BLOOD GLUCOSE LEVELS AND GENETIC FACTORS AS PREDICTORS OF NEUROCOGNITIVE OUTCOMES

by

Alexandra M. V. Wennberg

A dissertation submitted to Johns Hopkins University in conformity with the requirements for the degree of Doctor of Philosophy

Baltimore, Maryland

May 7, 2015

ABSTRACT

Alzheimer's disease (AD), a neurodegenerative disease characterized by progressive cognitive decline, is becoming increasingly prevalent as the population ages. There is no effective treatment for AD, so manipulating modifiable risk factors before clinical symptoms of dementia develop may be the most effective course to prevent, delay, or modify the course of AD. Type II diabetes mellitus (DM), characterized by hyperglycemia and insulin resistance, affects over a quarter of older adults and has been linked with AD, cognitive decline, and brain atrophy. Because DM is preventable and treatable, it represents an intervention target for AD. This dissertation investigated the association between glucose levels and three outcomes – performance on a battery of cognitive tests over time, time to symptom onset of mild cognitive impairment (MCI) or dementia, and cortical thickness in AD-signature regions (i.e., entorhinal cortex, inferior temporal gyrus, parahippocampal gyrus pars triangularis, precuneus, superior frontal and parietal gyri, supramarginal gyrus, and temporal pole). Additionally, it investigated how two genes – apolipoprotein E (APOE) and translocase of the outer mitochondrial membrane (TOMM) 40 – may modify the relationship between glucose and these outcomes. Both the APOE ε4 allele and the TOMM40 very long/very long (VL/VL) genotype (versus the short/short (S/S) genotype) have been associated with increased risk of dementia, cognitive decline, and brain atrophy, and some evidence suggests that these genetic factors may further increase the risk in individuals with DM. This research was conducted using data from the BIOCARD Study (n=349), which

enrolled primarily middle-aged individuals, three-quarters of whom had a first-degree relative with dementia. The goal of the study was to examine the early signs and symptoms of AD in a high-risk cohort. For this dissertation, baseline blood glucose level, which was ascertained from a blood draw in a clinical exam, was the primary predictor and was available for 333 participants. APOE and TOMM40 genotypes were also determined from blood samples. Age of MCI or dementia symptom onset was determined through consensus diagnosis. At annual visits, participants completed a neuropsychological test battery, and at baseline and bi-annually, participants completed magnetic resonance imaging (MRI) scans. The first paper in this study used linear mixed effects models (LMMs) and generalized estimating equation (GEE) models to investigate the association between glucose and cognitive performance over time. We used confirmatory factor analysis (CFA) to create one factor based on performance on tests of executive function (the executive function factor) and one factor based on performance on tests of memory (the memory factor). We found that higher baseline glucose was associated with greater decline on the executive function factor score in both LMMs (B=-0.005; 95% CI -0.008, -0.001) and GEE models (B=-0.004; 95% CI -0.007, -0.001) that controlled for age, sex, race, education, depression, and medical conditions (i.e., cardiovascular conditions, hypertension, hypercholesterolemia, traumatic brain injury (TBI)). Interaction analyses using LMMs found that higher glucose was associated with significant decline in executive function score in ε4 carriers (B=-0.013; 95% CI -0.020, -0.006), but not in non-carriers (B=-0.002, 95% CI -0.006, 0.002). In LMMs, higher glucose was also associated with poorer memory factor score over time in

subjects with the S/S genotype (B=-0.013; 95% CI -0.024, -0.002), versus the VL/VL genotype (B=0.004; 95% CI -0.003, 0.010). The second paper in this study used Cox proportional hazard models to investigate the association between baseline blood glucose level and time to MCI or dementia symptom onset, as well as the association between the interaction of APOE and glucose and TOMM40 and glucose and time to symptom onset. We did not find an association between glucose level and time to symptom onset; additionally, we did not find that APOE or TOMM40 modified this relationship. In the final paper, linear regression analyses showed that glucose level was associated with reduced cortical thickness in the parahippocampal gyrus (B=-0.002; 95% CI -0.003, -0.0001) and temporal pole (B=-0.002; 95% CI -0.003, -0.0002) in a fully-adjusted model. Additionally, higher glucose levels were associated with thinner measures of the superior parietal gyri (B=-0.001; 95% CI -0.002, 0.0005 vs. B=0.0007; 95% CI $3.05e^{-6}$, 0.001) and temporal pole (B=-0.004; 95% CI -0.008, -0.0009 vs. B=-0.0003; 95% CI -0.002, 0.001) in ε4 allele carriers versus non-carriers. This evidence suggests that even in middle-aged, cognitively healthy samples, higher levels of glucose, even in those without DM, can negatively impact cognitive performance and cortical thickness. Maintaining normal blood glucose levels may be important in middle age for reducing risk of cognitive decline, AD, and brain atrophy.

Advisor: Dr. Adam P. Spira

Thesis Committee:

Dr. Marilyn Albert (Chair)

Dr. George Rebok

Dr. Jennifer Schrack

Dr. Adam Spira (Advisor)

Dr. Vadim Zipunnikov

Alternates:

Dr. Jeanine Parisi

Dr. Judith Kasper

ACKNOWLEDGEMENTS

As they say, “it takes a village,” and completing my doctorate would not have been possible without the mentorship, support, and encouragement of so many people.

First, thank you to my advisor, Dr. Adam Spira. I am so fortunate to have a mentor that continuously supports, encourages, and motivates me. Thank you for all of the guidance you have given me and for always having an open door.

Thank you to Dr. Marilyn Albert and Dr. George Rebok who have also been incredible mentors to me. Dr. Albert, thank you for giving me the opportunity to work with the BIOCARD data; I have learned so much. Thank you also to Dr. Vadim Zipunnikov, Dr. Jennifer Schrack, Dr. Anja Soldan, and Dr. Corinne Pettigrew, without whom this dissertation would not have been completed. Vadim, thank you for all of the hours you put in helping me with my analyses. Anja and Corinne, thank you for helping me navigate the BIOCARD data.

Thank you also to Chris Kaufmann and to Dr. Lenis Chen-Edinboro for being great lab mates and always helping me out.

I would also like to thank my earlier mentors and teachers, including Dr. Echo Leaver, Dr. Bernie Possidente, Dr. Steve Heilig, and Rebecca McCammon. Thank you for fostering my love of learning and research.

Finally, thank you to my family. Mom, Dad, and Anders, I will never be able to put into words how much I love you and how important your support has been to me. Nick and Marjhan, thank you for always being there for me and making me smile.

TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

INTRODUCTION

PROBLEM STATEMENT

The population is aging; adults over the age of 65 are the fastest growing segment of the population (Administration on Aging, 2013). Although the older adult population suffers from several chronic conditions and diseases, two of the most burdensome and prevalent – Alzheimer's disease (AD) and type II diabetes mellitus (DM) – appear to be connected. Currently, there is no cure for AD, so identifying AD earlier in the disease course and intervening on modifiable risk factors may be the most effective path to mediate the burden of the disease.

Alzheimer's Disease

Over five million older adults in the United States have dementia, and AD is the leading cause of dementia, accounting for approximately 60 to 80 percent of cases (Alzheimer's Association, 2014; Hebert, Weuve, Scherr, & Evans, 2013). By 2050, the number of people over the age of 65 with AD is expected to triple to 13.8 million (Hebert et al., 2013). Worldwide, the number of people with AD is expected to double every 20 years, which means it will affect 81.1 million people by 2040 (Ferri et al., 2005). AD is a chronic neurodegenerative disease that is both emotionally and economically burdensome. The hallmark of AD is progressive decline in memory and cognition (American Psychiatric Association, 2013). AD patients also have significant functional decline and often have behavioral symptoms (e.g., agitation, aggression, and inappropriate affect), and as the disease progresses patients also have problems speaking, walking, and eating (Alzheimer's

Association, 2014). AD is the fifth leading cause of death in older adults; however, due to reporting error, it is likely that the number of people who die with or due to AD is underestimated (Alzheimer's Association, 2014; Murphy, Xu, & Kochanek, 2013).

Extracellular amyloid plaques, intracellular neurofibrillary tangles, and neuron death are the pathological characteristics of AD (Alzheimer's Association, 2014). The brains of AD patients accumulate beta-amyloid protein fragments, which form the plaques, and tau protein, which are a major component of the tangles; as these proteins accumulate they lead to neurotoxicity and apoptosis (G. V. Johnson & Stoothoff, 2004; Sun, Bromley-Brits, & Song, 2012). The accumulation of beta-amyloid and tau is thought to occur several years or decades before the symptoms of AD appear (Jack et al., 2013). While many individuals go undiagnosed until they develop clinical AD, some are diagnosed with mild cognitive impairment (MCI), which is often a prodromal stage of AD (Petersen, 2004). Like AD, MCI patients have cognitive decline, though it is not as substantial as in AD patients, and MCI patients do not have significant functional impairment (Petersen, 2004). Not all MCI patients progress to AD; however, approximately 10 to 20 percent of MCI patients convert to AD annually (Busse, Hensel, Guhne, Angermeyer, & Riedel-Heller, 2006; Fischer et al., 2007; Petersen et al., 1999).

Beyond the mortality rates associated with AD, it is a very difficult disease for patients, family and friends, and the health care system to manage (Alzheimer's Association, 2013). AD is also very costly to the health care system. It has been

estimated that in 2014 Medicare and Medicaid will pay 150 billion dollars for the care of AD patients, and total costs are expected to reach 214 billion dollars (Alzheimer's Association, 2014; Bynum, 2011). AD patients often need long-term care, skilled nursing care, medication, and hospital visits, all of which are costly. Further, AD patients often have other medical conditions (e.g., kidney disease, cardiovascular disease, diabetes), and compared to patients who have these conditions but do not have AD, those with AD have longer hospital stays and higher healthcare costs (Bynum, 2011).

As the prevalence of AD increases, the need to treat and/or prevent it is urgent. The strain that AD is placing on patients, families, and the healthcare system is unsustainable (Alzheimer's Association, 2013). Currently, there is no treatment for AD, so it is important to identify pathways for reducing the symptoms and decline, and possibly preventing onset of AD, earlier in the disease course.

Type II Diabetes Mellitus

DM disproportionately affects older adults; over a quarter of the older adult population has DM and approximately half of the older adult population is thought to have pre-diabetes (American Diabetes Association, 2014b; Centers for Disease Control and Prevention, 2014). It should be noted that a recent article has suggested that the diagnostic criteria for pre-diabetes is too broad, and is leading to over-diagnosis (Yudkin & Montori, 2014). However, it is also estimated that nearly 30 percent of people with DM are undiagnosed (Centers for Disease Control and Prevention, 2014). Type II diabetes accounts for 90 percent of all diabetes cases

(World Health Organization, 2012). Type II diabetes begins with insulin resistance; this is a condition in which insulin, a hormone, cannot effectively enter cells and/or the pancreas is not able to produce enough insulin. Due to insulin resistance, glucose is not able to enter the cells to be used for energy, and therefore accumulates in the blood leading to high blood sugar (American Diabetes Association, 2014b). The diagnostic level for DM is a glycated hemoglobin (HbA1C) level of 6.5 percent or higher or fasting blood glucose of 126 mg/dL or higher. Pre-diabetes, the prodromal stage of DM, is diagnosed at a HbA1c level between 5.7 and 6.4 percent or a fasting blood glucose between 100 and 125 mg/dL (American Diabetes Association, 2014a). DM increases the risk of dementia, as well as hypertension, hypercholesterolemia, cardiovascular disease, stroke, neuropathy, and kidney disease, and it is the seventh leading cause of death across all age groups (American Diabetes Association, 2014b). In addition to the negative health outcomes associated with DM, it is also a very costly disease. The estimated total cost of DM, including both direct and indirect costs, was 245 billion dollars, in 2012, 176 billion dollars of this were direct medical costs (American Diabetes Association, 2014b).

Fortunately, type II diabetes is preventable and treatable. Many factors, including race, sex, age, diet, and activity level affect the risk of developing DM (American Diabetes Association, 2014b). However, DM can be prevented by avoiding insulin resistance and maintaining a healthy blood glucose level, generally through diet and moderate levels of physical activity (Knowler et al., 2002). Type II diabetes can be treated through diet and exercise, oral medication (e.g., metformin,

pioglitazone), or, if necessary, insulin injections (American Diabetes Association, 2011). While it does not occur very often, DM can be treated so effectively that it is possible to revert to a prediabetic or normal state (Gregg et al., 2012).

DM has been identified as a risk factor for AD. A recent study found that as many as one third of AD cases may be prevented by preventing risk factors for AD, including DM (Norton, Matthews, Barnes, Yaffe, & Brayne, 2014). Given that DM is so prevalent among older adults, and that it is so easily preventable and treatable, it may be that by targeting individuals who have or are at risk of developing DM, we may be able to delay the onset of AD or even prevent a significant number of AD cases from developing. As stated, DM is a risk factor of AD; it has been found that having DM increases the risk for AD by at least 50 percent (Cheng, Huang, Deng, & Wang, 2012). Further, DM has been found to negatively impact cognition and the rate of age-related cognitive decline (Lamport, Lawton, Mansfield, & Dye, 2009; Tuligenga et al., 2014). DM patients have also been found to have greater volumetric loss in several brain areas associated with cognitive function and that are affected in AD (Bryan et al., 2014; de Bresser et al., 2010; Falvey et al., 2013; Raber, Huang, & Wesson Ashford, 2004; Roberts, Knopman, Przybelski, et al., 2014; van Harten, de Leeuw, Weinstein, Scheltens, & Biessels, 2006).

Two genetic factors have also been found to increase the risk of AD, and there is evidence suggesting that they may also be associated with DM. The apolipoprotein E (APOE) ε4 allele and the translocase of the outer mitochondrial membrane (TOMM40) long and very long alleles and genotypes (i.e., heterozygotes

or homozygotes) have both been linked to an increased risk of AD (Caselli et al., 2012; Chartier-Harlin et al., 1994; Hayden et al., 2012; S. C. Johnson et al., 2011; Kim et al., 2011; Lutz, Crenshaw, Saunders, & Roses, 2010; Mastaglia et al., 2013; Roses et al., 2010; Shen et al., 2010). Additionally, studies have shown that individuals who have both DM and an ε4 allele have a greater risk of developing AD than those who have just one of those risk factors (Dore, Elias, Robbins, Elias, & Nagy, 2009; Vagelatos & Eslick, 2013). Similarly, physiological evidence suggests that a similar risk association may exist between DM, longer length of a poly-T nucleotide repeat of TOMM40, and AD (Hayden et al., 2012; Potkin et al., 2009).

Apolipoprotein E

There are three allelic variations of the apolipoprotein E (APOE) gene – ε2, ε3, and ε4. The ε4 allele has long been associated with increased risk of AD (Chartier-Harlin et al., 1994; Shen et al., 2010). Approximately 14 percent of the population are carriers of the ε4 allele, but 40 percent of AD patients are carriers (Farrer et al., 1997). Both the heterozygous and homozygous genotypes have been associated with increased risk of AD, and the number of ε4 alleles is positively correlated with greater amounts of Aβ in the brain (Chartier-Harlin et al., 1994; Reiman et al., 2009; Shen et al., 2010). Evidence suggests that those with the ε4 allele have impaired clearance of Aβ from the brain (Bales, Dodart, DeMattos, Holtzman, & Paul, 2002).

The ε4 allele is associated with both risk of AD and earlier age of onset of sporadic AD (Corder et al., 1993; Farrer et al., 1997; Rebeck, Reiter, Strickland, & Hyman, 1993). Compared to individuals who are ε3 homozygotes, individuals who

are ε2/ε4 have a 2.6 times increased risk of developing AD; those who are ε3/ε4 have a 3.2 times increased risk; and those who are ε4 homozygotes have a 14.9 times increased risk (Farrer et al., 1997). However, it is important to note that these odds ratios vary by race and ethnic group, such that individuals who are ε4/ε4 have greater or smaller risk of developing AD based on their race/ethnicity. For example, African Americans with an e4 allele (odds ratio = 5.7) and Hispanics (odds ratio = 2.2) are at substantially decreased risk compared to Whites (odds ratio = 12.5), whereas the Japanese (odds ratio = 33.1) have a substantially greater risk of AD (Farrer et al., 1997). The ε4 allele has also been found to be more prevalent amongst MCI patients (Pa et al., 2009), and MCI patients who have an ε4 allele have been shown to perform worse on memory tests and have faster cognitive decline across a number of domains compared to MCI patients without an ε4 allele (Cosentino et al., 2008; Farlow et al., 2004; G. E. Smith et al., 1998; Whitehair et al., 2010). It has also been found that an ε4 allele is associated with greater memory deficits in cognitively normal persons (Caselli et al., 2007; Caselli et al., 2004).

These findings have also been supported by imaging studies; AD and MCI patients who are ε4 carriers have greater medial temporal lobe, and particularly hippocampal, atrophy compared to non-carriers (Hashimoto et al., 2001; Korf, Wahlund, Visser, & Scheltens, 2004; Petersen et al., 1995). Similarly, magnetic resonance imaging (MRI) studies have found that ε4 carriers have faster declines in cortical thickness and hippocampal volume, which are associated with poorer cognitive performance (Espeseth et al., 2008; Fennema-Notestine et al., 2011; Reiman et al., 1998). Functional MRI (fMRI) studies have found that even ε4 carriers

who do not exhibit Aβ aggregation have disrupted resting-state connectivity (Machulda et al., 2011; Sheline et al., 2010). Additionally, fMRI has shown that presence of an ε4 allele is associated with greater hippocampal activation during memory tasks, suggesting that ε4 carriers must use compensatory efforts in order to complete these tasks (Bondi, Houston, Eyler, & Brown, 2005; Bookheimer et al., 2000; Filippini et al., 2009). Finally, presence of an ε4 allele may negatively impact synaptic plasticity; animal studies have found that ε4 is associated with fewer and shorter denditric spines (Dumanis et al., 2009; Klein, Mace, Moore, & Sullivan, 2010).

The role of APOE in the central nervous system is to take-up lipids, and reallocate them to cells for myelin generation or membrane repair; APOE is also involved in regulating glutamate receptor function and synaptic plasticity (Y. Chen, Durakoglugil, Xian, & Herz, 2010; Hauser, Narayanaswami, & Ryan, 2011). As previously mentioned, it is thought that individuals with an ε4 allele do not clear Aβ from the brain as efficiently as individuals without an ε4 allele. This may be because the protein Apoe-ε4 binds to Aβ with a lower affinity, as compared to Apoe-ε3 or Apoe-ε2 lipoproteins (LaDu et al., 1994). Aggregates of Aβ cause synaptic injury, apoptosis, and are thought to lead to dementia (Biennow, de Leon, & Zetterberg, 2006; Hardy & Selkoe, 2002). It has also been found that ε4 carriers may be more susceptible to the types of neuronal stress that lead to tau phosphorylation, and therefore tangles (Aboud, Mrak, Boop, & Griffin, 2012; Liu, Kanekiyo, Xu, & Bu, 2013). Further, ε4 animal models have shown that the ε4 allele is associated with a greater inflammatory response; this finding may explain why anti-inflammatory AD drugs are most effective in ε4 carriers (Lynch et al., 2003; C. A. Szekely et al., 2008).

Translocase of the Outer Mitochondrial Membrane (TOMM) 40

More recently, another gene, TOMM40, has been linked to AD risk. Within intron 6 of the TOMM40 homolog gene, the length of a poly-T nucleotide repeat (rs10524523, or "523" for short) has been found to be associated with risk of AD (Lutz et al., 2010; Roses et al., 2010). TOMM40 has three allelic variations – short (S), long (L), and very long (VL). Longer poly-T repeats (i.e., ≥ 27) are associated with risk of AD and earlier AD onset, while shorter poly-T repeats (i.e., ≤ 16 repeats) may be protective (Abraham et al., 2008; Grupe et al., 2007; Harold et al., 2009; G. Li et al., 2013; Roses et al., 2010).

Studies in the Alzheimer's Disease Neuroimaging Initiative have shown that the very long/very long genotype is twice as prevalent in AD patients, and more common in MCI and is associated with hippocampal atrophy (Potkin et al., 2009; Shen et al., 2010). A case-control study found that, compared to those with the short/short genotype, individuals with one long allele had a 2.96 times increased risk of AD, and those with two long alleles had a 5.66 times increased risk of AD; additionally, those with long alleles had onset three years earlier as compared to those with the short/short genotype (G. Li et al., 2013). Further, the number of poly-T alleles (i.e., one or two) was positively associated with the number of neuritic tangles present in the brain (i.e., Braak stages V-VI) (G. Li et al., 2013). Additional studies have found that longer TOMM40 poly-T length is also associated with poorer performance on episodic memory tasks (S. C. Johnson et al., 2011) and smaller hippocampal volume (Bis et al., 2012; Shen et al., 2010; Vounou et al., 2012).

However, some studies have not found an association between TOMM40 and AD (Cruchaga et al., 2011; Guerreiro & Hardy, 2012; Jun et al., 2012). It is also unknown whether TOMM40 is independently associated with risk of AD, or if it is tied to the risk associated with APOE genotype (Ferencz et al., 2013). It is difficult to separate the effect of the two genes because they are in linkage disequilibrium, meaning they recombine less frequently than if they were in random formation (Mastaglia et al., 2013; Roses et al., 2010).

Although evidence is somewhat limited by small sample sizes, it appears that individuals with the APOE ε4/ ε4 genotype tend to have long poly-T repeats (between 21 and 30, with an average of 26.78 repetitions), while individuals with the APOE ε3/ ε3 genotype have a bimodal distribution of poly-T repeats, with peaks at 15.71 repetitions and 33.15 repetitions (Roses et al., 2010). To help determine the role of TOMM40 independently of APOE, some studies have examined only individuals with the APOE ε3/ ε3 genotype (S. C. Johnson et al., 2011; G. Li et al., 2013). Despite this entanglement, some studies have found that TOMM40 is associated with APOE levels in the cerebrospinal fluid and expression of APOE in the hippocampus (Bekris, Galloway, Montine, Schellenberg, & Yu, 2010; Bekris et al., 2008), suggesting TOMM40 plays a functional role in risk and development of AD (G. Li et al., 2013). Further, due to the relatively small body of research on TOMM40 and that most studies have been conducted in primarily Caucasian samples, the extent to which TOMM40 expression and length of the poly-T repeats vary by race or ethnic group is unknown (Roses et al., 2014). However, early evidence suggests, that like APOE, the risk association with AD and the length of poly-T repeats varies by race

and/or ethnic group. For example, in Caucasian populations the number of poly-T repeats spans from 14 to 39, but in African American populations the number of poly-T repeats spans from 14-54; it is not yet clear how these differences affect risk of LOAD and age of onset of LOAD (Roses et al., 2014). Future research conducted in more diverse samples is needed to elucidate any racial or ethnic group differences in TOMM40 expression. In conclusion, TOMM40 is important as it suggests that individuals who do not have an ε4 allele may also have increased genetic risk of developing AD.

The role of TOMM40 is to encode the mitochondrial pore protein, Tom40, which is, in part, involved in transporting Aβ into mitochondria (Hansson Petersen et al., 2008). Buildup of Aβ in the mitochondria may disrupt neuronal function (Caspersen et al., 2005). It has been suggested that the amyloid cascade begins with neurotoxic effects caused by both Tom40 and Apoe, or perhaps the interaction of the two, which releases cytochrome C (a protein involved in the electron transport chain) (Roses, 2010; Roses et al., 2010).

BACKGROUND – Diabetes and Alzheimer's Disease

DM is thought to increase the risk for AD and other dementias (Vagelatos & Eslick, 2013), be associated with age-related cognitive decline (Biessels, Strachan, Visseren, Kappelle, & Whitmer, 2014), and be associated with volumetric decline in several brain areas (van Elderen et al., 2010; van Harten et al., 2006).

Cross-sectional and longitudinal evidence has shown that DM and insulin resistance are associated with cognitive impairment and incident AD (Cukierman, Gerstein, & Williamson, 2005; Irie et al., 2008; Peila, Rodriguez, & Launer, 2002). As aforementioned, insulin resistance is caused by prolonged hyperinsulinemia, and is present in both diabetics and pre-diabetics. Having DM in mid-life has been associated with a three times increased risk of developing dementia, including AD, in later life (Schanider Beeri et al., 2004). In recent meta-analyses, it was estimated that DM is associated with a 1.33 to 2.05 times increased risk of AD (Lu, Lin, & Kuo, 2009; Vagelatos & Eslick, 2013). Another meta-analysis examined 16 longitudinal studies that investigated the association between DM and AD; 10 of the studies found a significant association, while 6 did not; however, when all 16 studies were pooled together, there was a significant association between DM and risk of AD (G. Cheng et al., 2012). A study that used population attributable fraction calculations estimated that 6 to 10 percent of dementia cases are directly attributable to DM (Kloppenborg, van den Berg, Kappele, & Biessels, 2008). DM is also associated with risk of MCI. A meta-analysis that pooled two studies found that in the total of 2,484 people, DM was significantly associated with increased risk of MCI (G. Cheng et al., 2012). Additionally, DM may negatively affect MCI patients as well; it was found that

patients with both DM and MCI had faster cognitive decline than those with MCI, but without DM (Ravona-Springer et al., 2010).

Diabetes and cognitive decline in specific domains

Several studies have also found that DM is associated with cognitive decline in those without dementia or MCI. Evidence suggests that developing DM in midlife and longer duration of DM is more greatly associated with decline in a range of cognitive domains. One study found that having DM in midlife was associated with diminished global cognition and executive function in late life (Roberts, Knopman, Przybelski, et al., 2014). Further, longer duration of DM, and the interaction between duration and poorly controlled DM, as measured by HbA1c levels, has been associated with poorer performance on attention and working memory, executive function, verbal fluency, and global cognition tasks (West et al., 2014). A recent study also examined rates of decline in DM patients (Tuligenga et al., 2014). The authors found that cognitively normal individuals (i.e., without a diagnosis of dementia or MCI) with DM had a 45 percent faster decline in memory, a 29 percent faster decline in reasoning, and a 24 percent faster decline in global cognition compared to cognitively normal persons without DM. These findings are substantial, as they translate to an equivalent 3.3 years of aging in memory, 2.9 years of aging in reasoning, and 4.4 years of aging in global cognition. These findings were further amplified in persons with poor glycemic control; those individuals showed even faster decline (Tuligenga et al., 2014).

The association between glycemic control and neuropsychological test performance has also been widely examined. Hyperglycemia and hyperinsulinemia, below the threshold for a DM diagnosis, have also been found to be associated with poorer cognition. For instance, higher insulin levels are associated with worse global cognition, memory, executive functioning, attention, and verbal fluency; these findings were more substantial in persons without AD with higher insulin levels as compared to AD patients with higher insulin levels (Burns et al., 2012). Similarly, higher glucose levels were associated with poorer verbal and logical memory, delayed, immediate, and working memory, processing speed, and verbal and spatial ability in persons with neither DM nor dementia (Lamport et al., 2009; Seetharaman et al., 2014). Recently, a longitudinal study found that higher HbA1c levels in midlife are associated with cognitive decline over a 20-year period, and that more poorly controlled DM is associated with greater decline (Rawlings et al., 2014). These findings lend support to the theory that cognitive decline associated with DM begins in pre-diabetic stages, and then continues gradually (Biessels et al., 2014). It may be that cognitive decline caused by DM, hyperinsulinemia, and/or hyperglycemia lowers the threshold for the onset of dementia (Biessels et al., 2014). This may mean that DM truly starts to affect cognition in middle age, and later onset (i.e., past the age of 65) of DM may not affect risk of dementia or cognitive decline (Tuligenga et al., 2014). Therefore, treating or preventing DM in middle age is critical in order to reduce the risk or perhaps even prevent age-related cognitive decline and AD (American Diabetes Association, 2013; Vagelatos & Eslick, 2013).

