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Herpes Simplex-1 As An Additive Risk Factor for Cognitive Decline in Apolipoprotein E4 Carriers

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Dedication

For my grandmother, Myumi S. Kalinowski, a source of limitless inspiration and support.

Herpes Simplex-1 As An Additive Risk Factor for Cognitive Decline in Apolipoprotein E4 Carriers

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The identification of early, modifiable risk factors for cognitive decline presents the most promising opportunity for intervention. To date one of the most robustly replicated risk factors for the most common form of dementia, Alzheimer's disease (AD), is the Apolipoprotein E4 (ApoE4) allele. Risk for sporadic and familial late onset AD increases nearly threefold for each E4 allele an individual carries. However some E4 carriers do not develop cognitive decline, and many non-E4 carriers do, highlighting the role of environmental variables in the progression to clinical symptoms. There is evidence that herpes simplex-1 (HSV-1), a common neurotropic viral infection with affinity for the same brain structures affected in AD, is an acquired risk factor that may compound the genetic risk associated with ApoE4. We examined the interaction between the ApoE4 allele and HSV-1 in cognitively normal middle-aged adults using neuropsychological testing and structural and functional neuroimaging. Neuropsychological assessments were used to determine cognitive differences between groups. Structural neuroimaging was used to measure group differences in bilateral hippocampal volumes, and cortical thickness in brain regions most likely to be affected by AD and HSV-1. Functional neuroimaging was used to examine differences in resting-state brain activity within the default mode network (DMN), a network known for alterations in functional connectivity during the progression from normal aging to AD. With regard to cognition we found that ApoE4 carriers performed significantly lower on tests of executive functioning when they were infected with HSV-1. HSV-1 infection

alone also correlated with significantly lower full scale IQ (FSIQ). Within the structural domain we found that individuals with ApoE4 had significantly smaller bilateral hippocampal volumes compared to individuals without the virus, regardless of HSV-1 status. Within the functional domain we failed to find any group differences in functional connectivity within the DMN. Together these findings suggest that HSV-1 may contribute to cognitive changes linked to cognitive vulnerability, and that ApoE4 may contribute to structural brain vulnerability. Because these factors are identifiable prior to the onset of frank cognitive decline, antiviral intervention could be considered as a means of mitigating risk for cognitive decline.

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Chapter 1: General Introduction

1.0 Overview

Cognition is the most important determinant of functional ability and quality of life (Gaugler et al., 2009). Intact cognitive function is crucial for successful aging; yet, our understanding of how inherited and acquired risk factors converge over the lifespan to result in brain vulnerability is poor, and cures for dementia are non-existent. By the time clinical symptoms of cognitive decline emerge, mitigating intervention is limited to marginally slowing their progression. Hence, identification and modification of risk factors in midlife, well before clinical symptoms appear, offers the most promising path for preventing functional loss in later life.

Of particular interest is the interaction between genetic vulnerability and acquired risk factors such as infectious disease, and the potential for such interactions to promote brain changes early in life that may increase the odds of future cognitive decline. One promising area of research is the relationship between the E4 allele of the Apolipoprotein E gene (ApoE4), a known genetic risk factor for Alzheimer's disease (AD), and herpes simplex-I (HSV-1), the virus that causes cold sores. There is evidence to suggest that ApoE4 and HSV-1 interact to promote deleterious brain changes beginning in midlife, but to date no studies have used neuroimaging to examine the brains of clinically normal HSV-1-positive ApoE4 carriers. This study seeks to identify early cognitive, structural and functional changes in the brains of healthy middle-aged HSV-1 positive ApoE4 carriers. If HSV-1 does interact with the ApoE4 allele type, viral suppression early in the lifespan may be a critical approach to preventing late life functional loss in at-risk individuals.

1.1 THE APOLIPOPROTEIN E GENE: FUNCTION AND EVOLUTION

The Apolipoprotein E (ApoE) gene expresses apolipoprotein E, a 229 amino acid glycoprotein synthesized primarily in liver and secondarily in brain. Physiologically apolipoproteins bind and transport lipids to make them available to cells. They are the most abundant lipoproteins expressed in the brain and are involved in a range of functions including cholesterol homeostasis, neuroplasticity, immunomodulation, and amyloid deposition and clearance (Rebeck et al., 2002). The ApoE gene has three alleles resulting in three protein isoforms: ApoE2, -E3, and -E4. Individuals carry two copies of the gene and are either homo- or heterozygous for the alleles they carry.

