studied using a neuroimaging genetics approach more than any other risk genes (7–17). Also, family history of AD can serve as a proxy for genetic risk and has been used in neuroimaging genetics studies to identify characteristics of a high risk group (Xu et al., 2009; Berti et al., 2011; Honea et al., 2012; Wang et al., 2012). Each of these factors, *APOE*, *CLU*, *PICALM* and family history of AD, has been previously shown to be related to hippocampal structure or function as measured by MRI-based techniques in humans (10; 12; 14; 15; 22–24). Thus, we selected these components to calculate a genetic risk score (GRS) based on their statistical association with AD risk and their previous association with the hippocampus in neuroimaging genetics studies.

The use of high-resolution structural MRI to calculate the thickness of the strip of gray matter within the convoluted hippocampus allows for sensitive measurement of changes in morphology (25). This approach is preferable to measuring the gross volume of the hippocampus because it focuses on the compartment of the hippocampal complex where cell bodies reside and thus is designed to measure morphological changes that may be related to neuronal loss. Using hippocampal thickness measurements, subregions of the hippocampal complex including entorhinal cortex, subiculum, CA3 and the dentate gyrus have been shown to be thinner in APOEε4 carriers compared to non-carriers (26–28). In this work we take these findings further by expanding our focus to include additional genetic risk factors for AD.

The present study is the first to find evidence of an association of an AD GRS and cortical thinning of the hippocampus over time in healthy adults. By focusing our GRS development on genetic factors that have been shown to associate with hippocampal structure or function in healthy older adults, we were able to boost our power to detect a link between genetic risk for AD and changes in hippocampal gray matter. Our findings support the validity of a neuroimaging genetics approach to studying genetic risk for disease in healthy, preclinical populations. Identifying quantitative neuroimaging endophenotypes associated with genetic risk for AD in healthy adults will increase our ability to identify healthy individuals who are at greatest risk of developing AD and target them for intervention. In the present study, we hypothesized that the AD GRS would be related to baseline hippocampal morphology when controlling for confounding factors like age and sex. We further hypothesized that the GRS would predict longitudinal thinning in the hippocampal complex, especially the entorhinal cortex and subiculum subregions, over two year follow-up.

Materials and Methods

Participants

Participants for this study were Caucasian individuals of either sex recruited as part of an ongoing initiative to study aging, AD genetic risk and dementia by the UCLA Longevity Center. The recruitment strategy focused on older adult community centers, relatives of AD patients referred by the local Alzheimer's Association chapter, memory groups, and other groups catering to older adults with age-related memory concerns. This strategy resulted in the recruitment of approximately 40-50% of participants carrying at least one copy of the APOEε4 allele, which is greater than the 20-25% that would be expected from purely random recruitment (29; 30). Participants were categorized as having a positive family history of AD if at least one first-degree relative had been diagnosed with AD based on standard criteria (31). All participants in the present study were healthy and cognitively intact at the time of enrollment. In our study, participants were defined as non-demented if they were cognitively intact based on clinical examination, the results of the Mini Mental State Exam (MMSE; for gross cognition, threshold≥27) and standard criteria for AAMI (Age Associated Memory Impairment); specifically, participants were excluded if they had scores more than two standard deviations below normal on two or more of the memory tests described in the next section. In addition, participants with clinical anxiety, depression or any neuropsychiatric or neurological illness were excluded. This study was performed in accordance with UCLA Institutional Review Board (IRB) protocols and approved by the UCLA Human Subjects Protection Committee. All participants gave written informed consent upon enrollment in this study.

Neuropsychological Assessment

A 3-hour neuropsychological battery was administered to each participant. The battery included tests of the following: General Intelligence (Subtests of the WAIS-III) (32), Fluency (Fruits and Vegetables) (33), Attention (Digits Forward and Backward) (32), Language (Boston

Naming Test) (34), Verbal Memory (Buschke-Fuld Selective Reminding Task) (35), WMS-III Logical Memory and Verbal Paired Associates learning (32) and Visual Memory (Rey-Osterrieth Figure test) (36). Participants also completed a family history questionnaire (37), a memory complaints self-report questionnaire (38), the Hamilton Depression and Anxiety Inventories (Hamilton 1959; Hamilton 1960), the Neuropsychiatric Inventory (41) and the MMSE (42).

Genotyping

A trained phlebotomist at the UCLA Clinical and Translational Research Laboratory drew a blood sample from each participant. Leukocytes from 10ml of the sample were frozen and stored at -80°C. 200µg of genomic DNA were isolated from the remaining 10ml and screened using a PCR-based mutation detection assay and a microsatellite marker based genotyping. Real Time PCR on an Applied Biosystems 7900HT Real Time PCR machine was used to perform *APOE* SNP (rs429358 and rs7412) genotyping. In addition to a standard curve amplification protocol, an allelic discrimination step was added to facilitate the contrast between the two alleles and their respective reporter dyes. These dyes are incorporated into a Taqman SNP Genotyping Assay with identification numbers C___3084793_20 and C___904973_10 for rs429358 and rs7412, respectively (Applied Biosystems, Foster City, CA). Results were confirmed by repeating the experiment. Single nucleotide polymorphism (SNP) genotyping data was analyzed using SDS software (version 2.3, Applied Biosystems). This program calculates the affinity of the sample to one of the two reporter dyes that, in turn, represents one allele over the other. *CLU* (rs11136000) and *PICALM* (rs3851179) SNPs were genotyped using iPLEX chemistry on the massARRAY platform (Sequenom, San Diego, CA) as per the manufacturer's instructions. The assay was based on primer extension and allowed for a locus specific PCR reaction followed by an extension reaction in which the primer anneals immediately upstream of the polymorphic site being genotyped. Through the use of MALDI-TOF mass spectrometry, the mass of the extended primer is determined. Sequenom Typer software automatically translates

the mass of the observed primers into a genotype. Positive controls were included on every chip to ensure genotyping accuracy. The results of all genotyping protocols are strictly confidential and are never revealed to the research participant.

Genetic Risk Scores

A GRS for AD was calculated for each participant (Figure 4.1). The GRS measured genetic risk load for AD across *APOE*, *CLU* and *PICALM* as well as taking into account family history of AD. We calculated two sets of GRS: unweighted and weighted. The unweighted risk score (URS) was the sum of risk factors including a family history of AD (0 if negative history or 1 if positive history), APOEε4 alleles (0, 1, or 2), *CLU* risk alleles (0, 1, or 2) and *PICALM* risk alleles (0, 1, or 2). For the weighted risk scores (WRS) we used the logarithm of published odds ratios (OR) to weight the relative contribution of these risk factors before summing: positive family history OR=2, APOEε4 OR=3, *CLU* minor allele OR=0.9, *PICALM* minor allele OR=0.9 (6). We chose to focus our GRS on these risk factors because they are among the most consistently reproduced genetic risk factors associated with late-onset sporadic AD. In addition, each of these factors has been previously shown to be related hippocampal structure or function as measured using MRI-based techniques in humans (10; 12; 14; 15; 22–24).

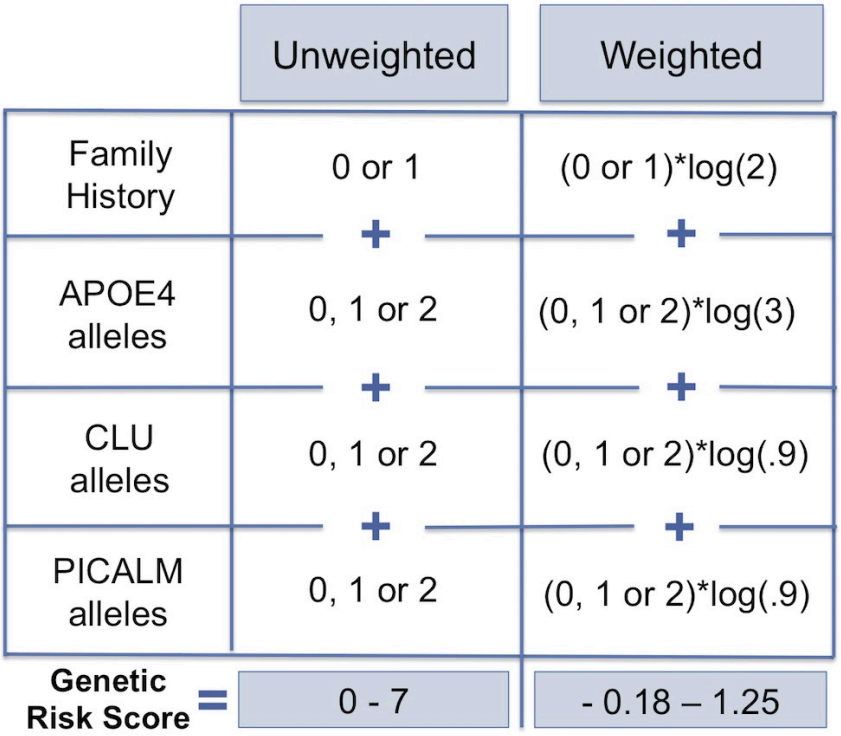


Figure 4.1: Genetic Risk Score Calculation. An unweighted risk score (URS) for each participant was calculated by adding family history of AD (0 if negative history or 1 if positive history), number of APOEε4 alleles (0,1, or 2), CLU risk alleles (0,1, or 2) and PICALM risk alleles (0,1, or 2). A weighted risk score (WRS) for each participant was calculated using the logarithm of published odds ratios (OR) to weight the relative contribution of the factors before summing: positive family history OR=2, APOEε4 OR=3, CLU minor allele OR=0.9, PICALM minor allele OR=0.9. Possible unweighted risk scores range from 0 to 7 and weighted risk scores range from -0.18 – 1.25.

Imaging Acquisition

MRI acquisition was completed using a Siemens 3T Trio magnet located at the UCLA Staglin IMHRO Center for Cognitive Neuroscience (scans acquired 2010-2012; n=8 baseline, n=13 follow-up) or a Siemens Allegra 3T located at the UCLA Brain Mapping Center (scans acquired 2006-2010). Whole-brain 3D T1-weighted magnetization prepared rapid acquisition gradient-echo (MPRAGE) volumetric scans and high-resolution oblique coronal T2-weighted fast spin echo (FSE) sequences were acquired with each participant. Scan parameters are as follows for the MPRAGE (Allegra parameters in parentheses): axial slicing, TR=1900ms (2300ms), TE=2.26ms (2.93ms), FOV=250x218mm (256x256mm), flip angle=9°, matrix=256x215mm, 176 slices (160 slices), slice thickness=1mm, zero-filled to a matrix of

256x224 resulting in a voxel size=1x0.976x0.976 mm^3 (1x1.3x1.3mm^3). For the high-resolution hippocampal structural imaging sequence parameters are as follows: TR= 5200ms, TE= 107ms (105ms), FOV= 200mmx200mm, flip angle= 139°, matrix=512mmx512mm, slice thickness= 3mm, spacing 0mm, 19 slices, in-plane voxel size= 0.39x0.39mm. Some participants' whole brain or high-resolution hippocampal structural imaging data have been used in previous publications (5; 23; 43–46).

Statistical and Imaging Analyses

Neuropsychological Performance

To test whether participants in the baseline group differed from the subset with follow-up data, two-tailed *t*-tests were used to examine age, sex, education and general cognition. We examined potential relationships between genetic risk load and sex or age using a *t*-test and Pearson correlation, respectively. These tests were completed using tools from R Project for Statistical Computing (http://www.r-project.org).

Whole-Brain Structural Imaging

Whole-brain structural MRI scans were processed using Freesurfer (47). This computational neuroanatomy software suite uses tissue contrast to determine the boundary between gray and white matter as well as the pial surface of the brain and calculates the distance between vertices plotted as a mesh on each surface across the whole cortex. After completing the FreeSurfer automated pipeline, each participant's scan was visually checked for accuracy. Minimal manual edits were completed when necessary by a single individual (TMH). Intracranial volume (ICV) estimates from FreeSurfer were used to normalize hippocampal thickness estimates from baseline scans for baseline only analyses. We used the following formula in order to normalize: ICV-corrected thickness = [(thickness in mm / ICV in mm^3) * 10^6]. Multiplying the quotient by 10^6 results in values at the same order of magnitude as the original thickness estimates.

High-Resolution Hippocampal Structural Imaging

A cortical segmentation and unfolding procedure was used to measure thickness of the gray matter of the hippocampal complex (HC) (23; 25; 26; 45) (Figure 4.2). First, the white matter and cerebrospinal fluid (CSF) within the medial temporal lobe (MTL) were manually traced on oblique coronal slices. Slices were acquired from the scanner at intervals of 3mm perpendicular to the longitudinal axis of the HC to maximize the resolution where anatomical variability is greatest. To account for slice thickness, a procedure that creates six linearly interpolated slices between each acquired pair was used to increase resolution along the longitudinal axis of the HC (48). The interpolation procedure resulted in a final voxel size of 0.39x0.39x0.43mm (for two subjects final voxel size was 0.39mmx0.39mmx0.56mm due to a thicker slice interval of 3.9mm). Next, up to 18 contiguous layers of gray matter were created using a region-expansion algorithm starting at the white matter boundary and continuing to the CSF boundary. This results in a HC gray matter strip which contains cornu ammonis (CA) fields 1, 2 and 3, the dentate gyrus (DG), subiculum (SUB), entorhinal cortex (ERC), perirhinal cortex (PRC), parahippocampal cortex (PHC), and the fusiform gyrus (FUS). Our resolution is not high enough to reliably distinguish between DG and CA fields 2 and 3 so we combine these regions into a single subregion denoted CA23DG. Next, gray matter strip was flattened using an iterative algorithm based on multidimensional scaling that has been previously used by our group (25). Demarcations indicating the boundaries between different HC subregions were drawn on each slice based on anatomical landmarks in histological and MRI atlases (49–51). Demarcations were placed in accordance with guidelines and findings produced by the Hippocampal Subfields Group (52). The demarcations are extended to form continuous boundaries between subregions in 2D space. ROIs are drawn in 2D space and transformed back into in-plane space where gray matter thickness measurements were calculated. To calculate thickness, we computed the distance to the closest non-gray matter voxel. Specifically,

in 2D space, for each middle point voxel the maximum distance value for all the corresponding 3D voxels across the layers of the gray matter strip was multiplied by two. Mean thickness was calculated by averaging this value across all the 2D voxels within a given subregion. We averaged each subregion across left and right hemispheres as we did not have any specific hypotheses regarding the laterality of an association between longitudinal change in hippocampal structure and genetic risk for AD.

Manual segmentations of baseline and follow-up scans for each participant were inspected by a single individual (ZM) to ensure consistency and minimize noise in our thickness estimates. During image processing, investigators were blinded to the demographic and genetic information corresponding to each image.

Associations between baseline thickness estimates corrected for ICV and the GRS were tested using Pearson correlation. To examine thinning over time, percent change in cortical thickness between baseline and follow-up scans was calculated for each participant with longitudinal data. The formula for calculating percent change was as follows: [((thickness at follow-up / thickness at baseline) - 1) * 100]. Percent change statistics were not corrected for ICV as measuring percent change in thickness within subjects obviates the need to control for normal variation in brain size. We also calculated partial correlations between GRS and baseline thickness or percent change in thickness controlling for the effects of age, sex and time between visits, when appropriate.

Corrections for multiple comparisons were done within each GRS because they were highly correlated and not independent ($r=0.72$, $p<0.0001$). We used a Bonferroni correction for two independent tests ($p = 0.05/2 = 0.025$) to control for multiple testing in entorhinal cortex and subiculum, the two regions in which we hypothesized thinning would be related to genetic risk for AD. These tests were simple effects tests following whole hippocampal complex analysis. Because entorhinal cortex and subiculum are subregions of the whole hippocampal complex,

these are not independent tests. Tests restricted to subfields other than entorhinal cortex and subiculum were exploratory only.

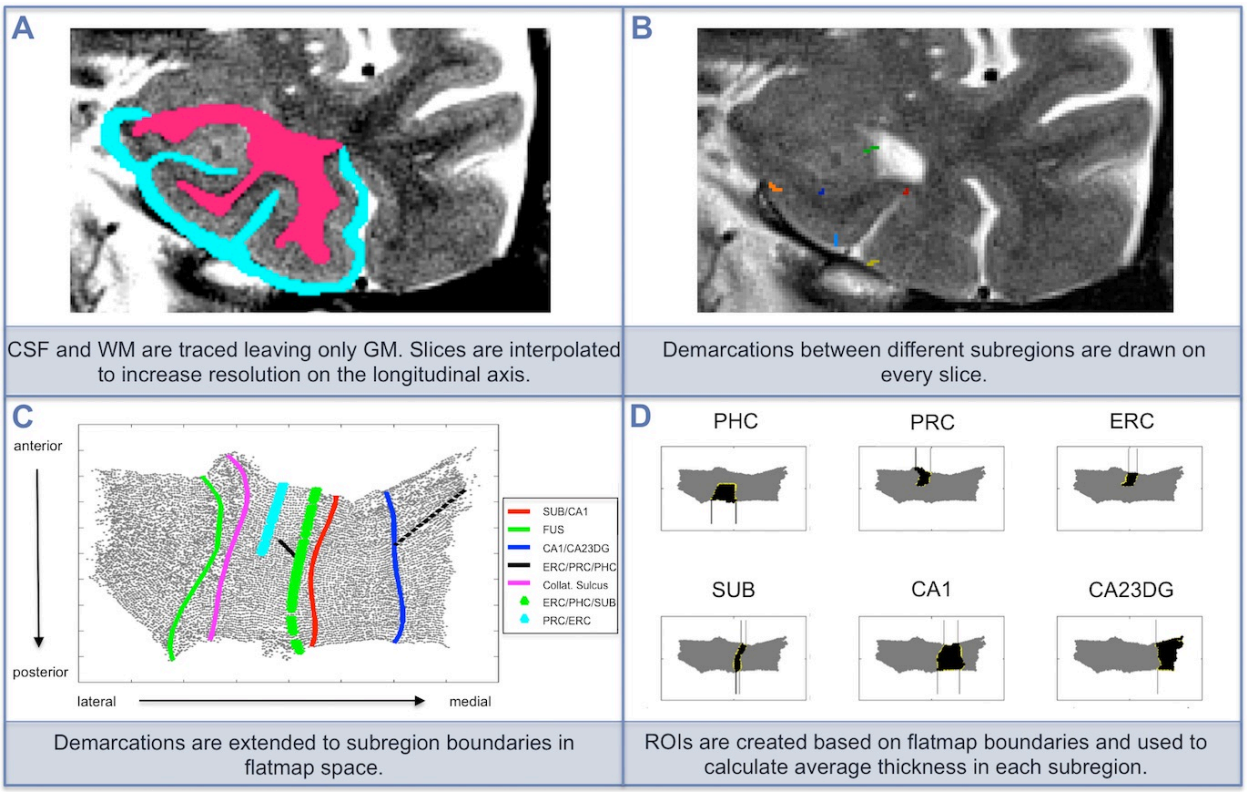


Figure 4.2: High-Resolution Hippocampal Imaging Processing Steps. A) Manual segmentation results in three distinct compartments: cerebrospinal fluid (teal), white matter (pink) and gray matter in between. B) The boundaries between hippocampal complex subregions are marked according to anatomical landmarks. Demarcations include CA23DG | CA1 (green), CA1 | subiculum (dark blue), subiculum | entorhinal cortex (orange), perhinal cortex | entorhinal (light blue), collateral sulcus (red) and fusiform gyrus (yellow). C) These demarcations are extended along the longitudinal axis of the hippocampal complex to form complete and smooth boundaries between subregions. D) Each subregion is then considered separately as a region of interest and average thickness is calculated for each.

Results

Participants

In the current study, 66 participants aged 48 and older were recruited. For 45 of our participants, two-year follow-up data were available. There were no differences in sex composition (p=0.42), age (p=0.95), education (p=0.42) or MMSE (p=0.31) between our larger baseline group and the subset with longitudinal data (Table 4.1). In order to ensure there were

no confounds of age or sex that would make interpreting the GRS signal difficult, we tested for a difference in genetic risk load between men and women (baseline: p=0.82, follow-up: p=0.48) and for a correlation between age and risk score (baseline: r=-0.10, p=0.42, follow-up: r=0.01, p=0.94) and detected no significant confounds.