Diabetes and brain changes

The associations between DM and dementia and cognitive decline are further supported by imaging and autopsy studies. In DM patients' brains there is substantial glucose dysregulation, inflammation, reduced perfusion, increased protein deposition, and increased oxidative stress, all of which negatively impact brain health (Kodl & Seaquist, 2008; L. Li & Holscher, 2007). Cross-sectional studies have consistently found that DM is associated with smaller brain volume (Bryan et al., 2014; Tiehuis et al., 2008; van Harten et al., 2006). Longitudinal evidence has shown that brain volume loss in DM patients is three times what is seen in persons without DM (de Bresser et al., 2010; Espeland et al., 2013; Kooistra et al., 2013; van Elderen et al., 2010). Specifically, middle-aged diabetics and pre-diabetics have greater atrophy in the hippocampus (Benedict et al., 2012; Convit, Wolf, Tarshish, & de Leon, 2003; den Heijer et al., 2003; Raji et al., 2010; Rasgon et al., 2011; Tan et al., 2011; Tiehuis et al., 2008; Willette et al., 2013), orbitofrontal cortex (Raji et al., 2010), and prefrontal cortex (Benedict et al., 2012), compared to non-diabetics. In addition, longitudinal studies have found that DM patients have greater declines in volume, which are associated with cognitive decline; further, these volumetric and cognitive performance declines are comparable to three to five years of aging (de Bresser et al., 2010; Kooistra et al., 2013; van Elderen et al., 2010).

While the extent to which DM affects white matter volume is unclear, studies have found that DM is associated with declining white matter volumes and increasing presence of infarcts, which are linked with cognitive decline (Manschot et al., 2006; Reijmer et al., 2011; Saczynski et al., 2009). In the Women's Health Initiative Memory Study cohort, women with DM had significantly more total grey

matter volume atrophy, as well as focused grey matter volume loss in the parietal and temporal lobes, and larger ventricles and greater ischemic load as compared to women without DM (Espeland et al., 2013). Further, it seems that duration and severity of DM affects brain volume. Those who have had DM longer have reduced grey and white matter, and a greater number of white matter lesions (Saczynski et al., 2009). Similarly, diabetics who took medications or insulin (which can be considered a marker for more severe DM), have reduced grey and white matter as compared to DM patients who did not need to take these medications (Saczynski et al., 2009)

Studies that have examined connectivity in the brains of DM patients have found that they have decreased connectivity both locally and globally (Reijmer, Leemans, Brundel, Kappelle, & Biessels, 2013), and studies have shown that DM patients have reduced functional connectivity between regions of the default mode network (Musen et al., 2012). Further, even persons with increased insulin levels have decreased functional connectivity in areas associated with cognitive function, specifically between the medial prefrontal cortex and hippocampus (Kenna et al., 2013). These findings indicate that DM patients have both disrupted structural and functional connectivity. Insulin resistance has also been linked with impaired Aβ clearance and tau phosphorylation, which are thought to contribute to the development of the plaques and tangles characteristic of AD (Cholerton, Baker, & Craft, 2013; Cook et al., 2003; Farris et al., 2003; Ho et al., 2004; Mayeux et al., 2003; Neumann et al., 2008; Perez, Morelli, Cresto, & Castano, 2000). This body of evidence suggests that prolonged hyperglycemia and hyperinsulinemia contribute

to the pathological development of dementia, and the longer a person has these conditions the greater the risk for negative cognitive outcomes becomes.

Diabetes, APOE and TOMM40, and decline

The risk relationship between DM and cognitive decline and AD may be impacted by APOE and TOMM40, such that diabetics with either an ε4 allele or the TOMM40 long allele are at an even greater risk of developing cognitive decline and AD compared to those with just one of these risk factors. Studies have found that ε4 and DM have a synergistic relationship, such that those with both are 5.5 times more likely to have AD whereas those with just DM and without an ε4 allele are 1.8 times more likely to have AD and those with an ε4 allele without DM are approximately twice as like to have AD (Haan, Shemanski, Jagust, Manolio, & Kuller, 1999; Peila et al., 2002). However, these risk associations vary by study; a recent meta-analysis examined the interaction between DM and APOE on clinical AD, and found that risk rates ranged from 1.44 to 4.99 times increased risk (Vagelatos & Eslick, 2013). Autopsy studies have also supported this interaction. The brains of diabetics with an ε4 allele have more plaques in the hippocampus and tangles in the cortex, compared to those of individuals with just one of these risk factors (Peila et al., 2002). It seems that hyperglycemia, hyperinsulinemia, and insulin resistance support the formation of plaques (Matsuzaki et al., 2010) particularly in those with an ε4 allele. Studies have also examined the relationship between insulin resistance and ε4 and risk of AD. Two community-based studies have found that impaired insulin function at midlife interacted with ε4 to increase risk of AD (Ronnemaa et al., 2008; W. L. Xu,

Qiu, Wahlin, Winblad, & Fratiglioni, 2004); however, these findings have not been reported consistently (Schrijvers et al., 2010; W. Xu, Qiu, Winblad, & Fratiglioni, 2007).

When considering the risk relationship between cerebrovascular and/or metabolic conditions (e.g., DM), there is reason to believe that TOMM40, because it affects mitochondrial function, modifies this relationship (Hayden et al., 2012; Potkin et al., 2009). Multiple studies have found a decreased number of mitochondria and decreased brain glucose metabolism early in the AD course (Hirai et al., 2001; Mosconi, 2005). Fluoro-2-deoxy-D-glucose positron emission tomography (FDG-PET) imaging studies have even shown that there is decreased glucose metabolism in areas commonly affected early in the AD process, specifically the parieto-tempral and posterior cingulate cortices, as well as the frontal cortex, which is affected later in the course of AD (Mosconi, 2005). A recent study has found that older diabetics with the VL/VL genotype exhibit poorer cognitive performance, compared to those with the S/S genotype (Greenbaum et al., 2014). While it is still unclear exactly how TOMM40 may interact with DM to affect risk of dementia and cognitive decline, it may prove to be an important modifier because of its role in mitochondrial functioning and evidence showing it increases risk of AD.

Mechanisms

As previously mentioned, prolonged hyperglycemia and hyperinsulinemia may contribute to the development of AD decades prior to clinical onset (Launer, 2005; Roberts, Knopman, Cha, et al., 2014; W. Xu et al., 2009); however, several

other mechanisms may link DM to dementia and cognitive decline. Hyperinsulinemia may be detrimental to the otherwise positive effects normal levels of insulin have on cognition (Cholerton et al., 2013). Impaired insulin receptor signaling is a substantial component of the pathophysiology of both DM and AD (Balaraman, Limaye, Levey, & Srinivasan, 2006; Lee & Kim, 2007). Studies in mouse models have shown that disrupted insulin signaling increases levels of hyperphosphorylated tau and decreases cognitive function (Lucas et al., 2001). It has also been found that insulin works to regulate neurotransmitter release and synaptic plasticity (Ahmadian et al., 2004; Jonas et al., 1997; Skeberdis, Lan, Zheng, Zukin, & Bennett, 2001; Wan et al., 1997; Wang & Linden, 2000). Further, insulin receptors are concentrated in the hippocampus and medial frontal cortex, suggesting that chronically high levels of insulin might readily affect memory (Cholerton, Baker, & Craft, 2011; Craft, 2006; Zhao & Alkon, 2001). Animal studies have shown that long-term cognitive impairment results when insulin is depleted in mouse model brains, and that this impairment may be prevented if the animals are given insulin treatment (Biessels et al., 1998; Grunblatt, Salkovic-Petrisic, Osmanovic, Riederer, & Hoyer, 2007; Lannert & Hoyer, 1998). Hyperglycemia and hyperinsulinemia have also been shown to downregulate the actions of insulin-degrading enzyme (IDE) (Cholerton et al., 2013). Downregulation of IDE is associated with the formation of neuritic plaques and neurofibrillary tangles, through its involvement in Aβ clearance and prevention of tau phosphorylation (Alvarez, Munoz, & Maccioni, 2001; Alvarez, Toro, Caceres, & Maccioni, 1999; Maccioni, Otth, Conca, & Munoz, 2001; Pandini et al., 2013). In vivo studies suggest

that higher levels of insulin in the brain promote the release of Aβ, and may compete with Aβ as a target for IDE activity, which hinders breakdown of Aβ (Gasparini et al., 2001; Watson et al., 2003).

Advanced glycation end products (AGES) have also been identified as a possible mechanism linking DM and AD (Launer, 2005). Both diabetics and AD patients have an increased number of AGES compared to normal controls, and AGES are at the root of many diabetes complications (e.g., neuropathy, vision loss) (Goh & Cooper, 2008; M. A. Smith et al., 1994; Vitek et al., 1994). An increased number of AGES is associated with oxidative stress, endothelial and vascular dysfunction, increased inflammation, and increased protein, DNA, and mitochondrial damage (Mosconi, 2005; Singh, Barden, Mori, & Beilin, 2001). As previously mentioned, mitochondrial damage has been found to occur very early in the AD process. Studies have also found that in the brains of AD patients, AGES co-localize with apoE, which points towards involvement in the formation of neuritic plaques (Y. M. Li & Dickson, 1997). Additionally, a hormone known as insulinotropic glucagon-like pedptide-1 (GLP-1), which enables insulin release in hyperglycemic states, reacts in hyperglycemic conditions by improving insulin signaling, and, like AGES, defends against oxidative stress and neurotoxicity (Holscher, 2010). This suggests that GLP-1 may also play a role in the risk relationship between DM and AD. Studies have found that treatment with GLP-1 may improve cognition and amyloid burden in both animals and humans (Bomfim et al., 2012; Sivertsen, Rosenmeier, Holst, & Vilsboll, 2012). This evidence suggests that in DM patients metabolic control may be a therapeutic target for reducing risk of cognitive decline and dementia.

Oxidative stress and mitochondrial dysfunction are also implicated in the relationship between DM and AD. In mouse models, mitochondrial dysfunction occurs before plaques and tangles are present, perhaps suggesting that it is a causal factor in AD pathogenesis (Chou et al., 2011). Additionally, oxidative stress, which is pervasive in both DM and AD patients (Nunomura et al., 2004; Rosen et al., 2001), contributes to mitochondrial dysfunction. Oxidative stress promotes Aβ production, the Aβ then interacts with mitochondrial proteins, which causes a disruption in the electron transport chain, thereby promoting mitochondrial dysfunction and production of reactive oxygen species (Oda, Tamaoka, & Araki, 2010; Reddy et al., 2010)

It is likely that mitochondrial dysfunction is the link between DM, genetic factors, and AD, as well. As discussed, the APOE- ε4 allele is associated with greater risk of AD. Studies have also shown that individuals with an ε4 allele have increased mitochondrial dysfunction, which is thought to be caused specifically by ε4 fragments (Gibson et al., 2000), leading to neuronal damage (S. Chang et al., 2005; H. K. Chen et al., 2012). Because TOMM40 affects mitochondrial function, and because it appears mitochondria are involved even in the early stages of AD development, TOMM40 may also affect the risk relationship between DM and AD (Hayden et al., 2012; Potkin et al., 2009).

Psychiatric and medical conditions may act as mediators or confounders in the relationship between DM and AD. For example, major depression is associated with both DM and AD, and may act as a major confounder in the risk relationship

(Stewart & Liolitsa, 1999). Additionally, vascular conditions (e.g., hypertension, stroke, and obesity) are often co-morbid with DM, and even pre-date onset of DM in many patients. Because these conditions may contribute to AD, they may be confounding observed associations between DM and AD (Stewart & Liolitsa, 1999).

Potential for Treatment and Prevention

Unfortunately, AD treatment efforts, including nonsteroidal anti-inflammatory drugs (NSAIDs), γ-secretase inhibitors, β-secretase inhibitors, and an Aβ binding agent, tramiprosate have been largely unsuccessful to this point (Arvanitakis, Bennett, Wilson, & Barnes, 2010; D'Onofrio et al., 2012; Fourrier, Letenneur, Begaud, & Dartigues, 1996; Henley, May, Dean, & Siemers, 2009; Kaether, Haass, & Steiner, 2006; Morihara, Chu, Ubeda, Beech, & Cole, 2002; Mullard, 2012; Szekely, Green, & Breitner, 2008; Wolfe, 2012; Zandi et al., 2002). Some studies have suggested that treatment may only be effective prior to Aβ deposition (Lambracht-Washington & Rosenberg, 2013). Currently, there is no reliable biomarker that precedes Aβ deposition that can be used to determine the risk of developing AD (Jack et al., 2013). Given these limitations, it may be most effective to focus treatment and prevention efforts on persons with known modifiable risk factors, such as DM (Kravitz, Schmeidler, & Schnaider Beeri, 2013).

AD pathology begins to manifest in the brain years or decades prior to clinical symptoms become apparent (Amieva et al., 2008; Braak, Thal, Ghebremedhin, & Del Tredici, 2011; Jorm, Korten, & Henderson, 1987; Ohm, Muller, Braak, & Bohl, 1995). Therefore, introducing interventions in middle age, during or

just prior to the prodromal stage of AD, may be the most effective route for slowing the progression, delaying onset, or even preventing the disease (Carlson et al., 2008; C. Y. Chang & Silverman, 2004). DM is an example of a mid-life modifiable risk factor, and it is highly prevalent, so it may be a promising target for altering the course of AD (Kravitz et al., 2013). Indeed, diabetics that have received aggressive treatment for hyperinsulinemia and blood glucose control showed improvements in cognitive performance (Naor, Steingruber, Westhoff, Schottenfeld-Naor, & Gries, 1997; Ryan et al., 2006). In the ACCORD-MIND study cohort, the rate of brain atrophy on repeated MRI scans was slowed in DM patients who received intensive glycemic control treatment compared to patients who received standard treatment; however, these volumetric differences were not associated with improved cognitive function (Launer et al., 2011).

Similarly, treating dementia patients and those with cognitive impairment with intranasal insulin and rosiglitazone has been shown to improve cognitive function in these populations. (Craft et al., 2003; Craft et al., 1999; Craft et al., 1996; Craft et al., 1998; Park, Seeley, Craft, & Woods, 2000; Risner et al., 2006). Recent clinical trials investigating the potential benefits of the common type II diabetes oral medication pioglitazone have found that adults over age 60 taking the drug reduced their risk of developing AD by 6 percent; however, more extensive clinical trials are needed to determine the potential benefits of the drug (German Center for Neurodegenerative Diseases, 2014). Thus, DM pharmaceuticals may work to alter the course of AD in both diabetics and cognitively impaired individuals. Nonetheless, some studies have found that these treatments may not be effective in ε4 allele

carriers (Craft et al., 2003; Reger et al., 2008). Further studies are needed to understand the association between DM and cognitive decline, and how genetics impact this relationship. Such studies will help identify therapeutic targets for preventing or altering the course of AD.

PUBLIC HEALTH SIGNIFICANCE

Given the high and increasing prevalence of both DM and AD, and the myriad negative outcomes associated with both diseases, it is becoming increasingly important to find ways to treat and prevent DM and AD. Although DM is associated with an increased risk of AD (Vagelatos & Eslick, 2013), the nature and mechanisms of this risk relationship are still unconfirmed. Using population attributable risk analyses, one study found that approximately 30 percent of AD cases are attributable to modifiable risk factors (Barnes & Yaffe, 2011). The authors proposed that interventions at mid-life that target these factors, including vascular and metabolic variables (e.g., physical inactivity, smoking, hypertension, obesity, and diabetes), may reduce the prevalence of AD (Norton et al., 2014). The research presented here investigates DM as a risk factor for cognitive decline, AD or MCI symptom onset, and grey matter atrophy, and genetic factors that may affect these risk relationships in a sample at high-risk of developing AD. Prior studies indicate that individuals with both DM or pre-diabetes and a genetic risk factor are at elevated risk for developing AD or MCI, will have steeper trajectories of cognitive decline, and greater brain atrophy in areas associated with AD, as compared to individuals with one or neither of these risk factors, (Benedict et al., 2012; Chartier-Harlin et al., 1994; Peila et al., 2002; Potkin et al., 2009; Raji et al., 2010; Schanider Beeri et al., 2004; Shen et al., 2010). This research aims to elucidate the role of DM and genetic factors in the progression of AD, from the prodromal phase to clinical onset (Amieva et al., 2008; Braak et al., 2011).

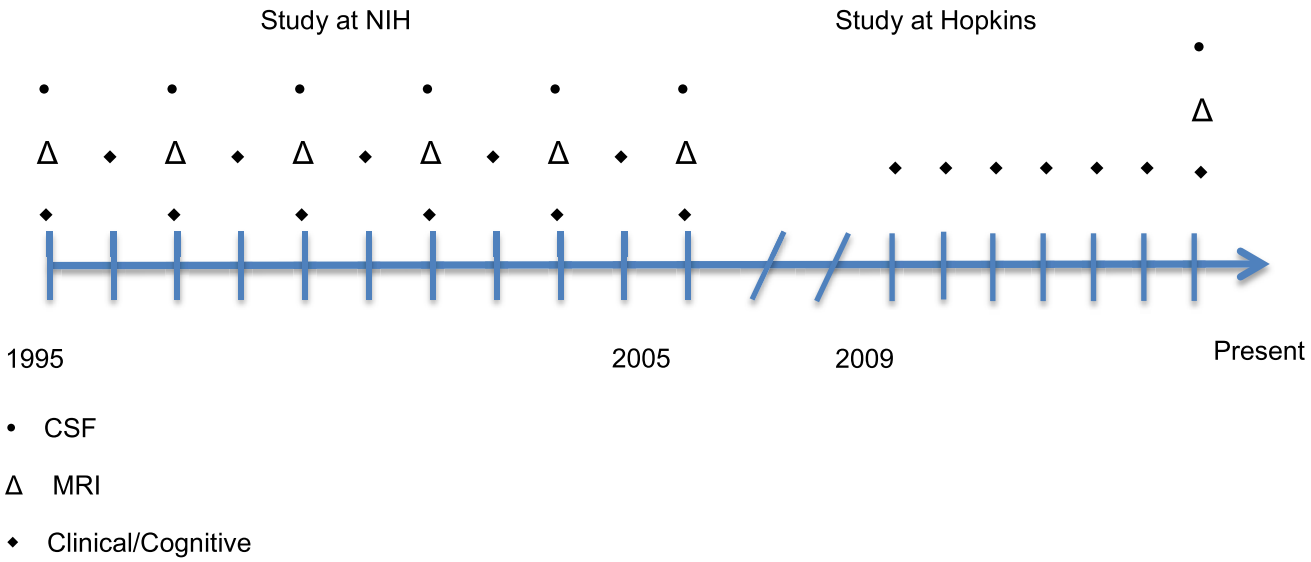
Results of this research may have implications for future treatment targets and intervention strategies. In light of the epidemic levels of DM and increasing prevalence of AD, and the apparent connection between the two diseases, it is essential to understand the relationship between the diseases and how this link may be leveraged to alter the course and prevalence of AD. Because pharmacological treatments of AD have been largely unsuccessful, targeting modifiable risk factors may be the most promising route for altering the course of, delaying the onset of, or even preventing AD (Craft et al., 2003; Giacobini & Gold, 2013; Kravitz et al., 2013). Further, genotyping is becoming more accessible and affordable, so it is becoming easier to identify individuals who are at heightened risk of cognitive outcomes, based both on their genotype and lifestyle risk factors. Therefore, if the hypotheses guiding this research are supported, clinicians may be able to design treatment strategies, including tighter metabolic control in DM patients, and other individuals at increased risk based on these risk factors. This would have implications for reducing the prevalence of AD, and subsequently reducing the healthcare costs of both diseases.

DATA: THE BIOCARD COHORT

This study used data from the Biomarkers of Cognitive Decline Among Normal Individuals (BIOCARD) cohort. The BIOCARD study began at the National Institutes of Health (NIH) in 1995; it was discontinued in 2005 due to administrative reasons. The study was re-initiated 2009 at the Johns Hopkins University School of Medicine via a U01 mechanism (PI: Marilyn Albert, PhD). The study initially enrolled and followed 349 participants; they were mainly middle-aged and all were cognitively normal. The cohort was considered at high-risk of developing dementia as nearly 75 percent of participants had a first-degree relative with dementia. Approximately half of the participants were female, the majority of participants were White, and they were, on average, highly educated (Table 1). Participants were recruited via local and national advertisements, word-of-mouth, and educational lectures. Exclusion criteria included significant cognitive impairment (e.g., dementia, MCI), or presence of significant medical, physical, psychiatric or neurological disorders (e.g., severe cardiovascular disease, schizophrenia, or seizure disorders), which was based on a clinical exam (Moghekar et al., 2013; Soldan et al., 2013). Presently, 89 percent of the initial cohort have been reenrolled; 38 participants are now deceased. On average, participants have been followed for 12.2 years (range 8 to 18 years). BIOCARD was designed to identify risk factors for dementia and to study the prodromal stages of dementia. Since re-initiation, the study is analyzing existing data, and is continuing to collect data from enrolled participants; it is not enrolling any new participants.

Participants completed a two-day comprehensive exam, including a physical and neurological exam, a neuropsychological battery, structural magnetic resonance imaging (MRI), and standard blood work (e.g., complete blood count, cholesterol count, B_{12} levels), at the Clinical Center of the NIH at enrollment. A subset of participants also consented to have their cerebrospinal fluid (CSF) drawn via lumbar puncture; 307 participants had their CSF drawn once, and 199 participants had their CSF drawn more than once (Moghekar, Goh, Li, Albert, & O'Brien, 2012). During the annual follow-up appointments between 1995 and 2005, BIOCARD participants completed the neuropsychological battery, and a physical and neurological exam. Every other year, participants completed MRI scans, blood work, and CSF draws (if consented) (Figure 1). Since re-initiation at Johns Hopkins University in 2009, annual exams have included a physical and neurological exam; assessment of past and present medication use; mood assessment with the Geriatric Depression Scale (Yesavage et al., 1982); assessment of family history of dementia; a similar neuropsychological assessment; and history of clinical symptom onset based on participant and informant reports (e.g., a family member) derived from the Clinical Dementia Rating Scale (CDR) interview (Morris, 1993).

Figure 1: BIOCARD Study Design and Follow-up



Adapted from (Moghekar et al., 2013)

Genotyping

Both APOE and TOMM40 genotype were determined using blood samples. APOE genotype was determined at the NIH; TOMM40 genotype was determined at Johns Hopkins University using stored blood samples.

Diabetes Diagnosis

DM diagnosis was determined during the BIOCARD annual exams. This information, along with use of DM medications, was recorded in the participants' BIOCARD records. Additionally, at the BIOCARD clinical examinations fasting blood glucose measures were ascertained through routine blood tests. There are 329 participants with complete baseline blood glucose data. Blood glucose level was collected at all of the clinical examinations; on average, there are 2.4 blood glucose

measures (range 1 to 7) per participant.

Neuropsychological Battery

From 1995 to 2005, and from 2009 to present, BIOCARD participants have completed a neuropsychological battery annually. The battery includes tests that measure a range of cognitive domains (i.e., memory executive function, language, visuospatial ability, attention, speed of processing, and psychomotor speed). The tests include the Mini-Mental State Exam (Folstein, Folstein, & McHugh, 1975), Letter and Category Fluency (Brenton & Hamsher, 1976), the Boston Naming Test (30-item version) (Kaplan, Goodglass, & Weintraub, 1983), the Logical Memory and Paired Associates subtests from the Wechsler Memory Scale – Revised (Wechsler, 1987), the California Verbal Learning Test (Delis, Kramer, Kaplan, & Ober, 1987), the Digit Span Backward and Forwards tests from the Wechsler Memory Scale – Revised (Wechsler, 1987), the Rey-Osterreith Figure, copy and recall (Rey, 1941), the Trail Making Test, Parts A and B (Reitan, 1958), the Block Design subtest of the Wechsler Adult Intelligence Scale – Revised (Wechsler, 1981), the Digit Symbol Substitution Test from the Wechsler Adult Intelligence Scale – Revised (Wechsler, 1981), and the Grooved Pegboard test (Klove, 1963).

On average, participants have completed at least a partial portion of the battery a mean of 7.3 assessments each. In total, 2,029 follow-up assessments have been completed. Since 2009, when the study was moved to Johns Hopkins University, the battery was shortened slightly due to time constraints.

AD and MCI consensus diagnosis

The BIOCARD Clinical Core at Johns Hopkins University performs an annual consensus diagnosis for each participant. The Clinical Core is comprised of neurologists, neuropsychologists research nurses, and research assistants. For each participant, the team considers changes in performance on the BIOCARD neuropsychological battery, reports from the participant and informant (e.g., a family member) regarding changes in cognition using the CDR, and other relevant clinical data in order to determine if the participant has MCI or dementia, or is cognitively normal. If a participant is diagnosed with MCI or dementia, the team also determines the age of onset and the cause of the impairment (e.g., Parkinson's disease, AD, or vascular dementia). This diagnostic method has been applied posthumously to the 38 deceased participants. To date, 49 participants have been diagnosed with MCI, and 14 participants have been diagnosed with AD. This research only included participants with late-onset or sporadic, as opposed to early-onset or familial AD and MCI in the analyses (Albert et al., 2013).

MRI Scans

MRI images were obtained using a 1.5 Tesla General Electric scanner. The scanning protocol involved localizer scans, axial Fast Spin Echo sequence (TR = 4250, TE = 108, FOV = 512 × 512, thickness/gap = 5.0/0.0 mm, flip angle = 90, 28 slices), Axial Flair sequence (TR = 9002, TE = 157.5, FOV = 256 × 256, thickness/gap = 5.0/0.0 mm, flip angle = 90, 28 slices), Coronal Spoiled Gradient Echo sequence (TR=24, TE- 2 FOV = 256 × 256, thickness/gap = 2.0/0.0 mm, flip angle = 20, 124 slices), and Sagittal Spoiled Gradient Echo sequence (TR = 24, TE = 3, FOV = 256 ×

256, thickness/gap = 1.5/0.0 mm, flip angle = 45, 124 slices) (Miller et al., 2013). As described by Miller and colleagues, the analysis of the scans focused on measuring the volume and thickness of the hippocampus, entorhinal cortex, and amygdala (Miller et al., 2013). The BIOCARD imaging core used the process of diffeomorphometry to analyze the scans (Csernansky et al., 2000; Qiu, Crocetti, et al., 2009; Qiu, Fennema-Notestine, Dale, & Miller, 2009). Diffeomorphometry consists of three steps: 1) segmentation of the target structures; 2) creation of a single template coordinate system from the baseline scan; and 3) mapping the template onto the target structure using triangulated meshes. Intracranial volume was calculated using coronal SPGR scans in FreeSurfer 5.1.0 (Segonne et al., 2004); the aforementioned structures were compared against intracranial volume in order to normalize specific structure volumes.

Additionally, cortical thickness in AD-signature regions was calculated with FreeSurfer. AD signature regions, as identified by Dickerson and colleagues (2009; 2011), include the entorhinal cortex, inferior temporal gyrus, parahippocampal gyrus, pars triangularis of the inferior frontal gyrus, precuneus, superior frontal gyrus, superior parietal gyrus, supramarginal gyrus, and temporal pole. Cortical thickness was measured using MRI scans, and by calculating the space from the boundary of grey matter and white matter to the boundary between grey matter and CSF (Fischl & Dale, 2000); the measures were based on both spatial intensity gradients and signal intensity. This method of measurement tessellates the boundary between grey and white matter, uses automated topology correction (Fischl, Liu, & Dale, 2001; Segonne, Pacheco, & Fischl, 2007), and accounts for

surface deformation (Dale, Fischl, & Sereno, 1999; Dale & Sereno, 1993; Fischl & Dale, 2000). This method of measurement has good validity and reliability (Han et al., 2006; Kuperberg et al., 2003; Rosas et al., 2002; Salat et al., 2004). Participants completed scans every other year, on average, while the study was being conducted at the NIH (1995 to 2005). There were 335 participants who underwent an MRI scan at enrollment; during follow-up 470 additional scans have been completed.

