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.

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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
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
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. Prediabetes, 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 ε4 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 dendritic 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).
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

*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-temporal 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\textsubscript{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).
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.
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 BI OCARD 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.
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/m² (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/m²). 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 ($B=-0.0007; 95\% \text{ CI} -0.001, -0.0001$) (Table 9). In Model 2, which controlled for age, education, race, sex, and medical conditions, glucose was associated with thinner parahippocampal gyrus ($B=-0.002; 95\% \text{ CI} -0.003, -0.0001$) and temporal pole ($B=-0.002; 95\% \text{ CI} -0.003, -0.0002$) measures. In Model 1, we found that the association between glucose and grey matter atrophy was stronger among those with an APOE $\varepsilon4$ allele. Among those without an e4 allele, higher glucose levels were associated with thicker superior parietal gyri ($B=0.0007, 95\% \text{ CI } 3.05e^{-6}, 0.001$), compared to those with an e4 allele ($B=-0.001; 95\% \text{ CI} -0.002, 0.0005$; interaction term $p=0.035$). Further, higher glucose levels were associated with significantly thinner temporal pole measures in e4 carriers ($B=-0.004; 95\% \text{ CI} -0.008, -0.0009$) versus non-carriers ($B=-0.0003; 95\% \text{ CI} -0.002, 0.001$; 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 \( \varepsilon4 \) alleles, as compared to those without an \( \varepsilon4 \) 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 prediabetes 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.


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*Diabetes Care, 27*(10), 2335-2340.

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

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

TBI=traumatic brain injury.
Table 2. Executive function factor structure matrix: Correlations between variables and promax rotated common factors

<table>
<thead>
<tr>
<th>Variable</th>
<th>Factor Loading</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digit Span Back</td>
<td>0.4168</td>
</tr>
<tr>
<td>DSST</td>
<td>0.6338</td>
</tr>
<tr>
<td>Verbal Fluency “A”</td>
<td>0.4280</td>
</tr>
<tr>
<td>WAIS Block Design</td>
<td>0.6506</td>
</tr>
</tbody>
</table>

Table 3. Memory factor structure matrix: Correlations between variables and promax rotated common factors

<table>
<thead>
<tr>
<th>Variable</th>
<th>Factor Loading</th>
</tr>
</thead>
<tbody>
<tr>
<td>Logical Memory, immediate</td>
<td>0.8769</td>
</tr>
<tr>
<td>Logical Memory, delay</td>
<td>0.8789</td>
</tr>
<tr>
<td>Pair Association, learning</td>
<td>0.4964</td>
</tr>
<tr>
<td>Pair Association, delay</td>
<td>0.4290</td>
</tr>
</tbody>
</table>
Table 4. LMM: Association between baseline glucose (mg/dL) and change in factor score

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th>Model 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B (95% CI)</td>
<td>p</td>
</tr>
<tr>
<td>Executive Function</td>
<td>-0.004 (-0.007, -0.0005)</td>
<td><strong>0.022</strong></td>
</tr>
<tr>
<td>Memory</td>
<td>0.0005 (-0.003, 0.004)</td>
<td>0.805</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>VL/VL (n=41)</th>
<th>S/S (n=38)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B (95% CI)</td>
<td>p</td>
</tr>
<tr>
<td>Executive Function</td>
<td>0.004 (-0.003, 0.010)</td>
<td>0.283</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th>Model 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B (95% CI)</td>
<td>p</td>
</tr>
<tr>
<td>Executive Function</td>
<td>-0.007 (-0.010, -0.004)</td>
<td><strong>&lt;0.001</strong></td>
</tr>
<tr>
<td>Memory</td>
<td>0.0001 (-0.003, 0.003)</td>
<td>0.942</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>APOE ε4 Positive (n=93)</th>
<th>APOE ε4 Negative (n=189)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B (95% CI)</td>
<td>p</td>
</tr>
<tr>
<td>Executive function</td>
<td>-0.013 (-0.020, -0.006)</td>
<td><strong>&lt;0.001</strong></td>
</tr>
</tbody>
</table>