Humans are alone among primates to demonstrate multiple ApoE alleles, suggesting that genetic polymorphisms favoring alterations in cholesterol metabolism emerged in early hominid evolution, probably following migration into regions with increased temperature variability. The ApoE4 allele occurs in ~15% (+/- 8.5) of the human population and is over-represented in individuals whose genetic ancestors lived further from the equator in colder environments with greater metabolic demands (for a full review of ApoE population rates, see Eisenberg et al., 2010). The E4 allele also has a higher incidence in pre-industrialized populations, for reasons discussed below.

The move from torrid to temperate zones likely made reduced cholesterol clearance selectively advantageous, but not without a tradeoff. ApoE4 carriers also demonstrate increased vulnerability to certain infectious diseases (Amouyel et al., 1994; Burgos et al., 2003; Burt et. al., 2008) and poorer outcome following brain injury (Friedman et al., 1999; Teasdale et al., 1997), indicating that the ApoE gene modulates immune function in addition to lipid metabolism. The E4 allele confers increased vulnerability following infections of the nervous system, but has been shown to have a protective effect against diarrheal diseases and liver damage associated with hepatitis C

(Oria et al., 2005 & 2007; Wozniak et al., 2002). Therefore, the ApoE4 allele may have provided advantageous resistance to pathogens that were, and are, more prevalent in pre-industrialized settings, but this advantage is lost in industrialized populations where chronic non-infectious disorders are the leaders in mortality.

1.2 THE APOE GENE AND THE CENTRAL NERVOUS SYSTEM

Different ApoE genotypes are linked to varying levels of brain vulnerability, identified for the purposes of this paper as risk for developing cognitive impairment later in life (Laws et al., 2003; Lindsay, J., 2002). The ApoE4 allele is the most widely accepted susceptibility gene for late-onset Alzheimer's disease (AD), the most common type of dementia (Lindsay, J. 2002). Genetic risk for AD increases in a dose-dependent manner, with E4 homozygotes having a tenfold higher risk for developing dementia than "risk neutral" E3 homozygotes (Corder et al., 1993; Laws et al., 2003; Slooter et al., 1998).

1.2.1 ApoE, lipid metabolism and amyloid deposition

Amyloid-beta (Aβ) is a peptide resulting from enzymatic cleavage of the amyloid precursor protein (APP). Aβ exists in several different isoforms depending on where cleavage of APP occurs. Certain isoforms, particularly Aβ42, can aggregate to form flexible oligomers. For unknown reasons these oligomers sometimes become misfolded and further aggregate to form senile plaques in brain tissue; these plaques are the neuropathological hallmark of AD (for a full review of the role of Aβ in AD, see Haass et al., 2007).

Mechanisms linking ApoE to Aβ deposition have yet to be fully elucidated, but one hypothesis implicates inefficiencies in cholesterol and amyloid clearance from brain parenchyma among E4 carriers. Owing to structural differences ApoE isoforms vary in

their lipid binding affinity. Specifically, ApoE4 binds preferentially to very large low-density lipoproteins (VLDLs), whereas ApoE2 and E3 bind preferentially to high-density lipoproteins (HDLs) (Hatters et al., 2006). Studies in humans and animals have revealed that avid binding of ApoE4 to VLDLs slows their metabolic clearance and results in higher serum LDL levels (Bergeron et al., 1996; Demant et al., 1991; Hatters et al., 2006; Knouff et al., 1999; Mahley et al., 2009; 19). Greater LDL cholesterol availability may upregulate the enzymatic cleavage of APP and partially explain why high cholesterol is linked to increased amyloidogenesis (Frears et al., 1999; Laws et al., 2003). Once formed, amyloid deposits bind with ApoE4-cholesterol complexes, which readily aggregate into denser plaques than those formed by ApoE3 (Sanan et al., 1994). Colocalization of Aβ, ApoE, and cholesterol within the core of plaques has been demonstrated in animal models and in human brain tissue taken from AD patients (Burns et al., 2003; Deane et al., 2008; Mori et al., 2001).