Table 4.1: Cohort Characteristics. The cohort with baseline data did not differ from the subset of participants with follow-up data in sex, age, education or general cognition. MMSE= Mini Mental State Exam

Characteristic	Baseline Participants (n=66)	Follow-Up Participants (n=45)	*P*-value
Sex (M/F)	21/45	18/27	0.421
Age (years; mean+/-SD)	63.0 +/- 10 .4	63.2 +/- 7.8	0.953
Education (years; mean+/-SD)	16.4 +/- 2.4	18.0 +/- 5.7	0.417
MMSE (mean+/-SD)	29.2 +/- 0.84	28.9 +/- 0.86	0.313
Time Between Visits (years; mean+/-SD)	*N/A*	2.12 +/- 0.68	*N/A*

Genetic Risk Scores

In our cohort URS ranged from 1.0 to 6.0 (Figure 4.3). No participant had zero risk factors nor the maximum of 7.0. WRS ranged from -0.09 to 1.15 (Figure 4.3). As expected, there was a high correspondence between URS and WRS within subjects (r=0.72, p<0.0001). Distributions of risk scores between our baseline group and follow-up group were not significantly different. We included the WRS in our analyses for transparency, so the effect of weighting could be fairly assessed alongside the additive URS approach. Our focus, however, was on the URS as this score is most easily and reliably reproduced across research sites.

We tested for an association of verbal memory scores (logical memory delay total and delay total change over two-years) with GRS and found no significant relationship between behavior and URS (baseline: r=0.14, p=0.13; follow-up: r= r=-0.06, p=0.34) or WRS (baseline: r=-0.06, p=0.34; follow-up: r=0.05, p=0.37). The lack of an association between cognition and genetic risk score highlights the preclinical focus of this work, which is to identify biomarkers that are associated with genetic risk for AD in cognitively healthy older adults.

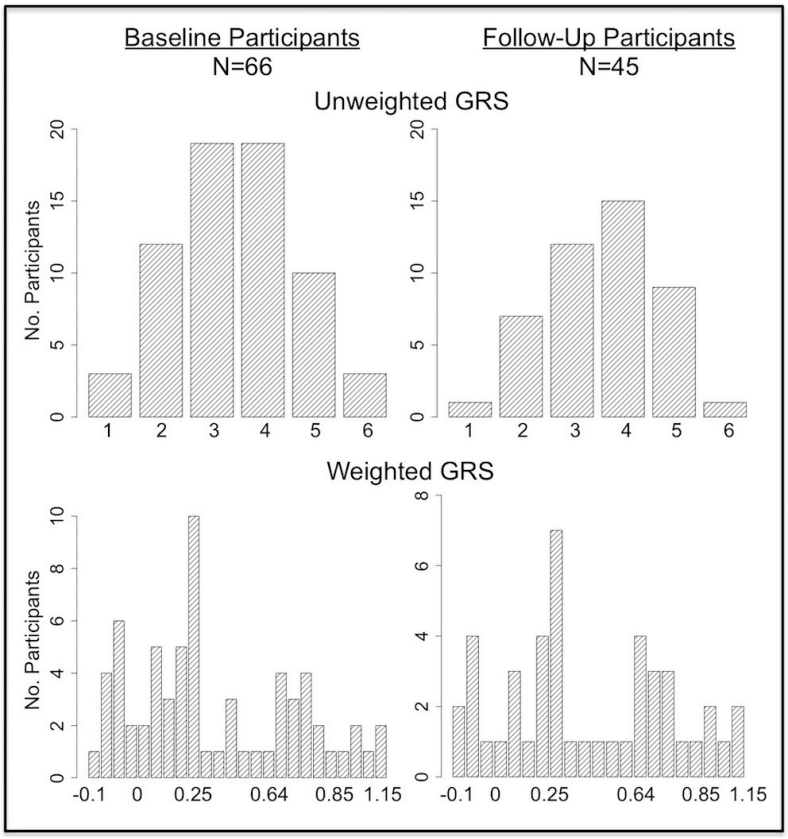


Figure 4.3: Distribution of Genetic Risk Scores. Participants risk scores were normally distributed across the range of possible scores. No participants had zero genetic risk factors nor did any have the maximum of 7 risk factors. There were no differences in either URS or WRS distributions between the baseline cohort (n=66) and the subset of participants with longitudinal data (n=45).

High Resolution Hippocampal Structural Imaging: Baseline

Baseline hippocampal complex subfield thickness was corrected for overall differences in size by normalizing each participant's thickness values by their ICV. There was no significant relationship between GRS and ICV-normalized thickness across the entire hippocampus (URS: r=0.15, p=0.16; WRS: r=0.02, p=0.44) (Figure 4.4). Next, we examined entorhinal cortex and subiculum, two regions affected early in AD, and again found no association between GRS and ICV-normalized thickness (URS: r=0.14, p=0.13; WRS: r=0.05, p=0.35). As an exploratory analysis, we examined the remaining subfields and did not find any significant relationship between thickness and genetic risk. Finally, we ran partial correlations controlling for the effects

of age and sex in the whole hippocampus and in each subregion individually. These partial correlations again showed no significant association between thickness and genetic risk.

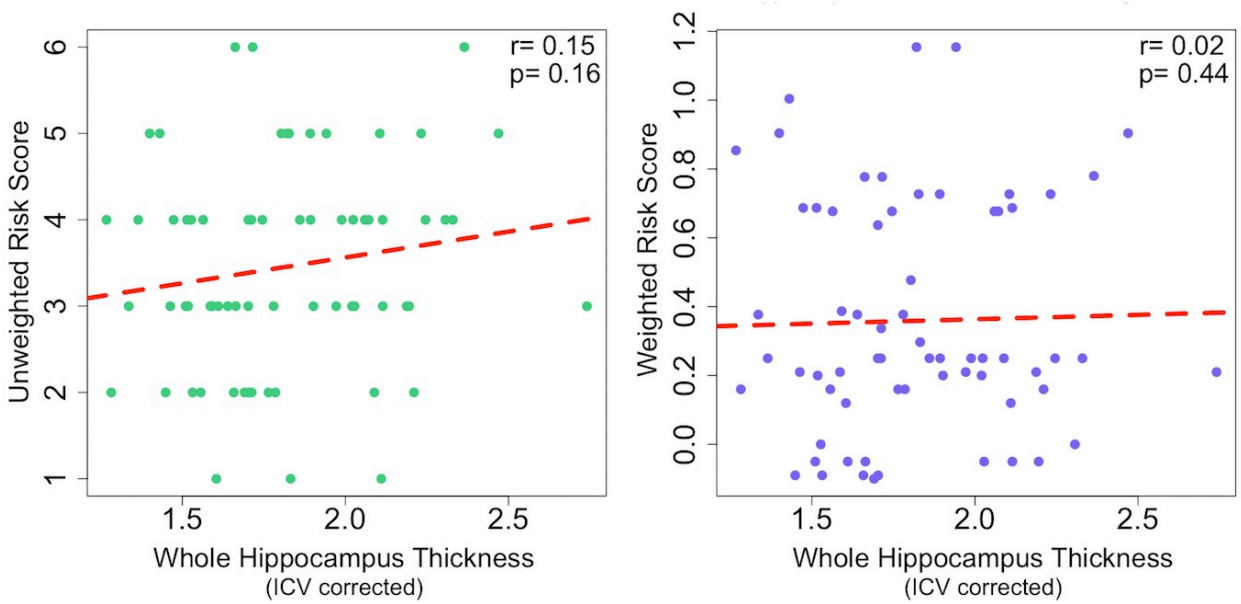


Figure 4.4: Baseline Scatterplots Show No Association Between Genetic Risk Scores and Hippocampal Complex Thickness. Baseline whole hippocampal complex thickness estimates were corrected for normal variation in size using intracranial volume (ICV). There was no significant correlation between unweighted or weighted risk scores and ICV-corrected whole hippocampal complex thickness in our baseline cohort of 66 cognitively healthy older adults.

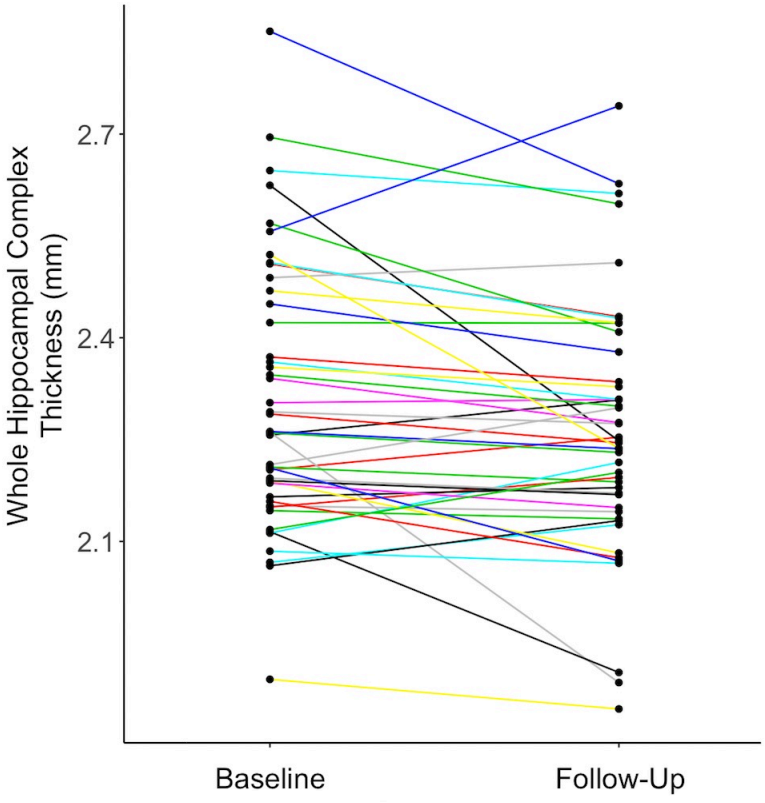


Figure 4.5: Longitudinal Hippocampal Complex Gray Matter Thickness. Each participant's mean thickness across the whole hippocampal complex is plotted at baseline and at follow-up. Most subjects experienced modest changes in thickness while fewer subjects had more dramatic changes in thickness, usually as decreases in thickness. Only one subject had an increase in mean thickness greater than 0.15 mm.

High Resolution Hippocampal Structural Imaging: Longitudinal Change

Across the entire cohort, the average change in whole hippocampal thickness was -1.91 (+/- 4.7)% over an average of 2.13 (+/- 0.68) years. This is slightly higher than previously published estimates of hippocampal atrophy using volumes estimates, but we are using a more sensitive technique and we include perihippocampal cortical regions in our whole hippocampal complex average (53; 54). Individual trajectories varied relatively widely, accounting for the large standard deviation in thickness percent change. Most people experienced mild changes in thickness, but a subset had more dramatic changes, usually thinning over time (Figure 4.5). There were some individuals whose thickness increased between baseline and follow-up.

We found a significant negative correlation between increasing GRS and more negative percent change in cortical thickness across the entire hippocampal complex (URS: r=-0.40, p=0.003; WRS: r=-0.25, p=0.048) (Figure 4.6). We hypothesized that this effect was driven by entorhinal cortex and the subiculum, two regions of the hippocampal complex that are affected early by AD pathology. In entorhinal cortex, thickness correlated with both GRS types (URS: r=-0.35, p=0.009; WRS: r=-0.35, p=0.009) (Figure 4.6). In subiculum, the association was significant but not as strong (URS: r=-0.31, p=0.01; WRS: r=-0.22, p=0.07). We also ran partial correlations controlling for the effects of age, sex and time between baseline and follow-up scans. Partial correlation coefficients were still significant for whole hippocampal complex cortical thickness and URS (URS: r=-0.34, p=0.028; WRS: r= -0.27 p=0.086) and entorhinal cortex thickness with both risk scores (URS: r=-0.32 p=0.038; WRS: r=-0.34 p=0.025). As exploratory analyses, we examined each remaining hippocampal complex subfield and found additional significant relationships to URS with fusiform gyrus (r=-0.35, p=0.009), parahippocampal cortex (r=-0.26, p=0.042), and CA1 (r=-0.25, p=0.048) thickness (Figure 4.7). Finally, we compared a multiple regression model using our URS to predict change in whole hippocampal complex thickness to a model that included only *APOE* as the genetic risk regressor (homozygous carrier=2, heterozygous carrier=1, non-carrier=0) (Table 4.2). Age, sex and time between baseline and follow-up visits were included in both models. We found that the URS model overall was highly significant ($p<0.001$) and that URS was a significant predictor within the model (p=0.028), along with time between visits (p=0.002) and a trend for sex (p=0.059). In contrast, the *APOE*-alone overall model was significant (p=0.003) but *APOE* itself was not a significant predictor of thickness (p=0.15). Instead the model was driven by time between visits (p=0.002) and sex (p=0.004) (Table 4.2). We used Akaike information criterion (AIC) and Bayesian information criterion (BIC) to directly compare models. Comparing the URS model to the model with *APOE*-alone reveals that the URS model is a better fit to our data (URS model: AIC=258.0, BIC=268.9; *APOE* model: AIC=261.2, BIC=272.1). The URS model was also

a better fit when compared to a model that used family history (FH) of AD to quantify genetic risk (URS model: AIC=258.0, BIC=268.9; FH model: AIC=263.0, BIC=273.9).

Table 4.2: Multivariate Models Predicting Percent Change in Hippocampal Complex Thickness.

	Predictors	Coefficients		t-value	p-value
		Betas	Std. Error		
Model 1: Unweighted Risk Score	(Constant)	17.153	5.994	2.862	0.007**
	Age	-0.102	0.079	-1.291	0.204
	Sex	-2.730	1.409	-1.938	0.060
	Yrs Btwn Visits	-2.863	0.893	-3.207	0.003**
	Unweighted Risk Score	-1.322	0.583	-2.270	0.028*
				R^2	p-value
				0.364	<0.001
	Predictors	Coefficients		t-value	p-value
		Betas	Std. Error		
Model 2: Weighted Risk Score	(Constant)	17.032	6.144	2.772	0.008**
	Age	-0.144	0.079	-1.826	0.075
	Sex	-3.735	1.337	-2.793	0.008**
	Yrs Btwn Visits	-2.901	0.915	-3.172	0.003**
	Weighted Risk Score	-3.101	1.763	-1.758	0.086
				R^2	p-value
				0.333	0.002
	Predictors	Coefficients		t-value	p-value
		Betas	Std. Error		
Model 3: *APOE*	(Constant)	17.227	6.255	2.754	0.009**
	Age	-0.150	0.080	-1.872	0.068
	Sex	-4.080	1.349	-3.024	0.004**
	Yrs Btwn Visits	-2.981	0.932	-3.199	0.003**
	APOE	-1.249	0.869	-1.437	0.158
				R^2	p-value
				0.317	0.003
	Predictors	Coefficients		t-value	p-value
		Betas	Std. Error		
Model 4: Family History	(Constant)	15.945	6.310	2.527	0.016*
	Age	-0.146	0.082	-1.782	0.082
	Sex	-3.702	1.435	-2.579	0.014*
	Yrs Btwn Visits	-2.699	0.960	-2.810	0.008**
	FH	-0.895	1.404	-0.637	0.528
				R^2	p-value
				0.289	0.007

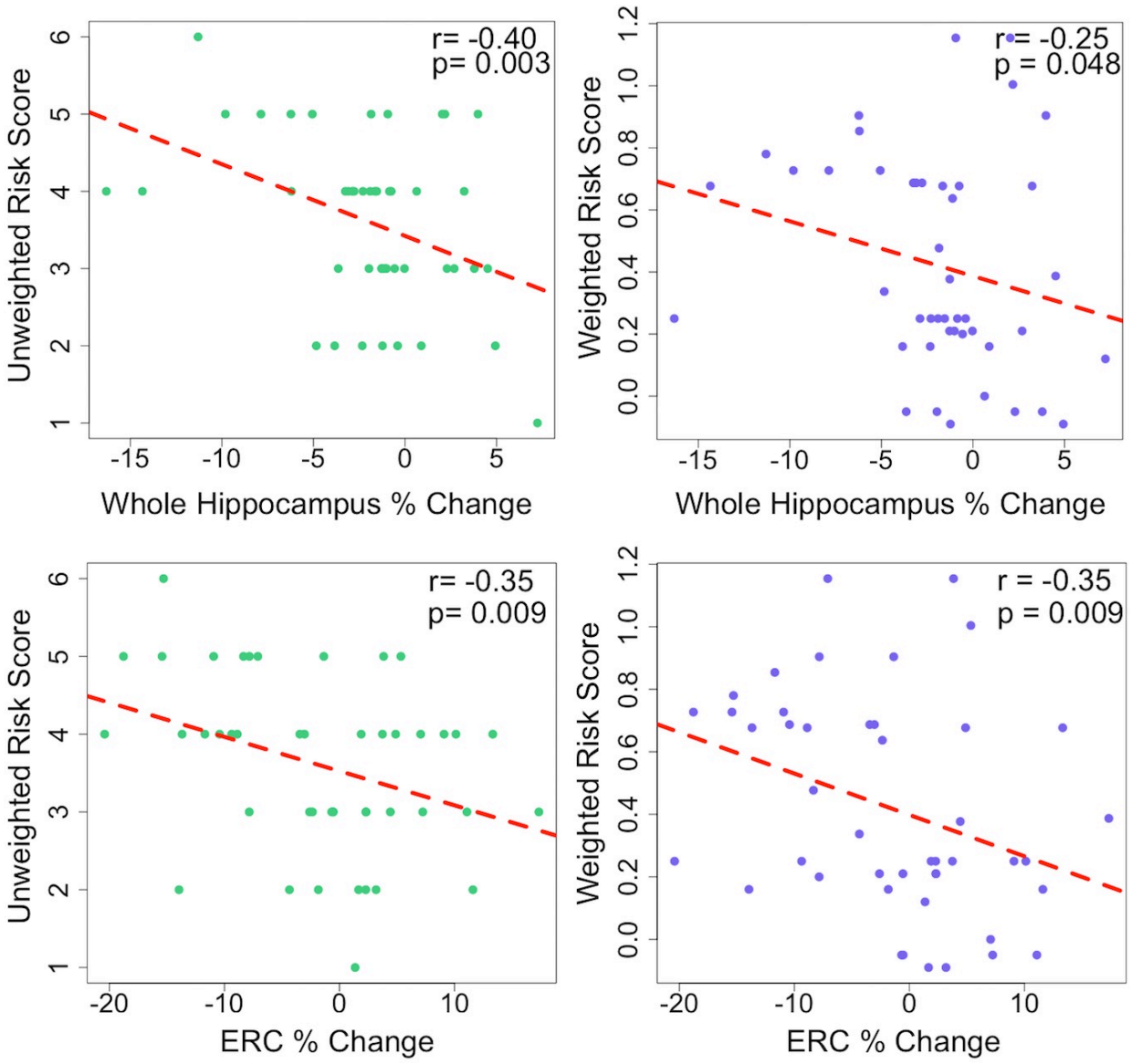


Figure 4.6: Greater Genetic Risk Score Predicts Thinning Across the Hippocampal Complex and Especially in Entorhinal Cortex. There is a significant relationship between both weighted and unweighted risk scores and percent change of bilateral hippocampal complex thickness over two years. This effect was particularly strong in entorhinal cortex (ERC), a region adjacent to the anterior portion of the hippocampus proper that is affected early in the course of AD.