STATEMENT OF STUDY AIMS

This study aims to elucidate the relationship between glucose and AD and cognitive outcomes, and how genetic risk factors affect this risk relationship, in a sample that is considered high-risk for developing dementia. In particular, this study investigated how baseline glucose levels were associated with cognitive test performance over time, time to dementia or MCI symptom onset, and cortical thickness measures at baseline. Additionally, it investigated how the APOE ε4 allele and TOMM40 genotype may modify the relationship between glucose and these outcomes. The TOMM40 analyses were restricted in order to be sufficiently conservative in their assumptions. Specifically, these analyses were limited to subjects who were ε3 homozygous. This was done because the S allele may have a negative impact on cognitive outcomes in ε3/4 carriers (Gold, Johnson, Powell, & Smith, 2012; Gold, Powell, Andersen, & Smith, 2010; Greenbaum et al., 2014; Lyall et al., 2014), and this assured that the outcomes were not affected by the presence of either the ε4 or ε2 allele (Gold et al., 2012; Gold et al., 2010; Greenbaum et al., 2014; Lyall et al., 2014). Further, this study only compared VL homozygotes to S homozygotes, because the L allele is in high linkage disequilibrium with the ε4 allele (Greenbaum et al., 2014). Finally, all TOMM40 interaction analyses were restricted on only Caucasian participants, because TOMM40 poly-T length and associated risk with dementia and cognitive performance outcomes varies by race and ethnic group (Roses et al., 2014). This study was conducted using data from participants in the BIOCARD Study. The aims of this thesis were as follows:

Aim 1

To examine the association between baseline glucose and time to onset of MCI or dementia symptoms, cognitive test performance over time, and cortical thickness in AD-signature regions at baseline.

Hypothesis 1a. Higher baseline glucose will be associated with greater risk of and earlier onset of MCI or dementia symptoms.

Hypothesis 1b. Higher baseline glucose will be associated with declining performance on a battery of cognitive tests over time.

Hypothesis 1c. Higher baseline glucose will be associated with reduced cortical thickness measures in AD-signature regions (i.e., entorhinal cortex, inferior temporal gyrus, parahippocampal gyrus pars triangularis, precuneus, superior frontal and parietal gyri, supramarginal gyrus, and temporal pole*) at baseline.*

Aim 2

To examine the interaction of baseline glucose level with APOE and TOMM40 genotypes on cognitive decline, as measured by a battery of neuropsychological tests.

Hypothesis 2a. The APOE-ε4 allele and glucose level will interact, such that the association between glucose and poorer cognitive performance over time is stronger among those with an APOE- ε4 allele than among those without.

Hypothesis 2b. The TOMM40 VL/VL genotype and glucose level will interact, such that the association between glucose and both poorer cognitive performance over time is stronger among those with the TOMM40 VL/VL genotype than among those with the TOMM40 S/S genotype.

Aim 3

To examine the interaction of baseline glucose level with APOE and TOMM40 genotypes on time to dementia or MCI symptom onset.

Hypothesis 3a. The APOE-ε4 allele and glucose will interact, such that the association between glucose level and dementia or MCI symptom onset is stronger among those with an APOE-ε4 allele than among those without.

Hypothesis 3b. The TOMM40 VL/VL genotype and glucose will interact, such that the association between glucose level and dementia or MCI symptom onset is stronger among those with the TOMM40 VL/VL genotype than among those with the TOMM40 S/S genotype.

Aim 4

To examine the interaction of APOE and TOMM40 genotypes with glucose level on cortical thickness measures in AD-signature regions at baseline.

Hypothesis 4a. The APOE-ε4 allele and glucose will interact such that higher glucose levels will be more strongly associated with thinner cortical measures among those with an APOE-ε4 allele than among those without.

Hypothesis 4b. The TOMM40 VL/VL genotype and glucose will interact such that higher glucose levels will be more strongly associated with thinner cortical measures among those with the TOMM40 VL/VL genotype than among those with the TOMM40 S/S genotype.

PAPER 1

Baseline blood glucose and genetic factors as predictors of cognitive decline

ABSTRACT

Type II diabetes mellitus (DM) has been associated with cognitive decline. There may be an interaction between DM and the apolipoprotein E (APOE) ε4 allele, such that those with both are at greater risk for cognitive decline. There may be a similar relationship between DM and the translocase of the outer mitochondrial membrane (TOMM) 40 very long (VL) homozygous genotype with respect to cognitive performance, whereas short (S) homozygotes may be protected. Here, we investigated the association between baseline fasting blood glucose level and cognitive test performance over time in 333 participants from the BIOCARD Study, a cognitively healthy, middle-aged cohort. Glucose level was measured at the baseline clinical exam. We used confirmatory factor analysis (CFA) to generate latent variables measuring executive function and memory based on a battery of neuropsychological tests. We found that higher baseline blood glucose was associated with declining executive function using linear mixed effects models (LMM) (B=-0.005; 95% CI -0.008, -0.001) and generalized estimating equation (GEE) models (B=-0.004; 95% CI -0.007, -0.001) adjusted for sex, age, education, depression, and medical conditions. This association was stronger amongst ε4 carriers (B=-0.013; 95% CI -0.020, -0.006), versus non-carriers (B=-0.002, 95% CI -

0.006, 0.002). In LMMs, glucose was associated with declining executive function factor score in subjects with the S/S genotype (B=-0.013; 95% CI -0.024, -0.002), versus the VL/VL genotype (B=0.004; 95% CI -0.003, 0.010). Findings suggest that glucose control is important for maintaining cognitive function over time, even in healthy middle-aged individuals.

INTRODUCTION

Type II diabetes mellitus (DM) and pre-diabetes affect approximately half of the older adults in the United States (American Diabetes Association, 2014b). DM is associated with a number of negative physical and mental health outcomes (e.g., neuropathy, kidney problems, depression) (American Diabetes Association, 2014b), and there is broad empirical evidence that DM is also associated with greater age-related cognitive decline in multiple cognitive domains (Cukierman et al., 2005; Schrijvers et al., 2010; Tan et al., 2011). Epidemiological studies in large national cohorts have found that DM is associated with poorer performance on memory and visuospatial tasks (Elias et al., 1997; Wennberg et al., 2014). Recent studies have also found that DM and higher insulin and blood sugar levels are associated with poorer performance on tasks measuring global cognition, memory, speed of processing, verbal fluency, attention and working memory, and executive function (Burns et al., 2012; Lamport et al., 2009; West et al., 2014). Further, evidence suggests that longer duration of DM or poor glycemic control is associated with greater cognitive deficits and faster decline (Rawlings et al., 2014; Roberts, Knopman, Przybelski, et al., 2014; West et al., 2014). It also appears that cognitive decline associated with insulin resistance and higher glucose levels begins during the prediabetic stage in later midlife (i.e., 55 to 65 years of age), and that the greatest amount of damage due to insulin resistance and disrupted glucose levels occurs prior to diagnosis of AD (Biessels et al., 2014; Tuligenga et al., 2014).

The apolipoprotein-E (APOE) ε4 allele has been established as a risk factor for AD and associated cognitive decline; many studies have shown that carriers of the ε4 allele are at greater risk of both of these outcomes (Chartier-Harlin et al., 1994; Mormino et al., 2014). There is also evidence that there may be a synergistic relationship between DM and the APOE ε4 allele, such that individuals who have both DM and an ε4 allele are more likely to develop AD and experience greater atrophy in brain areas associated with cognition and more neuritic plaques in the hippocampus and neurofibrillary tangles in the cortex compared to those with either DM or an ε4 allele (den Heijer et al., 2003; Peila et al., 2002). It has also been shown that both MCI patients and cognitively normal individuals who have both DM and an ε4 allele have faster cognitive decline and poorer performance on memory tasks (Caselli et al., 2007; Caselli et al., 2004; Cosentino et al., 2008; Farlow et al., 2004; Ravona-Springer et al., 2014; Whitehair et al., 2010).

Similarly, pathophysiological evidence suggests that there may be a similar relationship between DM and the translocase of the outer mitochondrial membrane (TOMM) 40 gene (Caspersen et al., 2005; Hansson Petersen et al., 2008). TOMM40 has three allelic variations – short (S), long (L), and very long (VL). The number of poly-T repeats in the chromosome determines allele length. Longer poly-T repeats (i.e., ≥ 27) have been found to be associated with risk of AD and earlier onset of AD, while shorter poly-T repeats (i.e., ≤ 16 repeats) may be protective (Abraham et al., 2008; Grupe et al., 2007; Harold et al., 2009; G. Li et al., 2013; Roses et al., 2010). Studies have found that carriers of the TOMM40 VL allele have an increased risk of AD; this is the case in both those who carry just one VL allele (odds ratio = 2.96), and

in those who carry two VL alleles (odds ratio = 5.66) (G. Li et al., 2013). Further, the VL allele has also been associated with increased risk of MCI and hippocampal atrophy (Potkin et al., 2009; Shen et al., 2010). Given this evidence, and the aforementioned risk association of APOE ε4 and DM with cognitive outcomes, it is reasonable to believe that the TOMM40 VL/VL genotype modifies the relationship between DM and cognitive performance.

The current study investigated the association between DM and cognitive performance using participants in a study known as the Biomarkers of Cognitive Decline Among Normal Individuals: the BIOCARD cohort. We hypothesized that higher baseline blood glucose levels would be associated with declining performance on neuropsychological tests over time. Further, we hypothesized that those with both higher glucose and an ε4 allele or higher glucose and a TOMM40 long allele would have even greater cognitive decline.

METHODS

BIOCARD Study

In 1995 the BIOCARD Study began at the National Institutes of Health (NIH). It was discontinued in 2005 due to administrative reasons, but was re-initiated at the Johns Hopkins University School of Medicine in 2009 (PI: Marilyn Albert, PhD). At the NIH, 349 participants were enrolled in the study. Since re-initiation, 89 percent of the original participants have been reenrolled; 38 participants are deceased. The primary goal of the study was to identify risk factors for cognitive impairment and dementia in a mainly middle-aged, high-risk cohort. The sample

was characterized as such because participants were recruited based on family history of dementia; nearly three-quarters had a first-degree relative with dementia. The goal of the ongoing research at Johns Hopkins is to analyze existing data, and to continue to collect data from participants without enrolling new participants (Moghekar et al., 2013; Soldan et al., 2013).

When participants were enrolled at the NIH, they underwent a two-day comprehensive exam that included a physical and neurological assessment, a neuropsychological battery, structural magnetic resonance imaging (MRI), and blood draw with common lab assays (e.g., glucose, albumin, creatinine). A subset of participants also volunteered to have their cerebrospinal fluid (CSF) collected via lumbar puncture (Moghekar et al., 2012). Participants had annual follow-up visits; at each visit participants underwent a clinical and neurological exam, as well as a neuropsychological battery. While the study was conducted at the NIH, participants completed MRI scans, CSF draws, and blood draws every other year.

Participants

Participants were excluded from the BIOCARD Study if they presented with any significant cognitive impairment, or medical, psychological, or neurological disorder (e.g., severe cardiovascular disease, schizophrenia, or seizure disorder). Participants were mostly middle aged at recruitment. The sample is a high-risk sample for dementia, as approximately three-quarters of participants had a first-degree relative with dementia. This analysis included 333 participants from the BIOCARD Study. Informed consent was obtained from all participants.

Glucose and Diabetes Measures

Fasting blood glucose was collected during routine blood draws at the BIOCARD clinical appointments. In some instances, blood glucose was collected more frequently (e.g., for participants enrolled in different studies, at routine medical appointments, etc.), and these data were also included in our analysis. Of the 349 participants, 333 had baseline blood glucose data. During the clinical exam, it was also determined if the participant had a history of DM, and if they were using any medications (e.g., insulin, Metformin) to manage their DM.

Genotyping

Participant's APOE genotype was determined using blood samples collected at the NIA. TOMM40 genotype was determined using blood samples collected at Johns Hopkins University. Genotyping was performed using restriction endonuclease digestion of polymerase chain reaction amplified genomic DNA; this was completed by Athena Diagnostics (Worcester, MA). For statistical analyses, we coded participants with one or more ε4 alleles as '1,' and those without were coded as '0.' Participants with the TOMM40 VL/VL genotype were also coded as '1,' while those with the S/S genotype were coded as '0.'

For the purposes of this study, we restricted the TOMM40 analyses in order to take a more conservative approach. Although the S/S genotype is usually considered protective, as compared to VL/VL, studies have shown that the S allele may confer risk among individuals with an APOE ε3/4 genotype (Gold et al., 2012; Gold et al., 2010; Greenbaum et al., 2014; Lyall et al., 2014). Therefore, we restricted

analyses to APOE ε3 homozygotes. This also reduced the likelihood that any association between glucose and cognition we observed was caused by either the ε2 or ε4 APOE alleles (Gold et al., 2012; Gold et al., 2010; Greenbaum et al., 2014; Lyall et al., 2014). We only compared VL homozygotes to S homozygotes, because the L allele is in high linkage disequilibrium with the ε4 allele, and we did not want to inadvertently capture any effect of APOE (Greenbaum et al., 2014). Finally, we restricted TOMM40 analyses to only include White participants because it is thought that, as in APOE, TOMM40 poly-T repeats and associated risk varies by race and ethnic group, but these variations are not yet well understood (Roses et al., 2014).

Cognitive outcomes

The BIOCARD participants (n=349) completed a battery of neuropsychological tests on an annual basis. The tests include the Mini-Mental State Exam (Folstein et al., 1975), Letter and Category Fluency (Brenton & Hamsher, 1976), the Boston Naming Test (30-item version) (Kaplan et al., 1983), the Logical Memory and Paired Associates subtests from the Wechsler Memory Scale – Revised (Wechsler, 1987), the California Verbal Learning Test (Delis et al., 1987), the Digit Span Forward and Backward tests from the Wechsler Memory Scale – Revised (Wechsler, 1987), the Rey-Osterreith Figure, copy and recall (Rey, 1941), the Trail Making Test, Parts A and B (Reitan, 1958), the Block Design subtest of the Wechsler Adult Intelligence Scale – Revised (Wechsler, 1981), the Digit Symbol Substitution Test from the Wechsler Adult Intelligence Scale – Revised (Wechsler, 1981), and the Grooved Pegboard test (Klove, 1963). Participants completed at least some of the

tests at 7.3 visits, on average. All participants completed the battery upon study entry, and an additional 2,029 follow-up assessments have been completed.

Additional Variables

Participants also reported their age, the number of years of education they completed, and their race (i.e., White, African American, Hispanic, Other). Participants were also asked if they had a first-degree relative with dementia, and if so, how that relative was related to them (e.g., mother, father, sibling, etc.). During the clinical exam, participants were weighed and measured, and this information was used to calculate body mass index (BMI; kg/m^2). Participants were also asked if about their history of medical conditions, including cardiovascular disease, cerebrovascular disease, neurological conditions, metabolic conditions, mental health conditions (e.g., depression, anxiety), and smoking and substance use history. Participants also completed a number of standard clinical tests, a blood panel (e.g., cholesterol, potassium, bilirubin, etc.), was performed on drawn blood, and participants were asked about past and present medication use.

Statistical Analysis

We used Chi-squared and t-tests to determine if there were any differences in participant characteristics by ε4 status or TOMM40 genotype. We used linear mixed effects models (LMMs) with an autoregressive correlation matrix and generalized estimating equations (GEE) with an unstructured correlation matrix to examine the association between glucose at baseline and cognitive test scores over time. We also created interaction terms between glucose and APOE and glucose and

TOMM40 to determine the association between the interaction of glucose and ε4 status and cognitive performance, as well as the association between the interaction of glucose and TOMM40 genotype and cognitive performance. *A priori* to the analyses, we decided that if the interaction terms had a p-value ≤ 0.10 we would stratify the results. We chose to use an unstructured correlation matrix in the GEE models because when we attempted the analyses using an autoregressive correlation matrix, the pattern of missingness caused the model to drop many of the observations, greatly diminishing power. Therefore, the results from the GEE models may be asymptotically robust due to misspecification of the correlation structure. The results examining the association between baseline glucose and individual cognitive test scores over time were not robust, so we chose to combine the test scores to create more sensitive measures of cognition. We did this with confirmatory factor analysis (CFA).

Confirmatory Factor Analysis

CFA is based on structural equation modeling, and factors can be created based on *a priori* hypotheses (Jöreskog, 1969). Based on theory and *a priori* hypotheses, we grouped tests based on the cognitive domain they test (e.g., executive function, memory). We used orthogonal rotation, maximum likelihood estimation with robust variance estimation to estimate the models, and determined which tests loaded to which factors using a cut-off factor loading of 0.4 or greater. The number of factors was determined using an Eigenvalue of 1 or greater; this was verified using a scree plot. For these analyses, we used two factors. The first we

called “executive function,” and it included scores from the Digit Span backward, DSST, Verbal Fluency “A,” and WAIS Block Design (Table 2). The second we named “memory;” it included scores from Logical Memory immediate and delayed recall and Paired Associates learning and delay (Table 3). We used LMMs with autoregressive correlation matrices and GEE models with unstructured correlation matrices to determine the association between baseline glucose and factor score over time. We examined these associations in a model that controlled for age and sex (Model 1), and a fully-adjusted model that controlled for age, sex, education, race, medical conditions (i.e., cardiovascular disease, hypertension, hypercholesterolemia, traumatic brain injury (TBI)), and depression (Model 2). We again used interaction terms to determine if the association between glucose and factor score was modified by ε4 status or TOMM40 genotype. All analyses were completed using Stata 12.0 (StataCorp, 2009 College Station, TX).

RESULTS

Participant Characteristics

There were 333 participants with a baseline blood glucose measure. Of these, 58% were female, 98% were White, and they were on average 56.9 years old (SD=10.2). They had a high mean level of education (17.1 years, SD=2.4). As would be expected, given BIOCARD exclusion criteria, participants were relatively healthy; few reported history of or current medical conditions (i.e., cardiovascular disease, hypertension, hypercholesterolemia, TBI) (Table 1). Approximately one-third of participants had at least one ε4 allele. Those with an ε4 allele were more likely to

have hypercholesterolemia and depression (Table 1). Of the participants who were White and were APOE ε3 homozygous, 55 had the TOMM40 VL/VL genotype and 56 had the S/S genotype; there were no differences in demographic variables between the two genotypes (Table 1).

Association between Glucose and Factor Scores: LMMs

In a model that included sex and age as covariates (Model 1), we found that higher baseline blood glucose was associated with a declining score on the executive function factor (B=-0.004; 95% CI -0.007, -0.0005). This association remained significant (B=-0.004; 95% CI -0.008, -0.001) after also controlling for education, race, and medical conditions (Model 2). There was no association between baseline glucose and memory factor score over time in either Model 1 or Model 2 (Table 4). Although there was a significant interaction term (p=0.015) between glucose and APOE for the memory factor in Model 2, this was not significant after stratification. However, among those with the S/S genotype, higher baseline blood glucose was associated with decreasing executive function factor score (B=-0.013; 95% CI -0.024, -0.002), as compared to those with the VL/VL genotype (B=0.004; 95% CI -0.003, 0.010), in a model that controlled for sex and age (p for interaction=0.009) (Table 5). There was not an interaction between glucose and TOMM40 genotype in Model 2.

The Association between Glucose and Factor Scores: GEE Models

Similar to results from LMMs, we found that in both Models 1 (B=-0.007; 95% CI -0.010, -0.004) and 2 (B=-0.004; 95% CI -0.008, -0.001), higher baseline blood glucose was associated with declining executive function factor score in GEE

models, as well (Table 6). Again, baseline blood glucose was not associated with the memory factor in either of the two models. In interaction analyses investigating the association between glucose and memory factor, the interaction term between glucose and TOMM40 was not significant (p=0.204). The interaction term between glucose and TOMM40 for executive function was significant (p=0.015), but this association did not remain significant after stratification. Again, there was no significant interaction between glucose and TOMM40 in Model 2. However, in the fully adjusted model, higher baseline blood glucose was associated with decreasing executive function score amongst those with one or more ε4 alleles (B=-0.013; 95% CI -0.020, -0.006), as compared to those without an ε4 allele (B=-0.005; 95% CI -0.009, -0.002) (p for interaction = 0.019) (Table 7). The interaction term was also significant (p=0.019) for the memory factor, but associations between glucose and the memory factor were not significant after stratification by APOE ε4 status.

DISCUSSION

This study examined the association between baseline blood glucose and subsequent change in cognitive performance over time in a middle-aged cohort at elevated risk for developing dementia. Higher baseline blood glucose was associated with declining executive function factor score. In some analyses, this association was stronger among those with one or more APOE ε4 alleles. Additionally, our analyses using LMMs indicated that this relationship was more robust among TOMM40 S homozygotes. However, analyses using GEE models showed that the

interaction between glucose and TOMM40 was only significant for the memory factor score.

This study used CFA to create latent factors as a means of measuring cognitive change. This was done to create a more sensitive measure of cognitive domains. However, using these factors makes interpretation of the coefficients more difficult. On the executive function factor, most participants had a score between -2 and 2 (Figure 2). To put this into context, those with a score of -2 on the executive function factor had a mean score of 29.8 on the DSST, 6.6 on the verbal fluency test, 13.4 on the WAIS block design test, and 3.9 on the Digit Span backward test. Conversely, those with a score of 2 had mean scores of 87.7 on the DSST, 20.7 on the verbal fluency test, 48.8 on the WAIS block design test, and 11.3 on the Digit Span backward test. Although our coefficients may seem small, over time a coefficient of -0.004 is clinically significant. Blood glucose levels tend to increase with age, and can have great variation during the aging process. For example, a study in a nationally representative cohort found that from the ages of 40 to 70, fasting blood glucose increases by 20 mg/dL, on average (Yashin et al., 2009). This translates into appreciable decline on the executive function factor (i.e., 20 x -0.004= -0.08), and this decline would be even greater for those who experience greater than average increases in blood glucose level over time, even if they are never diagnosed with DM.

These findings are consistent with past studies. It has been found that DM patients have 45% faster decline in memory, 29% in reasoning, and 24% in global cognition, as compared to normal controls; this translates to 3.3 years aging in

memory, 2.9 years in reasoning, and 4.4 years in global cognition (Tuligenga et al., 2014). A recent study also found that higher glucose in later middle-aged subjects without DM was associated with decline in perceptual speed, verbal and spatial ability, and global cognition (Seetharaman et al., 2014). It is thought that DM and hyperglycemia begins to affect cognition as early as midlife (Tuligenga et al., 2014; West et al., 2014). Hyperglycemia damages neurons, produces glutamate toxicity, and increases oxidative stress, which produces mitochondrial damage (Nunomura et al., 2004; Rosen et al., 2001; West et al., 2014). Studies in animal models show that mitochondrial dysfunction occurs prior to plaque and tangle formation, and may contribute to AD pathogenesis (Chou et al., 2011). Oxidative stress-induced mitochondrial dysfunction caused by hyperglycemia may also be a link between DM, genetic factors, and neurocognitive outcomes. Carriers of the ε4 allele have increased mitochondrial dysfunction caused by ε4 fragmentation (Gibson et al., 2000), which causes neuronal damage (S. Chang et al., 2005; H. K. Chen et al., 2012). TOMM40 is also linked with mitochondrial and metabolic function, and because mitochondrial function is important in the early stages of AD development, TOMM40 may also affect the risk relationship between hyperglycemia and cognitive decline (Hayden et al., 2012; Potkin et al., 2009). This suggests that elevated glucose levels, even in people without a DM diagnosis, may be susceptible to the negative impact that hyperglycemia has on cognitive function, and individuals with an ε4 allele or the TOMM40 VL/VL genotype may be even more vulnerable.

The findings of this study must be viewed alongside its limitations. We hypothesized that the VL/VL group would have greater decline than the S/S group.

This was based on other findings that have shown the VL/VL allele is associated with poorer cognitive performance in diabetics (Greenbaum et al., 2014). However, we found that subjects with the S/S genotype had declining factor scores over time, but we suspect that this may have been due to our limited sample size (VL/VL n=41; S/S n=38). Given that the decline in factor score (memory versus executive function) depended on the type of model (GEE versus LMMs) used, we suspect that statistical power was an issue. Second, due to missingness in the dataset, we were unable to control for BMI, which has been associated with poorer cognitive performance (Gunstad et al., 2007). However, some evidence shows that BMI is associated with cognitive decline via hyperglycemia (Luchsinger, 2008), so by including it in the models as a covariate we may have risked over-adjustment. Third, we may have found a more robust association had we used a more accurate measure of glucose, such as HbA1c measures, which reflect glucose levels over the previous three months. Finally, the findings of this study may not be generalizable to the general population, because the majority of the BIOCARD cohort is White, they are very highly educated, and most were considered high-risk for dementia because they had a first-degree relative with the disease.

In this middle-aged, cognitively healthy cohort, we found that baseline blood glucose level was associated with declining executive function factor score. We found that this relationship was stronger amongst APOE ε4 carriers, as compared to non-carriers, in more robust models. This suggests that even moderate difference in blood glucose in middle age may impact cognitive function over time. Future research should work to determine how glucose impacts cognitive function in

younger cohorts, and larger studies should determine how this relationship is modified by TOMM40 genotype. Additionally, efforts to improve glucose control through diet and physical activity, even in non-diabetics, may be important for maintaining cognitive health throughout the aging process.

PAPER 2

Blood glucose levels and genetic factors as predictors of age of onset of mild cognitive impairment (MCI) and dementia symptoms

ABSTRACT

Several studies have found that type II diabetes mellitus (DM) increases the risk of dementia. Further, studies have found that there is a synergistic interaction between DM and the apolipoprotein-E (APOE) ε4 allele and DM, such that those with both risk factors have an even greater risk of developing dementia, compared to those with neither or only one risk factor. The translocase of the outer mitochondrial membrane (TOMM) 40 gene has also been linked to dementia risk, and there is evidence to suggest it may interact with DM. Here, we investigated the association between baseline blood glucose and age of clinical mild cognitive impairment (MCI) and dementia symptom onset; the association between the interaction of APOE and blood glucose and symptom onset; and the association between the interaction of glucose and TOMM40 very long/very long (VL/VL) genotype as a risk factor for dementia. This research was conducted using longitudinal data from the Biomarkers of Cognitive Decline Among Normal Individuals: the BIOCARD cohort (n=333), which enrolled mainly middle-aged participants, most of whom were considered high risk because many had a first degree relative with dementia. We did not find an association between baseline blood glucose and age of onset of clinical symptoms in a multivariable-adjusted Cox

proportional hazards model (Hazard Ratio (HR)=1.00; 95% Confidence Interval (CI)= 0.993, 1.01). Nor did we find an interaction between glucose level and genotype. Future studies should more closely exam the mechanistic link between DM and dementia, and how APOE or TOMM40 genotype may modify this relationship.

INTRODUCTION

As the population ages, both type II diabetes mellitus (DM) and Alzheimer's disease (AD) are becoming greater public health concerns (Administration on Aging, 2013; Alzheimer's Association, 2014; American Diabetes Association, 2014b). Approximately 25 percent of older adults have DM, and it is estimated that 50 percent of older adults are pre-diabetic (American Diabetes Association, 2014b). Similarly, AD and mild cognitive impairment (MCI), which is commonly a prodrome of AD, are both highly prevalent. Approximately 5 million older adults have been diagnosed with AD, and this number is expected to increase three-fold by 2050 (Herbert, Scherr, Bienias, Bennett, & Evans, 2003). Further, it has been estimated that 10 to 20 percent of people over the age of 65 have MCI (Hanninen, Hallikainen, Tuomainen, Vanhanen, & Soininen, 2002; Lopez et al., 2003; Roberts et al., 2008), of whom 10 to 30 percent are estimated to progress to dementia annually (Fischer et al., 2007; Petersen et al., 1999).