Model controlled sex, age, education, race, depression, and medical conditions. Interaction term, \( p=0.019 \).
Figure 2 Executive Function Factor Scores
<table>
<thead>
<tr>
<th>Model</th>
<th>Hazard Ratio (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td>1.00 (0.996, 1.01)</td>
<td>0.655</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.00 (0.996, 1.01)</td>
<td>0.653</td>
</tr>
<tr>
<td>Model 3</td>
<td>1.00 (0.993, 1.01)</td>
<td>0.928</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th></th>
<th>Model 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B (95% CI)</td>
<td>p</td>
<td>B (95% CI)</td>
<td>p</td>
</tr>
<tr>
<td>Entorhinal cortex</td>
<td>-0.0006 (-0.002, 0.002)</td>
<td>0.943</td>
<td>-0.001 (-0.003, 0.0005)</td>
<td>0.171</td>
</tr>
<tr>
<td>Inferior temporal gyrus</td>
<td>-0.0006 (-0.0007, 0.0006)</td>
<td>0.846</td>
<td>0.0002 (-0.0005, 0.0009)</td>
<td>0.577</td>
</tr>
<tr>
<td>Parahippocampal gyrus</td>
<td>-0.001 (-0.002, 0.0003)</td>
<td>0.119</td>
<td>-0.002 (-0.003, -0.0001)</td>
<td>0.036</td>
</tr>
<tr>
<td>Pars triangularis</td>
<td>-0.0007 (-0.001, -0.00001)</td>
<td>0.045</td>
<td>-0.0007 (-0.001, 0.0001)</td>
<td>0.092</td>
</tr>
<tr>
<td>Precuneus</td>
<td>-0.00009 (-0.0007, 0.0005)</td>
<td>0.776</td>
<td>-0.00001 (-0.0008, 0.0006)</td>
<td>0.764</td>
</tr>
<tr>
<td>Superior frontal gyrus</td>
<td>-0.0000004 (-0.00007, 0.00008)</td>
<td>0.993</td>
<td>-0.00002 (-0.0001, 0.00007)</td>
<td>0.702</td>
</tr>
<tr>
<td>Superior parietal gyrus</td>
<td>0.0003 (-0.00002, 0.00009)</td>
<td>0.251</td>
<td>0.0003 (-0.0004, 0.00009)</td>
<td>0.433</td>
</tr>
<tr>
<td>Supramarginal gyrus</td>
<td>0.0001 (-0.0005, 0.0007)</td>
<td>0.712</td>
<td>-0.000009 (-0.00008, 0.00006)</td>
<td>0.811</td>
</tr>
<tr>
<td>Temporal pole</td>
<td>-0.0009 (-0.002, 0.0004)</td>
<td>0.162</td>
<td>-0.002 (-0.003, -0.0002)</td>
<td>0.027</td>
</tr>
<tr>
<td>Average thickness</td>
<td>-0.0003 (-0.0009, 0.0003)</td>
<td>0.359</td>
<td>-0.0006 (-0.001, 0.00009)</td>
<td>0.090</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>APOE ε4 Positive (n=79)</th>
<th></th>
<th>APOE ε4 Negative (n=168)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B (95% CI)</td>
<td>p</td>
<td>B (95% CI)</td>
<td>p</td>
</tr>
<tr>
<td>Superior parietal gyrus</td>
<td>-0.001 (-0.002, 0.0005)</td>
<td>0.183</td>
<td>0.0007 (3.05e^-6, 0.001)</td>
<td>0.049</td>
</tr>
<tr>
<td>Temporal pole</td>
<td>-0.004 (-0.008, -0.0009)</td>
<td>0.014</td>
<td>-0.0003 (-0.002, 0.001)</td>
<td>0.711</td>
</tr>
</tbody>
</table>

Model controlled sex and age. Interaction term p-value=0.049
CURRICULUM VITAE

Alexandra M. V. Wennberg
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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

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**


**Presentations**


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


**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