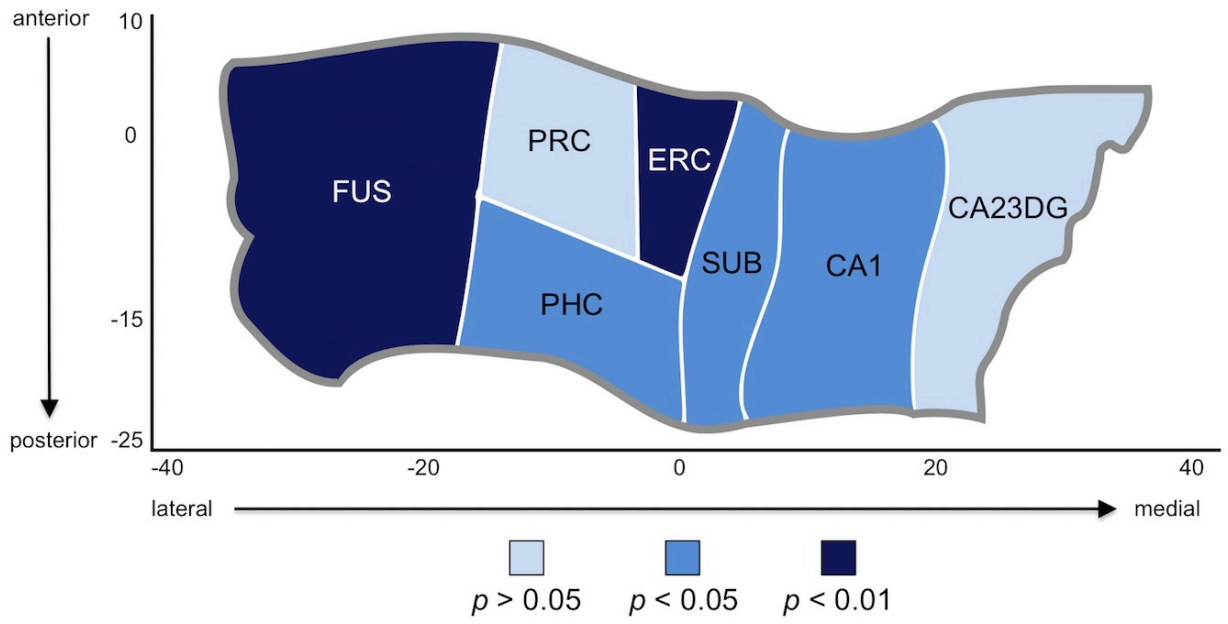


Figure 4.7: Hippocampal Complex Unfolded to Reveal Region-Wise Relationships to Unweighted Genetic Risk Score. A cortical unfolding procedure is used to produce a flat map of the hippocampal complex. Regions are colored according to the statistical strength of the association between unweighted risk score (URS) and percent change in thickness between baseline and follow-up scans. In addition to entorhinal cortex (ERC), the fusiform gyrus (FUS) showed a significant relationship to URS at $p < 0.01$. Parahippocampal cortex (PHC), subiculum (SUB) and CA1 all showed a significant relationship to URS at $p < 0.05$. The only regions in the hippocampal complex where change in thickness was not associated to genetic risk were CA23-dentate gyrus (CA23DG) and perirhinal cortex (PRC).

Discussion

We have shown that a GRS for AD is associated with hippocampal thinning over two years, but not with baseline morphology, in cognitively healthy older adults. Our findings provide evidence that genetic risk screening might be a valuable tool for predicting trajectories of endophenotypes and, ultimately, disease. By showing that greater genetic risk is associated with greater thinning in the hippocampus, a region that is particularly vulnerable to AD pathology, we demonstrate the power of working with a neuroimaging genetics approach in cognitively healthy individuals. There were no associations between GRS and baseline hippocampal morphology, which was not in line with our hypotheses. This highlights the importance of longitudinal data, especially when studying healthy older volunteers, for identifying differences in atrophy rates that are likely more sensitive that baseline differences (2;

3). There were also no associations between GRS and verbal memory performance in our participants, which supports the idea that neuroimaging endophenotypes for AD may be more sensitive markers of risk for disease progression during the preclinical phase. Our study identified a predictive relationship between genetic risk for AD and hippocampal complex thinning that is not mediated by cognition. Thus, our findings demonstrate of a truly preclinical potential biomarker for AD.

Other investigators have taken polygenic AD risk score approaches in neuroimaging studies. One of the first of these studies reported that a GRS that included all of the then-known AD risk genes predicted cortical thinning in regions particularly vulnerable to AD including entorhinal, lateral temporal and posterior cingulate cortices (55). Another more recent study used a similar approach, combining all known AD risk genes into a single score, and examined several structural measures in a large cohort of cognitively normal subjects (56). The authors found that a higher genetic risk score was significantly associated with hippocampal volume, but not with intracranial volume or whole brain volume. Our results, like these studies, support the existence of a predictive link between genetic risk for AD and hippocampal complex morphology.

The present study design has two unique strengths. The first is the two-level selection criteria we used in creating our GRS. While there is certainly a defensible rationale for creating risk scores that include every known genetic locus with significant association to disease, we argue in favor of a hypothesis driven approach restricted to genes and factors for which evidence links them to the biomarker of interest. It unlikely that every genetic risk factor associated with AD incidence is also significantly associated with a given AD biomarker, such as hippocampal integrity. It is more likely that many of the genetic loci associated with AD incidence have non-overlapping molecular mechanisms leading to increased risk for disease and, therefore, would likely drive changes in some biomarkers for AD and not others. For example, studies have shown that *CLU* variants are related to structural and functional MRI

biomarkers, but no association to positron emission tomography (PET)-measured amyloid deposition or to AD-relevant CSF analytes has been reported. Our approach of using only genetic variants associated with AD incidence and also hippocampal structure or function strengthens our ability to detect a significant association with hippocampal atrophy. A second strength of our study is the process by which we measure our biomarker, hippocampal complex cortical thickness. Volumetric measurements of the hippocampus based on whole-brain structural imaging are less sensitive to subtle changes in gray matter morphology than the semi-manual hippocampal complex segmentation process with high-resolution, partial-field-of-view imaging employed in this work. In preclinical AD, specific cortical laminae experience neuronal loss, which affects total volume only subtly while exerting a greater affect on gray matter thickness measurements (57; 58). Also, cortical thickness measurements are calculated at hundreds of points across the gray matter of the hippocampal complex, making averages more robust and less likely to be influenced by noise or error than lower-resolution volume estimates that include regions of white matter and, sometimes, CSF.

Our method of hippocampal subfield segmentation is one of several such techniques. There is an ongoing effort to create a harmonized protocol for hippocampal subfield segmentation which we are actively supporting (52; 59). These efforts are essential to ensure that findings from different research groups are comparable and, therefore, better serve to enhance our understanding of hippocampal morphology and pathological changes to hippocampal structure. However, our lab has been consistently and successfully using versions of our current method for over 10 years and it is the most reliable method available, especially as pertains the segmentation of the most anterior hippocampal subfields, including entorhinal cortex (5; 25; 45). In future studies, we plan to adopt the automated techniques resulting from the Hippocampal Subfields Working Group efforts. In the present study, we chose not to interrogate left and right hippocampal complexes separately because we did not have a

hypothesis regarding laterality of the association between an AD GRS and hippocampal thinning.

We recognize that the factors included in our genetic risk score are not entirely independent. For example, carriers of the APOEε4 allele often have a higher incidence of positive family history of AD when compared to APOEε4 non-carriers (60). However, due to our recruitment strategy targeting the worried-well and older adults with a family history of AD, APOEε4 non-carriers in our cohort are enriched for other genetic risk factors for AD, such as family history of AD, despite their lack of an APOEε4 allele. In our cohort, there were no significant differences in family history in carriers (60.7% with positive family history) versus non-carriers (65.8%) of the APOEε4 allele (p=0.80).

There are several ways to attempt to identify genetic risk factors associated with a particular endophenotype, including data reduction techniques such as principal component analysis and regression techniques such as logistic regression in genome-wide association studies. In the present study, we chose to use the two-level selection criteria approach due to its conceptual novelty. Our use of an OR-weighted GRS and an unweighted GRS side-by-side was meant to illustrate the advantage of one over the other, if present. However, we found that in our GRS composed of 4 genetic risk factors, it was at least equally effective to use a simple linear additive risk score as it was to use a weighted approach. Because odds ratios change slightly with each genome-wide associate study (GWAS), a simple additive approach might be best to ensure comparability and reliability of a GRS across labs and in clinical trials.

In addition to hippocampal integrity, another potential biomarker of preclinical AD is amyloid and tau deposition as measured by PET. We do not have amyloid- or tau-PET data available on these subjects, so it is not possible to rule out the presence of these pathologies in these subjects. We are also not able to estimate the tau-positivity rate based on the literature as tau-PET is a relatively new tool, but there is evidence that, like amyloid, tau is sometimes present in high levels in the brains of clinically normal individuals (61). According to Doraiswamy

and colleagues (2014), approximately 14% of cognitively normal older subjects are amyloid positive (62). Of course, the cut-off to define amyloid positivity is not precise and varies across studies, so this positivity rate is just an estimate. Still, assuming this rate is accurate, it would indicate that 9-10 (9.24) participants in our cohort are likely to be amyloid positive. Thus, we feel that even with the potential noise introduced into our sample by possibly including subjects with amyloid, we still have a large enough sample of amyloid negative participants to detect the significant effect between GRS and hippocampal morphology.

Mechanistic insights from neuroimaging genetics studies are inherently limited by the lack of known causal variants driving many of the significant GWAS signals in AD. Saykin and colleagues (2015) describe a multi-step process to move from these genetic signals to targeted therapeutics (63). In their model genetics and neuroimaging intersect at the first step (discovering genetic loci that are robustly associated with a relevant trait) and at the final step (identifying individuals most likely to benefit from experimental therapies). The steps linking these two together include, first, the identification of causal variants, then testing hypothesized mechanisms in model systems and, finally, developing mechanism-targeted therapeutics. In the present study we have demonstrated an additive effect of multiple genetic risk factors on an AD biomarker, indicating that there might be different mechanisms affecting the same outcome measure, in this case hippocampal complex cortical thickness.

The present study provides the first evidence that a hypothesis-driven AD GRS predicts increased hippocampal complex subfield thinning over two years in healthy, older adults. This work is extremely relevant to clinical trial design because of the short, two-year follow-up time along with the ease of collecting genetic and MRI data. Both are minimally invasive and can be repeated as needed. We argue that prescreening preclinical, cognitively normal individuals to maximize genetic risk will increase power to detect changes in related biomarkers. Indeed, preliminary work designed to assess the increased power of genetic pre-screening in clinically impaired cohorts has been promising. Kohannim and colleagues (2013) report up to a 50%

decrease in sample size needed to detect an effect in atrophy over two years (64). Genetic pre-screening paired with neuroimaging-based outcome measures is going to be a critical component of future AD clinical trials focused on cognitively healthy, preclinical individuals for which traditional pencil-and-paper outcome measures will not be sensitive enough to detect drug effects.

Chapter 4 References

1. Bateman RJ, Xiong C, Benzinger TLS, Fagan AM, Goate A, Fox NC, *et al.* (2012): Clinical and biomarker changes in dominantly inherited Alzheimer's disease. *N Engl J Med* 367: 795–804.
2. Chincarini A, Sensi F, Rei L, Gemme G, Squarcia S, Longo R, *et al.* (2015): Integrating longitudinal information in hippocampal volume measurements for the early detection of Alzheimer's disease. *Neuroimage* 125: 834–847.
3. Lu PH, Thompson PM, Leow A, Lee GJ, Lee A, Yanovsky I, *et al.* (2011): Apolipoprotein E genotype is associated with temporal and hippocampal atrophy rates in healthy elderly adults: a tensor-based morphometry study. *J Alzheimers Dis* 23: 433–42.
4. Jak AJ, Houston WS, Nagel BJ, Corey-Bloom J, Bondi MW (2007): Differential cross-sectional and longitudinal impact of APOE genotype on hippocampal volumes in nondemented older adults. *Dement Geriatr Cogn Disord* 23: 382–9.
5. Donix M, Burggren AC, Suthana NA, Siddarth P, Ekstrom AD, Krupa AK, *et al.* (2010): Longitudinal changes in medial temporal cortical thickness in normal subjects with the APOE-4 polymorphism. *Neuroimage* 53: 37–43.
6. Lambert JC, Ibrahim-Verbaas C a, Harold D, Naj a C, Sims R, Bellenguez C, *et al.* (2013): Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. *Nat Genet* 45: 1452–8.
7. Bralten J, Franke B, Arias-Vásquez A, Heister A, Brunner HG, Fernández G, Rijpkema M (2011): CR1 genotype is associated with entorhinal cortex volume in young healthy adults. *Neurobiol Aging* 32: 2106.e7–11.
8. Stevens BW, DiBattista AM, William Rebeck G, Green AE (2014): A gene-brain-cognition pathway for the effect of an Alzheimer's risk gene on working memory in young adults. *Neuropsychologia* 61: 143–9.
9. Braskie MN, Jahanshad N, Stein JL, Barysheva M, McMahon KL, de Zubicaray GI, *et al.* (2011): Common Alzheimer's disease risk variant within the CLU gene affects white matter microstructure in young adults. *J Neurosci* 31: 6764–70.
10. Erk S, Meyer-Lindenberg A, Opitz von Boberfeld C, Esslinger C, Schnell K, Kirsch P, *et al.* (2011): Hippocampal function in healthy carriers of the CLU Alzheimer's disease risk variant. *J Neurosci* 31: 18180–4.
11. Green AE, Gray JR, Deyoung CG, Mhyre TR, Padilla R, Dibattista AM, William Rebeck G (2014): A combined effect of two Alzheimer's risk genes on medial temporal activity during executive attention in young adults. *Neuropsychologia* 56: 1–8.
12. Zhang P, Qin W, Wang D, Liu B, Zhang Y, Jiang T, Yu C (2014): Impacts of PICALM and CLU variants associated with Alzheimer's disease on the functional connectivity of the hippocampus in healthy young adults. *Brain Struct Funct*. doi: 10.1007/s00429-014-0738-4.
13. Hohman TJ, Koran ME, Thornton-Wells T (2013): Epistatic genetic effects among Alzheimer's candidate genes. *PLoS One* 8: e80839.
14. Biffi A, Anderson CD, Desikan RS, Sabuncu M, Cortellini L, Schmansky N, *et al.* (2010): Genetic variation and neuroimaging measures in Alzheimer disease. *Arch Neurol* 67: 677–85.
15. Furney SJ, Simmons A, Breen G, Pedroso I, Lunnon K, Proitsi P, *et al.* (2011): Genome-

wide association with MRI atrophy measures as a quantitative trait locus for Alzheimer's disease. *Mol Psychiatry* 16: 1130–8.

16. Harrison TM, Bookheimer SY (2016): Neuroimaging Genetic Risk for Alzheimer's Disease in Preclinical Individuals: From Candidate Genes to Polygenic Approaches. *Biol Psychiatry Cogn Neurosci Neuroimaging* 1: 14–23.
17. Harrison TM, Burggren AC, Bookheimer SY (2015): Neuroimaging Genetics of Alzheimer's Disease. In: Bigios KL, Hariri AR, Weinberger DR, editors. *Neuroimaging Genet Princ Pract*Oxford University Press, pp 357–397.
18. Honea RA, Vidoni ED, Swerdlow RH, Burns JM (2012): Maternal family history is associated with Alzheimer's disease biomarkers. *J Alzheimers Dis* 31: 659–68.
19. Wang L, Roe CM, Snyder AZ, Brier MR, Thomas JB, Xiong C, *et al.* (2012): Alzheimer disease family history impacts resting state functional connectivity. *Ann Neurol* 72: 571–7.
20. Xu G, McLaren DG, Ries ML, Fitzgerald ME, Bendlin BB, Rowley HA, *et al.* (2009): The influence of parental history of Alzheimer's disease and apolipoprotein E epsilon4 on the BOLD signal during recognition memory. *Brain* 132: 383–91.
21. Berti V, Mosconi L, Glodzik L, Li Y, Murray J, De Santi S, *et al.* (2011): Structural brain changes in normal individuals with a maternal history of Alzheimer's. *Neurobiol Aging* 32: 2325.e17–26.
22. Okonkwo OC, Xu G, Dowling NM, Bendlin BB, Larue A, Hermann BP, *et al.* (2012): Family history of Alzheimer disease predicts hippocampal atrophy in healthy middle-aged adults. *Neurology* 78: 1769–76.
23. Donix M, Burggren AC, Suthana NA, Siddarth P, Ekstrom AD, Krupa AK, *et al.* (2010): Family history of Alzheimer's disease and hippocampal structure in healthy people. *Am J Psychiatry* 167: 1399–406.
24. Honea RA, Swerdlow RH, Vidoni ED, Burns JM (2011): Progressive regional atrophy in normal adults with a maternal history of Alzheimer disease. *Neurology* 76: 822–9.
25. Ekstrom AD, Bazih AJ, Suthana NA, Al-Hakim R, Ogura K, Zeineh M, *et al.* (2009): Advances in high-resolution imaging and computational unfolding of the human hippocampus. *Neuroimage* 47: 42–9.
26. Burggren AC, Zeineh MM, Ekstrom AD, Braskie MN, Thompson PM, Small GW, Bookheimer SY (2008): Reduced cortical thickness in hippocampal subregions among cognitively normal apolipoprotein E e4 carriers. *Neuroimage* 41: 1177–83.
27. Mueller SG, Schuff N, Raptentsetsang S, Elman J, Weiner MW (2008): Selective effect of Apo e4 on CA3 and dentate in normal aging and Alzheimer's disease using high resolution MRI at 4 T. *Neuroimage* 42: 42–8.
28. Mueller SG, Weiner MW (2009): Selective effect of age, Apo e4, and Alzheimer's disease on hippocampal subfields. *Hippocampus* 19: 558–64.
29. Bookheimer SY, Strojwas MH, Cohen MS, Saunders AM, Pericak-Vance MA, Mazziotta JC, Small GW (2000): Patterns of brain activation in people at risk for Alzheimer's disease. *N Engl J Med* 343: 450–6.
30. Small GW, Ercoli LM, Silverman DH, Huang SC, Komo S, Bookheimer SY, *et al.* (2000): Cerebral metabolic and cognitive decline in persons at genetic risk for Alzheimer's disease. *Proc Natl Acad Sci U S A* 97: 6037–42.
31. Dubois B, Feldman HH, Jacova C, Dekosky ST, Barberger-Gateau P, Cummings J, *et al.*

(2007): Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS-ADRDA criteria. *Lancet Neurol* 6: 734–46.

32. Wechsler D (1997): *Wecshler Adult Intelligence Scale, 3rd Edition*. . San Antonio, TX: Harcourt Assessement.
33. Cauthen NR (1978): Verbal fluency: normative data. *J Clin Psychol* 34: 126–9.
34. Goodglass HP, Kaplan EP (2001): Boston Naming Test, 3rd EditionLippincott Williams & Wilkins.
35. Buschke H, Fuld PA (1974): Evaluating storage, retention, and retrieval in disordered memory and learning. *Neurology* 24: 1019–25.
36. Osterrieth PA (1944): Le test de copie d'une figure complex: Contribution à l'étude de la perception et de la memoir. *Arch Psychol (Geneve)*286–356.
37. Breitner JC, Folstein MF (1984): Familial Alzheimer Dementia: a prevalent disorder with specific clinical features. *Psychol Med* 14: 63–80.
38. Gilewski MJ, Zelinski EM, Schaie KW (1990): The Memory Functioning Questionnaire for assessment of memory complaints in adulthood and old age. *Psychol Aging* 5: 482–90.
39. Hamilton M (1959): The assessment of anxiey states by rating. *Br J Med Psychol* 32: 50–55.
40. Hamilton M (1960): A rating scale for depression. *J Neurol Neurosurg Psychiatry* 23: 56–62.
41. Cummings JL, Mega M, Gray K, Rosenberg-Thompson S, Carusi DA, Gornbein J (1994): The Neuropsychiatric Inventory: comprehensive assessment of psychopathology in dementia. *Neurology* 44: 2308–14.
42. Folstein MF, Robins LN, Helzer JE (1983): The Mini-Mental State Examination. *Arch Gen Psychiatry* 40: 812.
43. Brown JA, Terashima KH, Burggren AC, Ercoli LM, Miller KJ, Small GW, Bookheimer SY (2011): Brain network local interconnectivity loss in aging APOE-4 allele carriers. *Proc Natl Acad Sci U S A* 108: 20760–5.
44. Burggren A, Brown J (2013): Imaging markers of structural and functional brain changes that precede cognitive symptoms in risk for Alzheimer's disease. *Brain Imaging Behav*. doi: 10.1007/s11682-013-9278-4.
45. Burggren AC, Renner B, Jones M, Donix M, Suthana NA, Martin-Harris L, *et al.* (2011): Thickness in entorhinal and subicular cortex predicts episodic memory decline in mild cognitive impairment. *Int J Alzheimers Dis* 2011: 956053.
46. Donix M, Burggren AC, Scharf M, Marschner K, Suthana NA, Siddarth P, *et al.* (2013): APOE associated hemispheric asymmetry of entorhinal cortical thickness in aging and Alzheimer's disease. *Psychiatry Res* 214: 212–20.
47. Fischl B, Dale AM (2000): Measuring the thickness of the human cerebral cortex from magnetic resonance images. *Proc Natl Acad Sci U S A* 97: 11050–5.
48. Zeineh MM, Engel SA, Thompson PM, Bookheimer SY (2001): Unfolding the human hippocampus with high resolution structural and functional MRI. *Anat Rec* 265: 111–20.
49. Amaral DG, Insausti R (1990): *The Human Nervous System*. . . *Hum Nerv Syst*Elsevier. doi: 10.1016/B978-0-12-547625-6.50026-X.
50. Mai JK, Paxinos G (1997): *Atlas of the human brain*. . Academic Press.
51. Duvernoy HM (2005): *The Human Hippocampus: Functional Anatomy, Vascularization and Serial Sections with MRI*. . Springer Science & Business Media.