Many studies, both cross-sectional and longitudinal, have found that DM is associated with an increased risk for MCI and AD (Cukierman et al., 2005; Irie et al., 2008; Peila et al., 2002). Meta-analyses have estimated that DM is associated with a 1.33 to 2.05 increased risk of AD (Lu et al., 2009; Vagelatos & Eslick, 2013). A study that used population attributable risk calculations found that 6 to 10 percent of dementia cases are directly attributable to DM (Kloppenborg et al., 2008). Further, studies have found that DM significantly increases the risk of MCI (G. Cheng et al., 2012). Diabetics have extended periods of hyperglycemia and hyperinsulinemia,

which may play a factor in the development of AD many years before clinical symptoms develop (Launer, 2005; W. Xu et al., 2009). Further, diabetics who have had DM for longer and who have more poorly controlled glucose and insulin levels are at even greater risk of developing AD (Roberts, Knopman, Przybelski, et al., 2014; Saczynski et al., 2009; West et al., 2014).

Two genetic factors have also been linked to an increased risk of AD. The apolipoprotein-E (APOE) ε4 allele has been shown to be one of the most reliable and strongest risk factors for risk of AD (Chartier-Harlin et al., 1994). More recently, translocase of the outer mitochondrial membrane (TOMM) 40 long (L) and very long (VL) alleles, have also been associated with increased risk of AD and earlier age of onset of late onset AD compared to the short (S) allelic variation (Roses, 2010; Roses et al., 2010). Studies have also found that there may be an interaction between these genetic factors and DM. Findings from a meta-analyses show that individuals with both an ε4 allele and DM have an even greater risk of developing AD, compared to individuals with just one or neither of those risk factors (Vagelatos & Eslick, 2013). One study found that persons with both DM and one ε4 allele have a 5.5 times increased risk of AD (Haan et al., 1999), whereas those with DM without an ε4 allele have approximately twice the risk of AD (Vagelatos & Eslick, 2013), and those with one ε4 allele without DM have a two to three times increased risk (Farrer et al., 1997). To our knowledge, only one study has investigated the risk relationship of TOMM40 in diabetics on cognitive outcomes (Greenbaum et al., 2014). Pathophysiological evidence suggests a potential synergistic interaction between TOMM40 and diabetes on dementia. TOMM40 is involved in mitochondrial function

and metabolic changes, suggesting that it plays a role in both AD and DM (Caspersen et al., 2005; Hansson Petersen et al., 2008; Hayden et al., 2012; Hirai et al., 2001; Mosconi, 2005; Potkin et al., 2009).

This study used data from participants in the Biomarkers of Cognitive Decline Among Normal Individuals: the BIOCARD cohort. We investigated the association between baseline glucose level and age of onset of symptoms of MCI or AD; the association between the interaction between baseline glucose level and APOE genotype and the age of onset of symptoms; and the association between the interaction between baseline glucose level and TOMM40 genotype and the age of onset of symptoms. We hypothesized that higher glucose levels would increase the risk of developing MCI or AD, and that higher glucose levels in combination with a genetic risk factor (i.e., an ε4 allele or a TOMM40 long allele) would further increase this risk.

METHODS

BIOCARD Study

At baseline, the BIOCARD cohort (n=349) was middle-aged and high risk for dementia; it was designed to investigate risk factors for and the very early stages of cognitive impairment and dementia. The study was initially conducted at the National Institutes of Health (NIH) from 1995 to 2005; in 2005 the study was discontinued for administrative reasons. The study was re-initiated at the Johns Hopkins University School of Medicine in 2009 (PI: Marilyn Albert, PhD). A total of 89 percent of the participants have been reenrolled; 38 participants are now

deceased. No new participants are currently being enrolled, but the BIOCARD research team is continuing to collect data on the original cohort and analyze data that were collected at the NIH (Moghekar et al., 2013; Soldan et al., 2013).

When enrolled, participants underwent a two-day comprehensive exam at the Clinical Center of the NIH. They completed a clinical and neurological exam, as well as a neuropsychological battery, structural magnetic resonance imaging (MRI) scans, and standard blood work. Some participants also volunteered to have samples of their cerebrospinal fluid (CSF) collected via a lumbar puncture. Participants had annual follow-up exams. Every year participants completed clinical and neurological exams and a neuropsychological battery, and every other year they also completed MRI scans and CSF draws.

Participants

By design, BIOCARD participants were generally considered high-risk for dementia, as approximately three-quarters of participants had a first-degree relative with dementia. At enrollment, most participants were middle-aged (mean=57.2, standard deviation (SD)=10.3). Participants were excluded from the study if they had cognitive impairment, or a significant medical, psychiatric, or neurological disorder (e.g., severe cardiovascular disease, substance abuse, or epilepsy). Informed consent was obtained from all participants.

Glucose and Diabetes Measures

During the clinical exam, BIOCARD participants had a routine blood draw from which fasting blood glucose was measured. Of the 349 participants, 333 had a baseline glucose level recorded (mean=99.1 mg/dL, SD=24.6). DM diagnosis was self-reported. At the clinical exam each participant was asked if a doctor had ever diagnosed them with DM. Participants were also asked about medication use, including diabetes medication (e.g., Metformin, injectable insulin, etc.).

Genotyping

Both APOE and TOMM40 genotypes were determined from blood samples using restriction endonuclease digestion of polymerase chain reaction amplified genomic DNA; this was completed by Athena Diagnostics (Worcester, MA). APOE genotype was ascertained at the NIH, and TOMM40 genotype was determined after the study was re-initiated at Johns Hopkins University, using stored blood samples.

Time to MCI or Dementia Symptom Onset

Consensus dementia diagnosis was performed annually for each participant at Johns Hopkins University. The BIOCARD clinical core, which consists of expert neurologists, neuropsychiatrists, research nurses, and research assistants, completed the diagnoses. This team reviewed changes in performance on the BIOCARD neuropsychological battery, reports from the participant and informants (e.g., a family member) regarding changes in cognition using the Clinical Dementia Rating (CDR) scale, and other potentially relevant clinical data. This information was used to determine if the participant was cognitively normal, or had MCI or dementia. If the participant was diagnosed with MCI or dementia, the team also worked to

determine the age of onset and cause of the impairment (e.g., AD, Parkinson's disease, vascular dementia, etc.). This same method of diagnosis was applied posthumously to deceased participants. Currently, 63 participants have been diagnosed with either MCI or late-onset AD dementia (MCI *n*=49; AD dementia *n*=14) (Albert et al., 2013).

Additional Variables

Participants also reported their age, the number of years of schooling they completed, and their race. Additionally, participants were asked if they had a first degree relative who had dementia (e.g., parent, sibling). Participants were also asked about their medical history, their mental health conditions, and smoking and substance use history. The clinical exam also included height and weight measurements, blood pressure measurements, heart rate, a standard blood panel (e.g., cholesterol, potassium, bilirubin), and a standard neurological exam.

Statistical Analyses

To identify differences in participant characteristics by genotype, we used chi-squared analyses and Student's t-test. We used Cox proportional hazards models to determine the association between baseline glucose level and time to onset of clinical MCI and dementia symptoms, as determined by consensus diagnosis. We chose this time point as the failure time because of the four-year gap in the study between when the study was discontinued at the NIH (2005) and reinitiated at Johns Hopkins (2009), during which the participants were not contacted; therefore,

by using the estimated age of onset we were able to in essence eliminate the time gap from the analyses (Albert et al., 2014).

Similarly, we used Cox regression analyses to determine the association between baseline glucose level and APOE or TOMM40 genotype and time to symptom onset by creating interaction terms between glucose and APOE and glucose and TOMM40. We chose to use a p-value cut-off of 0.10 or less for our interaction terms to determine whether to stratify the results. To examine the interaction between glucose and APOE, we first examined the unadjusted association (Model 1). In Model 2, we included sex, education, and race. Model 3 included the same covariates as Model 2 plus medical conditions (i.e., cardiovascular conditions, traumatic brain injury (TBI), hypertension, hypercholesterolemia, and depression). These variables were coded dichotomously (i.e., '0' or '1'), based on whether the participant did not or did have the medical or mental condition.

To examine the interaction between glucose and TOMM40, we restricted the sample to only those participants who were APOE ε3 homozygotes. This was done because some studies have shown that the TOMM40 S allele is associated with poor cognitive outcomes in APOE ε3/4 carriers (Gold et al., 2012; Gold et al., 2010; Greenbaum et al., 2014; Lyall et al., 2014) and by excluding APOE ε2 and ε4 carriers we were able to further isolate the effect of TOMM40; ε2 and ε4 have also been linked to cognitive outcomes (Gold et al., 2012; Gold et al., 2010; Greenbaum et al., 2014; Lyall et al., 2014). We also chose to only compare S allele homozygotes to VL allele homozygotes, because the L allele is in such high linkage disequilibrium with

the ε4 allele that, again, it would be difficult to determine the independent association of TOMM40 (Greenbaum et al., 2014). Finally, we restricted TOMM40 analyses to Caucasians, because early research has shown that non-Caucasian groups have a different number of poly-T repeats at the '523' locus, which is the length that determines allelic length (i.e., S, L, VL) associated with cognitive outcomes. Future research in other race and ethnic groups will provide more information about poly-T length and risk association in more diverse samples, but at this time it is more conservative to restrict analyses to Caucasians (Roses et al., 2014). For the purposes of statistical analyses, we coded participants with an ε4 as '1' and those without were coded as '0.' Similarly, we coded participants with the VL/VL genotype as '1' and those with the S/S genotype were coded as '0.' All analyses were completed using Stata 12.0 (StataCorp, 2009 College Station, TX).

RESULTS

Participant Characteristics

Of the participants with a blood glucose measure at baseline, over half (58%) were female, most (98%) were White, they had a mean age of 56.9 years (SD=10.2), and a high mean level of education (mean=17.1 years, SD=2.4). Participants were also generally healthy; the mean body mass index (BMI) was 26.4 kg/m^2 (SD=5.8), and there was a relatively low prevalence of medical conditions (i.e., cardiovascular disease, hypertension, hypercholesterolemia, traumatic brain injury (TBI)) (Table 1). Of the 333 participants with complete glucose data, 109 (32.7%) were ε4 carriers. Those with one or more ε4 alleles were more likely to have hypercholesterolemia

and depression, compared to those without an ε4 allele. Of the White participants with complete glucose data and the APOE ε3/ε3 genotype, 55 had the TOMM40 VL/VL genotype and 56 had the S/S genotype. There were no differences in participant characteristics between those with the VL/VL genotype and those with the S/S genotype (Table 1).

Effect of baseline glucose level on symptom onset

There was no association between baseline glucose level and age of clinical symptom onset in an unadjusted model (Hazard Ratio (HR)= 1.00, 95% confidence interval (CI)=0.996, 1.01). Nor was glucose associated with symptom onset after adjustment for years of education, sex, and race (HR=1.00; 95% CI 0.996, 1.01). The association remained non-significant when we added medical conditions as covariates (HR=1.00; 95% CI 0.993, 1.03) (Table 8). The association between glucose and age of symptom onset did not vary by APOE (for all 3 models, interaction term p-values>0.10) or TOMM40 (for all 3 models, interaction term p-values>0.10) genotype.

DISCUSSION

This study examined the association between baseline glucose level and onset of clinical symptoms of MCI or dementia in the BIOCARD cohort. Further, we investigated the association between the interaction between glucose and APOE and glucose and TOMM40 and clinical symptom onset. We found no association between glucose levels and clinical symptom onset and no association between interactions between glucose and genotype, either APOE or TOMM40, and age of symptom onset.

Several studies have found that higher levels of glucose, insulin resistance, and DM are all associated with risk of MCI and dementia (Vagelatos & Eslick, 2013). It has also been established that the ε4 allele is one of the strongest risk factors for MCI and dementia (Chartier-Harlin et al., 1994). Further, studies have found a positive interaction between DM and the ε4 allele with respect to cognitive outcomes, suggesting that those with the ε4 risk allele are at greater risk of developing MCI or dementia if they also have higher blood glucose levels, insulin resistance, or confirmed DM, compared to those with either the risk allele or DM (S. T. Cheng et al., 2012; Vagelatos & Eslick, 2013). Finally, new evidence suggests that the VL TOMM40 allele is associated with earlier age of onset of late onset AD (Roses, 2010; Roses et al., 2010). To our knowledge, this is the first study examining the association between the interaction of glucose level and TOMM40 and onset of clinical MCI and dementia symptoms; however, past studies in samples of diabetics have shown that those with the VL/VL genotype have worse cognitive performance than those with the S/S genotype (Greenbaum et al., 2014).

This study has many strengths, including its longitudinal design and unique cohort; however, the study also has some limitations. The BIOCARD study enrolled middle-aged, healthy subjects, so relatively few participants developed MCI or dementia at the time of analysis. Therefore, there may be limited power for detecting an association between baseline glucose and clinical symptom onset. We may be able to observe an association between glucose and onset of clinical symptoms when more participants develop MCI or dementia. Additionally, although blood glucose was measured in this study, more reliable measures of DM, such as

HbA1c, were not collected. These limitations may have attenuated the observed effect that glucose, and by extension DM, had on age of onset of clinical symptoms. We were not able to control for BMI in this study, as few participants had complete baseline height and weight data. Evidence has shown that BMI may be associated with risk of dementia (Tolppanen et al., 2014); conversely, it may be that BMI is associated with increased risk of dementia through hyperglycemia, so if we had controlled for BMI it would have been over-adjustment (Luchsinger, 2008). Finally, the findings of this study may not be generalizable to the general population, because participants had a high mean level of education and most were Caucasian.

Future studies are needed to further examine the link between DM and dementia and mechanisms that may connect the two conditions. Further, although the relationship between DM and risk of dementia by APOE genotype has been well defined (Vagelatos & Eslick, 2013). Further studies are needed in order to determine whether and how the association between DM and risk of dementia is modified by TOMM40 genotype. It has been suggested that as much as a quarter of dementia cases—the majority of which are due to AD—might be avoided by addressing modifiable risk factors, such as DM (Barnes & Yaffe, 2011). DM may be of particular importance because it is so prevalent amongst older adults, but it also a treatable and preventable disease (American Diabetes Association, 2014b). Given that there is no current effective treatment for AD, research that better defines the risk and mechanisms between modifiable risk factors and dementia will play an important role in decreasing the burden of the disease.

PAPER 3

Blood glucose levels and genetic factors as predictors of cortical thinning

ABSTRACT

Currently, there is no cure for Alzheimer's disease (AD), so identifying biomarkers to diagnose and intervene in the preclinical stages may be the most effective way to alter the course of AD. Cortical thinning in the entorhinal cortex, inferior temporal gyrus, parahippocampal gyrus pars triangularis, precuneus, superior frontal and parietal gyri, supramarginal gyrus, and temporal pole is thought to occur early in AD, thus representing a non-invasive marker for predicting progression to AD. It is also thought that modifiable risk factors for dementia, including type II diabetes mellitus (DM), may represent an important target for reducing the prevalence of AD. Additionally, genetic markers, including the apolipoprotein E (APOE) ε4 allele and the translocase of the outer mitochondrial membrane (TOMM) 40 very long (VL) allele, may modify this relationship. Here, we investigated the cross-sectional association between baseline blood glucose level and cortical thinning in a sample of middle-aged, cognitively normal adults. We also investigated the interaction between glucose and APOE and glucose and TOMM40, to determine whether genotype affected this relationship. At baseline, 333 participants in the Biomarkers of Cognitive Decline Among Normal Individuals: the BIOCARD cohort had a baseline fasting glucose measure, and a subset (n=269) had cortical thickness measures in

AD signature regions as measured by magnetic resonance imaging (MRI) scans. In a model adjusted for age, sex, race, education, and medical conditions, higher blood glucose was associated with a thinner parahippocampal gyrus (B=-0.002; 95% CI -0.003, -0.0001) and temporal pole (B=-0.002; 95% CI -0.003, -0.0002). When we examined the association between glucose and cortical thickness in a model that controlled for sex and age alone, we found that ε4 carriers versus non-carriers had thinner superior parietal gyri (B=-0.001; 95% CI -0.002, 0.0005 vs. B=0.0007; 95% CI $3.05e^{-6}$, 0.001) and temporal pole (B=-0.004; 95% CI -0.008, -0.0009 vs. B=-0.0003; 95% CI -0.002, 0.001) measures. These findings suggest that higher blood glucose levels may be associated with the presence of gray matter atrophy consistent with that observed in AD as early as middle age. Future studies should work to further elucidate this relationship.

INTRODUCTION

Alzheimer's disease (AD) is a growing problem in the United States. Over five million older adults have AD, and this number is expected to triple by 2050 (Herbert et al., 2003). Currently, there is no cure for AD, so it may be that intervening during the prodromal or preclinical stages is the most effective course for slowing cognitive decline associated with AD (Barnes & Yaffe, 2011; Deschaintre, Richard, Leys, & Pasquier, 2009). Many types of biomarkers, including blood biomarkers, cerebrospinal fluid (CSF) biomarkers, and imaging markers, are being explored with the hopes of diagnosing AD earlier in the disease course, and altering the trajectory of the decline (Lewczuk, Mroczko, Fagan, & Kornhuber, 2014; Mielke & Haughey, 2012). Cortical thickness, which is a measure of gray matter atrophy, can be measured by magnetic resonance imaging (MRI) scans, is one marker that may be prognostic of AD (Desikan et al., 2009; Dickerson et al., 2009).

Past studies have found that cortical thinning occurs in the prodromal stages of AD (Desikan et al., 2009; Desikan et al., 2008; Dickerson et al., 2009). There have been few studies that have investigated cortical thinning in the preclinical stages of AD. However, it has been found that in preclinical patients, cortical thinning in the entorhinal cortex, inferior temporal gyrus, parahippocampal gyrus pars triangularis, precuneus, superior frontal and parietal gyri, supramarginal gyrus, and temporal pole is observed in those with substantial amyloid burden (Dickerson et al., 2009), and that this thinning is associated with greater risk of cognitive decline and transition to dementia (Dickerson et al., 2011; Dickerson & Wolk, 2012).

Additionally, findings from the BIOCARD study have shown that lower average cortical thickness at baseline was associated with an increased risk of developing AD, and cortical thinning in AD-specific regions is associated with earlier onset of AD (Pettigrew et al., 2014).

Some modifiable risk factors for AD have also been implicated as risk factors for cortical thinning. Type II diabetes mellitus (DM) is one such factor. An estimated one-third of older adults have DM, and one-half are pre-diabetic (American Diabetes Association, 2014b). DM is associated with an increased risk of AD; diabetics have been shown to have 33% to 200% increased risk of developing AD, compared to non-diabetics (Vagelatos & Eslick, 2013). Further, studies in cognitively normal participants have shown that diabetics have reduced mean cortical thickness has compared to non-diabetics (Brundel, van den Heuvel, de Bresser, Kappelle, & Biessels, 2010). Cross-sectional analyses have shown that higher glucose and HbA1c levels are associated with grey matter atrophy in the left anterior cingulate region and the bilateral occipital regions (Z. Chen et al., 2015; Leritz et al., 2011). Another cross-sectional study in a middle-aged, cognitively normal cohort showed the diabetics had greater cortical thinning globally, and in the frontal and parietal lobes (van Velsen et al., 2013).

Two genes have also been linked with risk of AD, and may modify the relationship between DM and cognitive decline, risk of dementia, and brain atrophy. The first gene, APOE, is an established risk factor for late onset AD (LOAD). The ε4 allele, as compared to the ε2 or ε3 alleles, is associated with increased risk of AD

(Farrer et al., 1997), cognitive decline (Caselli et al., 2007; Caselli et al., 2004), and smaller brain volumes and cortical thinning (Espeseth et al., 2008; Fennema-Notestine et al., 2011). More recently, TOMM40, which has three allelic variations – short (S), long (L), and very long (VL), – has also been linked to LOAD. Compared to the TOMM40 short (S) allele, the very long (VL) allele is associated with earlier onset of LOAD (Roses et al., 2010), smaller brain volumes, and poorer cognitive performance (Ferencz et al., 2013). Further, there is evidence to suggest an interaction between DM and APOE and DM and TOMM40 that compounds the risk for poor cognitive outcomes (Greenbaum et al., 2014; Hayden et al., 2012; Potkin et al., 2009; Vagelatos & Eslick, 2013).

We determined the cross-sectional association between baseline glucose and cortical thickness in the Biomarkers of Cognitive Decline Among Normal Individuals: the BIOCARD cohort, a middle-aged, healthy, cognitively normal sample. We also examined how APOE and TOMM40 modify the relationship between glucose and cortical thickness. We hypothesized that higher baseline blood glucose would be associated with greater grey matter atrophy, and that this relationship would be stronger among those with the APOE and TOMM40 risk alleles.

METHODS

BIOCARD Study

The BIOCARD (n=349) Study started at the National Institutes of Health (NIH) in 1995. The study was designed in order to investigate risk factors for cognitive decline and dementia in a healthy, mainly middle-aged, high-risk cohort.

The study was discontinued in 2005, and re-initiated in 2009 at the Johns Hopkins University School of Medicine (PI: Marilyn Albert, PhD). Currently, 89% of the participants have been reenrolled, and 38 participants have died (Moghekar et al., 2013; Soldan et al., 2013).

At baseline, participants completed a two-day exam at the Clinical Center of the NIH where they completed a clinical and neurological exam, cognitive testing, MRI, and standard blood assays. A subset of participants also completed a lumbar puncture in order to draw samples of CSF. At annual follow-up exams, participants completed physical and neurological exams and cognitive testing; every two years they also completed MRI scans and CSF collection while the study was being conducted at the NIH.

Participants

At baseline, most participants were middle-aged (mean=57.2, standard deviation (SD)=10.3). The cohort was also considered high-risk for developing dementia, because approximately three-quarters of participants had a first-degree relative with dementia. Exclusion criteria included cognitive impairment, and significant medical, psychiatric, or neurological disorder (e.g., severe cardiovascular disease, Parkinson's disease, or epilepsy). The current study used data from 333 of the participants who had complete data for fasting blood glucose at baseline. Informed consent was obtained from all participants.

Glucose and Diabetes Measures

Fasting blood glucose was obtained from standard blood draws completed at baseline and follow-up visits. A total of 333 participants had complete data for baseline blood glucose level (mean=99.1 mg/dL, SD=24.4). DM status was ascertained during the clinical exam, when participants were also asked to report medication use, including DM medication (e.g., Metformin, injectable insulin, etc.).

Genotyping

APOE genotyping was completed at the NIH. TOMM40 genotyping was completed using stored blood samples after the study had been reinitiated at Johns Hopkins. Genotyping was completed at Athena Diagnostics (Worcester, MA), using restriction endonuclease digestion of polymerase chain reaction amplified genomic DNA. In our analyses, we coded participants with one or more ε4 alleles as '1' and those without an ε4 allele as '0.' In the TOMM40 analyses, those with the VL/VL genotype were coded as '1,' while those with the S/S genotype were coded as '0.'

Cortical Thickness Measures

While the study was being conducted at the NIH (1995-2005), MRI scans were completed every other year. At baseline, 335 participants completed an MRI scan. Scans were completed on a 1.5 Tesla General Electric scanner. The scanning protocol involved localizer scans, axial Fast Spin Echo sequence (TR = 4250, TE = 108, FOV = 512 × 512, thickness/gap = 5.0/0.0 mm, flip angle = 90, 28 slices), Axial Flair sequence (TR = 9002, TE = 157.5, FOV = 256 × 256, thickness/gap = 5.0/0.0 mm, flip angle = 90, 28 slices), Coronal Spoiled Gradient Echo sequence (TR=24, TE-2 FOV = 256 × 256, thickness/gap = 2.0/0.0 mm, flip angle = 20, 124 slices), and

Sagittal Spoiled Gradient Echo sequence (TR = 24, TE = 3, FOV = 256 × 256, thickness/gap = 1.5/0.0 mm, flip angle = 45, 124 slices) (Miller et al., 2013). Cortical thickness measures were obtained from MRI scans using FreeSurfer Version 5.0 for 269 participants at baseline. This study examined AD-signature regions, which were based on findings from the research conducted by Dickerson and colleagues (2009; 2011). Thickness of AD-signature regions considered both spatial intensity gradients and signal intensity of the scans, and were determined by estimating the space from the boundary of grey matter and white matter to the boundary between grey matter and CSF (Fischl & Dale, 2000). These methods have shown good validity and reliability (Han et al., 2006; Kuperberg et al., 2003; Rosas et al., 2002; Salat et al., 2004).

Additional Variables

During the clinical exams, a standard demographic profile and medical history were recorded, including age, number of years of education, race, family history of dementia, and current or past medical conditions (e.g., cardiovascular disease, metabolic conditions, and smoking and substance abuse). Participants' height and weight were also measured, and these measures were used to calculate body mass index (BMI; kg/m^2). Additionally, blood pressure measurements, heart rate, a standard blood panel (e.g., glucose, potassium, bilirubin), and a standard neurological exam were completed in the course of the clinical exam.

Statistical Analyses

We used chi-squared tests and Student's t-tests to compare participant demographic variables by genotype (i.e., ε4-carrier versus non-carrier and S/S versus VL/VL). We used linear regression models to determine the association between baseline blood glucose level and cortical thickness. In Model 1, we controlled for age, years of education, and sex. In Model 2, we controlled for age, education, sex, and baseline medical conditions (i.e., cardiovascular disease, hypertension, hypercholesterolemia, traumatic brain injury, and depression). To examine the interaction between glucose and APOE and the interaction between glucose and TOMM40, we created two interaction terms. We conducted these analyses including these interaction terms, and if the interaction coefficient was significant at a level of $p<=0.10$, we stratified the analyses to determine the difference in association between the genotypes.

To examine the interaction between glucose and TOMM40, we restricted the sample to only those participants who were APOE ε3 homozygous. This is because evidence has suggested that the S allele is associated with poorer cognitive outcomes in ε3 heterozygotes (Gold et al., 2012; Gold et al., 2010; Greenbaum et al., 2014; Lyall et al., 2014). This restriction also helped to nullify any effect that the ε2 and ε4 alleles might have on cortical thickness (Gold et al., 2012; Gold et al., 2010; Greenbaum et al., 2014; Lyall et al., 2014). Additionally, because the L allele is in high linkage disequilibrium with the ε4 allele, we compared people with the VL/VL genotype to people with the S/S genotype (Greenbaum et al., 2014). TOMM40 analyses also did not include any non-White participants, because early evidence has suggested that, like APOE, TOMM40 alleles and associated risk vary by race and

ethnic group. However, these variations are not yet well understood, so we chose to take the conservative approach of limiting the sample (Roses et al., 2014). Additionally, some participants were excluded from analyses because the FreeSurfer measures were not reliable. All analyses were completed using Stata 12.0 (StataCorp, 2009).

RESULTS

Participant Characteristics

Over half (58%) of participants were female, and participants were generally middle-aged at baseline (mean=56.9, SD=10.2). The majority of participants were White (98%), and they had a high mean level of education (17.1 years, SD=2.4). The average body mass index (BMI) was 26.4 kg/m^2 (SD=5.8), and the sample was relatively healthy, with low prevalence of medical conditions (i.e., cardiovascular disease, hypertension, hypercholesterolemia, or traumatic brain injury) (Table 1). There were 109 participants with one or more APOE ε4 alleles, and 222 without an ε4 allele. Those with an ε4 allele were more likely to have history of hypercholesterolemia and depression, as compared to non-carriers. Of the White participants who were APOE ε3 homozygous, 55 had the TOMM40 VL/VL genotype and 56 had the S/S genotype. There were no other significant differences by genotype. At baseline, the average fasting blood glucose level was 99.1 mg/dL (SD=24.6).