Animal models have also shown that the E4 allele inhibits Aβ clearance across the blood brain barrier (BBB) (Sanan et al., 1994), allowing it to collect in brain tissue. This may be due to the structural instability of ApoE4, making it more likely to assume a partially unfolded form conducive to self-aggregation and plaque formation (Hatters et al., 2006; Mahley et al., 2009). Once cholesterol and Aβ accumulation begins, the innate immune response initiates an inflammatory cascade that can further aggravate the neurotoxic effects of Aβ deposition (Rogers & Shen, 2000). These findings suggest that genetic differences in cholesterol transport and inflammatory response contribute to a feedback loop resulting in Aβ-mediated neurotoxicity.

1.2.2. ApoE and Immunomodulation

The ApoE4 allele's deleterious role in cases of cerebral infection and injury stems in part from inefficient clearance of neurotoxic products, but also from a stronger and more persistent inflammatory response compared to other allele types (Frautschy et al., 1998). Under normal physiologic conditions activated microglia, the brain's resident immune cells, survey the brain for pathogens and cellular debris, and induce inflammatory mediators that assist in the destruction and removal of "non-self" material. However, left unchecked, the chronic release of inflammatory mediators can lead to autotoxicity, synaptic degradation, and neuronal death (Fuller et al., 2010). Microglia with an E4 genotype maintain an activated, pro-inflammatory morphology even in the absence of inflammatory antigens (Frautschy et al., 1998; McGeer, P., 2001; McGeer & McGeer, 1995). In keeping with this finding, post mortem tissue samples from the brains of AD patients have shown local upregulation of inflammatory markers produced in response to damage resulting from the formation of Aβ plaques (Breitner et al., 1995; McGeer & McGeer, 2002 & 2007; Mcintosh et al., 2001; Nagele et al., 2004). Such an aggressive inflammatory response may be salutary in cases of acute diarrheal infection, but becomes pathogenic within the brain, especially in the presence of chronic irritants such as Aβ.

In a manner similar to the development of atherosclerotic lesions (which are also more common in ApoE4 carriers), the brain's pro-inflammatory response to Aβ plaques exacerbates lesioning and fosters a chronic inflammatory condition in ApoE4 carriers (Mcintosh et al., 2001). Evidence from in vitro and animal models suggests that non-steroidal anti-inflammatory drugs (NSAIDs) may retard the development of plaque formation and symptoms of AD by suppressing inflammation (Breitner et al., 1995), but support for this claim is less clear in human experimental and epidemiological literature

(Mcgeer & Mcgeer, 2007). Nevertheless, these data have contributed to the opinion that AD is foremost an inflammatory condition abetted by genetic vulnerability.

1.3 HERPES SIMPLEX-1 VIRUS (HSV-1)

Herpes viruses are comparatively large, species-specific DNA viruses infecting a variety of reptiles, birds and mammals. The larger coding capacity of DNA viruses compared to RNA viruses allows them to express a broader array of proteins capable of interfering with normal host immune function. This allows them to extend the period for replication and shedding, and to establish non-productive infection, or latency, within the host (Bunzli et al., 2004). In humans, HSV-1 is highly prevalent, thought to affect more than 70% of the general population after age 50 (Miller et al., 1998). Typically HSV-1 resides in the peripheral nervous system (PNS) (usually the trigeminal ganglia) and periodically reactivates to a productive infection in response to immunosuppressive factors such as stress. Host cells are destroyed during productive infection, visible in the form of cold sores, which occur approximately 40% of the time during viral reactivation (Wang et al., 2006).

1.4 HSV-1 AND THE CENTRAL NERVOUS SYSTEM

In rare instances of herpes simplex encephalitis (HSE) the virus travels along the trigeminal nerve in a retrograde fashion and produces lesions in the temporal lobes of the brain rather than moving in an anterograde fashion to produce cold sores on the lips; see Figure 1.1. It is unclear under what circumstances HSV-1 moves from the PNS to the CNS, but latent HSV-1 infection has been documented in the CNS of animals (Rock, 1983; Schlitt et al., 1986) and humans (Adams & Miller, 1973; Damasio et al., 1985; Jamieson et al., 1992; Kennedy et al., 1988; Wozniak et al., 2005). Animal studies have focused on olfactory and trigeminal tracts as potential pathways by which HSV-1

accesses the orbitofrontal regions of the brain (Johnson, R., 1964; Schlitt et al., 1986; Stroop et al., 1986). However, HSV-1 infiltration of the brain is most often associated with HSE and not asymptomatic latent infection. Whether this is because CNS infiltration is actually rare, or because it only becomes apparent in cases of florid disease, is not known. However, there is evidence that HSE can occur in a mild, subacute form (DeVincenzo & Thorne, 1994; Fodor et al., 1998; Klapper et al., 1984).