52. Yushkevich PA, Amaral RSC, Augustinack JC, Bender AR, Bernstein JD, Boccardi M, *et al.* (2015): Quantitative comparison of 21 protocols for labeling hippocampal subfields and parahippocampal subregions in in vivo MRI: towards a harmonized segmentation protocol. *Neuroimage* 111: 526–41.

53. Das SR, Avants BB, Pluta J, Wang H, Suh JW, Weiner MW, *et al.* (2012): Measuring longitudinal change in the hippocampal formation from in vivo high-resolution T2-weighted MRI. *Neuroimage* 60: 1266–79.

54. Fraser MA, Shaw ME, Cherbuin N (2015): A systematic review and meta-analysis of longitudinal hippocampal atrophy in healthy human ageing. *Neuroimage* 112: 364–74.

55. Sabuncu MR, Buckner RL, Smoller JW, Lee PH, Fischl B, Sperling RA (2012): The association between a polygenic Alzheimer score and cortical thickness in clinically normal subjects. *Cereb Cortex* 22: 2653–61.

56. Chauhan G, Adams HHH, Bis JC, Weinstein G, Yu L, Töglhofer AM, *et al.* (2015): Association of Alzheimer disease GWAS loci with MRI-markers of brain aging. *Neurobiol Aging*. doi: 10.1016/j.neurobiolaging.2014.12.028.

57. Hyman BT, Van Hoesen GW, Damasio AR, Barnes CL (1984): Alzheimer's disease: cell-specific pathology isolates the hippocampal formation. *Science* 225: 1168–70.

58. Gómez-Isla T, Price JL, McKeel DW, Morris JC, Growdon JH, Hyman BT (1996): Profound loss of layer II entorhinal cortex neurons occurs in very mild Alzheimer's disease. *J Neurosci* 16: 4491–500.

59. Boccardi M, Ganzola R, Bocchetta M, Pievani M, Redolfi A, Bartzokis G, *et al.* (2011): Survey of protocols for the manual segmentation of the hippocampus: preparatory steps towards a joint EADC-ADNI harmonized protocol. *J Alzheimers Dis* 26 Suppl 3: 61–75.

60. Zintl M, Schmitz G, Hajak G, Klünemann H-H (2009): ApoE genotype and family history in patients with dementia and cognitively intact spousal controls. *Am J Alzheimers Dis Other Demen* 24: 349–52.

61. Johnson KA, Schultz A, Betensky RA, Becker JA, Sepulcre J, Rentz D, *et al.* (2015): Tau PET imaging in aging and early Alzheimer's disease. *Ann Neurol* 79: 110–9.

62. Doraiswamy PM, Sperling RA, Johnson K, Reiman EM, Wong TZ, Sabbagh MN, *et al.* (2014): Florbetapir F 18 amyloid PET and 36-month cognitive decline: a prospective multicenter study. *Mol Psychiatry* 19: 1044–51.

63. Saykin AJ, Shen L, Yao X, Kim S, Nho K, Risacher SL, *et al.* (2015): Genetic studies of quantitative MCI and AD phenotypes in ADNI: Progress, opportunities, and plans. *Alzheimers Dement* 11: 792–814.

64. Kohannim O, Hua X, Rajagopalan P, Hibar DP, Jahanshad N, Grill JD, *et al.* (2013): Multilocus genetic profiling to empower drug trials and predict brain atrophy. *NeuroImage Clin* 2: 827–35.

CHAPTER 5

Discussion: Neuroimaging Genetic Risk for Alzheimer's Disease in Preclinical Individuals

Abstract

Better characterization of the preclinical phase of Alzheimer's disease (AD) is needed in order to develop effective interventions. Neuropathological changes in AD, including neuronal loss and the formation of proteinaceous deposits, begin up to 20 years before the onset of clinical symptoms. As such, the emergence of cognitive impairment should not be the sole basis used to diagnose AD nor to evaluate individuals for enrollment in clinical trials for preventative AD treatments. Instead, early preclinical biomarkers of disease and genetic risk should be used to determine most likely prognosis and enroll individuals in appropriate clinical trials. Neuroimaging-based biomarkers and genetic analysis together present a powerful system for classifying preclinical pathology in patients. Disease modifying interventions are more likely to produce positive outcomes when administered early in the course of AD. In this review, we examine the utility of the neuroimaging genetics field as it applies to AD and early detection during the preclinical phase. Neuroimaging studies focused on single genetic risk factors are summarized. However, we particularly focus on the recent increased interest in polygenic methods and discuss the benefits and disadvantages of these approaches. We discuss challenges in the neuroimaging genetics field, including limitations of statistical power arising from small effect sizes and the over-use of cross-sectional designs. Despite the limitations, neuroimaging genetics has already begun to influence clinical trial design and will play a major role in the prevention of AD.

Introduction

A long prodrome precedes the emergence of the clinical symptoms of Alzheimer's disease (AD) (1–3). Increasingly, the time between the first silent pathological changes in the brain and the earliest stages of cognitive impairment is understood to be a critical window during which prevention and treatment strategies may be most effective (4). This preclinical phase of AD pathogenesis that occurs before clinical symptoms emerge is not well characterized. By definition, individuals with preclinical AD are not aware that they are affected by any neurological pathology, nor are their deficits detectable with cognitive testing. Preclinical AD is distinct from mild cognitive impairment (MCI), which is characterized by subtle cognitive decline and can sometimes progress to a clinical diagnosis of AD (5; 6). In the absence of detectable cognitive decline, we have access to a limited set of research tools to explore preclinical AD in humans. These include neuroimaging, genetic testing, and biochemical assays of the blood and CSF. Thus, neuroimaging genetics research is poised to play a critical role in improving the characterization of the earliest phases of AD pathophysiology. In the following sections, we will discuss the important role of neuroimaging genetics in AD prevention and treatment with a particular focus on the preclinical phase of the disease. Specifically, we will review findings resulting from both candidate gene and polygenic approaches to neuroimaging genetics studies in AD. The goal of this chapter is to survey the status of the field, including its many limitations, and to argue that neuroimaging genetics research utilizing polygenic approaches will lead to better characterization of preclinical AD, which is necessary to achieve effective AD prevention.

Neuroimaging Preclinical Alzheimer's Disease

A common approach for studying preclinical AD is to use a group at increased risk for AD as a potential preclinical cohort and compare them to a cohort of controls without the risk factor. Increased risk can be defined by the presence of a particular genetic risk variant, such as the apolipoprotein E ε4 (*APOE*ε4) allele, a positive family history of AD, subjective memory

impairment as well as the presence of an early neuroimaging or cerebral spinal fluid (CSF) biomarker. Well validated neuroimaging-based biomarkers for AD in these types of cohorts include hippocampal volume loss or thinning, cortical thinning of key AD-related cortical regions, beta-amyloid positivity measured by positron emission tomography (PET) and default mode network (DMN) dysfunction measured by resting state functional MRI (rs-fMRI) (7–16). There is evidence from familial AD patients that these biomarkers precede the emergence of clinical symptoms by at least 3-5 and up to 20 years (1). A thorough description of the literature supporting these biomarker data is outside our focus and there are many excellent reviews available on these topics (17–21).

Clinical neuroimaging positive for biomarker changes, such as thinning of the hippocampus as measured with structural MRI, have been added to the updated AD diagnostic criteria (22). The acquisition of MRI-based biomarkers is minimally invasive, making these methods preferable to lumbar punctures. Both MRI and PET imaging can and have been used in longitudinal studies and provide a quantitative measure of change over time that is not influenced by cognitive performance, which can be affected by sleep patterns, illness, stress and other confounding factors. However, characteristics of imaging biomarkers are not yet sufficient for a preclinical AD diagnosis on the individual level. This is due to several factors, including the lack of extensive longitudinal data to map biomarker changes over time in an individual as well as the limitations in resolution and measurement of modern imaging techniques. Combining known biomarker trajectories with genetic risk stratification may increase prediction power, especially in clinical trial settings, giving greater relative importance to possible disease-related changes in individuals at the highest genetic risk for AD.

Neuroimaging and AD Candidate Genes

In 2000, the first study to combine neuroimaging and genetic risk for AD in healthy subjects found that carriers of the *APOE*ε4 allele had higher activation across several cortical

regions during a memory task compared to non-carriers (Figure 5.1; (23)). This approach, examining a selected variant(s) within a single gene and the association of that variant with brain structure and function, is a type of candidate gene study. Candidate gene studies in neuroimaging are very common, but they are controversial due to difficulties in both interpretation and replication of results (24). The now common practice of restricting candidates to genes for which a disease association has already been demonstrated has helped to make findings more robust. Still, a gene with a relatively large effect on disease incidence in a genome-wide association study (GWAS) is not necessarily related to neuroimaging phenotypes to the same degree. *APOE* is the most commonly studied candidate gene for AD. Because of the large proportion of the variance in AD heritability that is accounted for by *APOE*, investigators have been successful in identifying differences in many neuroimaging modalities based on *APOE* genotype (Figure 5.1; see (17–20); for updated review including recent findings see *Chapter 1*).

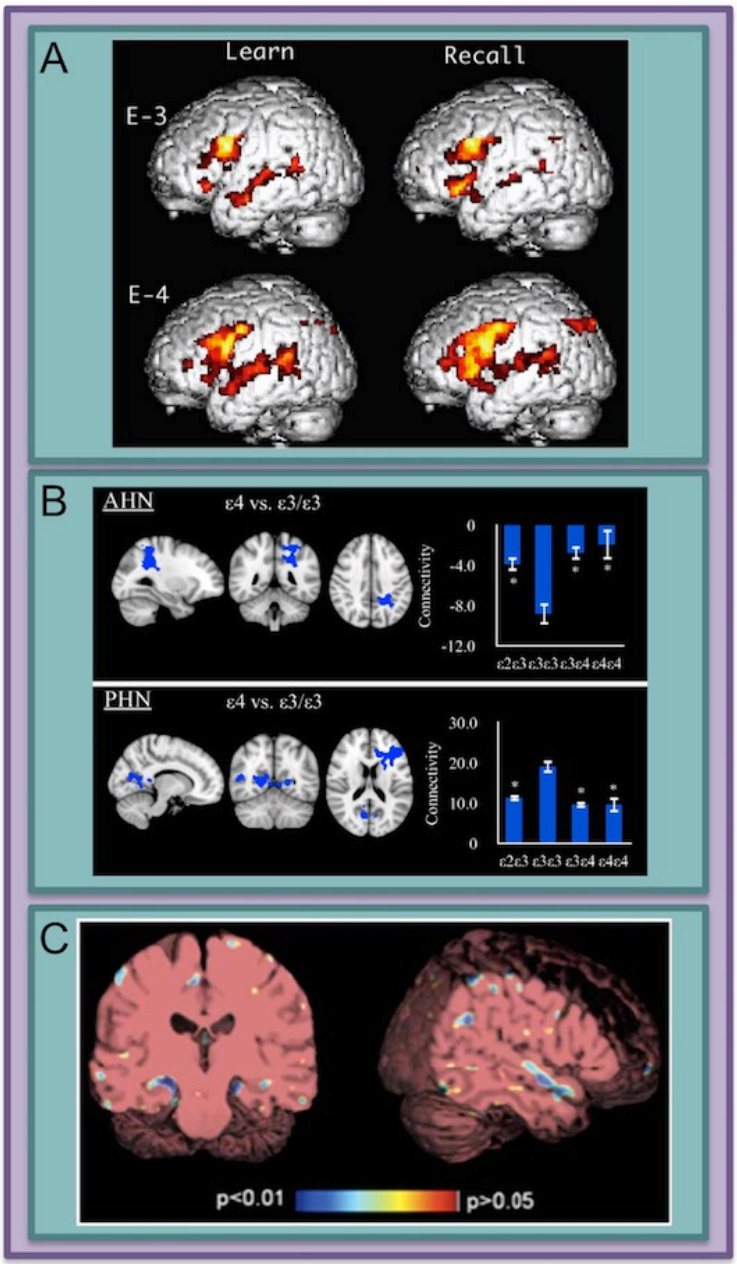


Figure 5.1: Differences between carriers and non-carriers of the *APOE* ε4 (APOEε4) allele have been shown using both structural and functional neuroimaging. The association between APOEε4 and AD risk has a moderate effect size. This may increase the likelihood of observing differences in neuroimaging phenotypes, which are relatively gross measures of neural structure and function. A) Carriers of the APOEε4 risk allele show potentially compensatory cortical activity in language areas during the learning and recall phases of a word-based paired-associates task. B) The anterior hippocampal network (AHN) and posterior hippocampal network (PHN) connectivity is modulated by *APOE* genotype. Bar graphs represent the network as a region of interest and denotes average connectivity in each genotype group. C) Structural MRI shows that healthy older carriers of APOEε4 have a greater atrophy rate over time in hippocampus and superior temporal gyrus when compared to non-carriers. Panels reprinted: A (23), B (95) and C (7).

In addition to *APOE*, other GWAS-identified AD risk genes have been studied using a candidate gene approach. These include *CLU*, *PICALM*, and *CR1* as well as *BIN1*, *ABCA7* and *EPHA1*. Of these genes, the one that has received the most attention in the neuroimaging literature is *CLU*. First linked to AD by May and colleagues in 1990, the coincident discovery of *CLU* in two independent GWASs in 2009 renewed the interest in *CLU* and its role in AD (25–27). The association of *CLU* SNP rs11136000 to AD has been replicated several times (28–30).

Several functional imaging studies have reported an effect of *CLU* genotype in both task-based and resting functional MRI (fMRI) paradigms. One fMRI experiment that tested for additive effects of *CLU* and *APOE* on blood-oxygen-level dependent (BOLD) signal during an executive attention task found a negative correlation between genetic risk and the BOLD signal associated with executive attention in the medial temporal lobe, as well as other regions (31). In another study, healthy older carriers of the *CLU* risk variant showed decreased coupling of the hippocampus and prefrontal cortex during memory retrieval tasks (recall and recognition) (32). In a resting-state fMRI experiment, subjects who were homozygous for the *CLU* risk allele had the same general pattern of positive and negative functional connectivity compared to carriers of the protective allele, but the magnitude of the connectivity was stronger in both the positive and negative directions (33). Taken together, these studies indicate a modulatory relationship between BOLD signal and *CLU* genotype (Figure 5.2).

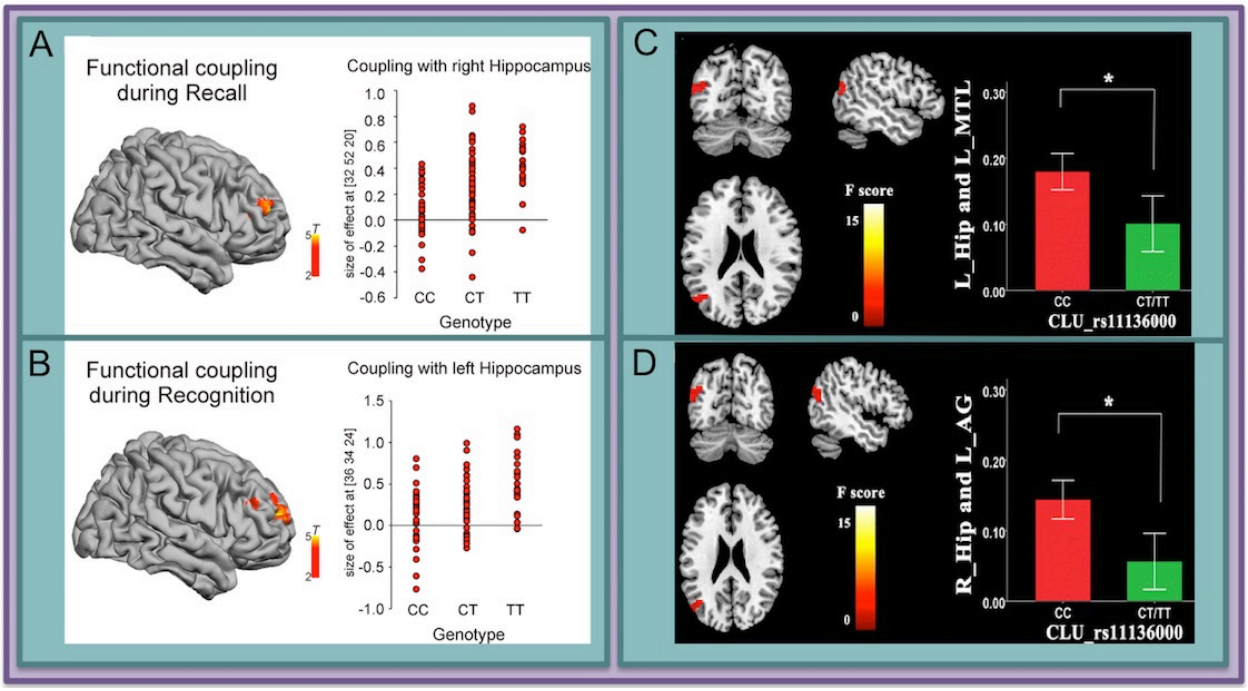


Figure 5.2: A single nucleotide polymorphism within the gene *CLU* that is associated with higher risk for AD has been associated with decreased functional connectivity of the hippocampus in two distinct studies. Functional connectivity between the hippocampus and frontal regions during both recall (A) and recognition (B) is modulated by *CLU* genotype such that individuals who carry the risk allele show lower connectivity in a dose-dependent manner. I another study, individuals who are homozygous for the *CLU* risk allele show greater connectivity between left hippocampus and left medial temporal lobe, as well as higher connectivity between right hippocampus and angular gyrus (D). Panels A and B reprinted (32). Panels C and D reprinted (33).

PICALM, a gene whose protein product is involved in synaptic transmission, has also been linked to imaging phenotypes in both structural and functional imaging (33–36). An epistatic effect of *PICALM* and *BIN1*, another gene involved in synaptic transmission, on amyloid deposition has been reported (36). *BIN1* was also linked to smaller entorhinal cortex and temporal pole volume in a structural imaging study (35). *CR1* has been shown in several studies to be associated with smaller entorhinal cortex volume in both young and older healthy adult subjects (35; 37). Finally, a positron emission tomography (PET) study found that there was a relationship between amyloid deposition and polymorphisms in *ABCA7* and *EPHA1* such that carrying the risk variant of *ABCA7* increases likelihood of amyloid positivity while the low-risk polymorphism of *EPHA1* decreases likelihood of amyloid positivity (38). A more complete

description of imaging studies focused on these GWAS-identified risk genes can be found in Table 5.1. See *Chapter 1* for more details.