Blood Glucose and Cortical Thickness

In a model that controlled for age and sex, higher fasting blood glucose levels were associated with lower cortical thickness in the pars triangularis of the inferior frontal gyrus (B=-0.0007; 95% CI -0.001, -0.0001) (Table 9). In Model 2, which controlled for age, education, race, sex, and medical conditions, glucose was associated with thinner parahippocampal gyrus (B=-0.002; 95% CI -0.003, -0.0001) and temporal pole (B=-0.002; 95% CI -0.003, -0.0002) measures. In Model 1, we found that the association between glucose and grey matter atrophy was stronger among those with an APOE ε4 allele. Among those without an e4 allele, higher glucose levels were associated with thicker superior partietal gyri (B=0.0007 95% CI $3.05e^{-6}$, 0.001), compared to those with an e4 allele (B=-0.001; 95% CI -0.002, 0.0005; interaction term p=0.035). Further, higher glucose levels were associated with significantly thinner temporal pole measures in e4 carriers (B=-0.004; 95% CI -0.008, -0.0009) versus non-carriers (B=-0.0003; 95% CI-0.002, 0.001; interaction term p=0.049) (Table 10). This association was no longer significant after stratification in fully adjusted models (interaction term p=0.041). There was no interaction between glucose and TOMM40 genotype (all interaction term p-values>0.10).

DISCUSSION

This study investigated the cross-sectional association between blood glucose level and cortical thickness, and how this association is affected by APOE and TOMM40 genotype, in a health, cognitively normal, middle-aged sample. We found that higher baseline glucose was significantly associated with decreased

cortical thickness in three AD signature regions, including the parahippocampal gyrus, pars triangularis, and temporal pole. Additionally, we found that higher glucose levels were associated with thinner superior parietal gyrus and temporal pole measures in subjects with one or more ε4 alleles, as compared to those without an ε4 allele. The parahippocampal gyrus, which surrounds the hippocampus and is important for memory encoding and retrieval, is affected early in AD (Dickerson et al., 2009; Dickerson et al., 2011). Similarly, the pars triangularis, which partially makes up Broca's area and is associated with semantic tasks, has been implicated as a region of interest that might be affected early in the course of AD (Dickerson et al., 2009; Dickerson et al., 2011). The temporal pole is involved in executive functioning tasks, such as theory of mind, which allows an individual to understand another individual's perspective (Olson, Plotzker, & Ezzyat, 2007), and the superior parietal gyrus is involved in attention shifting (Vandenberghe, Gitelman, Parrish, & Mesulam, 2001).

Previous studies have found that among AD patients, those with cardiovascular conditions, including DM, have greater grey matter atrophy in the hippocampus (Donix et al., 2013), suggesting that treatment of cardiovascular conditions might slow the cognitive decline in AD patients, and possibly in the prodromal and preclinical stages of AD (Deschaintre et al., 2009). In fact, studies have found that among diabetics with cortical thinning, one year of insulin therapy results in increases in cortical thickness (Z. Chen et al., 2015). The findings from our study suggest that even before any clinical symptoms of AD manifest, glucose may have a measureable negative effect on brain integrity. Early identification of

biomarkers, such as cortical thickness, related to AD may present an opportunity to significantly alter the course of the disease. Evidence shows that intervening after AD symptoms present is not effective; additionally, treatment efforts for AD have failed to this point (Lambracht-Washington & Rosenberg, 2013). However, intervening on modifiable risk factors (e.g., DM) in middle age or later middle age may significantly reduce the risk of or delay the onset of AD (Barnes & Yaffe, 2011). Identifying individuals at greatest risk for or in the earliest stages of AD via biomarkers, like grey matter atrophy, may help clinicians to better focus these intervention efforts.

This study has multiple limitations. First, we used blood glucose levels, as opposed to more accurate HbA1c measures, which are a measure of glucose over a period of three months. However, it is noteworthy that we found an association between glucose and cortical thickness, because this suggests that even modest fluctuations in glucose may affect cortical thickness. Second, we did not observe interaction between glucose and TOMM40. This may have been because of the relatively small sample size used when comparing VL homozygotes (n=55) to S homozygotes (n=58). Future research should work to elucidate any interaction between glucose and TOMM40 in larger samples, and investigate how other TOMM40 genotypes affect the relationship between glucose or DM and cortical thickness. Third, many participants were missing BMI data at baseline, so we were not able to include it as a covariate in the models. BMI has been linked with brain atrophy (Gunstad et al., 2008), but this association may be through hyperglycemia (Luchsinger, 2008). Therefore, had we included BMI as a covariate, we would have

risked over-adjustment. Fourth, this study sample was primarily White and had a high mean level of education, and most participants had a first-degree relative with dementia, making it a high-risk cohort. These characteristics may mean that the findings are not generalizable to the population as a whole. Finally, because this study was cross-sectional, we cannot infer causality from our findings.

Our results suggest glucose levels may promote cortical thinning, which may be a useful biomarker for identifying individuals in the preclinical stages of AD. Without a cure for AD, treatment for modifiable risk factors, such as DM, may be the most effective way to slow or, even halt, the progression of AD. Moving forward, it will be important to better understand how early cortical thinning predicts AD onset, and how blood glucose control may be able to slow this development.

CONCLUSION

This research investigated the association between baseline blood glucose and cognitive test performance over time, time to MCI or AD symptom onset, and grey matter atrophy in AD-signature regions. Results indicate that even modest increases in blood glucose in mid-life are associated with reduced cortical thickness in AD-signature regions and decline in executive function. These findings suggest that controlling blood glucose levels even in non-diabetics may be important for maintaining brain health and cognitive function with advancing age.

There is substantial evidence showing that hyperglycemia is associated with poorer cognitive function. Impaired glucose tolerance has been widely associated with poorer verbal, logical, working, and visuospatial memory (Lamport et al., 2009). Studies have also shown that acute hyperglycemia in type II diabetics is associated with poorer performance on tasks involving information processing, working memory, and attention (Sommerfield, Deary, & Frier, 2004). Hyperglycemia may result in cognitive decline through oxidative stress, cerebral blood flow changes, and neural toxicity (Biessels et al., 2014; West et al., 2014). In the same line as these previous studies, this study found an association between baseline glucose and declining executive function factor score. Additionally, this study found that this association was stronger amongst subjects with one or more ε4 alleles, compared to those with none. This is consistent with findings from previous studies (Ravona-Springer et al., 2014), and mechanistic evidence suggesting that the APOE ε4 allele is associated with increased neuronal stress (e.g., hyperexcitability, neuronal DNA

damage) and inflammation (Aboud et al., 2012; Liu et al., 2013; Lynch et al., 2003). Findings from this research were opposite from the expected direction in the analyses that examined the association between then interaction of glucose and TOMM40 and cognitive performance. We found that higher glucose levels predicted decline on executive function factor score in those with the S/S genotype, but not in those with the VL/VL genotype; however this may have been due to issues with statistical power. Mechanistic evidence suggests that the TOMM40 VL/VL allele may modify the association between glucose and cognitive decline via its role in mitochondrial function (Hayden et al., 2012; Mosconi, 2005; Potkin et al., 2009). The findings from this study are important, because few studies have examined the association between glucose and cognitive decline in younger, highly educated cohorts; this evidence suggests that hyperglycemia may negatively impact cognition regardless of cognitive reserve, and may do so more strongly among e4 carriers.

Although this study did not find an association between blood glucose and dementia or MCI symptom onset, this may have been because the BIOCARD cohort is still relatively young, and few (n=63) have been diagnosed with dementia or MCI at this point in time. In the future, an association between glucose and symptom onset may be detectable in this sample, which would be consistent with findings from earlier studies. Higher glucose levels have been associated with risk of dementia (Ronnemaa et al., 2009), though not consistently (Schrijvers et al., 2010). Studies have also shown that insulin resistance is associated with risk of dementia (Schrijvers et al., 2010). While some studies have shown an interaction effect with APOE (Haan et al., 1999; Peila et al., 2002), others have not (Schrijvers et al., 2010).

Based on the currently available literature, there is no evidence indicating whether a similar interaction effect is observed with TOMM40.

The cross-sectional finding that blood glucose levels are associated with thinner parahippocampal gyrus and temporal pole measures suggests that hyperglycemia may impact brain tissue integrity as early as middle age. Additionally, findings from this study showed that higher glucose levels were associated with greater grey matter atrophy of the superior parietal gyrus and temporal pole among APOE ε4 carriers, compared to non-carriers, which may indicate that individuals with an ε4 allele are more susceptible to the negative effects of hyperglycemia. Dickerson and colleagues (2009; 2011) found that cortical thinning occurs in AD-signature regions in the prodromal stages of AD. Studies in preclinical populations, including the BIOCARD cohort, have found that cortical thinning in AD-signature regions predicts risk of dementia and MCI symptom onset and earlier age of onset (Pettigrew et al., 2014). Studies in somewhat younger samples have shown that DM is associated with cortical thinning independent of macrovascular conditions (Brundel et al., 2010; Z. Chen, Li, Sun, & Ma, 2012; van Velsen et al., 2013), and that insulin therapy induces cortical thickening (Z. Chen et al., 2015). Studies have also found that hyperinsulinemia, independent of DM, is associated with cortical thinning in the frontal, temporal, and parietal lobes in a cognitively normal cohort (Yoon et al., 2014). This evidence suggests that DM is associated with substantial grey matter atrophy, but that better glucose control may prevent or reverse these effects. Additionally, while past studies have found that the APOE ε4 allele is associated with hippocampal volume loss (Mueller, Schuff, Raptentsetsang, Elman, &

Weiner, 2008), they have not found an interaction between APOE allelic variation and the association between DM and cortical thinning (Donix et al., 2013). This is the first study, to our knowledge, that has found an association between glucose levels and cortical thinning in a healthy, cognitively unimpaired cohort, and has shown that the ε4 allele may modify this relationship.

Limitations

Although this study had several strengths, including the unique cohort (e.g., middle aged at baseline, high risk for dementia due to family history), its longitudinal design, and wealth of measures (e.g., genotyping, multiple cognitive tests), the findings must be viewed alongside the limitations of the study. First, glucose was used as the main predictor. However, HbA1C is a more stable measure of blood glucose, and it may have been possible to better detect patterns of cognitive decline, risk of dementia, and grey matter atrophy if HbA1C measures had been used as the main predictor (Leritz et al., 2011; Marder et al., 2014). Second, there was a large amount of missingness of body mass index (BMI) data, so it was not possible to control for BMI as a potential confounder. BMI has been linked to brain shrinkage (Gunstad et al., 2008), cognitive decline (Gunstad et al., 2007), and risk of dementia (Tolppanen et al., 2014). However, other studies suggest that it is likely that BMI is actually associated with dementia and cognitive performance outcomes through hyperglycemia and insulin resistance (Luchsinger, 2008). Therefore, in this scenario, hyperglycemia would be a mediator and adjustment for BMI would be over-adjustment. Third, this study may have lacked significant power to detect an

interaction between glucose and TOMM40. The TOMM40 analyses were restricted to subjects who were APOE ε3 homozygotes and White, and only compared the VL/VL genotype to the S/S genotype, in order to be as conservative as possible; however, this substantially limited the sample size. Studies in larger samples may be able to detect an association between the interaction of glucose and TOMM40 and cognitive decline, risk of dementia, and brain atrophy. Finally, because the BIOCARD cohort is predominantly White, highly educated, and high-risk for dementia, the findings from this study may not be generalizable to the general population.

Future Directions

The findings from this study indicate that more research should be done to better understand the association between glucose levels and risk of dementia and associated cognitive decline in preclinical AD samples. Future studies should investigate a more detailed history of glucose or HbA1c in non-diabetic subjects, in order to better understand how fluctuations in glucose levels impact risk of dementia, cognitive decline, and cortical thinning. The cross-sectional findings from this study that show glucose is associated with cortical thinning indicate that hyperglycemia may be an important risk factor for grey matter atrophy. However, longitudinal evidence is needed to confirm this association. Fortunately, as more data become available, it will be possible to investigate these associations in the BIOCARD study. It will also be important for larger studies to investigate a potential glucose-TOMM40 interaction. The emerging evidence that shows APOE is not the only gene that impacts risk of dementia (G. Li et al., 2013) is intriguing; however,

because genotype is not modifiable, it is important to understand how modifiable risk factors (e.g., DM), impact the relationship between genotype and risk of dementia, and the associated cognitive decline and cortical thinning. Finally, clinicians should be made aware that higher levels of glucose, even without a pre-diabetes or DM diagnosis, may be putting their patients at risk for cognitive decline and dementia. Maintaining normal glucose levels through diet and physical activity may prove to be an important step for delaying the onset of or preventing cognitive decline and dementia in the general population.

BIBLIOGRAPHY

Aboud, O., Mrak, R. E., Boop, F., & Griffin, S. T. (2012). Apolipoprotein epsilon 3 alleles are associated with indicators of neuronal resilience. *BMC Med, 10*, 35. doi: 10.1186/1741-7015-10-35

Abraham, R., Moskvina, V., Sims, R., Hollingworth, P., Morgan, A., Georgieva, L., . . . Kirov, G. (2008). A genome-wide association study for late-onset Alzheimer's disease using DNA pooling. *BMC Med Genomics, 1*, 44. doi: 10.1186/1755-8794-1-44

Administration on Aging. (2013). A Profile of Older Americans: 2013. Washington, DC: U.S. Department of Health and Human Services

Ahmadian, G., Ju, W., Liu, L., Wyszynski, M., Lee, S. H., Dunah, A. W., . . . Wang, Y. T. (2004). Tyrosine phosphorylation of GluR2 is required for insulin-stimulated AMPA receptor endocytosis and LTD. *EMBO J, 23*(5), 1040-1050. doi: 10.1038/sj.emboj.7600126

Albert, M., Soldan, A., Gottesman, R., McKhann, G., Sacktor, N., Farrington, L., . . . Selnes, O. (2014). Cognitive changes preceding clinical symptom onset of mild cognitive impairment and relationship to ApoE genotype. *Curr Alzheimer Res, 11*(8), 773-784.

Albert, M., Soldan, A., Gottesman, R., McKhann, G., Sacktor, N., Farrington, L., . . . Selnes, O. (2013). Cognitive changes preceding clinical symptom onset of mild cognitive decline. *Submitted for review*.

Alvarez, A., Munoz, J.P., & Maccioni, R. B. (2001). A Cdk5-p35 stable complex is involved in the β -amyloid-induced deregulation of Cdk5 activity in hippocampal neurons. *Exp Cell Res, 264*(2), 266-274.

Alvarez, A., Toro, R., Caceres, A., & Maccioni, R. B. (1999). Inhibition of tau phosphorylating protein kinase cdk5 prevents beta-amyloid-induced neuronal death. *FEBS Lett, 459*(3), 421-426.

Alzheimer's Association. (2013). Alzheimer's Disease Facts and Figures. *Alzheimer's & Dementia, 9*(2), 1-69.

Alzheimer's Association. (2014). 2014 Alzheimer's Disease Facts and Figures. *Alzheimer's & Dementia, 10*(2).

American Diabetes Association. (2011). National Diabetes Fact Sheet.

American Diabetes Association. (2013, 20 August 2013). Diabetes Basics: Diabetes Statistics. Retrieved October 10, 2013, from http://www.diabetes.org/diabetes-basics/diabetes-statistics/

American Diabetes Association. (2014a). Diagnosis and classification of diabetes mellitus. *Diabetes Care, 37 Suppl 1*, S81-90. doi: 10.2337/dc14-S081

American Diabetes Association. (2014b). National Diabetes Statistics Report, 2014. Atlanta, GA: Centers for Disease Control

American Psychiatric Association. (2013). *Diagnositic and statistical manual of mental disorders* (5th ed.). Arlington, VA: American Psychiatric Publishing

Amieva, H., Le Goff, M., Millet, X., Orgogozo, J. M., Peres, K., Barberger-Gateau, P., . . . Dartigues, J. F. (2008). Prodromal Alzheimer's disease: successive emergence

of the clinical symptoms. *Ann Neurol, 64*(5), 492-498. doi: 10.1002/ana.21509

Arvanitakis, Z., Bennett, D. A., Wilson, R. S., & Barnes, L. L. (2010). Diabetes and cognitive systems in older black and white persons. *Alzheimer Dis Assoc Disord, 24*(1), 37-42. doi: 10.1097/WAD.0b013e3181a6bed5

Balaraman, Y., Limaye, A. R., Levey, A. I., & Srinivasan, S. (2006). Glycogen synthase kinase 3beta and Alzheimer's disease: pathophysiological and therapeutic significance. *Cell Mol Life Sci, 63*(11), 1226-1235. doi: 10.1007/s00018-005-5597-y

Bales, K. R., Dodart, J. C., DeMattos, R. B., Holtzman, D. M., & Paul, S. M. (2002). Apolipoprotein E, amyloid, and Alzheimer disease. *Mol Interv, 2*(6), 363-375, 339. doi: 10.1124/mi.2.6.363

Barnes, D. E., & Yaffe, K. (2011). The projected effect of risk factor reduction on Alzheimer's disease prevalence. *Lancet Neurol, 10*(9), 819-828. doi: 10.1016/s1474-4422(11)70072-2

Bekris, L. M., Galloway, N. M., Montine, T. J., Schellenberg, G. D., & Yu, C. E. (2010). APOE mRNA and protein expression in postmortem brain are modulated by an extended haplotype structure. *Am J Med Genet B Neuropsychiatr Genet, 153B*(2), 409-417. doi: 10.1002/ajmg.b.30993

Bekris, L. M., Millard, S. P., Galloway, N. M., Vuletic, S., Albers, J. J., Li, G., . . . Yu, C. E. (2008). Multiple SNPs within and surrounding the apolipoprotein E gene influence cerebrospinal fluid apolipoprotein E protein levels. *J Alzheimers Dis, 13*(3), 255-266.

Benedict, C., Brooks, S. J., Kullberg, J., Burgos, J., Kempton, M. J., Nordenskjold, R., . . . Schioth, H. B. (2012). Impaired insulin sensitivity as indexed by the HOMA score is associated with deficits in verbal fluency and temporal lobe gray matter volume in the elderly. *Diabetes Care, 35*(3), 488-494. doi: 10.2337/dc11-2075

Biennow, K., de Leon, M. J., & Zetterberg, H. (2006). Alzheimer's disease. *Lancet, 368*, 387-403.

Biessels, G. J., Kamal, A., Urban, I. J., Spruijt, B. M., Erkelens, D. W., & Gispen, W. H. (1998). Water maze learning and hippocampal synaptic plasticity in streptozotocin-diabetic rats: effects of insulin treatment. *Brain Res, 800*(1), 125-135.

Biessels, G. J., Strachan, M. W., Visseren, F. L., Kappelle, L. J., & Whitmer, R. A. (2014). Dementia and cognitive decline in type 2 diabetes and prediabetic stages: towards targeted interventions. *Lancet Diabetes Endocrinol, 2*(3), 246-255. doi: 10.1016/s2213-8587(13)70088-3

Bis, J. C., DeCarli, C., Smith, A. V., van der Lijn, F., Crivello, F., Fornage, M., . . . Seshadri, S. (2012). Common variants at 12q14 and 12q24 are associated with hippocampal volume. *Nat Genet, 44*(5), 545-551. doi: 10.1038/ng.2237

Bomfim, T.R., Forny-Germano, L., Sathler, L.B., Brito-Moreira, J., Houzel, J-C, Decker, H., . . . De Felice, F.G. (2012). An anti-diabetes agent protects the mouse brain from defective insulin signaling caused by Alzheimer's disease-associated A β oligomers. *Journal of Clinical Investigation, 112*(4), 1339-1353.

Bondi, M. W., Houston, W. S., Eyler, L. T., & Brown, G. G. (2005). fMRI evidence of compensatory mechanisms in older adults at genetic risk for Alzheimer disease. *Neurology, 64*(3), 501-508. doi: 10.1212/01.wnl.0000150885.00929.7e

Bookheimer, S. Y., Strojwas, M. H., Cohen, M. S., Saunders, A. M., Pericak-Vance, M. A., Mazziotta, J. C., & Small, G. W. (2000). Patterns of brain activation in people at risk for Alzheimer's disease. *N Engl J Med, 343*(7), 450-456. doi: 10.1056/nejm200008173430701

Braak, H., Thal, D. R., Ghebremedhin, E., & Del Tredici, K. (2011). Stages of the pathologic process in Alzheimer disease: age categories from 1 to 100 years. *J Neuropathol Exp Neurol, 70*(11), 960-969. doi: 10.1097/NEN.0b013e318232a379

Brenton, A.L., & Hamsher, K.S. (1976). Multilingual Aphasia Examination. Iowa City: University of Iowa Press.

Brundel, M., van den Heuvel, M., de Bresser, J., Kappelle, L. J., & Biessels, G. J. (2010). Cerebral cortical thickness in patients with type 2 diabetes. *J Neurol Sci, 299*(1-2), 126-130. doi: 10.1016/j.jns.2010.08.048

Bryan, R. N., Bilello, M., Davatzikos, C., Lazar, R. M., Murray, A., Horowitz, K., . . . Launer, L. J. (2014). Effect of diabetes on brain structure: the action to control cardiovascular risk in diabetes MR imaging baseline data. *Radiology, 272*(1), 210-216. doi: 10.1148/radiol.14131494

Burns, J. M., Honea, R. A., Vidoni, E. D., Hutfles, L. J., Brooks, W. M., & Swerdlow, R. H. (2012). Insulin is differentially related to cognitive decline and atrophy in

Alzheimer's disease and aging. *Biochim Biophys Acta, 1822*(3), 333-339. doi: 10.1016/j.bbadis.2011.06.011

Busse, A., Hensel, A., Guhne, U., Angermeyer, M. C., & Riedel-Heller, S. G. (2006). Mild cognitive impairment: long-term course of four clinical subtypes. *Neurology, 67*(12), 2176-2185. doi: 10.1212/01.wnl.0000249117.23318.e1

Bynum, J. (2011). *Unpublished tabulations based on data from the National 20% Sample Medicare Fee-for-Service Beneficiaries for 2009*. Dartmouth Medical School.

Carlson, N.E., Moore, M.M., Dame, A., Howieson, D., Silbert, L.C., Quinn, J.F., & Kaye, J.A. (2008). Trajectories of brain loss in aging and the development of cognitive impairment. *Neurology, 70*, 828-833.

Caselli, R. J., Dueck, A. C., Huentelman, M. J., Lutz, M. W., Saunders, A. M., Reiman, E. M., & Roses, A. D. (2012). Longitudinal modeling of cognitive aging and the TOMM40 effect. *Alzheimers Dement, 8*(6), 490-495. doi: 10.1016/j.jalz.2011.11.006

Caselli, R. J., Reiman, E. M., Locke, D. E., Hutton, M. L., Hentz, J. G., Hoffman-Snyder, C., . . . Osborne, D. (2007). Cognitive domain decline in healthy apolipoprotein E epsilon4 homozygotes before the diagnosis of mild cognitive impairment. *Arch Neurol, 64*(9), 1306-1311. doi: 10.1001/archneur.64.9.1306

Caselli, R. J., Reiman, E. M., Osborne, D., Hentz, J. G., Baxter, L. C., Hernandez, J. L., & Alexander, G. G. (2004). Longitudinal changes in cognition and behavior in asymptomatic carriers of the APOE e4 allele. *Neurology, 62*(11), 1990-1995.

Caspersen, C., Wang, N., Yao, J., Sosunov, A., Chen, X., Lustbader, J. W., . . . Yan, S. D. (2005). Mitochondrial Abeta: a potential focal point for neuronal metabolic dysfunction in Alzheimer's disease. *FASEB J, 19*(14), 2040-2041. doi: 10.1096/fj.05-3735fje

Centers for Disease Control and Prevention. (2014). National Diabetes Statistics Report: Estimates of Diabetes and Its Burden in the United States, 2014. Atlanta, GA: US Department of Health and Human Services; 2014.

Chang, C. Y., & Silverman, D. H. (2004). Accuracy of early diagnosis and its impact on the management and course of Alzheimer's disease. *Expert Rev Mol Diagn, 4*(1), 63-69. doi: 10.1586/14737159.4.1.63

Chang, S., ran Ma, T., Miranda, R. D., Balestra, M. E., Mahley, R. W., & Huang, Y. (2005). Lipid- and receptor-binding regions of apolipoprotein E4 fragments act in concert to cause mitochondrial dysfunction and neurotoxicity. *Proc Natl Acad Sci U S A, 102*(51), 18694-18699. doi: 10.1073/pnas.0508254102

Chartier-Harlin, M. C., Parfitt, M., Legrain, S., Perez-Tur, J., Brousseau, T., Evans, A., . . . et al. (1994). Apolipoprotein E, epsilon 4 allele as a major risk factor for sporadic early and late-onset forms of Alzheimer's disease: analysis of the 19q13.2 chromosomal region. *Hum Mol Genet, 3*(4), 569-574.

Chen, H. K., Liu, Z., Meyer-Franke, A., Brodbeck, J., Miranda, R. D., McGuire, J. G., . . . Mahley, R. W. (2012). Small molecule structure correctors abolish detrimental effects of apolipoprotein E4 in cultured neurons. *J Biol Chem, 287*(8), 5253-5266. doi: 10.1074/jbc.M111.276162

Chen, Y., Durakoglugil, M. S., Xian, X., & Herz, J. (2010). ApoE4 reduces glutamate receptor function and synaptic plasticity by selectively impairing ApoE receptor recycling. *Proc Natl Acad Sci U S A, 107*(26), 12011-12016. doi: 10.1073/pnas.0914984107

Chen, Z., Li, L., Sun, J., & Ma, L. (2012). Mapping the brain in type II diabetes: Voxel-based morphometry using DARTEL. *European Journal of Radiology, 81*(8), 1870-1876. doi: http://dx.doi.org/10.1016/j.ejrad.2011.04.025

Chen, Z., Sun, J., Yang, Y., Lou, X., Wang, Y., Wang, Y., & Ma, L. (2015). Cortical thinning in type 2 diabetes mellitus and recovering effects of insulin therapy. *J Clin Neurosci, 22*(2), 275-279. doi: 10.1016/j.jocn.2014.07.014

Cheng, G., Huang, C., Deng, H., & Wang, H. (2012). Diabetes as a risk factor for dementia and mild cognitive impairment: a meta-analysis of longitudinal studies. *Intern Med J, 42*(5), 484-491. doi: 10.1111/j.1445-5994.2012.02758.x

Cheng, S. T., Lau, R. W., Mak, E. P., Ng, N. S., Lam, L. C., Fung, H. H., . . . Lee, D. T. (2012). A benefit-finding intervention for family caregivers of persons with Alzheimer disease: study protocol of a randomized controlled trial. *Trials, 13*, 98. doi: 10.1186/1745-6215-13-98

Cholerton, B, Baker, LD, & Craft, S. (2011). Insulin resistance and pathological brain ageing. *Diabet Med, 28*(12), 1463-1475. doi: 10.1111/j.1464-5491.2011.03464.x

Cholerton, B, Baker, LD, & Craft, S. (2013). Insulin, cognition, and dementia. *European Journal of Pharmacology, Epub ahead of print*. doi: 10.1016/j.ejphar.2013.08.008

Chou, J. L., Shenoy, D. V., Thomas, N., Choudhary, P. K., Laferla, F. M., Goodman, S. R., & Breen, G. A. (2011). Early dysregulation of the mitochondrial proteome in a mouse model of Alzheimer's disease. *J Proteomics, 74*(4), 466-479. doi: 10.1016/j.jprot.2010.12.012

Convit, A., Wolf, O. T., Tarshish, C., & de Leon, M. J. (2003). Reduced glucose tolerance is associated with poor memory performance and hippocampal atrophy among normal elderly. *Proc Natl Acad Sci U S A, 100*(4), 2019-2022. doi: 10.1073/pnas.0336073100

Cook, D. G., Leverenz, J. B., McMillan, P. J., Kulstad, J. J., Ericksen, S., Roth, R. A., . . . Craft, S. (2003). Reduced hippocampal insulin-degrading enzyme in late-onset Alzheimer's disease is associated with the apolipoprotein E-epsilon4 allele. *Am J Pathol, 162*(1), 313-319.

Corder, E. H., Saunders, A. M., Strittmatter, W. J., Schmechel, D. E., Gaskell, P. C., Small, G. W., . . . Pericak-Vance, M. A. (1993). Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science, 261*(5123), 921-923.

Cosentino, S., Scarmeas, N., Helzner, E., Glymour, M. M., Brandt, J., Albert, M., . . . Stern, Y. (2008). APOE epsilon 4 allele predicts faster cognitive decline in mild Alzheimer disease. *Neurology, 70*(19 Pt 2), 1842-1849. doi: 10.1212/01.wnl.0000304038.37421.cc

Craft, S. (2006). Insulin resistance syndrome and Alzheimer disease: pathophysiologic mechanisms and therapeutic implications. *Alzheimer Dis Assoc Disord, 20*(4), 298-301. doi: 10.1097/01.wad.0000213866.86934.7e

Craft, S., Asthana, S., Cook, D. G., Baker, L.D., Cherrier, M., Purganan, K., . . . Krohn, A.J. (2003). Insulin dose-response effects on memory and plasma amyloid precursor protein in Alzheimer's disease: interaction with apolipoprotein E genotype. *Psychoneuroendocrinology, 28*, 809-822.

Craft, S., Asthana, S., Newcomer, J. W., Wilkinson, C. W., Matos, I. T., Baker, L. D., . . . Veith, R. C. (1999). Enhancement of memory in Alzheimer disease with insulin and somatostatin, but not glucose. *Arch Gen Psychiatry, 56*(12), 1135-1140.

Craft, S., Newcomer, J., Kanne, S., Dagogo-Jack, S., Cryer, P., Sheline, Y., . . . Alderson, A. (1996). Memory improvement following induced hyperinsulinemia in Alzheimer's disease. *Neurobiol Aging, 17*(1), 123-130.

Craft, S., Peskind, E., Schwartz, M. W., Schellenberg, G. D., Raskind, M., & Porte, D., Jr. (1998). Cerebrospinal fluid and plasma insulin levels in Alzheimer's disease: relationship to severity of dementia and apolipoprotein E genotype. *Neurology, 50*(1), 164-168.

Cruchaga, C., Nowotny, P., Kauwe, J. S., Ridge, P. G., Mayo, K., Bertelsen, S., . . . Goate, A. M. (2011). Association and expression analyses with single-nucleotide polymorphisms in TOMM40 in Alzheimer disease. *Arch Neurol, 68*(8), 1013-1019. doi: 10.1001/archneurol.2011.155

Csernansky, J. G., Wang, L., Joshi, S., Miller, J. P., Gado, M., Kido, D., . . . Miller, M. I. (2000). Early DAT is distinguished from aging by high-dimensional mapping of the hippocampus. Dementia of the Alzheimer type. *Neurology, 55*(11), 1636-1643.

Cukierman, T., Gerstein, H. C., & Williamson, J. D. (2005). Cognitive decline and dementia in diabetes--systematic overview of prospective observational studies. *Diabetologia, 48*(12), 2460-2469. doi: 10.1007/s00125-005-0023-4

D'Onofrio, G., Panza, F., Frisardi, V., Solfrizzi, V., Imbimbo, B. P., Paroni, G., . . . Pilotto, A. (2012). Advances in the identification of gamma-secretase inhibitors for the treatment of Alzheimer's disease. *Expert Opin Drug Discov, 7*(1), 19-37. doi: 10.1517/17460441.2012.645534

Dale, A. M., Fischl, B., & Sereno, M. I. (1999). Cortical surface-based analysis. I. Segmentation and surface reconstruction. *Neuroimage, 9*(2), 179-194. doi: 10.1006/nimg.1998.0395

Dale, A. M., & Sereno, M. I. (1993). Improved Localizadon of Cortical Activity by Combining EEG and MEG with MRI Cortical Surface Reconstruction: A Linear Approach. *J Cogn Neurosci, 5*(2), 162-176. doi: 10.1162/jocn.1993.5.2.162

de Bresser, J., Tiehuis, A. M., van den Berg, E., Reijmer, Y. D., Jongen, C., Kappelle, L. J., . . . Biessels, G. J. (2010). Progression of cerebral atrophy and white matter hyperintensities in patients with type 2 diabetes. *Diabetes Care, 33*(6), 1309-1314. doi: 10.2337/dc09-1923

Delis, D.C., Kramer, J.H., Kaplan, E., & Ober, B.A. (1987). California Verbal Learning Test. San Antonio, TX: The Psychological Corporation.

den Heijer, T., Vermeer, S. E., van Dijk, E. J., Prins, N. D., Koudstaal, P. J., Hofman, A., & Breteler, M. M. (2003). Type 2 diabetes and atrophy of medial temporal lobe structures on brain MRI. *Diabetologia, 46*(12), 1604-1610. doi: 10.1007/s00125-003-1235-0

Deschaintre, Y., Richard, F., Leys, D., & Pasquier, F. (2009). Treatment of vascular risk factors is associated with slower decline in Alzheimer disease. *Neurology, 73*(9), 674-680. doi: 10.1212/WNL.0b013e3181b59bf3

Desikan, R. S., Cabral, H. J., Fischl, B., Guttmann, C. R., Blacker, D., Hyman, B. T., . . . Killiany, R. J. (2009). Temporoparietal MR imaging measures of atrophy in subjects with mild cognitive impairment that predict subsequent diagnosis of Alzheimer disease. *AJNR Am J Neuroradiol, 30*(3), 532-538. doi: 10.3174/ajnr.A1397

Desikan, R. S., Fischl, B., Cabral, H. J., Kemper, T. L., Guttmann, C. R., Blacker, D., . . . Killiany, R. J. (2008). MRI measures of temporoparietal regions show differential rates of atrophy during prodromal AD. *Neurology, 71*(11), 819-825. doi: 10.1212/01.wnl.0000320055.57329.34

Dickerson, B. C., Bakkour, A., Salat, D. H., Feczko, E., Pacheco, J., Greve, D. N., . . . Buckner, R. L. (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*(3), 497-510. doi: 10.1093/cercor/bhn113

Dickerson, B. C., Stoub, T. R., Shah, R. C., Sperling, R. A., Killiany, R. J., Albert, M. S., . . . Detoledo-Morrell, L. (2011). Alzheimer-signature MRI biomarker predicts AD

dementia in cognitively normal adults. *Neurology, 76*(16), 1395-1402. doi: 10.1212/WNL.0b013e3182166e96

Dickerson, B. C., & Wolk, D. A. (2012). MRI cortical thickness biomarker predicts AD-like CSF and cognitive decline in normal adults. *Neurology, 78*(2), 84-90. doi: 10.1212/WNL.0b013e31823efc6c

Donix, M., Scharf, M., Marschner, K., Werner, A., Sauer, C., Gerner, A., . . . Holthoff, V. A. (2013). Cardiovascular risk and hippocampal thickness in Alzheimer's disease. *Int J Alzheimers Dis, 2013*, 108021. doi: 10.1155/2013/108021

Dore, G. A., Elias, M. F., Robbins, M. A., Elias, P. K., & Nagy, Z. (2009). Presence of the APOE epsilon4 allele modifies the relationship between type 2 diabetes and cognitive performance: the Maine-Syracuse Study. *Diabetologia, 52*(12), 2551-2560. doi: 10.1007/s00125-009-1497-2

Dumanis, S. B., Tesoriero, J. A., Babus, L. W., Nguyen, M. T., Trotter, J. H., Ladu, M. J., . . . Hoe, H. S. (2009). ApoE4 decreases spine density and dendritic complexity in cortical neurons in vivo. *J Neurosci, 29*(48), 15317-15322. doi: 10.1523/jneurosci.4026-09.2009

Elias, P. K., Elias, M. F., D'Agostino, R. B., Cupples, L. A., Wilson, P. W., Silbershatz, H., & Wolf, P. A. (1997). NIDDM and blood pressure as risk factors for poor cognitive performance. The Framingham Study. *Diabetes Care, 20*(9), 1388-1395.

Espeland, M. A., Bryan, R. N., Goveas, J. S., Robinson, J. G., Siddiqui, M. S., Liu, S., . . . Resnick, S. M. (2013). Influence of type 2 diabetes on brain volumes and changes in brain volumes: results from the Women's Health Initiative

Magnetic Resonance Imaging studies. *Diabetes Care, 36*(1), 90-97. doi: 10.2337/dc12-0555

Espeseth, T., Westlye, L. T., Fjell, A. M., Walhovd, K. B., Rootwelt, H., & Reinvang, I. (2008). Accelerated age-related cortical thinning in healthy carriers of apolipoprotein E epsilon 4. *Neurobiol Aging, 29*(3), 329-340. doi: 10.1016/j.neurobiolaging.2006.10.030

Falvey, C. M., Rosano, C., Simonsick, E. M., Harris, T., Strotmeyer, E. S., Satterfield, S., & Yaffe, K. (2013). Macro- and microstructural magnetic resonance imaging indices associated with diabetes among community-dwelling older adults. *Diabetes Care, 36*(3), 677-682. doi: 10.2337/dc12-0814

Farlow, M. R., He, Y., Tekin, S., Xu, J., Lane, R., & Charles, H. C. (2004). Impact of APOE in mild cognitive impairment. *Neurology, 63*(10), 1898-1901.

Farrer, L. A., Cupples, L. A., Haines, J. L., Hyman, B., Kukull, W. A., Mayeux, R., . . . van Duijn, C. M. (1997). Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. *JAMA, 278*(16), 1349-1356.

Farris, W, Mansourian, S, Chang, Y, Lindsley, L, Eckman, E.A., Frosch, M.P., . . . Guenette, S. (2003). Insulin-degrading enzyme reulates the levels of insulin, amoyloid β-protein, and the β-amyloid precursor protein intracellular domain in vivo. *Proc Natl Acad Sci U S A, 100*, 4162-4167.

Fennema-Notestine, C., Panizzon, M. S., Thompson, W. R., Chen, C. H., Eyler, L. T., Fischl, B., . . . Kremen, W. S. (2011). Presence of ApoE epsilon4 allele

associated with thinner frontal cortex in middle age. *J Alzheimers Dis, 26 Suppl 3*, 49-60. doi: 10.3233/jad-2011-0002

Ferencz, B., Laukka, E. J., Lovden, M., Kalpouzos, G., Keller, L., Graff, C., . . . Backman, L. (2013). The influence of APOE and TOMM40 polymorphisms on hippocampal volume and episodic memory in old age. *Front Hum Neurosci, 7*, 198. doi: 10.3389/fnhum.2013.00198

Ferri, C. P., Prince, M., Brayne, C., Brodaty, H., Fratiglioni, L., Ganguli, M., . . . Scazufca, M. (2005). Global prevalence of dementia: a Delphi consensus study. *Lancet, 366*(9503), 2112-2117. doi: 10.1016/s0140-6736(05)67889-0

Filippini, N., MacIntosh, B. J., Hough, M. G., Goodwin, G. M., Frisoni, G. B., Smith, S. M., . . . Mackay, C. E. (2009). Distinct patterns of brain activity in young carriers of the APOE-epsilon4 allele. *Proc Natl Acad Sci U S A, 106*(17), 7209-7214. doi: 10.1073/pnas.0811879106

Fischer, P., Jungwirth, S., Zehetmayer, S., Weissgram, S., Hoenigschnabl, S., Gelpi, E., . . . Tragl, K. H. (2007). Conversion from subtypes of mild cognitive impairment to Alzheimer dementia. *Neurology, 68*(4), 288-291. doi: 10.1212/01.wnl.0000252358.03285.9d

Fischl, B., & Dale, A. M. (2000). Measuring the thickness of the human cerebral cortex from magnetic resonance images. *Proc Natl Acad Sci U S A, 97*(20), 11050-11055. doi: 10.1073/pnas.200033797

Fischl, B., Liu, A., & Dale, A. M. (2001). Automated manifold surgery: constructing geometrically accurate and topologically correct models of the human

cerebral cortex. *IEEE Trans Med Imaging, 20*(1), 70-80. doi: 10.1109/42.906426

Folstein, M.F., Folstein, S.E., & McHugh, P.R. (1975). A practical method for grading the cognitive state of patients for teh clinician *J Psychiatr Res, 12*, 189-198.

Fourrier, A., Letenneur, L., Begaud, B., & Dartigues, J. F. (1996). Nonsteroidal antiinflammatory drug use and cognitive function in the elderly: inconclusive results from a population-based cohort study. *J Clin Epidemiol, 49*(10), 1201.

Gasparini, L., Gouras, G. K., Wang, R., Gross, R. S., Beal, M. F., Greengard, P., & Xu, H. (2001). Stimulation of beta-amyloid precursor protein trafficking by insulin reduces intraneuronal beta-amyloid and requires mitogen-activated protein kinase signaling. *J Neurosci, 21*(8), 2561-2570.

German Center for Neurodegenerative Diseases. (2014). *Biomarker Qualification for Risk of Mild Cognitive Impairment (MCI) Due to Alzheimer's Disease (AD) and Safety and Efficacy Evaluation of Pioglitazone in Delaying Its Onset.* Paper presented at the Alzheimer's Association International Conference, Copenhagen, Denmark.

Giacobini, E., & Gold, G. (2013). Alzheimer disease therapy-moving from amyloid-beta to tau. *Nat Rev Neurol*. doi: 10.1038/nrneurol.2013.223

Gibson, G. E., Haroutunian, V., Zhang, H., Park, L. C., Shi, Q., Lesser, M., . . . Blass, J. P. (2000). Mitochondrial damage in Alzheimer's disease varies with apolipoprotein E genotype. *Ann Neurol, 48*(3), 297-303.

Goh, S. Y., & Cooper, M. E. (2008). Clinical review: The role of advanced glycation end products in progression and complications of diabetes. *J Clin Endocrinol Metab, 93*(4), 1143-1152. doi: 10.1210/jc.2007-1817

Gold, B. T., Johnson, N. F., Powell, D. K., & Smith, C. D. (2012). White matter integrity and vulnerability to Alzheimer's disease: preliminary findings and future directions. *Biochim Biophys Acta, 1822*(3), 416-422. doi: 10.1016/j.bbadis.2011.07.009

Gold, B. T., Powell, D. K., Andersen, A. H., & Smith, C. D. (2010). Alterations in multiple measures of white matter integrity in normal women at high risk for Alzheimer's disease. *Neuroimage, 52*(4), 1487-1494. doi: 10.1016/j.neuroimage.2010.05.036

Greenbaum, L., Springer, R. R., Lutz, M. W., Heymann, A., Lubitz, I., Cooper, I., . . . Beeri, M. S. (2014). The TOMM40 poly-T rs10524523 variant is associated with cognitive performance among non-demented elderly with type 2 diabetes. *Eur Neuropsychopharmacol, 24*(9), 1492-1499. doi: 10.1016/j.euroneuro.2014.06.002

Gregg, E. W., Chen, H., Wagenknecht, L. E., Clark, J. M., Delahanty, L. M., Bantle, J., . . . Bertoni, A. G. (2012). Association of an intensive lifestyle intervention with remission of type 2 diabetes. *JAMA, 308*(23), 2489-2496. doi: 10.1001/jama.2012.67929

Grunblatt, E., Salkovic-Petrisic, M., Osmanovic, J., Riederer, P., & Hoyer, S. (2007). Brain insulin system dysfunction in streptozotocin intracerebroventricularly

treated rats generates hyperphosphorylated tau protein. *J Neurochem, 101*(3), 757-770. doi: 10.1111/j.1471-4159.2006.04368.x

Grupe, A., Abraham, R., Li, Y., Rowland, C., Hollingworth, P., Morgan, A., . . . Williams, J. (2007). Evidence for novel susceptibility genes for late-onset Alzheimer's disease from a genome-wide association study of putative functional variants. *Hum Mol Genet, 16*(8), 865-873. doi: 10.1093/hmg/ddm031

Guerreiro, R. J., & Hardy, J. (2012). TOMM40 association with Alzheimer disease: tales of APOE and linkage disequilibrium. *Arch Neurol, 69*(10), 1243-1244. doi: 10.1001/archneurol.2012.1935

Gunstad, J., Paul, R. H., Cohen, R. A., Tate, D. F., Spitznagel, M. B., & Gordon, E. (2007). Elevated body mass index is associated with executive dysfunction in otherwise healthy adults. *Compr Psychiatry, 48*(1), 57-61. doi: 10.1016/j.comppsych.2006.05.001

Gunstad, J., Paul, R. H., Cohen, R. A., Tate, D. F., Spitznagel, M. B., Grieve, S., & Gordon, E. (2008). Relationship between body mass index and brain volume in healthy adults. *Int J Neurosci, 118*(11), 1582-1593. doi: 10.1080/00207450701392282

Haan, M. N., Shemanski, L., Jagust, W. J., Manolio, T. A., & Kuller, L. (1999). The role of APOE epsilon4 in modulating effects of other risk factors for cognitive decline in elderly persons. *JAMA, 282*(1), 40-46.

Han, X., Jovicich, J., Salat, D., van der Kouwe, A., Quinn, B., Czanner, S., . . . Fischl, B. (2006). Reliability of MRI-derived measurements of human cerebral cortical

thickness: the effects of field strength, scanner upgrade and manufacturer. *Neuroimage, 32*(1), 180-194. doi: 10.1016/j.neuroimage.2006.02.051

Hanninen, T., Hallikainen, M., Tuomainen, S., Vanhanen, M., & Soininen, H. (2002). Prevalence of mild cognitive impairment: a population-based study in elderly subjects. *Acta Neurol Scand, 106*(3), 148-154.

Hansson Petersen, C. A., Alikhani, N., Behbahani, H., Wiehager, B., Pavlov, P. F., Alafuzoff, I., . . . Ankarcrona, M. (2008). The amyloid beta-peptide is imported into mitochondria via the TOM import machinery and localized to mitochondrial cristae. *Proc Natl Acad Sci U S A, 105*(35), 13145-13150. doi: 10.1073/pnas.0806192105

Hardy, J., & Selkoe, D. J. (2002). The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science, 297*(5580), 353-356. doi: 10.1126/science.1072994

Harold, D., Abraham, R., Hollingworth, P., Sims, R., Gerrish, A., Hamshere, M. L., . . . Williams, J. (2009). Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. *Nat Genet, 41*(10), 1088-1093. doi: 10.1038/ng.440

Hashimoto, M., Yasuda, M., Tanimukai, S., Matsui, M., Hirono, N., Kazui, H., & Mori, E. (2001). Apolipoprotein E epsilon 4 and the pattern of regional brain atrophy in Alzheimer's disease. *Neurology, 57*(8), 1461-1466.

Hauser, P. S., Narayanaswami, V., & Ryan, R. O. (2011). Apolipoprotein E: from lipid transport to neurobiology. *Prog Lipid Res, 50*(1), 62-74. doi: 10.1016/j.plipres.2010.09.001

Hayden, K. M., McEvoy, J. M., Linnertz, C., Attix, D., Kuchibhatla, M., Saunders, A. M., . . . Chiba-Falek, O. (2012). A homopolymer polymorphism in the TOMM40 gene contributes to cognitive performance in aging. *Alzheimers Dement, 8*(5), 381-388. doi: 10.1016/j.jalz.2011.10.005

Hebert, L. E., Weuve, J., Scherr, P. A., & Evans, D. A. (2013). Alzheimer disease in the United States (2010-2050) estimated using the 2010 census. *Neurology, 80*(19), 1778-1783. doi: 10.1212/WNL.0b013e31828726f5

Henley, D. B., May, P. C., Dean, R. A., & Siemers, E. R. (2009). Development of semagacestat (LY450139), a functional gamma-secretase inhibitor, for the treatment of Alzheimer's disease. *Expert Opin Pharmacother, 10*(10), 1657-1664. doi: 10.1517/14656560903044982

Herbert, L.E., Scherr, P. A., Bienias, J.L., Bennett, D.A., & Evans, D.A. (2003). Alzheimer's disease in the U.S. population: Prevalence estimates using the 2000 Census. *Archives of Neurology, 60*(8), 1119-1122.

Hirai, K., Aliev, G., Nunomura, A., Fujioka, H., Russell, R. L., Atwood, C. S., . . . Smith, M. A. (2001). Mitochondrial abnormalities in Alzheimer's disease. *J Neurosci, 21*(9), 3017-3023.

Ho, L, Qin, W, Pompl, P.N., Xiang, Z, Wang, J, Zhao, Z, . . . Pasinetti, G.M. (2004). Diet induced insulin resistance promotes amyloidosis in a transgenic mouse model of Alzheimer's disease. *FASEB Journal, 18*, 902-904.

Holscher, C. (2010). The role of GLP-1 in neuronal activity and neurodegeneration. *Vitam Horm, 84*, 331-354.

Irie, F., Fitzpatrick, A. L., Lopez, O.L., Kuller, L. H., Peila, R., Newman, A. B., & Launer, L. J. (2008). Enhanced risk for Alzheimer disease in persons with type 2 diabets and APOE e4: The Cardiovascular Health Study Cognition Study. *Journal of the American Medical Association, 65*(1), 89-93.

Jack, C. R., Knopman, D. S., Jagust, W. J., Petersen, R. C., Weiner, M. W., Aisen, P. S., . . . Trojanowski, J. Q. (2013). Update on hypothetical model of Alzheimer's disease biomarkers. *Lancet Neurol, 12*(2), 207-216. doi: 10.1016/s1474-4422(12)70291-0

Johnson, G. V., & Stoothoff, W. H. (2004). Tau phosphorylation in neuronal cell function and dysfunction. *J Cell Sci, 117*(Pt 24), 5721-5729. doi: 10.1242/jcs.01558

Johnson, S. C., La Rue, A., Hermann, B. P., Xu, G., Koscik, R. L., Jonaitis, E. M., . . . Sager, M. A. (2011). The effect of TOMM40 poly-T length on gray matter volume and cognition in middle-aged persons with APOE epsilon3/epsilon3 genotype. *Alzheimers Dement, 7*(4), 456-465. doi: 10.1016/j.jalz.2010.11.012

Jonas, E. A., Knox, R. J., Smith, T. C., Wayne, N. L., Connor, J. A., & Kaczmarek, L. K. (1997). Regulation by insulin of a unique neuronal Ca2+ pool and of neuropeptide secretion. *Nature, 385*(6614), 343-346. doi: 10.1038/385343a0

Jöreskog, K. G. (1969). A general approach to confirmatory maximum likelihood factor analysis. *Psychometrika, 34*(2), 183-202. doi: 10.1007/BF02289343

Jorm, A. F., Korten, A. E., & Henderson, A. S. (1987). The prevalence of dementia: a quantitative integration of the literature. *Acta Psychiatr Scand, 76*(5), 465-479.

Jun, G., Vardarajan, B. N., Buros, J., Yu, C. E., Hawk, M. V., Dombroski, B. A., . . . Farrer, L. A. (2012). Comprehensive search for Alzheimer disease susceptibility loci in the APOE region. *Arch Neurol, 69*(10), 1270-1279. doi: 10.1001/archneurol.2012.2052

Kaether, C., Haass, C., & Steiner, H. (2006). Assembly, trafficking and function of gamma-secretase. *Neurodegener Dis, 3*(4-5), 275-283. doi: 10.1159/000095267

Kaplan, E., Goodglass, H., & Weintraub, S. (1983). Boston Naming Test. Philadelphia: Lee & Febiger.

Kenna, H., Hoeft, F., Kelley, R., Wroolie, T., DeMuth, B., Reiss, A., & Rasgon, N. (2013). Fasting plasma insulin and the default mode network in women at risk for Alzheimer's disease. *Neurobiol Aging, 34*(3), 641-649. doi: 10.1016/j.neurobiolaging.2012.06.006

Kim, S., Swaminathan, S., Shen, L., Risacher, S. L., Nho, K., Foroud, T., . . . Saykin, A. J. (2011). Genome-wide association study of CSF biomarkers Abeta1-42, t-tau, and p-tau181p in the ADNI cohort. *Neurology, 76*(1), 69-79. doi: 10.1212/WNL.0b013e318204a397

Klein, R. C., Mace, B. E., Moore, S. D., & Sullivan, P. M. (2010). Progressive loss of synaptic integrity in human apolipoprotein E4 targeted replacement mice

and attenuation by apolipoprotein E2. *Neuroscience, 171*(4), 1265-1272. doi: 10.1016/j.neuroscience.2010.10.027

Kloppenborg, R.P., van den Berg, E., Kappele, L.J., & Biessels, G. J. (2008). Diabetes and other vascular risk factors for dementia: which factor matters most? A systematic review. *European Journal of Pharmacology, 585*(1), 97-108.

Klove, H. (1963). Grooved Pegboard. Lafayette, IN.

Knowler, W. C., Barrett-Connor, E., Fowler, S. E., Hamman, R. F., Lachin, J. M., Walker, E. A., & Nathan, D. M. (2002). Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med, 346*(6), 393-403. doi: 10.1056/NEJMoa012512

Kodl, C. T., & Seaquist, E. R. (2008). Cognitive dysfunction and diabetes mellitus. *Endocr Rev, 29*(4), 494-511. doi: 10.1210/er.2007-0034

Kooistra, M., Geerlings, M. I., Mali, W. P., Vincken, K. L., van der Graaf, Y., & Biessels, G. J. (2013). Diabetes mellitus and progression of vascular brain lesions and brain atrophy in patients with symptomatic atherosclerotic disease. The SMART-MR study. *J Neurol Sci, 332*(1-2), 69-74. doi: 10.1016/j.jns.2013.06.019

Korf, E. S., Wahlund, L. O., Visser, P. J., & Scheltens, P. (2004). Medial temporal lobe atrophy on MRI predicts dementia in patients with mild cognitive impairment. *Neurology, 63*(1), 94-100.

Kravitz, E., Schmeidler, J., & Schnaider Beeri, M. (2013). Type 2 diabetes and cognitive compromise: potential roles of diabetes-related therapies.

Endocrinol Metab Clin North Am, 42(3), 489-501. doi: 10.1016/j.ecl.2013.05.009

Kuperberg, G. R., Broome, M. R., McGuire, P. K., David, A. S., Eddy, M., Ozawa, F., . . . Fischl, B. (2003). Regionally localized thinning of the cerebral cortex in schizophrenia. *Arch Gen Psychiatry, 60*(9), 878-888. doi: 10.1001/archpsyc.60.9.878

LaDu, M. J., Falduto, M. T., Manelli, A. M., Reardon, C. A., Getz, G. S., & Frail, D. E. (1994). Isoform-specific binding of apolipoprotein E to beta-amyloid. *J Biol Chem, 269*(38), 23403-23406.

Lambracht-Washington, D., & Rosenberg, R. N. (2013). Advances in the development of vaccines for Alzheimer's disease. *Discov Med, 15*(84), 319-326.

Lamport, D. J., Lawton, C. L., Mansfield, M. W., & Dye, L. (2009). Impairments in glucose tolerance can have a negative impact on cognitive function: a systematic research review. *Neurosci Biobehav Rev, 33*(3), 394-413. doi: 10.1016/j.neubiorev.2008.10.008

Lannert, H., & Hoyer, S. (1998). Intracerebroventricular administration of streptozotocin causes long-term diminutions in learning and memory abilities and in cerebral energy metabolism in adult rats. *Behav Neurosci, 112*(5), 1199-1208.