Table 5.1: GWAS-Identified Risk Genes for AD: Neuroimaging Modalities in the Literature and Representative References. OR = odds ratio, from (91) ; sMRI = structural magnetic resonance imaging; DWI = diffusion weighted imaging; t-fMRI = task-based functional MRI; rs-fMRI = resting state functional MRI; PET = positron emission tomography

Gene	OR	sMRI	DWI	t-fMRI	rs-fMRI	PET	Comment
CLU	0.86 (0.84 – 0.89)	Bralten et al., 2011a; Stevens et al., 2014 (37; 92)	Braskie et al., 2011 (93)	Erk et al., 2011; Green et al., 2014 (31; 32)	Zhang et al., 2014 (33)		Protein co-chaperone
PICALM	0.87 (0.85 – 0.89)	Biffi et al., 2010; Bralten et al., 2011a; Furney et al., 2011 (34; 35; 37)			Zhang et al., 2014 (33)	Hohman et al., 2013 (36)	Synaptic transmission
CR1	1.18 (1.14 – 1.22)	Biffi et al., 2010; Bralten et al., 2011b (35; 94)					Innate immunity
BIN1	1.22 (1.18 – 1.25)	Biffi et al., 2010 (35)				Hohman et al., 2013 (36)	Synaptic transmission
ABCA7	1.15 (1.11 – 1.19)					Hughes et al., 2014 (38)	Lipid homeostasis
EPHA1	0.90 (0.88 – 0.93)					Hughes et al., 2014 (38)	Adhesion and contact mediated signaling

Relatively little genetic variance is accounted for by differentiating experimental groups based on carrier status of a single risk variant. In the next sections, we will cover polygenic scores and regression-based polygenic modeling approaches. These efforts aim to measure genetic risk as a continuous metric or as a set of predictors capable of revealing important relationships between genetic risk, brain structure and function and preclinical AD.

Polygenic Risk Scores

Combining multiple genetic risk loci into a single metric or score is an attractive way to modernize the candidate gene approach by using the metric or score as your “candidate” rather

than a single gene. Associations between a risk score and, for example, an imaging endophenotype cannot be attributed to a single gene, but these associations may be clinically useful in the effort to better characterize preclinical AD (39). Such metrics are designed on one of two main theoretical bases: first, that multiple risk polymorphisms in the same disease-related biological pathway will be more likely to disrupt normal functioning of that pathway; or, second, that multiple risk polymorphisms affecting various neuronal functions will together predispose or lead to disease. A polygenic risk score (PRS) can be calculated in several ways. Unweighted approaches simply tally the number of known risk alleles carried by a given individual. Weighted risk scores apply a statistic that captures the strength of the relationship between the genetic variant and disease to differentially weight each risk allele. When GWAS data is available, odds ratios are often used to weight risk alleles in a polygenic risk score but other effect size measures can be used (39). Another method of quantifying polygenic risk is assessing genotype patterns and binning subjects by their genotypes at multiple loci. A limitation of this approach is that a large sample is needed in order to have large enough sub-groups for meaningful statistical analysis. Finally, testing for interaction effects, or epistatic effects, between two or more genes is also technically a polygenic approach, although it differs in that risk effects are not additive but rather emerge from specific interactions between loci.

Using a PRS weighted by GWAS-reported odds ratios, Sabuncu and colleagues showed that increased genetic risk for AD was associated with decreased cortical thickness in AD-vulnerable regions, including entorhinal, lateral temporal, inferior parietal and posterior cingulate cortices (Figure 5.3; (40)). In another structural imaging study a large cohort of over 8,000 cognitively healthy older individuals was used to assess the relationship between a GWAS-loci based weighted PRS and several measures including intracranial volume, total brain volume, and hippocampal volume (41). The authors reported that higher PRS was associated with smaller hippocampal volume, a result that remained significant even after removing *APOE* from the PRS. Decreases in fractional anisotropy (FA) have emerged in the *APOE* literature as a

possible early indicator of disease-susceptibility (42; 43). More work is needed to ascertain whether there is an additive effect of AD risk genes on FA, but preliminary efforts in polygenic approaches to account for white matter integrity are promising (44).

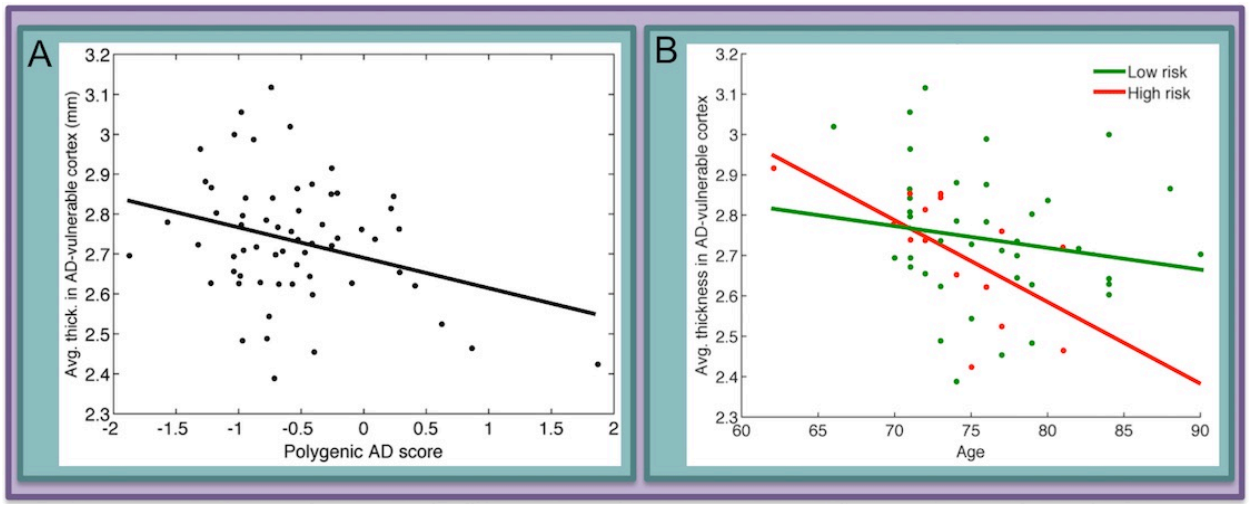


Figure 5.3: Polygenic risk scores have been used to show relationships between aggregate genetic risk for AD and morphological differences in AD-vulnerable cortical regions. A) A polygenic score for AD risk based on 26 common variants was negatively correlated with average thickness in a set of AD-vulnerable cortical regions in healthy older adults. The 26 variants, based on closest gene, were within or near *DAB1*, *CR1*, *BIN1*, *SSB*, *C6orf155*, *ARID18*, *CLU* (two SNPs), *KCNU1*, *MS4A6A*, *C11orf30*, *PICALM*, *CNTN5*, *BCL3* (two SNPs), *PVRL2* (5 SNPs), *TOMM40* (3 SNPs) and *APOE* (40; see Supplementary Table 2). B) The relationship between risk score and cortical thickness was driven by a strong age-associated decline in cortical thickness amongst individuals at highest genetic risk for AD. Panels reprinted (40).

There is also evidence from the functional imaging literature that epistatic effects are detectable. One study tested interactions between single nucleotide polymorphisms (SNPs) from 9 AD risk genes identified in GWASs and found that carrying *BIN1* risk variants and the *PICALM* protective variant was associated with increased amyloid deposition as measured by PET imaging (36). In young adults, it was reported that the effect of *APOE* and *CLU* risk on BOLD signal during an executive attention task was decreased activation of medial temporal structures as genetic risk increased (31). Another study of young adults using resting state fMRI found that an interaction effect between *PICALM* and *CLU* risk modulated hippocampal connectivity (33).

Regression Approaches to Polygenic Risk

The use of predictive regression models in clinical biostatistics is extremely common (45). Neuroimaging genetics presents a unique problem with millions of genetic markers (in whole genome data) that can be used as predictors and many outcome phenotypes of interest. Furthermore, linkage disequilibrium, or the tendency of certain genetic loci to be inherited together, must be considered when using any regression method since many of these models assume that predictors are independent (46). The numerous data reduction or selection methods used in regression analyses can be categorized as follows: stepwise regression, regularized regression, mixed linear modeling, projection and prior biological knowledge (47–51). While the methods are too numerous to review in detail, we highlight a few important perspectives with respect to AD.

Stepwise regression optimizes a linear model by successively removing, adding or alternating between adding and removing predictors. One study specifically demonstrated there is an advantage to using machine-learning based, cross-validated genetic algorithms over stepwise regression to predict conversion from MCI to AD (47). Regularized regression is similar to stepwise in that it assumes that a small number of the predictors will be the most informative. These approaches, like Lasso or sparse regression (e.g., ridge, elastic net), penalize larger models in favor of more parsimonious models. Silver and colleagues used sparse reduced-rank (Lasso) regression to model groups of SNPs that are all within a single biological pathway and calculate the strength of the relationship of that pathway to AD-related neuroimaging phenotypes (48). The authors reported that SNPs belonging to insulin signaling, vascular smooth muscle contract and focal adhesion pathways were the strongest predictors of structural change over 24 months of follow-up. Another study used an elastic net regularization method to explore genetic risk factors for AD affecting the hippocampal surface and found that

APOE and *TOMM40* were associated with hippocampal surface differences in anterior and middle regions (52).

Genome-wide complex trait analysis (GCTA; http://cnsgenomics.com/software/gcta/) is an example of an optimized linear modeling approach to polygenic risk for phenotypes. Developed to determine the portion of variability of a given trait that can be explained by all available SNPs rather than those that survive genome-wide significance, GCTA takes advantage of linear mixed effect modeling to combine fixed effects like age and sex with SNPs as random effects (53). A recent update to the approach ensures that this procedure can be completed in reasonable time despite the high computational demand of considering millions of SNPs and many phenotypes (54). The authors of the updated GCTA approach used a cohort of 1,320 subjects to compute heritability estimates for several structural neuroimaging measures including whole-brain cortical thickness (54). Ridge and colleagues used the GCTA approach to examine the proportion of the variance in AD status explained by 11 known, common genetic risk loci for AD and found that only 8% (standard error 0.03) of phenotypic variance was accounted for by these markers, while 33% (standard error 0.0072) of the variance was due to common SNPs, known and unknown (49). These results suggest that there are many more common AD-associated SNPs that have not been identified yet and that genetic variants that explain a large proportion of phenotypic variance are rare.

To test across many millions of SNP-SNP interactions it is necessary to apply a method that is capable of performing the computationally intensive task of high-dimensional predictor selection. Hibar and colleagues used a machine learning approach that was designed to perform well when the number of predictors is greater than the number of observations, as is the case when examining human SNP data, by ranking the normalized predictors by their correlation to the dependent variable (55). The authors discovered that the volume of a region of the temporal lobe was associated with the interaction between two SNPs across the clinical categories in the ADNI sample. Another study, also using ADNI, reduced the number of SNP-

SNP interactions they tested using a linear regression approach by only testing for interactions between SNPs that were members of a common biological pathway, such as calcium signaling or axon guidance, which were both associated with entorhinal cortex and hippocampal atrophy in their cohort (51). This approach based on prior biological knowledge has been shown to be an effective method of predictor selection (56). Similarly, SNP data reduction using projection techniques like independent component analysis has been used to identify independent groups of genes affecting a given trait (50). Post-hoc pathway analysis of the components then can reveal whether they are enriched for genes related to, for example, as Meda and colleagues found in their ADNI sample, inflammation, diabetes, obesity and cardiovascular disease (50).

Advanced Association Models

In addition to more traditional regression approaches, advanced association models can be used to confront the challenges of working with large datasets in neuroimaging genetics. Canonical correlation is a method for interpreting large cross-covariance matrices that maximizes correlation between linear combinations of pairs of vectors within a given matrix. Sparse canonical correlation takes this process a step further by minimizing the number of features used to find the maximum correlation structure using, for example, the well-known least squares approach (72). Sparse canonical correlation has been used to explore genetic risk factors for AD affecting the hippocampal surface (73). Looking only at AD risk genes as listed in the AlzGene database, the authors of that study found that *APOE* and *TOMM40* were associated with hippocampal surface differences in sparse regions, including anterior and middle areas (73). Variations of the sparse canonical correlation approach have been used in two other studies focused on AD (74; 75). The first used a “knowledge-guided” algorithm that accounted linkage disequilibrium and genetic co-expression networks and examined the relationship between SNPs within *APOE* and amyloid deposition as measured by florbetapir-PET (74). This study identified only a single SNP in *APOE* that was associated with amyloid

deposition, but they argue that their method can be scaled up to genome wide studies. The second study that used a similar approach and also examined *APOE*, discovering an association between a specific SNP and gray matter density in right hippocampus (75).

Limitations

Power: Effect Sizes and Variant Frequency

A major challenge in neuroimaging genetics is sufficiently powering studies to detect hypothesized effects. One problem is the low effect size of common genetic associations to disease in human polygenic disorders (57; 58). An exception to this pattern is the *APOE* locus where a commonly occurring variant is strongly associated to increased AD risk. In fact, *APOE* accounts for a larger amount of the variance in AD heritability than any single known genetic locus in another human neurobehavioral, polygenic disorder. Theoretically, because *APOE* accounts for a relatively large proportion of the heritability variance in AD, it is possible that accurately modeling polygenic risk for AD will be simpler than in other common polygenic neurobehavioral diseases. Thus, AD is an attractive neurological disorder to neuroimaging genetics investigators who are anxious to demonstrate that their field is uniquely positioned to identify early, preclinical predictors of disease.

Today, it is not clear if the underlying genetics of AD are best described as many high-effect rare variants (e.g., *TREM2* or *MAPT*) that, in different individuals, each lead to clinical AD or many low-effect common variants that together in a single individual can lead to clinical AD. To the neuroimaging genetics investigator, there are advantages and disadvantages to a common-variant or rare-variant theory of AD genetics. Of course, rare variants occur in so few individuals that it is difficult to amass a large cohort of carriers. However, increased emphasis on data sharing and access to continuously expanding reservoirs of pooled data means that reasonably sized samples of individuals with specific rare variants may be plausible (given a minor allele frequency of 0.002, a sample of 20,000 subjects would be needed to identify 40

carriers of the *TREM2* risk variant) (59). Often, rare variants associated with a particular disease have a relatively high effect size, which may make differences between carrier groups easier to detect, even at smaller sample sizes. In contrast, carriers of common variants are more easily amassed in large numbers, but investigators need extremely large cohorts to detect the low-effect size association that usually accompanies a disease-related common variant (Figure 5.4). As discussed in previous sections, methods for modeling multiple genetic risk factors in a single experiment are actively being developed and may help to exploit the synergistic predictive power of many low-effect-size common variants. In a thorough analysis of the PRS literature, Dudbridge used heritability estimate, sample size, locus significance threshold and PRS weighting method to generate formulae that allow investigators to estimate the likelihood that future studies will be sufficiently powered (39). The findings indicated that perhaps hundreds of thousands of subjects would be required to make PRS useful at the individual level. Sample sizes are generally not of this magnitude, but they are increasing quickly. Another simulation-based study based on 10,000 cases and controls reported that subjects in the top 5% of genetic risk for hypothetical disease are three to seven times more likely to be affected (60). A three to seven fold increase in risk is certainly clinically useful if not conclusive, as it suggests some individuals may be better candidates for clinical trials and that more frequent follow-up assessments are indicated.

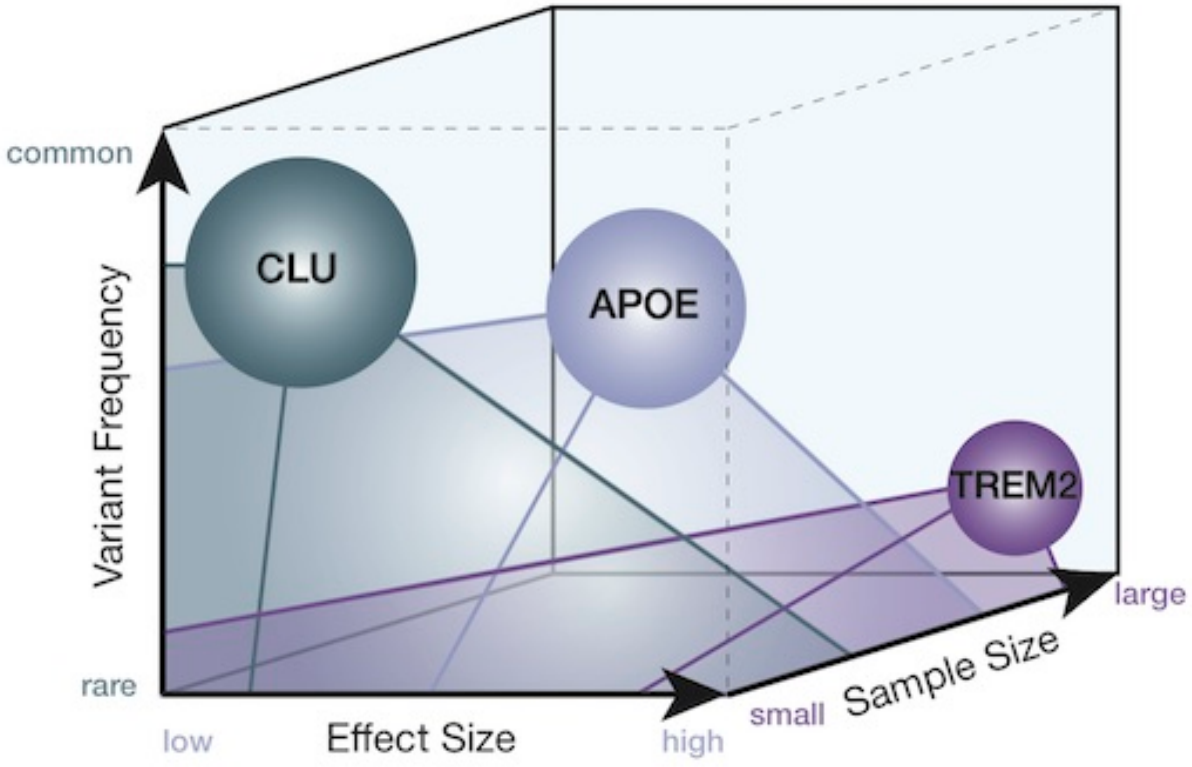


Figure 5.4: Practical and theoretical parameters of genetic risk factors in AD. The relationships between variant/allele frequency, effect size and sample size are such that designing adequately powered studies is challenging. *CLU*, *APOE* and *TREM2* are plotted as representative genes for the following three scenarios: first, a commonly occurring risk variant with a small effect size (*CLU*, risk allele is major allele with frequency at 60% and effect size of 0.86 (91)), second, a moderately common risk variant with a moderate effect size (*APOE* ε4 risk allele frequency is 12-14% with an effect size of 2.5 (27; 91)), and third, a rare variant with a relatively large effect size (*TREM2* risk variant is minor allele with a frequency of 0.2% and effect size of 3 or more (59; 96)). Note that there are no examples of genes in two extremes in this three dimensional space: high frequency variants that have large effect sizes and low frequency variants that have very small effect sizes. The lack of risk variants of the latter description could be due to the practical difficulties of measuring very small risk effects mediated by very uncommon variants.

Cross-sectional Versus Longitudinal Designs

Another major challenge in the field of neuroimaging genetics of AD is the predominant use of cross-sectional experimental designs to uncover the pathophysiological trajectory of AD. In the literature, inferences about the trajectory of AD are overwhelmingly made from cross-sectional studies in which data is collected from each subject only once and all the subjects are

randomly distributed across the age range under investigation with equal number of males and females. This approach makes it particularly difficult to make conclusions on the subject level because cross-sectional studies confound between-subject and within-subject variation (61). Given this limitation, drawing longitudinal conclusions based on cross-sectional evidence, even from many studies, is precarious and should be done cautiously (62).