Launer, L. J. (2005). Diabetes and brain aging: epidemiologic evidence. *Curr Diab Rep, 5*(1), 59-63.

Launer, L. J., Miller, M. E., Williamson, J. D., Lazar, R. M., Gerstein, H. C., Murray, A. M., . . . Bryan, R. N. (2011). Effects of intensive glucose lowering on brain

structure and function in people with type 2 diabetes (ACCORD MIND): a randomised open-label substudy. *Lancet Neurol, 10*(11), 969-977. doi: 10.1016/s1474-4422(11)70188-0

Lee, J., & Kim, M. S. (2007). The role of GSK3 in glucose homeostasis and the development of insulin resistance. *Diabetes Res Clin Pract, 77 Suppl 1*, S49-57. doi: 10.1016/j.diabres.2007.01.033

Leritz, E. C., Salat, D. H., Williams, V. J., Schnyer, D. M., Rudolph, J. L., Lipsitz, L., . . . Milberg, W. P. (2011). Thickness of the human cerebral cortex is associated with metrics of cerebrovascular health in a normative sample of community dwelling older adults. *Neuroimage, 54*(4), 2659-2671. doi: 10.1016/j.neuroimage.2010.10.050

Lewczuk, P., Mroczko, B., Fagan, A., & Kornhuber, J. (2014). Biomarkers of Alzheimer's disease and mild cognitive impairment: A current perspective. *Adv Med Sci, 60*(1), 76-82. doi: 10.1016/j.advms.2014.11.002

Li, G., Bekris, L. M., Leong, L., Steinbart, E. J., Shofer, J. B., Crane, P. K., . . . Yu, C. E. (2013). TOMM40 intron 6 poly-T length, age at onset, and neuropathology of AD in individuals with APOE epsilon3/epsilon3. *Alzheimers Dement, 9*(5), 554-561. doi: 10.1016/j.jalz.2012.06.009

Li, L., & Holscher, C. (2007). Common pathological processes in Alzheimer disease and type 2 diabetes: a review. *Brain Res Rev, 56*(2), 384-402. doi: 10.1016/j.brainresrev.2007.09.001

Li, Y. M., & Dickson, D. W. (1997). Enhanced binding of advanced glycation endproducts (AGE) by the ApoE4 isoform links the mechanism of plaque deposition in Alzheimer's disease. *Neurosci Lett, 226*(3), 155-158.

Liu, C. C., Kanekiyo, T., Xu, H., & Bu, G. (2013). Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy. *Nat Rev Neurol, 9*(2), 106-118. doi: 10.1038/nrneurol.2012.263

Lopez, O. L., Jagust, W. J., DeKosky, S. T., Becker, J. T., Fitzpatrick, A., Dulberg, C., . . . Kuller, L. H. (2003). Prevalence and classification of mild cognitive impairment in the Cardiovascular Health Study Cognition Study: part 1. *Arch Neurol, 60*(10), 1385-1389. doi: 10.1001/archneur.60.10.1385

Lu, F-P., Lin, K-P., & Kuo, H-K. (2009). Diabetes and the risk of multi-system aging phenotypes: A systematic review and meta-analysis. *PLoS One, 4*(1), e4144.

Lucas, J. J., Hernandez, F., Gomez-Ramos, P., Moran, M. A., Hen, R., & Avila, J. (2001). Decreased nuclear beta-catenin, tau hyperphosphorylation and neurodegeneration in GSK-3beta conditional transgenic mice. *EMBO J, 20*(1-2), 27-39. doi: 10.1093/emboj/20.1.27

Luchsinger, J. A. (2008). Adiposity, hyperinsulinemia, diabetes and Alzheimer's disease: an epidemiological perspective. *Eur J Pharmacol, 585*(1), 119-129. doi: 10.1016/j.ejphar.2008.02.048

Lutz, M. W., Crenshaw, D. G., Saunders, A. M., & Roses, A. D. (2010). Genetic variation at a single locus and age of onset for Alzheimer's disease. *Alzheimers Dement, 6*(2), 125-131. doi: 10.1016/j.jalz.2010.01.011

Lyall, D. M., Harris, S. E., Bastin, M. E., Munoz Maniega, S., Murray, C., Lutz, M. W., . . . Deary, I. J. (2014). Alzheimer's disease susceptibility genes APOE and TOMM40, and brain white matter integrity in the Lothian Birth Cohort 1936. *Neurobiol Aging, 35*(6), 1513 e1525-1533. doi: 10.1016/j.neurobiolaging.2014.01.006

Lynch, J. R., Tang, W., Wang, H., Vitek, M. P., Bennett, E. R., Sullivan, P. M., . . . Laskowitz, D. T. (2003). APOE genotype and an ApoE-mimetic peptide modify the systemic and central nervous system inflammatory response. *J Biol Chem, 278*(49), 48529-48533. doi: 10.1074/jbc.M306923200

Maccioni, R. B., Otth, C., Conca, I.I., & Munoz, J.P. (2001). The protein kinase Cdk5: Structural aspects, roles in neurogenesis and involvement in Alzheimer's pathology. *Eur J Biochem, 268*(6), 1518-1527.

Machulda, M. M., Jones, D. T., Vemuri, P., McDade, E., Avula, R., Przybelski, S., . . . Jack, C. R., Jr. (2011). Effect of APOE epsilon4 status on intrinsic network connectivity in cognitively normal elderly subjects. *Arch Neurol, 68*(9), 1131-1136. doi: 10.1001/archneurol.2011.108

Manschot, S. M., Brands, A. M., van der Grond, J., Kessels, R. P., Algra, A., Kappelle, L. J., & Biessels, G. J. (2006). Brain magnetic resonance imaging correlates of impaired cognition in patients with type 2 diabetes. *Diabetes, 55*(4), 1106-1113.

Marder, T. J., Flores, V. L., Bolo, N. R., Hoogenboom, W. S., Simonson, D. C., Jacobson, A. M., . . . Musen, G. (2014). Task-induced brain activity patterns in type 2

diabetes: a potential biomarker for cognitive decline. *Diabetes, 63*(9), 3112-3119. doi: 10.2337/db13-1783

Mastaglia, F. L., Rojana-Udomsart, A., James, I., Needham, M., Day, T. J., Kiers, L., . . . Roses, A. D. (2013). Polymorphism in the TOMM40 gene modifies the risk of developing sporadic inclusion body myositis and the age of onset of symptoms. *Neuromuscul Disord*. doi: 10.1016/j.nmd.2013.09.008

Matsuzaki, T., Sasaki, K., Tanizaki, Y., Hata, J., Fujimi, K., Matsui, Y., . . . Iwaki, T. (2010). Insulin resistance is associated with the pathology of Alzheimer disease: the Hisayama study. *Neurology, 75*(9), 764-770. doi: 10.1212/WNL.0b013e3181eee25f

Mayeux, R, Honig, LS, Tang, MX, Manly, J, Stern, Y, Schupf, N, & Mehta, P.D. (2003). Plasma A β -40 and A β -42 and Alzheimer's disease: relation to age, mortality, and risk. *Neurology, 61*(9), 1185-1190.

Mielke, M. M., & Haughey, N. J. (2012). Could plasma sphingolipids be diagnostic or prognostic biomarkers for Alzheimer's disease? *Clin Lipidol, 7*(5), 525-536. doi: 10.2217/clp.12.59

Miller, M. I., Younes, L., Tilak Ratnanather, J., Brown, T., Trinh, H., Postell, E., . . . The, BIOCARD Research Team. (2013). The diffeomophometry of temporal lobe structures in preclinical Alzheimer's disease. *NeuroImage: Clincal, In press*.

Moghekar, A, Goh, J, Li, M., Albert, M., & O'Brien, R.J. (2012). Cerebrospinal fluid A β and tau level fluctuation in an older clinical cohort. *Arch Neurol, 69*, 246-250.

Moghekar, A, Li, S, Lu, Y, Li, M, Wang, MC, Albert, M, & O'Brien, R. (2013). CSF biomarker changes precede symptom onset of mild cognitive impairment. *Neurology, 81*(20), 1753-1758. doi: 10.1212/01.wnl.0000435558.98447.17

Morihara, T., Chu, T., Ubeda, O., Beech, W., & Cole, G.M. (2002). Selective inhibition of Abeta42 production by NSAID R-enantiomers. *J Neurochem, 83*(4), 1009-1012.

Mormino, E. C., Betensky, R. A., Hedden, T., Schultz, A. P., Ward, A., Huijbers, W., . . . Sperling, R. A. (2014). Amyloid and APOE epsilon4 interact to influence short-term decline in preclinical Alzheimer disease. *Neurology, 82*(20), 1760-1767. doi: 10.1212/wnl.0000000000000431

Morris, J. C. (1993). The Clinical Dementia Rating (CDR): current version and scoring rules. *Neurology, 43*, 2412-2414.

Mosconi, L. (2005). Brain glucose metabolism in the early and specific diagnosis of Alzheimer's disease. FDG-PET studies in MCI and AD. *Eur J Nucl Med Mol Imaging, 32*(4), 486-510. doi: 10.1007/s00259-005-1762-7

Mueller, S. G., Schuff, N., Raptentsetsang, S., Elman, J., & Weiner, M. W. (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*(1), 42-48. doi: 10.1016/j.neuroimage.2008.04.174

Mullard, A. (2012). Sting of Alzheimer's failures offset by upcoming prevention trials. *Nat Rev Drug Discov, 11*(9), 657-660. doi: 10.1038/nrd3842

Murphy, S.L., Xu, J.Q., & Kochanek, K.D. (2013). Deaths: Final data for 2010. *National Vital Statistics Reports* (Vol. 61). Hyattsville, MD: National Center for Health Statistics.

Musen, G., Jacobson, A. M., Bolo, N. R., Simonson, D. C., Shenton, M. E., McCartney, R. L., . . . Hoogenboom, W. S. (2012). Resting-state brain functional connectivity is altered in type 2 diabetes. *Diabetes, 61*(9), 2375-2379. doi: 10.2337/db11-1669

Naor, M., Steingruber, H. J., Westhoff, K., Schottenfeld-Naor, Y., & Gries, A. F. (1997). Cognitive function in elderly non-insulin-dependent diabetic patients before and after inpatient treatment for metabolic control. *J Diabetes Complications, 11*(1), 40-46.

Neumann, K. F., Rojo, L., Navarrete, L. P., Farias, G., Reyes, P., & Maccioni, R. B. (2008). Insulin resistance and Alzheimer's disease: molecular links & clinical implications. *Curr Alzheimer Res, 5*(5), 438-447.

Norton, Sam, Matthews, Fiona E., Barnes, Deborah E., Yaffe, Kristine, & Brayne, Carol. (2014). Potential for primary prevention of Alzheimer's disease: an analysis of population-based data. *The Lancet Neurology, 13*(8), 788-794.

Nunomura, A., Chiba, S., Lippa, C. F., Cras, P., Kalaria, R. N., Takeda, A., . . . Perry, G. (2004). Neuronal RNA oxidation is a prominent feature of familial Alzheimer's disease. *Neurobiol Dis, 17*(1), 108-113. doi: 10.1016/j.nbd.2004.06.003

Oda, A., Tamaoka, A., & Araki, W. (2010). Oxidative stress up-regulates presenilin 1 in lipid rafts in neuronal cells. *J Neurosci Res, 88*(5), 1137-1145. doi: 10.1002/jnr.22271

Ohm, T. G., Muller, H., Braak, H., & Bohl, J. (1995). Close-meshed prevalence rates of different stages as a tool to uncover the rate of Alzheimer's disease-related neurofibrillary changes. *Neuroscience, 64*(1), 209-217.

Olson, I. R., Plotzker, A., & Ezzyat, Y. (2007). The Enigmatic temporal pole: a review of findings on social and emotional processing. *Brain, 130*(Pt 7), 1718-1731. doi: 10.1093/brain/awm052

Pa, J., Boxer, A., Chao, L. L., Gazzaley, A., Freeman, K., Kramer, J., . . . Johnson, J. K. (2009). Clinical-neuroimaging characteristics of dysexecutive mild cognitive impairment. *Ann Neurol, 65*(4), 414-423. doi: 10.1002/ana.21591

Pandini, G., Pace, V., Copani, A., Squatrito, S., Milardi, D., & Vigneri, R. (2013). Insulin has multiple antiamyloidgenic effects on human neuronal cells. *Endocrinology, 154*, 375-387.

Park, C. R., Seeley, R. J., Craft, S., & Woods, S. C. (2000). Intracerebroventricular insulin enhances memory in a passive-avoidance task. *Physiol Behav, 68*(4), 509-514.

Peila, R., Rodriguez, B. L., & Launer, L. J. (2002). Type 2 diabetes, APOE gene, and the risk for dementia and related pathologies: The Honolulu-Asia Aging Study. *Diabetes, 51*(4), 1256-1262.

Perez, A, Morelli, L, Cresto, J.C., & Castano, E.M. (2000). Degradation of soluble amyloid beta peptides 1-40, 1-42 by insulin degrading enzyme from Alzheimer disease and control brains. *Neurochem Res, 25*, 247-255.

Petersen, R. C. (2004). Mild cognitive impairment as a diagnostic entity. *J Intern Med, 256*(3), 183-194. doi: 10.1111/j.1365-2796.2004.01388.x

Petersen, R. C., Smith, G. E., Ivnik, R. J., Tangalos, E. G., Schaid, D. J., Thibodeau, S. N., . . . Kurland, L. T. (1995). Apolipoprotein E status as a predictor of the development of Alzheimer's disease in memory-impaired individuals. *JAMA, 273*(16), 1274-1278.

Petersen, R. C., Smith, G. E., Waring, S. C., Ivnik, R. J., Tangalos, E. G., & Kokmen, E. (1999). Mild cognitive impairment: clinical characterization and outcome. *Arch Neurol, 56*(3), 303-308.

Pettigrew, C., Soldan, A., Lu, Y., Wang, M.C., Brown, T., Selnes, O., . . . Albert, M. (2014). *Cortical regions are associated with risk of clinical symptom onset during preclinical Alzheimer's disease.* Paper presented at the The Annual Meeting of the Society for Neuroscience, Washington, D.C.

Potkin, S. G., Guffanti, G., Lakatos, A., Turner, J. A., Kruggel, F., Fallon, J. H., . . . Macciardi, F. (2009). Hippocampal atrophy as a quantitative trait in a genome-wide association study identifying novel susceptibility genes for Alzheimer's disease. *PLoS One, 4*(8), e6501. doi: 10.1371/journal.pone.0006501

Qiu, A., Crocetti, D., Adler, M., Mahone, E. M., Denckla, M. B., Miller, M. I., & Mostofsky, S. H. (2009). Basal ganglia volume and shape in children with attention

deficit hyperactivity disorder. *Am J Psychiatry, 166*(1), 74-82. doi: 10.1176/appi.ajp.2008.08030426

Qiu, A., Fennema-Notestine, C., Dale, A. M., & Miller, M. I. (2009). Regional shape abnormalities in mild cognitive impairment and Alzheimer's disease. *Neuroimage, 45*(3), 656-661.

Raber, J., Huang, Y., & Wesson Ashford, J. (2004). ApoE genotype accounts for the vast majority of AD risk and AD pathology. *Neurobiol Aging, 25*(5), 641-650.

Raji, C. A., Ho, A. J., Parikshak, N. N., Becker, J. T., Lopez, O. L., Kuller, L. H., . . . Thompson, P. M. (2010). Brain structure and obesity. *Hum Brain Mapp, 31*(3), 353-364. doi: 10.1002/hbm.20870

Rasgon, N. L., Kenna, H. A., Wroolie, T. E., Kelley, R., Silverman, D., Brooks, J., . . . Reiss, A. (2011). Insulin resistance and hippocampal volume in women at risk for Alzheimer's disease. *Neurobiol Aging, 32*(11), 1942-1948. doi: 10.1016/j.neurobiolaging.2009.12.005

Ravona-Springer, R., Heymann, A., Schmeidler, J., Sano, M., Preiss, R., Koifman, K., . . . Beeri, M. S. (2014). The ApoE4 genotype modifies the relationship of long-term glycemic control with cognitive functioning in elderly with type 2 diabetes. *Eur Neuropsychopharmacol, 24*(8), 1303-1308. doi: 10.1016/j.euroneuro.2014.05.001

Ravona-Springer, R., Luo, X., Schmeidler, J., Wysocki, M., Lesser, G., Rapp, M., . . . Schanider Beeri, M. (2010). Diabetes is associated with increased rate of cognitive decline in questionably demented elderly. *Dement Geriatr Cogn Disord, 29*, 68-74.

Rawlings, Andreea M., Sharrett, A. Richey, Schneider, Andrea L. C., Coresh, Josef, Albert, Marilyn, Couper, David, . . . Selvin, Elizabeth. (2014). Diabetes in Midlife and Cognitive Change Over 20 YearsA Cohort StudyDiabetes in Midlife and Cognitive Change Over 20 Years. *Ann Intern Med, 161*(11), 785-793. doi: 10.7326/M14-0737

Rebeck, G. W., Reiter, J. S., Strickland, D. K., & Hyman, B. T. (1993). Apolipoprotein E in sporadic Alzheimer's disease: allelic variation and receptor interactions. *Neuron, 11*(4), 575-580.

Reddy, P. H., Manczak, M., Mao, P., Calkins, M. J., Reddy, A. P., & Shirendeb, U. (2010). Amyloid-beta and mitochondria in aging and Alzheimer's disease: implications for synaptic damage and cognitive decline. *J Alzheimers Dis, 20 Suppl 2*, S499-512. doi: 10.3233/jad-2010-100504

Reger, M. A., Watson, G. S., Green, P. S., Baker, L. D., Cholerton, B., Fishel, M. A., . . . Craft, S. (2008). Intranasal insulin administration dose-dependently modulates verbal memory and plasma amyloid-beta in memory-impaired older adults. *J Alzheimers Dis, 13*(3), 323-331.

Reijmer, Y. D., Leemans, A., Brundel, M., Kappelle, L. J., & Biessels, G. J. (2013). Disruption of the cerebral white matter network is related to slowing of information processing speed in patients with type 2 diabetes. *Diabetes, 62*(6), 2112-2115. doi: 10.2337/db12-1644

Reijmer, Y. D., van den Berg, E., de Bresser, J., Kessels, R. P., Kappelle, L. J., Algra, A., & Biessels, G. J. (2011). Accelerated cognitive decline in patients with type 2

diabetes: MRI correlates and risk factors. *Diabetes Metab Res Rev, 27*(2), 195-202. doi: 10.1002/dmrr.1163

Reiman, E. M., Chen, K., Liu, X., Bandy, D., Yu, M., Lee, W., . . . Caselli, R. J. (2009). Fibrillar amyloid-beta burden in cognitively normal people at 3 levels of genetic risk for Alzheimer's disease. *Proc Natl Acad Sci U S A, 106*(16), 6820-6825. doi: 10.1073/pnas.0900345106

Reiman, E. M., Uecker, A., Caselli, R. J., Lewis, S., Bandy, D., de Leon, M. J., . . . Thibodeau, S. N. (1998). Hippocampal volumes in cognitively normal persons at genetic risk for Alzheimer's disease. *Ann Neurol, 44*(2), 288-291. doi: 10.1002/ana.410440226

Reitan, R.M. (1958). Validity of the trail making test as an indicator of organic brain damage. *Perceptual and Motor Skills, 8*, 271-276.

Rey, A. (1941). L'examen psychologique dans les cas d'encephalopathie traumatique. *Archives de Psychologie, 28*, 286-340.

Risner, M. E., Saunders, A. M., Altman, J. F., Ormandy, G. C., Craft, S., Foley, I. M., . . . Roses, A. D. (2006). Efficacy of rosiglitazone in a genetically defined population with mild-to-moderate Alzheimer's disease. *Pharmacogenomics J, 6*(4), 246-254. doi: 10.1038/sj.tpj.6500369

Roberts, R. O., Geda, Y. E., Knopman, D. S., Cha, R. H., Pankratz, V. S., Boeve, B. F., . . . Rocca, W. A. (2008). The Mayo Clinic Study of Aging: design and sampling, participation, baseline measures and sample characteristics. *Neuroepidemiology, 30*(1), 58-69. doi: 10.1159/000115751

Roberts, R. O., Knopman, D. S., Cha, R. H., Mielke, M. M., Pankratz, V. S., Boeve, B. F., . . . Lowe, V. J. (2014). Diabetes and elevated hemoglobin A1c levels are associated with brain hypometabolism but not amyloid accumulation. *J Nucl Med, 55*(5), 759-764. doi: 10.2967/jnumed.113.132647

Roberts, R. O., Knopman, D. S., Przybelski, S. A., Mielke, M. M., Kantarci, K., Preboske, G. M., . . . Jack, C. R., Jr. (2014). Association of type 2 diabetes with brain atrophy and cognitive impairment. *Neurology, 82*(13), 1132-1141. doi: 10.1212/wnl.0000000000000269

Ronnemaa, E., Zethelius, B., Sundelof, J., Sundstrom, J., Degerman-Gunnarsson, M., Berne, C., . . . Kilander, L. (2008). Impaired insulin secretion increases the risk of Alzheimer disease. *Neurology, 71*(14), 1065-1071. doi: 10.1212/01.wnl.0000310646.32212.3a

Ronnemaa, E., Zethelius, B., Sundelof, J., Sundstrom, J., Degerman-Gunnarsson, M., Lannfelt, L., . . . Kilander, L. (2009). Glucose metabolism and the risk of Alzheimer's disease and dementia: a population-based 12 year follow-up study in 71-year-old men. *Diabetologia, 52*(8), 1504-1510. doi: 10.1007/s00125-009-1393-9

Rosas, H. D., Liu, A. K., Hersch, S., Glessner, M., Ferrante, R. J., Salat, D. H., . . . Fischl, B. (2002). Regional and progressive thinning of the cortical ribbon in Huntington's disease. *Neurology, 58*(5), 695-701.

Rosen, P., Nawroth, P. P., King, G., Moller, W., Tritschler, H. J., & Packer, L. (2001). The role of oxidative stress in the onset and progression of diabetes and its complications: a summary of a Congress Series sponsored by UNESCO-MCBN,

the American Diabetes Association and the German Diabetes Society. *Diabetes Metab Res Rev, 17*(3), 189-212.

Roses, A. D. (2010). An inherited variable poly-T repeat genotype in TOMM40 in Alzheimer disease. *Arch Neurol, 67*(5), 536-541. doi: 10.1001/archneurol.2010.88

Roses, A. D., Lutz, M. W., Amrine-Madsen, H., Saunders, A. M., Crenshaw, D. G., Sundseth, S. S., . . . Reiman, E. M. (2010). A TOMM40 variable-length polymorphism predicts the age of late-onset Alzheimer's disease. *Pharmacogenomics J, 10*(5), 375-384. doi: 10.1038/tpj.2009.69

Roses, A. D., Lutz, M. W., Saunders, A. M., Goldgaber, D., Saul, R., Sundseth, S. S., . . . Welsh-Bohmer, K. A. (2014). African-American TOMM40'523-APOE haplotypes are admixture of West African and Caucasian alleles. *Alzheimers Dement, 10*(6), 592-601 e592. doi: 10.1016/j.jalz.2014.06.009

Ryan, C. M., Freed, M. I., Rood, J. A., Cobitz, A. R., Waterhouse, B. R., & Strachan, M. W. (2006). Improving metabolic control leads to better working memory in adults with type 2 diabetes. *Diabetes Care, 29*(2), 345-351.

Saczynski, J. S., Siggurdsson, S., Jonsson, P. V., Eiriksdottir, G., Olafsdottir, E., Kjartansson, O., . . . Launer, L. J. (2009). Glycemic status and brain injury in older individuals: the age gene/environment susceptibility-Reykjavik study. *Diabetes Care, 32*(9), 1608-1613. doi: 10.2337/dc08-2300

Salat, D. H., Buckner, R. L., Snyder, A. Z., Greve, D. N., Desikan, R. S., Busa, E., . . . Fischl, B. (2004). Thinning of the cerebral cortex in aging. *Cereb Cortex, 14*(7), 721-730. doi: 10.1093/cercor/bhh032

Schanider Beeri, M., Goldbourt, U., Silverman, J.M., Noy, S., Schmeidler, J., Ravona-Springer, R., . . . Davidson, M. (2004). Diabetes mellitus in midlife and the risk of dementia three decades later. *Neurology, 63*(10), 1902-1907.

Schrijvers, E. M., Witteman, J. C., Sijbrands, E. J., Hofman, A., Koudstaal, P. J., & Breteler, M. M. (2010). Insulin metabolism and the risk of Alzheimer disease: the Rotterdam Study. *Neurology, 75*(22), 1982-1987. doi: 10.1212/WNL.0b013e3181ffe4f6

Seetharaman, S., Andel, R., McEvoy, C., Dahl Aslan, A. K., Finkel, D., & Pedersen, N. L. (2014). Blood Glucose, Diet-Based Glycemic Load and Cognitive Aging Among Dementia-Free Older Adults. *J Gerontol A Biol Sci Med Sci*. doi: 10.1093/gerona/glu135

Segonne, F., Dale, A. M., Busa, E., Glessner, M., Salat, D., Hahn, H. K., & Fischl, B. (2004). A hybrid approach to the skull stripping problem in MRI. *Neuroimage, 22*(3), 1060-1075. doi: 10.1016/j.neuroimage.2004.03.032

Segonne, F., Pacheco, J., & Fischl, B. (2007). Geometrically accurate topology-correction of cortical surfaces using nonseparating loops. *IEEE Trans Med Imaging, 26*(4), 518-529. doi: 10.1109/tmi.2006.887364

Sheline, Y. I., Morris, J. C., Snyder, A. Z., Price, J. L., Yan, Z., D'Angelo, G., . . . Mintun, M. A. (2010). APOE4 allele disrupts resting state fMRI connectivity in the absence of amyloid plaques or decreased CSF Abeta42. *J Neurosci, 30*(50), 17035-17040. doi: 10.1523/jneurosci.3987-10.2010

Shen, L., Kim, S., Risacher, S. L., Nho, K., Swaminathan, S., West, J. D., . . . Saykin, A. J. (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*(3), 1051-1063. doi: 10.1016/j.neuroimage.2010.01.042

Singh, R., Barden, A., Mori, T., & Beilin, L. (2001). Advanced glycation end-products: a review. *Diabetologia, 44*(2), 129-146. doi: 10.1007/s001250051591

Sivertsen, J., Rosenmeier, J., Holst, J.J., & Vilsboll, T. (2012). The effect of glucagon-like peptide-1 on cardiovascular risk. *Nature Reviews Cardiology, 9*, 209-222.

Skeberdis, V. A., Lan, J., Zheng, X., Zukin, R. S., & Bennett, M. V. (2001). Insulin promotes rapid delivery of N-methyl-D- aspartate receptors to the cell surface by exocytosis. *Proc Natl Acad Sci U S A, 98*(6), 3561-3566. doi: 10.1073/pnas.051634698

Smith, G. E., Bohac, D. L., Waring, S. C., Kokmen, E., Tangalos, E. G., Ivnik, R. J., & Petersen, R. C. (1998). Apolipoprotein E genotype influences cognitive 'phenotype' in patients with Alzheimer's disease but not in healthy control subjects. *Neurology, 50*(2), 355-362.

Smith, M. A., Taneda, S., Richey, P. L., Miyata, S., Yan, S. D., Stern, D., . . . Perry, G. (1994). Advanced Maillard reaction end products are associated with Alzheimer disease pathology. *Proc Natl Acad Sci U S A, 91*(12), 5710-5714.