The importance of early detection in neurodegenerative diseases like AD is illustrated by the extensive neuronal loss already present in mildly symptomatic AD patients (63). In addition, recent work has established that AD risk genes are associated with differences in brain structure and function even in young people, including children and infants (64; 65). In light of this, how can investigators optimize experimental design for the study of AD risk and preclinical AD? Following subjects in longitudinal designs better allows for making inferences about disease trajectory but these studies are difficult in practice. In the modern pro-collaboration atmosphere though, multi-cohort longitudinal designs are feasible because many sites can each collect longitudinal data on a reasonably small number of subjects and then, assuming proper standardization and oversight is in place, these subjects can be combined to create a much larger cohort. The Alzheimer's Disease Neuroimaging Initiative (ADNI) is a good example of a multi-center effort in neuroimaging genetics of AD (66; 67). Optimized longitudinal mapping of AD progression will help identify individuals who are in the preclinical phase of AD. These individuals are likely to benefit the most from intervention, especially a progression-slowing or halting drug. Such a drug is not available today, but the accurate and precise definition of preclinical AD will be an essential component to the success of any candidate.

Clinical Utility of GWAS Loci: The Search for Causal Variants

The causal variants that give rise to the *APOE*ε4 allele are known polymorphisms at rs429358 and rs7412. Variants at these loci alter the structure and function of the translated APOE protein (68). In fact, *APOE*ε4 "structure correctors", which make protein products of

*APOE*ε4 behave like the more common protein products of *APOE*ε3 are currently being developed as a possible treatment for AD (69). In contrast, many of the GWAS-identified AD risk loci are located in intronic (*CLU*, *ABCA7*) or intragenic (*BIN1*, *EPHA1*) regions with no evidence that variants affect protein structure or function. An intragenic region may play some regulatory function, but in the cases of *EPHA1* and *BIN1* there is little evidence of conservation of these intragenic regions, which makes a regulatory role in genetic expression unlikely (70). There is even some debate over whether genes are correctly identified when significantly associated loci reside in non-coding regions. The common approach is to report the SNP as related to the nearest gene, but this is not necessarily the case. The search for the causal variants for these genes and for genes implicated in other common disease by GWASs is still ongoing (71). Ostensibly, the causal variant for one of these genetic risk loci will be a polymorphism in high linkage disequilibrium with the GWAS locus. In addition, the polymorphism should affect the downstream structure or function of the gene's RNA or protein product. The utility of GWAS-identified risk genes as potential drug targets is limited without first identifying the causal variants driving the association at each locus. One important step in this effort is the development of a functionally annotated genome and the tools to explore it, such as ENCODE (https://www.encodeproject.org). Using ENCODE investigators can quickly discover basic functional information about a locus of interest, perhaps one they identified in a GWAS. The functional elements annotated in the ENCODE project help investigators distinguish between, for example, regulatory elements, close-range promoters and genes that are likely to be transcribed (determined using RNA-seq and similar techniques). ENCODE is an excellent example of a large-scale collaborative project that will enhance the scientific community's ability to interpret genetic association signals.

Mechanistic Interpretations and Neuroimaging Genetics

The incorporation of human genetics into neuroimaging studies has identified brain traits that are associated with specific genetic variants or, more germane to this review, with genetic risk scores. However, as described in the text that precedes this section, the genetic loci are often identified via GWAS and thus, the causal variant is not known. This inherently limits the mechanistic insights researchers are able to gain from neuroimaging genetics studies of this kind. For *APOE*, for which there is no ambiguity about causal variants, neuroimaging has revealed that carriage of the *APOE*ε4 allele is related to increased amyloid deposition as measured by PET imaging which, in turn, is related to neuronal death and a higher rate of cortical thinning in AD-vulnerable regions when compared to matched controls who do not carry the risk variant. Saykin and colleagues (2015) describe a multi-step process to move from genetic signals to targeted therapeutics in which genetics and neuroimaging intersect at the first step (discovering genetic loci that are robustly associated with a relevant trait) and the final step (identifying individuals most likely to benefit from experimental therapies) (72). The middle steps include identification of causal genes, testing hypothesized mechanisms in model systems and developing therapeutics that act on these mechanisms. Thus, we believe the salient point is that neuroimaging genetics research is essential to the development and execution of therapeutic hypotheses, even if, in isolation, these studies do not always yield new mechanistic insights.

Generalizability Across Ancestries

It is important to recognize that all the largest AD GWASs used large cohorts of Caucasian European or American subjects. This creates a potential problem with generalizability to other ancestry groups, especially that of African ancestry (73). While there are published GWASs examining AD genetics in minority ancestral groups, one only of these, focused on African American participants, has topped 1,000 participants in the case and control groups ((74); see Table 1 in (75)). Thus, these groups remain understudied compared to the

very large GWASs with non-Hispanic Caucasian participants. The genetic loci implicated by studies of Caucasians might fail to replicate in a cohort of subjects from a different ethnic background due to population specific variants, differing patterns of linkage disequilibrium or even a heterogeneous genetic basis of AD in different ethnic groups (76). To illustrate this issue, consider that many small GWAS studies have tried to replicate the association of *CLU* with AD in non-Caucasian cohorts. The results of these studies indicate that there is an association between *CLU* and AD in Chinese cohorts, but not in cohorts of non-white Americans or Europeans (29; 77; 78). The limited generalization of results from large published GWASs in AD is a problem and a greater effort must be made to amass comparably large samples of different ancestral groups for new association studies. This effort may lead to the identification of certain genes that are associated with AD regardless of genetic background. These genes would be good candidates for increased research resources and drug targeting due to their greater generalizability. Also, importantly, further exploration of the genetic basis of AD in people of African and Hispanic descent may help elucidate any biological bases for the epidemiological differences observed in these ethnic groups, including higher incidence and earlier onset of AD (79).

Small Sample Sizes: Consequences for Neuroimaging Genetics

As eloquently described by Button and colleagues (2013), small sample sizes in neuroimaging studies decrease statistical power which leads to a decreased rate of detectable true positive results while leaving the rate of false positives unchanged (80). This has the effect of increasing the likelihood that a significant result is, in fact, spurious. Small sample sizes also bias studies toward large effect size results, as these are the only results that can be significant given the power limitations. The latter phenomenon has been dubbed the “winner’s curse” and leads to studies that are very difficult to replicate (80). Given these known problems, why are neuroimaging studies with small samples still (albeit less and less so) prevalent? This is related

to the relatively high cost of acquisition of neuroimaging data, the currently accepted need to "pilot" and publish new paradigms and techniques before formal funding for large-scale studies can be won and also immense pressure, especially on young investigators, to publish frequently (81). Taken together, it is clear that sample size is a very important consideration when performing a neuroimaging genetics study and robust power analyses are a crucial component of any research program.

Implications for Clinical Trials

Despite major challenges related to statistical power, polygenic risk modeling and generalizability, the field of neuroimaging genetics is poised to play a major role in the development of effective treatments for AD. Phase 3 AD treatment trials in humans have all had negative outcomes, not meeting endpoints despite promising data in model organisms and in preceding trial phases (82; 83). This high failure rate may be the result, in part, of heterogeneity across the study participants enrolled in these clinical trials. One source of heterogeneity is neuropathological variation. The clinical-neuropathological correspondence of AD (both pure and AD-vascular mixed pathology) occurs in about 87% of clinical AD cases that come to autopsy (84). Thus, more than 10% of clinically diagnosed AD patients actually suffer from some other neurodegenerative disorder, such as frontotemporal lobar degeneration (FTLD) or corticobasal degeneration (CBD). It is reasonable to assume that subjects with each of these diseases, from pure AD and mixed AD pathology to FTLC and CBD, will respond differently, if at all, to potential treatments that target a single molecular species, like Aβ oligomers or plaques. One way to help minimize neuropathological heterogeneity is through the use of PET imaging. The use of PET imaging of Aβ and tau as a pre-screening technique in clinical trials, while costly, will allow investigators to amass a more neuropathologically homogeneous cohort. Indeed, neuropathological pre-screening using PET imaging is currently being implemented for the first time as part of the Anti-Amyloid Treatment in Asymptomatic AD (A4) trial, the protocol of

which requires a positive Aβ florbetapir-PET scan for enrollment into the treatment arm (85). Another imaging-based method for neuropathological prescreening is diffusion-weighted MRI which can be used to estimate the severity of vascular pathology (86).

Neuropathological differences are not the only source of heterogeneity in clinical trial subjects. It is also important to consider the heterogeneity of the underlying genetics in each individual subject. Depending on the mechanism of the candidate drug, it is possible there will be some variation of response in trial participants with different genetic risk factors for AD. (87). Also, it is likely that by examining genetic risk, the ability to identify asymptomatic individuals who will progress to show cognitive decline is improved. Thus, investigators should consider implementing genetic prescreening measures that select for clinical trial participants that have certain genetic risk factors for AD (72). Clinical trials in AD have already started to use carriage of one or two risk variants (*APOE*, *TOMM40*) as a prescreening measure (88). Kohannim and colleagues published a study in which they tested the hypothesis that a *polygenic* screening protocol would decrease the sample size necessary to detect an effect in a hypothetical trial (89). The authors ranked 394 cognitively healthy and MCI ADNI subjects in order of decreasing polygenic risk score, calculated based on multiplying risk alleles for *APOE*, *CLU*, *CR1* and *PICALM* by the logarithm of the odds ratios reported for each gene in GWASs. They found that by selecting only the top 15% of subjects with highest genetic risk, the required sample size to show differences in temporal lobe atrophy decreased from 142 to 69 (89). This is excellent evidence that genetic pre-screening would increase statistical power in trials. Binning participants by genetic risk may well be the next frontier in AD clinical trial design.

Another important role for neuroimaging genetics in clinical trials is the development of hard, non-cognitive endpoints to assess treatment efficacy (90). Most AD trials to date have used soft endpoints such as paper-and-pencil memory measures or a composite dementia severity scores (82; 83). However, as trials shift their focus to preclinical individuals who are asymptomatic cognitive endpoints will no longer be appropriate. Thus, neuroimaging-based

biomarkers as well as others, such as CSF analyte levels, which capture pathological changes that precede cognitive decline, must be refined for use as clinical endpoints (90).

A neuroimaging genetics approach uses minimally invasive technologies to characterize the earliest pathophysiological changes in preclinical AD. In the effort to prevent and treat AD, the proximal goal of combining multiple genetic factors, neuroimaging biomarkers and other measures to estimate AD-risk is to pre-select clinical trial and research participants. The distal goal is to provide more detailed prognoses in the clinic during the preclinical phase that can be used to create optimized treatment plans and enroll ideal candidates in specific clinical trials.

Chapter 5 References

1. Bateman RJ, Xiong C, Benzinger TLS, Fagan AM, Goate A, Fox NC, *et al.* (2012): Clinical and biomarker changes in dominantly inherited Alzheimer's disease. *N Engl J Med* 367: 795–804.

2. Knopman DS, Jack CR, Wiste HJ, Weigand SD, Vemuri P, Lowe V, *et al.* (2012): Short-term clinical outcomes for stages of NIA-AA preclinical Alzheimer disease. *Neurology* 78: 1576–82.

3. Mormino EC, Betensky RA, Hedden T, Schultz AP, Ward A, Huijbers W, *et al.* (2014): Amyloid and APOE ε4 interact to influence short-term decline in preclinical Alzheimer disease. *Neurology* 82: 1760–7.

4. Sperling RA, Aisen PS, Beckett LA, Bennett DA, Craft S, Fagan AM, *et al.* (2011): Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* 7: 280–92.

5. Ganguli M, Lee C-W, Snitz BE, Hughes TF, McDade EM, Chang C-CHHow well do MCI criteria predict progression to severe cognitive impairment and dementia? *Alzheimer Dis Assoc Disord* 28: 113–21.

6. Albert MS, DeKosky ST, Dickson D, Dubois B, Feldman HH, Fox NC, *et al.* (2011): The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* 7: 270–9.

7. Lu PH, Thompson PM, Leow A, Lee GJ, Lee A, Yanovsky I, *et al.* (2011): Apolipoprotein E genotype is associated with temporal and hippocampal atrophy rates in healthy elderly adults: a tensor-based morphometry study. *J Alzheimers Dis* 23: 433–42.

8. Taylor JL, Scanlon BK, Farrell M, Hernandez B, Adamson MM, Ashford JW, *et al.* (2014): APOE-epsilon4 and aging of medial temporal lobe gray matter in healthy adults older than 50 years. *Neurobiol Aging* 35: 2479–85.

9. Donix M, Burggren AC, Suthana NA, Siddarth P, Ekstrom AD, Krupa AK, *et al.* (2010): Longitudinal changes in medial temporal cortical thickness in normal subjects with the APOE-4 polymorphism. *Neuroimage* 53: 37–43.

10. Honea RA, Swerdlow RH, Vidoni ED, Goodwin J, Burns JM (2010): Reduced gray matter volume in normal adults with a maternal family history of Alzheimer disease. *Neurology* 74: 113–20.

11. Heise V, Filippini N, Trachtenberg AJ, Suri S, Ebmeier KP, Mackay CE (2014): Apolipoprotein E genotype, gender and age modulate connectivity of the hippocampus in healthy adults. *Neuroimage* 98: 23–30.

12. Machulda MM, Jones DT, Vemuri P, McDade E, Avula R, Przybelski S, *et al.* (2011): Effect of APOE ε4 status on intrinsic network connectivity in cognitively normal elderly subjects. *Arch Neurol* 68: 1131–6.

13. Sheline YI, Morris JC, Snyder AZ, Price JL, Yan Z, D'Angelo G, *et al.* (2010): APOE4 allele disrupts resting state fMRI connectivity in the absence of amyloid plaques or decreased CSF Aβ42. *J Neurosci* 30: 17035–40.

14. Fleisher AS, Chen K, Liu X, Ayutyanont N, Roontiva A, Thiyyagura P, *et al.* (2013): Apolipoprotein E ε4 and age effects on florbetapir positron emission tomography in healthy aging and Alzheimer disease. *Neurobiol Aging* 34: 1–12.

15. Fleisher AS, Sherzai A, Taylor C, Langbaum JBS, Chen K, Buxton RB (2009): Resting-state BOLD networks versus task-associated functional MRI for distinguishing Alzheimer's disease risk groups. *Neuroimage* 47: 1678–90.

16. Wang L, Roe CM, Snyder AZ, Brier MR, Thomas JB, Xiong C, *et al.* (2012): Alzheimer disease family history impacts resting state functional connectivity. *Ann Neurol* 72: 571–7.

17. Cherbuin N, Leach LS, Christensen H, Anstey KJ (2007): Neuroimaging and APOE genotype: a systematic qualitative review. *Dement Geriatr Cogn Disord* 24: Karger Publishers348–62.

18. Bookheimer S, Burggren A (2009): APOE-4 genotype and neurophysiological vulnerability to Alzheimer's and cognitive aging. *Annu Rev Clin Psychol* 5: 343–62.

19. Trachtenberg AJ, Filippini N, Mackay CE (2012): The effects of APOE-ε4 on the BOLD response. *Neurobiol Aging* 33: 323–34.

20. Sheline YI, Raichle ME (2013): Resting state functional connectivity in preclinical Alzheimer's disease. *Biol Psychiatry* 74: 340–7.

21. Sperling R, Mormino E, Johnson K (2014): The Evolution of Preclinical Alzheimer's Disease: Implications for Prevention Trials. *Neuron* 84: 608–622.

22. McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR, Kawas CH, *et al.* (2011): The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* 7: 263–9.

23. Bookheimer SY, Strojwas MH, Cohen MS, Saunders AM, Pericak-Vance MA, Mazziotta JC, Small GW (2000): Patterns of brain activation in people at risk for Alzheimer's disease. *N Engl J Med* 343: 450–6.

24. Krystal JH, State MW (2014): Psychiatric disorders: diagnosis to therapy. *Cell* 157: 201–14.

25. May PC, Lampert-Etchells M, Johnson SA, Poirier J, Masters JN, Finch CE (1990): Dynamics of gene expression for a hippocampal glycoprotein elevated in Alzheimer's disease and in response to experimental lesions in rat. *Neuron* 5: 831–9.

26. Lambert J-C, Heath S, Even G, Campion D, Sleegers K, Hiltunen M, *et al.* (2009): Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. *Nat Genet* 41: 1094–9.

27. Harold D, Abraham R, Hollingworth P, Sims R, Gerrish A, Hamshere ML, *et al.* (2009): Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. *Nat Genet* 41: 1088–93.

28. Seshadri S, Fitzpatrick AL, Ikram MA, DeStefano AL, Gudnason V, Boada M, *et al.* (2010): Genome-wide analysis of genetic loci associated with Alzheimer disease. *JAMA* 303: 1832–40.

29. Jun G, Naj AC, Beecham GW, Wang L-S, Buros J, Gallins PJ, *et al.* (2010): Meta-analysis confirms CR1, CLU, and PICALM as alzheimer disease risk loci and reveals interactions with APOE genotypes. *Arch Neurol* 67: 1473–84.

30. Corneveaux JJ, Myers AJ, Allen AN, Pruzin JJ, Ramirez M, Engel A, *et al.* (2010): Association of CR1, CLU and PICALM with Alzheimer's disease in a cohort of clinically characterized and neuropathologically verified individuals. *Hum Mol Genet* 19: 3295–301.

31. Green AE, Gray JR, Deyoung CG, Mhyre TR, Padilla R, Dibattista AM, William Rebeck G (2014): A combined effect of two Alzheimer's risk genes on medial temporal activity during executive attention in young adults. *Neuropsychologia* 56: 1–8.

32. Erk S, Meyer-Lindenberg A, Opitz von Boberfeld C, Esslinger C, Schnell K, Kirsch P, *et al.* (2011): Hippocampal function in healthy carriers of the CLU Alzheimer's disease risk variant. *J Neurosci* 31: 18180–4.

33. Zhang P, Qin W, Wang D, Liu B, Zhang Y, Jiang T, Yu C (2014): Impacts of PICALM and CLU variants associated with Alzheimer's disease on the functional connectivity of the hippocampus in healthy young adults. *Brain Struct Funct*. doi: 10.1007/s00429-014-0738-4.

34. Furney SJ, Simmons A, Breen G, Pedroso I, Lunnon K, Proitsi P, *et al.* (2011): Genome-wide association with MRI atrophy measures as a quantitative trait locus for Alzheimer's disease. *Mol Psychiatry* 16: 1130–8.

35. Biffi A, Anderson CD, Desikan RS, Sabuncu M, Cortellini L, Schmansky N, *et al.* (2010): Genetic variation and neuroimaging measures in Alzheimer disease. *Arch Neurol* 67: 677–85.

36. Hohman TJ, Koran ME, Thornton-Wells T (2013): Epistatic genetic effects among Alzheimer's candidate genes. *PLoS One* 8: e80839.

37. Bralten J, Franke B, Arias-Vásquez A, Heister A, Brunner HG, Fernández G, Rijpkema M (2011): CR1 genotype is associated with entorhinal cortex volume in young healthy adults. *Neurobiol Aging* 32: 2106.e7–11.

38. Hughes TM, Lopez OL, Evans RW, Kamboh MI, Williamson JD, Klunk WE, *et al.* (2014): Markers of cholesterol transport are associated with amyloid deposition in the brain. *Neurobiol Aging* 35: 802–7.

39. Dudbridge F (2013): Power and predictive accuracy of polygenic risk scores. *PLoS Genet* 9: e1003348.

40. Sabuncu MR, Buckner RL, Smoller JW, Lee PH, Fischl B, Sperling RA (2012): The association between a polygenic Alzheimer score and cortical thickness in clinically normal subjects. *Cereb Cortex* 22: 2653–61.

41. Chauhan G, Adams HHH, Bis JC, Weinstein G, Yu L, Töglhofer AM, *et al.* (2015): Association of Alzheimer disease GWAS loci with MRI-markers of brain aging. *Neurobiol Aging*. doi: 10.1016/j.neurobiolaging.2014.12.028.

42. Westlye ET, Hodneland E, Haász J, Espeseth T, Lundervold A, Lundervold AJ (2012): Episodic memory of APOE ε4 carriers is correlated with fractional anisotropy, but not cortical thickness, in the medial temporal lobe. *Neuroimage* 63: 507–16.

43. Heise V, Filippini N, Ebmeier KP, Mackay CE (2011): The APOE ε4 allele modulates brain white matter integrity in healthy adults. *Mol Psychiatry* 16: Macmillan Publishers Limited908–16.

44. Kohannim O, Jahanshad N, Braskie MN, Stein JL, Chiang M-C, Reese AH, *et al.* (2012): Predicting white matter integrity from multiple common genetic variants. *Neuropsychopharmacology* 37: 2012–9.

45. Schrodi SJ, Mukherjee S, Shan Y, Tromp G, Sninsky JJ, Callear AP, *et al.* (2014): Genetic-based prediction of disease traits: prediction is very difficult, especially about the future. *Front Genet* 5: 162.

46. Devlin B, Roeder K, Wasserman L (2003): Analysis of multilocus models of association. *Genet Epidemiol* 25: 36–47.

47. Johnson P, Vandewater L, Wilson W, Maruff P, Savage G, Graham P, *et al.* (2014): Genetic algorithm with logistic regression for prediction of progression to Alzheimer's disease. *BMC Bioinformatics* 15 Suppl 1: S11.

48. Silver M, Janousova E, Hua X, Thompson PM, Montana G (2012): Identification of gene pathways implicated in Alzheimer's disease using longitudinal imaging phenotypes with sparse regression. *Neuroimage* 63: 1681–94.

49. Ridge PG, Mukherjee S, Crane PK, Kauwe JSK (2013): Alzheimer's disease: analyzing the missing heritability. *PLoS One* 8: e79771.

50. Meda SA, Narayanan B, Liu J, Perrone-Bizzozero NI, Stevens MC, Calhoun VD, *et al.* (2012): A large scale multivariate parallel ICA method reveals novel imaging-genetic relationships for Alzheimer's disease in the ADNI cohort. *Neuroimage* 60: 1608–21.

51. Meda SA, Koran MEI, Pryweller JR, Vega JN, Thornton-Wells TA (2013): Genetic interactions associated with 12-month atrophy in hippocampus and entorhinal cortex in Alzheimer's Disease Neuroimaging Initiative. *Neurobiol Aging* 34: 1518.e9–18.

52. Wan J, Kim S, Inlow M, Nho K, Swaminathan S, Risacheri SL, *et al.* (2011): Hippocampal surface mapping of genetic risk factors in AD via sparse learning models. *Med Image Comput Comput Assist Interv* 14: 376–83.

53. Yang J, Lee SH, Goddard ME, Visscher PM (2011): GCTA: a tool for genome-wide complex trait analysis. *Am J Hum Genet* 88: 76–82.

54. Ge T, Nichols TE, Lee PH, Holmes AJ, Roffman JL, Buckner RL, *et al.* (2015): Massively expedited genome-wide heritability analysis (MEGHA). *Proc Natl Acad Sci*201415603.

55. Hibar DP, Stein JL, Jahanshad N, Kohannim O, Toga AW, McMahon KL, *et al.* (2013): Exhaustive search of the SNP-sNP interactome identifies epistatic effects on brain volume in two cohorts. *Med Image Comput Comput Assist Interv* 16: 600–7.

56. Kerr WT, Douglas PK, Anderson A, Cohen MS (2014): The utility of data-driven feature selection: re: Chu et al. 2012. *Neuroimage* 84: 1107–10.

57. Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorff LA, Hunter DJ, *et al.* (2009): Finding the missing heritability of complex diseases. *Nature* 461: 747–53.

58. Wray NR, Yang J, Hayes BJ, Price AL, Goddard ME, Visscher PM (2013): Pitfalls of predicting complex traits from SNPs. *Nat Rev Genet* 14: Nature Publishing Group, a division of Macmillan Publishers Limited. All Rights Reserved.507–15.

59. Jonsson T, Stefansson H, Steinberg S, Jonsdottir I, Jonsson P V, Snaedal J, *et al.* (2013): Variant of TREM2 associated with the risk of Alzheimer's disease. *N Engl J Med* 368: 107–16.

60. Wray NR, Goddard ME, Visscher PM (2007): Prediction of individual genetic risk to disease from genome-wide association studies. *Genome Res* 17: 1520–8.

61. Thompson WK, Hallmayer J, O'Hara R (2011): Design considerations for characterizing psychiatric trajectories across the lifespan: application to effects of APOE-ε4 on cerebral cortical thickness in Alzheimer's disease. *Am J Psychiatry* 168: 894–903.

62. Kraemer HC, Yesavage JA, Taylor JL, Kupfer D (2000): How can we learn about developmental processes from cross-sectional studies, or can we? *Am J Psychiatry* 157: 163–71.

63. Jack CR, Knopman DS, Jagust WJ, Shaw LM, Aisen PS, Weiner MW, *et al.* (2010): Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. *Lancet Neurol* 9: 119–28.

64. Shaw P, Lerch JP, Pruessner JC, Taylor KN, Rose AB, Greenstein D, *et al.* (2007): Cortical morphology in children and adolescents with different apolipoprotein E gene polymorphisms: an observational study. *Lancet Neurol* 6: 494–500.

65. Dean DC, Jerskey BA, Chen K, Protas H, Thiyyagura P, Roontiva A, *et al.* (2014): Brain differences in infants at differential genetic risk for late-onset Alzheimer disease: a cross-sectional imaging study. *JAMA Neurol* 71: 11–22.

66. Manning EN, Barnes J, Cash DM, Bartlett JW, Leung KK, Ourselin S, Fox NC (2014): APOE ε4 is associated with disproportionate progressive hippocampal atrophy in AD. *PLoS One* 9: e97608.

67. Nho K, Corneveaux JJ, Kim S, Lin H, Risacher SL, Shen L, *et al.* (2013): Whole-exome sequencing and imaging genetics identify functional variants for rate of change in hippocampal volume in mild cognitive impairment. *Mol Psychiatry* 18: 781–7.

68. Mahley RW, Weisgraber KH, Huang Y (2009): Apolipoprotein E: structure determines function, from atherosclerosis to Alzheimer's disease to AIDS. *J Lipid Res* 50 Suppl: S183–8.

69. Chen H-K, Liu Z, Meyer-Franke A, Brodbeck J, Miranda RD, McGuire JG, *et al.* (2012): Small molecule structure correctors abolish detrimental effects of apolipoprotein E4 in cultured neurons. *J Biol Chem* 287: 5253–66.

70. Morgan K, Carrasquillo MM (2013): *Genetic Variants in Alzheimer's Disease*. . New York: Springer Science+Business Media.

71. Cirulli ET, Goldstein DB (2010): Uncovering the roles of rare variants in common disease through whole-genome sequencing. *Nat Rev Genet* 11: Nature Publishing Group415–25.

72. Saykin AJ, Shen L, Yao X, Kim S, Nho K, Risacher SL, *et al.* (2015): Genetic studies of quantitative MCI and AD phenotypes in ADNI: Progress, opportunities, and plans. *Alzheimers Dement* 11: 792–814.

73. Marigorta UM, Navarro A (2013): High trans-ethnic replicability of GWAS results implies common causal variants. *PLoS Genet* 9: e1003566.

74. Reitz C, Jun G, Naj A, Rajbhandary R, Vardarajan BN, Wang L-S, *et al.* (2013): Variants in the ATP-binding cassette transporter (ABCA7), apolipoprotein E ϵ4,and the risk of late-onset Alzheimer disease in African Americans. *JAMA* 309: American Medical Association1483–92.

75. Reitz C, Mayeux R (2014): Genetics of Alzheimer's disease in Caribbean Hispanic and African American populations. *Biol Psychiatry* 75: 534–41.

76. Tian C, Gregersen PK, Seldin MF (2008): Accounting for ancestry: population substructure and genome-wide association studies. *Hum Mol Genet* 17: R143–50.

77. Yu J-T, Li L, Zhu Q-X, Zhang Q, Zhang W, Wu Z-C, *et al.* (2010): Implication of CLU gene polymorphisms in Chinese patients with Alzheimer's disease. *Clin Chim Acta* 411: 1516–9.

78. Chen LH, Kao PYP, Fan YH, Ho DTY, Chan CSY, Yik PY, *et al.* (2012): Polymorphisms of CR1, CLU and PICALM confer susceptibility of Alzheimer's disease in a southern Chinese population. *Neurobiol Aging* 33: 210.e1–7.

79. Gurland BJ, Wilder DE, Lantigua R, Stern Y, Chen J, Killeffer EH, Mayeux R (1999): Rates of dementia in three ethnoracial groups. *Int J Geriatr Psychiatry* 14: 481–93.

80. Button KS, Ioannidis JPA, Mokrysz C, Nosek BA, Flint J, Robinson ESJ, Munafò MR (2013): Power failure: why small sample size undermines the reliability of neuroscience. *Nat Rev Neurosci* 14: 365–76.

81. Poline J-B, Breeze JL, Ghosh S, Gorgolewski K, Halchenko YO, Hanke M, *et al.* (2012): Data sharing in neuroimaging research. *Front Neuroinform* 6: 9.

82. Salloway S, Sperling R, Fox NC, Blennow K, Klunk W, Raskind M, *et al.* (2014): Two phase 3 trials of bapineuzumab in mild-to-moderate Alzheimer's disease. *N Engl J Med* 370: 322–33.

83. Doody RS, Thomas RG, Farlow M, Iwatsubo T, Vellas B, Joffe S, *et al.* (2014): Phase 3 trials of solanezumab for mild-to-moderate Alzheimer's disease. *N Engl J Med* 370: 311–21.

84. Schneider JA, Arvanitakis Z, Leurgans SE, Bennett DA (2009): The neuropathology of probable Alzheimer disease and mild cognitive impairment. *Ann Neurol* 66: 200–208.

85. Sperling RA, Rentz DM, Johnson KA, Karlawish J, Donohue M, Salmon DP, Aisen P (2014): The A4 study: stopping AD before symptoms begin? *Sci Transl Med* 6: 228fs13.

86. Mayzel-Oreg O, Assaf Y, Gigi A, Ben-Bashat D, Verchovsky R, Mordohovitch M, *et al.* (2007): High b-value diffusion imaging of dementia: application to vascular dementia and alzheimer disease. *J Neurol Sci* 257: Elsevier105–13.

87. Roden DM, George AL (2002): The genetic basis of variability in drug responses. *Nat Rev Drug Discov* 1: 37–44.

88. TOMMORROW Study (n.d.).: *Alzheimer's Prev Initiat*. Retrieved from http://tommorrowstudy.com.

89. Kohannim O, Hua X, Rajagopalan P, Hibar DP, Jahanshad N, Grill JD, *et al.* (2013): Multilocus genetic profiling to empower drug trials and predict brain atrophy. *NeuroImage Clin* 2: 827–35.

90. Cash DM, Rohrer JD, Ryan NS, Ourselin S, Fox NC (2014): Imaging endpoints for clinical trials in Alzheimer's disease. *Alzheimers Res Ther* 6: 87.

91. Lambert JC, Ibrahim-Verbaas CA, Harold D, Naj AC, Sims R, Bellenguez C, *et al.* (2013): Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. *Nat Genet* 45: Nature Publishing Group, a division of Macmillan Publishers Limited. All Rights Reserved.1452–8.

92. Stevens BW, DiBattista AM, William Rebeck G, Green AE (2014): A gene-brain-cognition pathway for the effect of an Alzheimer's risk gene on working memory in young adults. *Neuropsychologia* 61: 143–9.

93. Braskie MN, Jahanshad N, Stein JL, Barysheva M, McMahon KL, de Zubicaray GI, *et al.* (2011): Common Alzheimer's disease risk variant within the CLU gene affects white matter microstructure in young adults. *J Neurosci* 31: 6764–70.

94. Bralten J, Franke B, Arias-Vásquez A, Heister A, Brunner HG, Fernández G, Rijpkema M (2011): CR1 genotype is associated with entorhinal cortex volume in young healthy adults. *Neurobiol Aging* 32: 2106.e7–11.

95. Trachtenberg AJ, Filippini N, Ebmeier KP, Smith SM, Karpe F, Mackay CE (2012): The effects of APOE on the functional architecture of the resting brain. *Neuroimage* 59: 565–72.

96. Guerreiro R, Wojtas A, Bras J, Carrasquillo M, Rogaeva E, Majounie E, *et al.* (2013): TREM2 variants in Alzheimer's disease. *N Engl J Med* 368: 117–27.

CONCLUSION

Neurodegenerative diseases present unique challenges in biomedical research. Long preclinical prodromes precede the emergence of symptoms and, thus, clinical diagnosis is a mid-to-late event in the course of the disease (1). Because of this, there has been a rapid expansion of research on the preclinical phase of neurodegenerative diseases. The preclinical phase is likely the ideal time to provide interventions and therapy, but in the absence of clinical symptoms preclinical disease is difficult to identify. In Alzheimer's disease (AD), the study the dominantly inherited familial AD has been crucial to elucidating the preclinical changes that occur in the brain (2).

Familial AD, however, is much less common than late-onset, sporadic AD which accounts for 99% of all AD cases (3). In the research described in this volume, we used genetic risk for late-onset, sporadic AD to study healthy people at elevated risk for AD in the future. In Chapters 2 and 4 we found that greater genetic risk for AD is associated with functional and structural changes in key brain regions known to be involved in the earliest pathological changes in AD. The results of these studies, performed with healthy older adult volunteers, may be driven by preclinical changes in the elevated AD-risk group. We cannot, however, definitively determine the presence preclinical disease in our research participants, which limits our interpretation. In Chapter 3, we were able to uncover stable differences in intrinsic connectivity architecture in young adults based on genetic risk for AD. These results elucidate the functional effects of different *APOE* genotypes in young people, but they illustrate how challenging it is to interpret the results of neuroimaging genetics studies. It is clear, based on the work described in this volume, that characterizing patterns of biomarker change in longitudinal research across the lifespan is crucial to tracking gene-biomarker associations and identifying changes in these associations that might be signs of imminent clinical decline. When we discover the neural consequences of genetic risk for AD in young and middle-aged adults we will improve our ability

to use a neuroimaging genetics approach in older, healthy adults to identify the preclinical phase of AD.

Delaying or preventing the onset of AD would have a huge impact as AD is an extremely costly disease, requiring $226 billion in resources in the United States in 2015 (4). The path to effective AD intervention has two key elements: first, the development of effective drugs and second, the identification of individuals who will benefit from those drugs. The latter effort is the driving principle of the work described in this volume. By studying genetic risk for disease and preclinical populations, we aim to push the threshold for early diagnosis further and further toward the true, silent onset of the disease in the brain.

To best illustrate the pressing need for early diagnosis and effective interventions in AD, we must consider the disease in the context of the global aging phenomenon. Globalization and the demographics of aging are inextricably intertwined. Economic opportunity, increased emphasis on education (especially for women), health literacy and adequate medical care are all key features of a developed, globally competitive country. They are also the features that lead to rapid aging within a population. In the “developed” environment, fertility rates decline while people live increasingly longer lives, creating a population whose older subset is growing faster than any other age bracket. This phenomenon has been observed in the United States, many European countries and Japan. In addition to their ever-expanding elderly populations, these countries also share a method for adapting economically to demographic shifts toward older age groups. They look outward and utilize the resources of countries where young, cheap workers abound; countries like China and the Philippines, and now India. As these countries, full of younger, cheaper labor, reap the benefits of a growing economy, they begin to face some of the same aging-related challenges that countries like the United States are currently grappling with. China, for one, does not have a social security system and has chosen to not invest resources in the care of their elderly. This puts enormous pressure on adult children to provide for their aging parents. When these parents have AD or another form of dementia their needs

often will become too difficult for their families to manage. Thus, as the population of older adults in countries like China and India grows, the AD crisis in the United States and Europe will truly become a global crisis.

As people live longer and longer lives, increasing the expected human lifespan, it is essential to focus on increasing the duration of the "healthspan" or the length of time a person lives in the absence of severe illness or disability (5). This is the mission of AD researchers, advocates and doctors. Our primary aim is not to lengthen the lifespan but rather to prolong the healthspan. No person, at any age, should suffer the uniquely terrifying experience of the AD patient: the total loss of that which is most precious to us, our memories.

Conclusion References

1. Jack CR, Knopman DS, Jagust WJ, Shaw LM, Aisen PS, Weiner MW, *et al.* (2010): Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. *Lancet Neurol* 9: 119–28.

2. Bateman RJ, Xiong C, Benzinger TLS, Fagan AM, Goate A, Fox NC, *et al.* (2012): Clinical and biomarker changes in dominantly inherited Alzheimer's disease. *N Engl J Med* 367: 795–804.

3. Thies W, Bleiler L (2013): 2013 Alzheimer's disease facts and figures. *Alzheimers Dement* 9: 208–45.

4. Lewin Model Group (2015): *Changing the Trajectory of Alzheimer's Disease: How a Treatment by 2025 Saves Lives and Dollars*.

5. Nikolich-Žugich J, Goldman DP, Cohen PR, Cortese D, Fontana L, Kennedy BK, *et al.* (2016): Preparing for an Aging World: Engaging Biogerontologists, Geriatricians, and the Society. *J Gerontol A Biol Sci Med Sci* 71: 435–44.

APPENDIX

A Model For Teaching Advanced Neuroscience Methods: A Student-Run Seminar to Increase Practical Understanding and Confidence

Abstract

Neuroscience doctoral students must master specific laboratory techniques and approaches to complete their thesis work (hands-on learning). Due to the highly interdisciplinary nature of the field, learning about a diverse range of methodologies through literature surveys and coursework is also necessary for student success (hands-off learning). Traditional neuroscience coursework stresses what is known about the nervous system with relatively little emphasis on the details of the methods used to obtain this knowledge. Furthermore, hands-off learning is made difficult by a lack of detail in methods sections of primary articles, subfield-specific jargon and vague experimental rationales. We designed a student-taught course to enable first-year neuroscience doctoral students to overcome difficulties in hands-off learning by introducing a new approach to reading and presenting primary research articles that focuses on methodology. In our literature-based course students were encouraged to present a method with which they had no previous experience. To facilitate weekly discussions, “experts” were invited to class sessions. Experts were advanced graduate students who had hands-on experience with the method being covered and served as discussion co-leaders. Self-evaluation worksheets were administered on the first and last days of the 10-week course and used to assess students’ confidence in discussing research and methods outside of their primary research expertise. These evaluations revealed that the course significantly increased the students’ confidence in reading, presenting and discussing a wide range of advanced neuroscience methods.

Introduction

Today, doctoral students in the life sciences are trained in a highly interdisciplinary environment that requires mastery of diverse methodologies (1). This represents a departure from the traditional model of doctoral education that encouraged specialization (2). However, core curricula for doctoral programs rarely include formal, laboratory-based instruction on advanced methods. There are several reasons for this, including potentially large investments of time and resources required to ensure that each student receives sufficient instruction and experience with a given method. At the University of California, Los Angeles, doctoral students in neuroscience identified this lack of instruction in methodology as a weakness of the core, required curriculum. To help address this weakness, we developed a new seminar course called Neuroscientific Methods (hereafter "*Methods*") to be integrated into the required first-year academic schedule for Neuroscience doctoral students.

Doctoral students are practically motivated to become experts in several complementary approaches to address their research questions. A diverse skillset leads to better outcomes on grant applications and in manuscript submissions. One common way to obtain proficiency with a research method is through the laboratory training environment. Specifically, students often receive instruction from other laboratory members on methods central to the main focus of their mentor's research program. Students will also seek out training in additional techniques in other laboratories, especially those of collaborators on their campus.

It is less common for students to attempt to improve their understanding of techniques in the context of the classroom, without hands-on instruction. We believe this is a skill that, like any other, must be practiced to be improved. *Methods* offered an opportunity to introduce students to an approach to reading and presenting articles that focused on the methods sections, a part of primary research articles that non-experts often skim. The ability to evaluate and present unfamiliar topics is a necessary skill in academia where reviewing articles, grants and dossiers are required (3). We believe that the *Methods* course provides a supportive environment for

first-year doctoral students to begin to hone this skillset with the help of their peers, as well as more advanced students.