Soldan, A., Pettigrew, C., Li, S., Wang, M. C., Moghekar, A., Selnes, O. A., . . . O'Brien, R. (2013). Relationship of cognitive reserve and cerebrospinal fluid biomarkers to the emergence of clinical symptoms in preclinical Alzheimer's disease. *Neurobiol Aging, 34*(12), 2827-2834. doi: 10.1016/j.neurobiolaging.2013.06.017

Sommerfield, A. J., Deary, I. J., & Frier, B. M. (2004). Acute hyperglycemia alters mood state and impairs cognitive performance in people with type 2 diabetes. *Diabetes Care, 27*(10), 2335-2340.

StataCorp. (2009). Stata Statistical Software: Release 11. College Station, TX: StataCorp LP.

Stewart, R., & Liolitsa, D. (1999). Type 2 diabetes mellitus, cognitive impairment and dementia. *Diabet Med, 16*(2), 93-112.

Sun, X., Bromley-Brits, K., & Song, W. (2012). Regulation of beta-site APP-cleaving enzyme 1 gene expression and its role in Alzheimer's disease. *J Neurochem, 120 Suppl 1*, 62-70. doi: 10.1111/j.1471-4159.2011.07515.x

Szekely, C. A., Breitner, J. C., Fitzpatrick, A. L., Rea, T. D., Psaty, B. M., Kuller, L. H., & Zandi, P. P. (2008). NSAID use and dementia risk in the Cardiovascular Health Study: role of APOE and NSAID type. *Neurology, 70*(1), 17-24. doi: 10.1212/01.wnl.0000284596.95156.48

Szekely, C.A., Green, R.C., & Breitner, J.H. (2008). No advantage of A β 42-lowering NSAIDs for prevention of Alzheimer dementia in six pooled cohort studies. *Neurology, 70*, 2291-2298.

Tan, Z. S., Beiser, A. S., Fox, C. S., Au, R., Himali, J. J., Debette, S., . . . Seshadri, S. (2011). Association of metabolic dysregulation with volumetric brain magnetic resonance imaging and cognitive markers of subclinical brain aging in middle-aged adults: the Framingham Offspring Study. *Diabetes Care, 34*(8), 1766-1770. doi: 10.2337/dc11-0308

Tiehuis, A. M., van der Graaf, Y., Visseren, F. L., Vincken, K. L., Biessels, G. J., Appelman, A. P., . . . Mali, W. P. (2008). Diabetes increases atrophy and vascular lesions on brain MRI in patients with symptomatic arterial disease. *Stroke, 39*(5), 1600-1603. doi: 10.1161/strokeaha.107.506089

Tolppanen, A. M., Ngandu, T., Kareholt, I., Laatikainen, T., Rusanen, M., Soininen, H., & Kivipelto, M. (2014). Midlife and late-life body mass index and late-life dementia: results from a prospective population-based cohort. *J Alzheimers Dis, 38*(1), 201-209. doi: 10.3233/jad-130698

Tuligenga, R. H., Dugravot, A., Tabak, A. G., Elbaz, A., Brunner, E. J., Kivimaki, M., & Singh-Manoux, A. (2014). Midlife type 2 diabetes and poor glycaemic control as risk factors for cognitive decline in early old age: a post-hoc analysis of the Whitehall II cohort study. *Lancet Diabetes Endocrinol, 2*(3), 228-235. doi: 10.1016/s2213-8587(13)70192-x

Vagelatos, N.T., & Eslick, G.D. (2013). Type 2 diabetes as a risk factor for Alzheimer's disease: The counfounders, interactions, and neuropathology associated with this relationship. *Epidemiol Rev, 35*, 152-160.

van Elderen, S. G., de Roos, A., de Craen, A. J., Westendorp, R. G., Blauw, G. J., Jukema, J. W., . . . van der Grond, J. (2010). Progression of brain atrophy and cognitive decline in diabetes mellitus: a 3-year follow-up. *Neurology, 75*(11), 997-1002. doi: 10.1212/WNL.0b013e3181f25f06

van Harten, B., de Leeuw, F. E., Weinstein, H. C., Scheltens, P., & Biessels, G. J. (2006). Brain imaging in patients with diabetes: a systematic review. *Diabetes Care, 29*(11), 2539-2548. doi: 10.2337/dc06-1637

van Velsen, E. F., Vernooij, M. W., Vrooman, H. A., van der Lugt, A., Breteler, M. M., Hofman, A., . . . Ikram, M. A. (2013). Brain cortical thickness in the general elderly population: the Rotterdam Scan Study. *Neurosci Lett, 550*, 189-194. doi: 10.1016/j.neulet.2013.06.063

Vandenberghe, R., Gitelman, D. R., Parrish, T. B., & Mesulam, M. M. (2001). Functional specificity of superior parietal mediation of spatial shifting. *Neuroimage, 14*(3), 661-673. doi: 10.1006/nimg.2001.0860

Vitek, M. P., Bhattacharya, K., Glendening, J. M., Stopa, E., Vlassara, H., Bucala, R., . . . Cerami, A. (1994). Advanced glycation end products contribute to amyloidosis in Alzheimer disease. *Proc Natl Acad Sci U S A, 91*(11), 4766-4770.

Vounou, M., Janousova, E., Wolz, R., Stein, J. L., Thompson, P. M., Rueckert, D., & Montana, G. (2012). Sparse reduced-rank regression detects genetic associations with voxel-wise longitudinal phenotypes in Alzheimer's disease. *Neuroimage, 60*(1), 700-716. doi: 10.1016/j.neuroimage.2011.12.029

Wan, Q., Xiong, Z. G., Man, H. Y., Ackerley, C. A., Braunton, J., Lu, W. Y., . . . Wang, Y. T. (1997). Recruitment of functional GABA(A) receptors to postsynaptic domains by insulin. *Nature, 388*(6643), 686-690. doi: 10.1038/41792

Wang, Y. T., & Linden, D. J. (2000). Expression of cerebellar long-term depression requires postsynaptic clathrin-mediated endocytosis. *Neuron, 25*(3), 635-647.

Watson, G. S., Peskind, E. R., Asthana, S., Purganan, K., Wait, C., Chapman, D., . . . Craft, S. (2003). Insulin increases CSF Abeta42 levels in normal older adults. *Neurology, 60*(12), 1899-1903.

Wechsler, D. (1981). Wechsler Adult Intelligence Scale - Revised Manual New York: The Psychological Corporation.

Wechsler, D. (1987). Wechsler Memory Scale - Revised Manual. San Antonio, TX: Psychological Corporation.

Wennberg, A.M.V., Gottesman, R., Kaufmann, C.N., Albert, M., Rebok, G. W., Chen-Edinboro, L., & Spira, A. P. (2014). Diabetes and cognitive outcomes in a nationally representative sample: The National Health and Aging Trends Study (NHATS). *International Psychogeriatrics*. doi: 10.1017/S1041610214001380

West, R. K., Ravona-Springer, R., Schmeidler, J., Leroith, D., Koifman, K., Guerrero-Berroa, E., . . . Schnaider-Beeri, M. (2014). The Association of Duration of Type 2 Diabetes with Cognitive Performance is Modulated by Long-Term Glycemic Control. *Am J Geriatr Psychiatry*. doi: 10.1016/j.jagp.2014.01.010

Whitehair, D. C., Sherzai, A., Emond, J., Raman, R., Aisen, P. S., Petersen, R. C., & Fleisher, A. S. (2010). Influence of apolipoprotein E varepsilon4 on rates of cognitive and functional decline in mild cognitive impairment. *Alzheimers Dement, 6*(5), 412-419. doi: 10.1016/j.jalz.2009.12.003

Willette, A. A., Xu, G., Johnson, S. C., Birdsill, A. C., Jonaitis, E. M., Sager, M. A., . . . Bendlin, B. B. (2013). Insulin resistance, brain atrophy, and cognitive performance in late middle-aged adults. *Diabetes Care, 36*(2), 443-449. doi: 10.2337/dc12-0922

Wolfe, M. S. (2012). gamma-Secretase as a target for Alzheimer's disease. *Adv Pharmacol, 64*, 127-153. doi: 10.1016/b978-0-12-394816-8.00004-0

World Health Organization. (2012). Media centre: Diabetes fact sheet. Retrieved 6 August, 2014, from http://www.who.int/mediacentre/factsheets/fs312/en/

Xu, W. L., Qiu, C. X., Wahlin, A., Winblad, B., & Fratiglioni, L. (2004). Diabetes mellitus and risk of dementia in the Kungsholmen project: a 6-year follow-up study. *Neurology, 63*(7), 1181-1186.

Xu, W., Qiu, C., Gatz, M., Pedersen, N.L., Johansson, B., & Fratiglioni, L. (2009). Mid- and late-life diabestes in relation to the risk of dementia: a population-based twin study. *Diabetes, 58*, 71-77.

Xu, W., Qiu, C., Winblad, B., & Fratiglioni, L. (2007). The effect of borderline diabetes on the risk of dementia and Alzheimer's disease. *Diabetes, 56*(1), 211-216. doi: 10.2337/db06-0879

Yashin, A. I., Ukraintseva, S. V., Arbeev, K. G., Akushevich, I., Arbeeva, L. S., & Kulminski, A. M. (2009). Maintaining physiological state for exceptional survival: What is the normal level of blood glucose and does it change with age? *Mech Ageing Dev, 130*(9), 611-618. doi: 10.1016/j.mad.2009.07.004

Yesavage, J. A., Brink, T. L., Rose, T. L., Lum, O., Huang, V., Adey, M., & Leirer, V. O. (1982). Development and validation of a geriatric depression screening scale: a preliminary report. *J Psychiatr Res, 17*(1), 37-49.

Yoon, C. W., Kang, M., Shin, H. Y., Jeon, S., Yang, J. J., Kim, S. T., . . . Seo, S. W. (2014). Higher C-peptide levels are associated with regional cortical thinning in 1093 cognitively normal subjects. *Eur J Neurol, 21*(10), 1318-1323, e1380-1311. doi: 10.1111/ene.12485

Yudkin, John S, & Montori, Victor M. (2014). *The epidemic of pre-diabetes: the medicine and the politics* (Vol. 349).

Zandi, P. P., Anthony, J. C., Hayden, K. M., Mehta, K., Mayer, L., & Breitner, J. C. (2002). Reduced incidence of AD with NSAID but not H2 receptor antagonists: the Cache County Study. *Neurology, 59*(6), 880-886.

Zhao, W. Q., & Alkon, D. L. (2001). Role of insulin and insulin receptor in learning and memory. *Mol Cell Endocrinol, 177*(1-2), 125-134.

Table 1. Participant characteristics at baseline, n (%) or mean ± standard deviation

	Total (n=333)	APOE ε4 carriers (n=109)	APOE ε4 non-carriers (n=224)	*p*	TOMM40 VL/VL (n=55)	TOMM40 S/S (n=56)	*p*
Age, mean (SD)	56.9 ± 10.2	57.9 ± 7.9	56.4 ± 11.1	0.24	56.6 ± 11.8	57.1 ± 11.9	0.80
Sex, % female	192 (58)	67 (61)	125 (56)	0.57	32 (59)	31 (54)	0.68
Race, % Caucasian	327 (98)	105 (96)	222 (99)	0.18	N/A	N/A	N/A
Education, mean (SD)	17.1 ± 2.4	17.4 ± 2.2	16.9 ± 2.4	0.12	16.7 ± 2.4	17.1 ± 2.3	0.40
Baseline glucose (mg/dL), mean (SD)	99.1 ± 24.6	97.7 ± 25.2	99.9 ± 24.8	0.46	98.3 ± 29.5	101.9 ± 27.2	0.50
Diabetes, %	6 (2)	0 (0)	6 (3)	0.09	1 (2)	2 (4)	0.62
Cardiovascular conditions, %	14 (5)	6 (7)	8 (4)	0.57	5 (12)	1 (2)	0.07
Hypertension	37 (13)	13 (14)	24 (13)	0.60	4 (9)	6 (13)	0.63
Hypercholesterolemia	46 (17)	23 (26)	23 (12)	**0.003**	7 (16)	6 (13)	0.61
TBI	11 (4)	4 (4)	7 (4)	0.84	4 (9)	1 (2)	0.12
Depression, ever	53 (19)	25 (28)	28 (15)	**0.02**	9 (21)	6 (13)	0.28
Depression, within 2 yrs of baseline	38 (14)	19 (22)	19 (10)	**0.01**	9 (21)	4 (9)	0.09

TBI=traumatic brain injury.

Table 2. Executive function factor structure matrix: Correlations between variables and promax rotated common factors	
Variable	Factor Loading
Digit Span Back	0.4168
DSST	0.6338
Verbal Fluency “A”	0.4280
WAIS Block Design	0.6506

Table 3. Memory factor structure matrix: Correlations between variables and promax rotated common factors	
Variable	Factor Loading
Logical Memory, immediate	0.8769
Logical Memory, delay	0.8789
Pair Association, learning	0.4964
Pair Association, delay	0.4290

Table 4. LMM: Association between baseline glucose (mg/dL) and change in factor score				
	Model 1		Model 2	
	B (95% CI)	*p*	B (95% CI)	*p*
Executive Function	-0.004 (-0.007, -0.0005)	**0.022**	-0.004 (-0.008, -0.001)	**0.010**
Memory	0.0005 (-0.003, 0.004)	0.805	0.0009 (-0.003, 0.005)	0.665
Model 1 controlled for sex and age. Executive Function, n=307; Memory, n=323. Model 2 controlled for sex, age, education, race, depression, and medical conditions. Executive Function, n=282; Memory, n=298.				

Table 5. GLM Model 1: Association between baseline glucose (mg/dL) and change in factor score by TOMM40 status				
	VL/VL (n=41)		S/S (n=38)	
	B (95% CI)	*p*	B (95% CI)	*p*
Executive Function	0.004 (-0.003, 0.010)	0.283	-0.013 (-0.024, -0.002)	**0.021**
Model controlled for sex and age. Interaction term, *p*=0.009				

Table 6. GEE Model: Association between baseline glucose (mg/dL) and change in factor score				
	Model 1		Model 2	
	B (95% CI)	*p*	B (95% CI)	*p*
Executive Function	-0.007 (-0.010, -0.004)	**<0.001**	-0.004 (-0.008, -0.001)	**0.009**
Memory	0.0001 (-0.003, 0.003)	0.942	-0.0002 (-0.004, 0.004)	0.923
Model 1 controlled for sex and age. Executive Function, n=307; Memory, n=323. Model 2 controlled for sex, age, education, race, depression, and medical conditions. Executive Function, n=282; Memory, n=298.				

Table 7. GEE Model 2: Association between baseline glucose (mg/dL) and change in factor score by APOE status				
	APOE ε4 Positive (n=93)		APOE ε4 Negative (n=189)	
	B (95% CI)	*p*	B (95% CI)	*p*
Executive function	-0.013 (-0.020, -0.006)	**<0.001**	-0.005 (-0.009, -0.002)	**0.002**
Model controlled sex, age, education, race, depression, and medical conditions. Interaction term, *p*=0.019.				

Figure 2 Executive Function Factor Scores

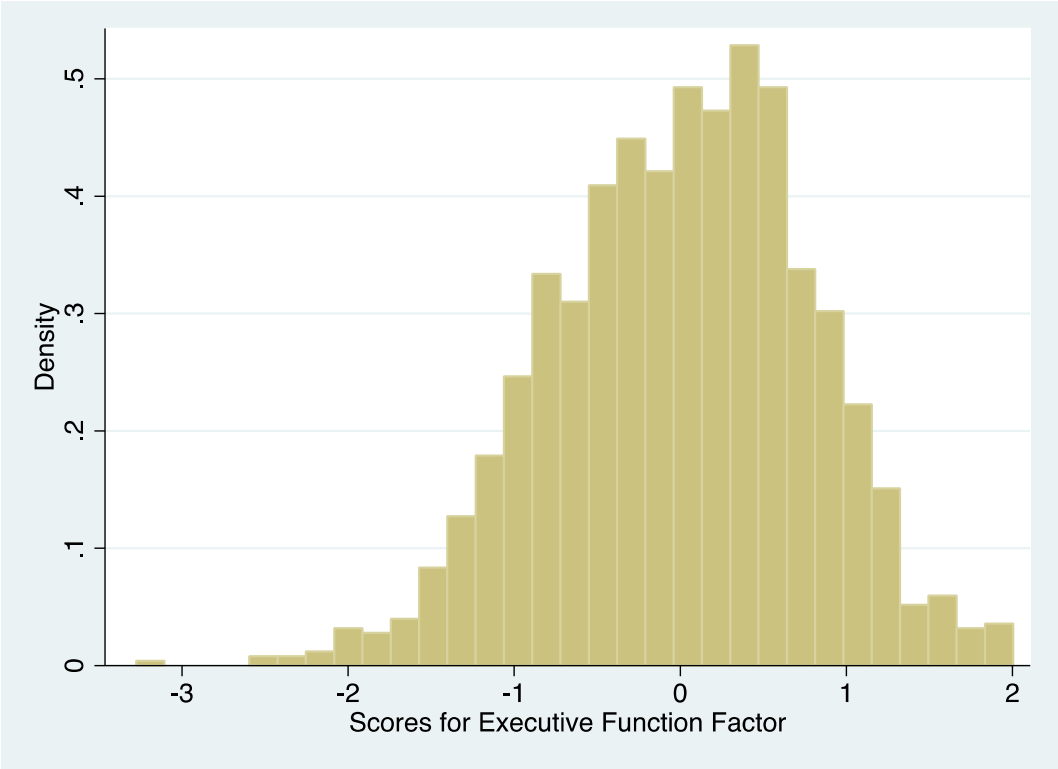


Table 8. Cox regression: Association between baseline glucose (mg/dL) and time to symptom onset		
	Hazard Ratio (95% CI)	*p*
Model 1	1.00 (0.996, 1.01)	0.655
Model 2	1.00 (0.996, 1.01)	0.653
Model 3	1.00 (0.993, 1.01)	0.928
Model 1 is unadjusted. Model 2 is adjusted for years of education, sex, and race. Model 3 is adjusted for the same covariates as Model 2 + depression and medical conditions.		

Table 9. Linear regression: Cross-sectional association between baseline glucose (mg/dL) and cortical thickness at baseline				
	Model 1		Model 2	
	B (95% CI)	*p*	B (95 % CI)	*p*
Entorhinal cortex	-0.0006 (-0.002, 0.002)	0.943	-0.001 (-0.003, 0.0005)	0.171
Inferior temporal gyrus	-0.0006 (-0.0007, 0.0006)	0.846	0.0002 (-0.0005, 0.0009)	0.577
Parahippocampal gyrus	-0.001 (-0.002, 0.0003)	0.119	-0.002 (-0.003, -0.0001)	**0.036**
Pars triangularis	-0.0007 (-0.001, -0.00001)	**0.045**	-0.0007 (-0.001, 0.0001)	0.092
Precuneus	-0.00009 (-0.0007, 0.0005)	0.776	-0.0001 (-0.0008, 0.0006)	0.764
Superior frontal gyrus	-0.000004 (-0.0007, 0.0008)	0.993	-0.0002 (-0.001, 0.0007)	0.702
Superior parietal gyrus	0.0003 (-0.0002, 0.0009)	0.251	0.0003 (-0.0004, 0.0009)	0.433
Supramarginal gyrus	0.0001 (-0.0005, 0.0007)	0.712	-0.00009 (-0.0008, 0.0006)	0.811
Temporal pole	-0.0009 (-0.002, 0.0004)	0.162	-0.002 (-0.003, -0.0002)	**0.027**
Average thickness	-0.0003 (-0.0009, 0.0003)	0.359	-0.0006 (-0.001, 0.00009)	0.090
Average thickness is over the AD-specified areas. Model 1 (n=253) adjusted for age and sex. Model 2 (n=235) adjusted for age, sex, race, education, depression, and medical conditions.				

Table 10. Cross-sectional association between baseline glucose (mg/dL) and cortical thickness at baseline by APOE status				
	APOE ε4 Positive (n=79)		APOE ε4 Negative (n=168)	
	B (95% CI)	*p*	B (95% CI)	*p*
Superior parietal gyrus	-0.001 (-0.002, 0.0005)	0.183	0.0007 ($3.05e^{-6}$, 0.001)	**0.049**
Temporal pole	-0.004 (-0.008, -0.0009)	**0.014**	-0.0003 (-0.002, 0.001)	0.711
Model controlled sex and age. Interaction term p-value=0.049				

CURRICULUM VITAE

Alexandra M. V. Wennberg

Email: awennbe1@jhu.edu

Phone (cell): 612/281-2344

Education

Johns Hopkins University Bloomberg School of Public Health

September 2011-present
Doctor of Philosophy (PhD), Department of Mental Health

Thesis: "Type II Diabetes Mellitus and genetic factors as predictors of cognitive decline and dementia" (Advisor: Adam Spira, PhD)

Expected Graduation: Summer 2015

Washington University School of Medicine, Alzheimer's Disease Research Center

Clinical Dementia Rating Certified, February 2015

Johns Hopkins Bloomberg School of Public Health
Certificate of Gerontology, May 2014

Skidmore College

September 2006-May 2010
Bachelor of Arts (BA), Neuroscience, Cum Laude

Highest Honors Fall 2008, Spring 2009, Fall 2009, Spring 2010; Honors Fall 2007, Spring 2008

Professional Experience

Johns Hopkins Hospital Center for Cerebral Spinal Fluid Disorders
December 2013-present

Research Assistant for Abhay Moghekar, MD: Completed cognitive testing and cerebrospinal fluid processing for a study of sleep disorders and amyloid beta in clinical patients.

Johns Hopkins Clinical Research Center and Kennedy Krieger Institute

September 2014-present

Research Assistant for Mark Wu, MD, PhD and George Capone, MD: recruited participants and collected and processed data for a study of sleep disordered breathing and amyloid beta in Down's syndrome patients.

Johns Hopkins Bloomberg School of Public Health, Department of Mental Health

October 2013-present

Research Assistant for Adam Spira, PhD: processed actigraphy data for study of sleep/wake disturbance in older adults with mild cognitive impairment and normal cognitive status.

Clemson University/Greenville Health System's Center for Success in Aging

May 2014-August 2014

Consultant: assisted in researching and writing a grant for the implementation of a program to provide assistance to local caregivers.

Clemson University/Greenville Health System's Center for Success in Aging

May 2013-August 2013

Consultant: researched and wrote a manuscript on Alzheimer's disease caregiver burden, and worked with clinicians and researchers to design a program to provide assistance to local caregivers.

Johns Hopkins University, Departments of Neurology and Mental Health

August 2011-August 2013

Aging and Dementia Training Program, Predoctoral Fellow

T32 AG027668 (PI: Marilyn Albert, PhD)

Skidmore College, Department of Biology

September 2009-May 2010

Research Collaborator: conducted a project investigating age-related circadian rhythm changes in normal aging and Alzheimer's disease using *Drosophila melanogaster* as a model organism.

Skidmore College, Department of Psychology

September 2008-August 2009

Research Collaborator: investigated the effects of lifestyle, specifically diet, physical activity, and sleep, on cognitive aging in community-dwelling older adults.

Publications

Wennberg, A. M. V., Zipunnikov, V., Kaufmann, C. N., Gottesman, R. F., Albert, M. S., Rebok, G. W., Kasper, J. D., & Spira, A. P. (In preparation). Longitudinal association between diabetes and cognitive decline in a nationally representative sample: The National Health and Aging Trends Study

Wennberg, A. M. V., Streetman-Loy, B., Dye, C. J., & Pham, H. T. (In press). Alzheimer's patient familial caregivers: A review of burden and interventions. *Health & Social Work.*

Kaufmann, C. N., Mojtabai, R., Hock, R. S., Thorpe Jr., R. J., Canham, S. L. Chen, L-Y, **Wennberg, A. M. V.**, Chen-Edinboro, L., & Spira, A. P. Racial/ethnic differences in insomnia trajectories among U.S. older adults. Submitted to *SLEEP.*

Wennberg, A. M. V., Kueider, A. M., Spira, A. P., Adams, G., Rager, R., & Rebok, G. W. (In press). Online attention training for older adults. *Cognitive Technology.*

Chen-Edinboro, L. P., Kaufmann, C. N., Augustinavicius, J. L., Mojtabai, R., Parisi, J. M., **Wennberg, A. M. V.**, Smith, M. T., Spira, A. P. (2015). Neighborhood physical disorder, social cohesion and insomnia: results from participants over age 50 in the Health and Retirement Study. *International Psychogeriatrics 27(2)*, 289-296.

Wennberg, A. M., Gottesman, R. F., Kaufmann, C. N., Albert, M. S., Chen-Edinboro, L. P., Rebok, G. W., Kasper, J. D. & Spira, A. P. (2014). Diabetes and cognitive outcomes in a nationally representative sample: the National Health and Aging Trends Study. *International Psychogeriatrics*, *26*(10), 1729-1735.

Wennberg, A. M., Canham, S. L., Smith, M. T., & Spira, A. P. (2013). Optimizing sleep in older adults: Treating insomnia. *Maturitas 76*(3), 247-252.

Presentations

Wennberg, A. M. V., Zipunnikov, V., Kaufmann, C. N., Albert, M. S., Rebok, G. W., Kasper, J. D., & Spira, A. P. (2015). "Longitudinal Association Between Diabetes and Cognitive Decline in a Nationally Representative Sample: The National Health and Aging Trends Study." Presentation at the American Association for Geriatric Psychiatry's 2015 Annual Meeting, New Orleans, LA.

Wennberg, A. M., Gottesman, R. F., Kaufmann, C. N., Albert, M. S., Chen-Edinboro, L. P., Rebok, G. W., Kasper, J. D. & Spira, A. P. (2014). "Diabetes and cognitive outcomes in a nationally representative sample: the National Health and Aging Trends Study."

Presentation at the Gerontological Society of America 67th Annual Conference, Washington, D.C.

Wennberg, A. M., Kueider, A. M., Spira, A. P., Rager, R., Adams, G. L., & Rebok, G. W. (2013). "Attention Workout™: Improving cognition in older adults online." Presentation at the Gerontological Society of America 66th Annual Conference, New Orleans, LA.

Kueider, A. M., **Wennberg, A.**, Rebok, G. W., Parisi, J. M., & Spira, A. P. (2013). "Marital status in older adults: Associations with memory and functional abilities in the ACTIVE study." Presentation at the American Public Health Association Conference, Boston, MA.

Possidente, B., Benway, T., Kidder, M., Veinoglu, A., **Wennberg, A.** & Crowther, D. (2010). "Beta-amyloid peptide mutations alter circadian locomotor behavior in a transgenic *Drosophila* model of Alzheimer's disease." Presentation at the Behavior Genetics Association, 40th Annual Meeting, Seoul, Korea.

Wennberg, A., & Leaver, E. (2010). "The effect of lifestyle on cognition and aging." Presentation at the Annual Cognitive Aging Conference, Atlanta, GA.

Teaching Assistant

Prevention of Mental Health Disorders: Public Health Interventions, Johns Hopkins Bloomberg School of Public Health, 2014

Psychopathology for Public Health, Johns Hopkins Bloomberg School of Public Health, 2014

Professional Activities

Gerontological Society of America, Member

American Heart Association, Member

Honors and Awards

Admitted to the Aging and Dementia Training Program, Johns Hopkins University, 2011-2013

Student Government Association Responsible Citizenship Internship Award recipient, Skidmore College, 2010

Awarded Student Opportunity Funds, Skidmore College, 2010

Student Government Association Senior Leadership Award recipient, Skidmore College, 2009-2010

Awarded funding for Summer Faculty/Student Research Program, Skidmore College, 2009

Awarded Conference Travel Funds Grant, Psychology Department, Skidmore College, 2009