At each meeting of *Methods*, we invited 1-3 “experts” to join the class to facilitate discussion. Experts were advanced graduate students with direct, often current, experience with the method being presented that week. Through participation in *Methods*, experts were able to practice discussing their own research and their informal teaching skills. In addition to the experts, the course was designed and facilitated by second-year neuroscience graduate students. For these aspects of the course, we were inspired by other studies demonstrating that graduate student-taught courses are successful models for graduate and upper-level undergraduate education (4; 5). In addition, the community-based teaching and support for this course gave the experts and facilitators a chance to engage in curriculum design and team-teaching, both important parts of academic life and professional development that are often overlooked in doctoral training (3; 6). Indeed, perceived quality of professional development training has been shown to have an effect on self-efficacy measures in graduate teaching assistants (7). Coalescing around a community-identified need for further instruction in neuroscience methods to keep pace with the highly diverse and interdisciplinary nature of neuroscience research has helped foster an environment where UCLA neuroscience doctoral students feel that efforts to provide a holistic graduate experience are supported and valued.

In the present study, we report the rationale, goals, design, implementation and assessment of a seminar course focused on increasing student confidence in their ability to comprehend new methodologies using a hands-off learning approach. The overarching goals of the course guided the design and implementation. Those goals were as follows:

1. Expose first-year graduate students to a wide range of neuroscience methods with a special focus on widely used and newly developed methods featured in recent high-impact publications.

2. Promote discourse and collaboration between first-year and advanced neuroscience doctoral students, especially for learning about new methods that could be applicable to their work or for assistance in the process of choosing a dissertation laboratory.

3. Emphasize practical considerations of experimental design with a focus on the advantages and limitations of each method.

4. Build students' confidence in their ability to prepare and present material outside their areas of first-hand expertise.

Materials and Methods

Participants

Methods was created for and has been implemented with first-year neuroscience doctoral students enrolled in the UCLA Neuroscience Interdepartmental Graduate Program (NSIDP). The course is now part of the required first-year curriculum for the program and is held in the winter quarter. In the context of the NSIDP, this is often the quarter when students are conducting their first laboratory rotation in search of a suitable mentor and research environment for their dissertation work. About 75% of the incoming students in the NSIDP start the program the fall after their undergraduate graduation, and most of the remaining incoming students are within three years of completion of their undergraduate studies.

In addition to the students enrolled in the course, *Methods* drew on the larger neuroscience community for participation and support. Two second-year NSIDP students served as the course instructors (CRKC, TMH). Their roll included developing the mission and goals of the course, creating the syllabus and recruiting advanced graduate students and faculty to take part in weekly discussions. The instructors met each week before class to discuss the articles that would be covered and to compile lists of questions and comments that might be useful in guiding the group discussion. Throughout the course the instructors gave informal feedback to students about their presentations. The student creators of the course also acted as

mentors for subsequent second-year student facilitators. Finally, *Methods* was overseen by a UCLA faculty member (AMA) who had designed a neuroscience methodology-focused seminar course during the previous year. *Methods* grew out of her course and she guided its year-to-year development and implementation.

Course Design

Methods was designed for weekly, 2-hour meetings over a 10-week quarter period and was listed as a seminar/literature review course. At each weekly session, two to three assigned readings were discussed: typically one review article focused on the method of interest and at least one experimental paper employing that technique. At the first meeting, the student course instructors gave an example presentation covering a review article on optogenetics and a primary research article that used an optogenetics study design. The instructors introduced an alternate approach to the standard journal-club style presentation that follows the structure of a primary research article (e.g., background, methods, results, discussion). Instead, student presenters were encouraged to 1) focus on the history, development, application, advantages and disadvantages of the assigned method; and 2) provide a critical interpretation of the results from the experimental paper (i.e., what might the study results mean in the context of known limitations of the method, was the method appropriate for the research question, how could the method have been used differently). Students were encouraged to use resources (e.g., JoVE, Wikipedia, textbooks) outside of the assigned reading to augment their understanding of the method and to prepare their presentations.

After the example presentation during week 1, students were asked to sign up to present on one of weeks 2-9. They were instructed to choose a week covering a method with which they had no past hands-on experience. To facilitate discussions, 1-3 “experts” were invited to class sessions. Experts were advanced UCLA graduate students who were actively engaged in using the method being covered that week. The experts served as co-leaders of the class

discussions, provided critical commentary and bridged gaps in understanding. They were also valuable for their ability to correct misinformation or misconceptions regarding the use of a particular method. Experts' contact information was made available to student presenters so that they could also be consulted during the presentation preparation phase.

At the last meeting during week 10, students were instructed to prepare a 5-minute long "elevator-pitch" style presentation with no more than 5 accompanying slides. For the pitches, students focused on a method that they had hands-on experience using either in a previous research position or during their concurrent rotation. They were instructed to use their pitch to convince the audience that a particular method was the best one to address their research question and/or to test their hypotheses. The purpose of the elevator pitches was to encourage students to use what they had learned about presenting science from a methods-focused perspective. In *Methods*, we asked the students to tackle the difficult tasks of learning about a neuroscience method in a hands-off fashion and then presenting that method to their peers in a critical way. We wanted the students to have the opportunity to apply what they learned about presenting a method to their own research. Our theory was that critically assessing unfamiliar methods would provide new insight into the familiar methods our students use in their laboratories. The elevator pitches were an excellent capstone for our students, who found the assignment fun and enjoyed hearing brief "pitches" by their peers.

Syllabus

The syllabus for the *Methods* course was separated into three modules: cellular, molecular and systems level neuroscience. Each module featured several corresponding neuroscience methods. The outline of the syllabus is included in the text that follows. The method discussed each week is underlined for clarity.

Week 1 - Introduction to the course and sample presentation on Optogenetics.

Cellular Module:

Week 2 – Innovative Approaches in Electrophysiology

Week 3 – Two-Photon Microscopy

Molecular Module:

Week 4 – Mapping Synaptic Contacts: Fun with Tracers

Week 5 – Genetic Manipulation of Model Organisms

Week 6 – Genomics and Bioinformatics

Systems Module:

Week 7 – Behavioral Assessment in Model Organisms

Week 8 – Structural MRI: DTI and Network Analysis

Week 9 – Functional MRI: The BOLD Signal and the Resting Brain

Week 10 – Elevator Pitches

Course Assessment

An anonymous self-evaluation worksheet was prepared to assess students' confidence with discussing research and methods outside of their primary research expertise. These worksheets where administered on the first and last day of class and focused on assessing each student's perceived confidence in reading, presenting and discussing advanced neuroscience methods.

There were 9 items on the self-evaluation and students were asked to use a 10-point scale to rate each statement where 1=not at all/little to none/probably not and 10=very/definitely/very confident. The items were as follows:

1. I am familiar with a wide variety of methods currently being employed by neuroscientists.

2. I am confident in my ability to decide which methods should be used to address a wide variety of neuroscience research questions.

3. I understand the relative pros and cons to using competing neuroscience methods to address a research question.

4. I am comfortable approaching posters for studies that use methods outside of my particular research experience.

5. I find the language/terminology used in some neuroscience research papers intimidating.

6. I feel I can read and understand the methods section from journal articles outside of my expertise.

7. I feel comfortable presenting neuroscience journal articles to colleagues/peers.

8. I am confident in my ability to critically analyze the findings of any neuroscience paper.

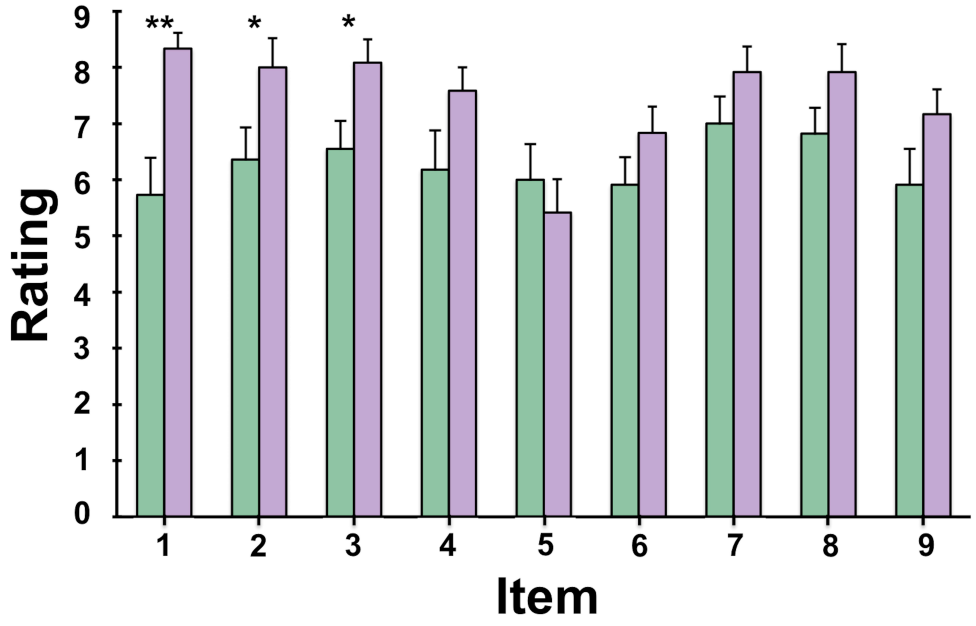
9. I am confident in my ability to spot weaknesses in methods outside my own research background.

Finally, there was a prompt where students were asked at the first meeting to "include suggestions and comments about what you would like to get out of this course" and at the last meeting to "include any suggestions and comments for improving the course; tell us about what you found useful and not so useful; which weeks/topics were your favorite/least favorite?"

Average ratings for each item on the first (timepoint 1; TP1) and the last day of class (timepoint 2; TP2) were compared using Wilcoxon rank sum tests. An average rating for each student was also calculated across all the items. Because the worksheets were completed anonymously we could not examine change in ratings for specific students. Instead, we used a Wilcoxon rank sum test to compare all student average ratings across items at TP1 and TP2. For item 5 lower scores indicated greater confidence so this item was reverse scored. All statistical analyses were completed using tools from the R Project for Statistical Computing (http://www.r-project.org)

Results

The data described here were collected during the winter quarter, January - March 2013, at UCLA during the first implementation of *Methods*. We collected self-report worksheets from 11 students at TP1 and 12 students at TP2. Average ratings on specific items ranged from 5.73 – 7.0 at TP1 on the 10-point scale. At TP1, average ratings across all the items by a single student ranged from 3.67 – 9.67. Thus, we learned that within our class confidence in using, understanding and presenting unfamiliar methods varied widely. Interestingly, the highest student average at TP2 was 9.11, which may have been the same student who reported the highest confidence ratings at TP1. The range in student average scores at TP2 was 5.22 – 9.11. We compared students' average rating across the 9 items at TP1 and TP2 and found that students' average rating increased over the course of *Methods* ($p=0.045$). Analysis of the self-evaluation individual item ratings at TP1 and TP2 revealed that the course significantly increased students' confidence in their familiarity with and ability to evaluate current advanced neuroscience methods (Appendix Figure 1). The average rating increased for each item from the first meeting to the last meeting of the course, except for item 5.



Appendix Figure 1. Average item ratings for each statement from the first (green, n=11) and last (purple, n=12) day of class. Mean ratings increased from the first to the last meeting for all statements except for item 5, for which we expected a decrease (see item in *Materials and Methods*). **p<0.01, *p<0.05

In response to our prompt for what students would like to gain from the course, students wrote about wanting to start with the basics and build their neuroscience knowledge on a solid foundation. One student wrote, “I would like to get a basic understanding of a variety of methods. It often seems assumed that we have some background in a variety of techniques for which I have no prior knowledge, especially in genetics”. Another student wrote that they hoped to gain “A more thorough understanding of the practical details/limitations of the methods we discuss”.

At the final meeting, students were asked what aspects of the course they found useful or not useful. In general, the new course was well received and thought to be useful. Constructive criticism included some frustration with being asked to learn new methods from peers who were not experts themselves. For example, one student wrote, “It was hard to learn sometimes from people with little expertise in the research they were presenting. Perhaps

assign two presentations to each person - one on a method in which they are expert and one where they are not an expert. Then a team of two can work together (one expert, one novice) on each methods presentation". Other students asked for more thorough coverage of certain topics they felt were underrepresented, like neurochemical techniques or nanoscience approaches to neuroscience topics.

Discussion

We have developed and implemented a seminar-style course to teach neuroscience methods to first-year doctoral students in a non-laboratory environment. The *Methods* course is student-run by two facilitators and supported by the larger community of neuroscience doctoral students at UCLA. This approach with doctoral students learning and teaching together has been shown to be effective in other classroom models (4; 5). The *Methods* course aims to augment the traditional curriculum and emphasizes skills critical to the development of successful academics. The NSIDP grants doctorates in Neuroscience, a broad and interdisciplinary subject that requires concerted effort to familiarize oneself with the breadth of the field. *Methods* quickly gives first-year graduate students an opportunity to span that breadth through a presentation and discussion based literature review course.

Responses from the anonymous self-evaluation worksheets revealed that the course was successful in increasing student confidence when presenting and discussing neuroscience research and methods outside of their primary research expertise. Across students, the ratings for each item improved from the first meeting to the last meeting of the course. The increased confidence in exploring unfamiliar methods and topics is anticipated to encourage student participation in journal clubs, to motivate communication across disciplines at scientific conferences, to encourage rotations in a variety of laboratories, to identify courses in which to serve as a teaching assistant and to influence students' choices to employ a diverse set of methodologies in their own research.

One limitation of the course assessment was that the self-evaluations were collected anonymously with no system to match individual student's ratings at the first meeting to their ratings at the final meeting (e.g., to run paired nonparametric analyses). Another limitation was that subsequent *Methods* course graduate student facilitators have used their own approaches to assessing the value and effectiveness of the course (8–10). That said, the focus of this report was introduce the novel course design and to include the initial data indicating its success, not to perform a meta-analysis. We are partnering with the NSIDP leadership to create a standardized *Methods* evaluation and assessment tool that will be implemented consistently and allow for data pooling and analysis across yearly implementations of the course moving forward.

One of the benefits of enabling each pair of facilitators the opportunity to improve and expand the course is the increased focus on presentation efficacy and skills. Now, as part of *Methods,* each student receives aggregated feedback from their peers on how clearly and effectively they presented their assigned technique. As one of the founding goals of the course was to build students' confidence in their ability to prepare and present material outside their areas of first-hand expertise we believe the addition of formal presentation feedback has strengthened the course overall. In addition, subsequent iterations of the course have, based on class size, required students to make two shorter presentations during the quarter so that presentation feedback might be directly applied to the second assignment. Finally, the elevator-pitch session at the end of the course has been modified to a 3-minute presentation with no accompanying slides. This approach encourages students to focus more on the content and style of their presentation instead of prepared slides while still allowing them to apply what they learned about presenting neuroscience methods to their own research experience.

The most common criticism of *Methods* was that a particular technique might have been effectively explained and taught by a student that had prior experience using that technique. We do not disagree with this feedback; it is true that individuals who have first-hand experience are

likely to present on a particular method more easily and effectively. However, the goal of this doctoral-level course was to also place students outside of their comfort zone and to illustrate how much could be gained by an in-depth and critical examination of an unfamiliar technique. Each week, presenters demonstrated an understanding of the basics and, sometimes, the subtleties of the method they were assigned. Experts assisted and their participation proved to be an effective way to focus the discussion when presenters struggled. We believe the challenges that students faced in the *Methods* course are similar to challenges they will face in their professional careers. The opportunity to practice these skills is an important part of the doctoral training experience that, if provided in the curriculum, often requires non-traditional approaches (4).

We believe *Methods* can be adapted to an upper-division undergraduate seminar course and would be a valuable addition to undergraduate science curricula. Our suggestions for this alternate design are based, in part, on the feedback we received from our first-year doctoral students. There is a well-described lack of instruction on the scientific process in undergraduate education (11; 12). *Methods* would serve as many undergraduates' first exposure to examining techniques as a primary aim while gaining exposure to primary research articles. This exposure would begin to address the need for a more comprehensive undergraduate science education that includes critical evaluation of experimental design and interpretation of data (13). By engaging in this type of critical thinking, undergraduates will be mentally stepping into the scientific process from the classroom. With graduate student co-facilitators and graduate student expert partners, *Methods* can be brought to a level appropriate for upper-division undergraduates. Students could pair or form groups to prepare joint presentations; preparation of presentations could be guided by graduate student experts. Thus, the burden of teaching the methods would not fall solely to the undergraduates. In addition to being a crucial part of an undergraduate version of the *Methods* course, the communication and discourse between doctoral students and undergraduates provides a low-barrier way for younger students to learn

more about post-graduate science education and life as a doctoral student.

We have been gratified to see *Methods* continue to thrive after its initiation in 2013. Beyond this specific course, *Methods* serves as an example of how existing courses can be developed by students into student-led activities. We believe that the process of creating curriculum is a social endeavor, with influences from the faculty, the students and the material itself (14; 15). In that spirit, each pair of subsequent student co-facilitators has added their own unique modifications, improvements and perspective to *Methods* such that it continues to grow organically. However, the founding mission and goals of the course have remained the same: to expose students to a diverse set of neuroscience research methods, to promote discourse between students at different stages of their degree and professional development, to encourage the practical analysis of current research and ultimately, to build student confidence in understanding neuroscience research outside their primary areas of expertise.

Appendix References

1. Holley K (2008): The challenge of an interdisciplinary curriculum: A cultural analysis of a doctoral-degree program in neuroscience. *High Educ* 58: 241–255.

2. McBride BB, Brewer CA, Bricker M, Machura M (2011): Training the next generation of renaissance scientists: The GK–12 ecologists, educators, and schools program at The University of Montana. *Bioscience* 61: Oxford University Press466–476.

3. Ullrich L, Dumanis SB, Evans TM, Jeannotte AM, Leonard C, Rozzi SJ, *et al.* (2014): From student to steward: The Interdisciplinary Program in Neuroscience at Georgetown University as a case study in professional development during doctoral training. *Med Educ Online* 19: 22623.

4. Needelman BA, Ruppert DE (2006): Graduate student–run course framework for comprehensive professional development. *J Nat Resour Life Sci Educ* 35: American Society of Agronomy62–71.

5. Ullrich LE, Krafnick AJ, Dumanis SB, Forcelli PA (2012): Drugs, the brain, and behavior: A graduate student-run comprehensive course in neuroscience. *J Undergrad Neurosci Educ* 10: A105–12.

6. Austin AE (2002): Preparing the next generation of faculty: Graduate school as socialization to the academic career. *J Higher Educ* 73: 94–122.

7. DeChenne SE, Koziol N, Needham M, Enochs L (2015): Modeling sources of teaching self-efficacy for science, technology, engineering, and mathematics graduate teaching assistants. *CBE Life Sci Educ* 14: . doi: 10.1187/cbe.14-09-0153.

8. Ching CR, Harrison TM, Einstein MC, Bonnano SL, Andrews AM, Levine M (2013): *A model for teaching advanced neuroscience methods to graduate students: A student-run seminar to increase practical understanding and onfidence*. . . *2013 Neurosci Meet Plan*New Orleans, LA: Society for Neuroscience, 2013.

9. Einstein M, Bonanno S, Ching CR, Harrison TM, Andrews AM (2014): *A new model for teaching advanced neuroscientific methods to graduate students*. . Washington, DC: Society for Neuroscience, 2014.

10. DiTullio D, He C, Andrews AM (2015): *A student-led graduate seminar familiarizes students with neuroscientific techniques and improves oral presentation skills*. . Chicago, IL: Society for Neuroscience, 2015.

11. Coil D, Wenderoth MP, Cunningham M, Dirks C (2010): Teaching the process of science: Faculty perceptions and an effective methodology. *CBE Life Sci Educ* 9: 524–35.

12. Handelsman J, Ebert-May D, Beichner R, Bruns P, Chang A, DeHaan R, *et al.* (2004): Education. Scientific teaching. *Science* 304: American Association for the Advancement of Science521–2.

13. Kozeracki CA, Carey MF, Colicelli J, Levis-Fitzgerald M, Grossel M (2006): An intensive primary-literature-based teaching program directly benefits undergraduate science majors

and facilitates their transition to doctoral programs. *CBE Life Sci Educ* 5: 340–7.

14. Tierney W (1989): *Curricular landscapes, democratic vistas: Transformative leadership in higher education*. . Westport, CY: Praeger Publishers.

15. Lindblom-Ylänne S, Trigwell K, Nevgi A, Ashwin P (2006): How approaches to teaching are affected by discipline and teaching context. *Stud High Educ* 31: Routledge285–298.