Possession of an ApoE4 allele facilitates HSV-1's neuroinvasiveness (Burgos et al., 2003 & 2006). Once HSV-1 breaches the CNS there are three lines of reasoning linking it to AD. First, ApoE4 carriers are overrepresented among HSV-1 positive AD patients, suggesting that central HSV-1 infection of ApoE4 carriers confers a higher risk of AD than ApoE4 alone or virus alone (Itzhaki et al., 1997). Second, ApoE4 carriers are more likely to exhibit cold sores (Dobson & Itzhaki, 1999; Itzhaki et al., 1997), indicating that E4 carriers are less successful at viral suppression, and that HSV-1 in E4 carriers is more likely to result in tissue destruction. Third, HSV-1 DNA is present in the same brain regions most vulnerable to AD: the medial temporal lobes, and temporal and frontal cortices (Dobson & Itzhaki, 1999; Itzhaki et al., 1997). The virus has not been found in occipital cortex, which is typically spared in AD (Dobson & Itzhaki, 1999; Itzhaki et al., 1997).

1.5 THE APOE GENE: EFFECTS ON COGNITION AND BRAIN IN HEALTHY ADULTS

The picture that emerges from these data suggests that possession of an E4 allele confers greater risk for pathogenic Aβ deposition, increased susceptibility to HSV-1 infection, and a pro-inflammatory immune response. Together these factors may assist in the development of chronic inflammatory conditions within the brain, amyloidosis, and eventually the synaptic loss and neurodegeneration associated with AD. These processes

begin decades before changes in cognition appear and are considered pathological only once they provoke clinical symptoms. However there is evidence to suggest that neuropsychological differences exist between E4 carriers and non-carriers beginning in early adulthood, long before the emergence of pathological behavioral changes. Neuropsychological trends observed in healthy ApoE4 carriers indicate that possession of an ApoE4 allele is associated with cognitive advantages in early adulthood followed by cognitive decrements beginning in midlife and progressing more steeply than other allele types through the sixth decade and beyond.

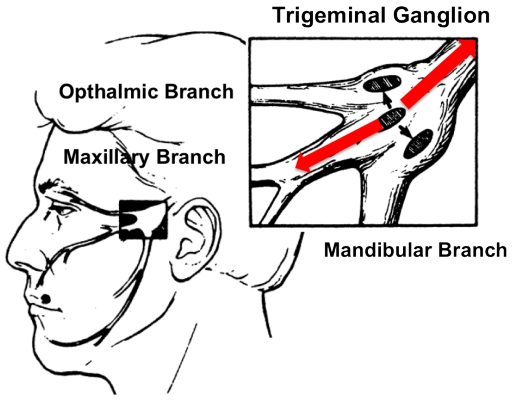

Figure 1.1 Herpes Simplex-1 infection showing anterograde and retrograde pathways

1.5.1 ApoE4 and Neuropsychological Test Performance

A meta-analysis of neuropsychological performance and ApoE status among cognitively normal adults reported that older E4 carriers perform significantly worse on measures of episodic memory, executive functioning, perceptual speed, and global cognitive ability (Wisdom et al., 2011). However, studies of young E4 carriers (between the ages of 18-30) have shown better performance on mental arithmetic tasks (Puttonen et al., 2003), episodic memory performance (Mondadori et al., 2006), and decision-making,

prospective memory, and verbal fluency (Marchant et al., 2010) compared to non-carriers. Yet by midlife these cognitive advantages begin to invert. For example, findings from a longitudinal repeated measures study conducted on individuals at age 20 and again at 55 found significantly higher general cognitive ability in E4 carriers compared to non-carriers at age 20, but significantly poorer episodic memory in E4 carriers at age 55 (Baxter et al., 2003). Another cross-sectional study of healthy adults with a mean age of 46 found that E4 carriers performed worse on learning and memory tests compared to non-carriers (Flory et al., 2000). Similarly, verbal learning ability declined over a two-year period in E4 carriers over age 60, but not those under age 60, and not in non-carriers (Caselli et al., 2004).

1.5.2 ApoE4 and Structural Brain Changes

Beyond differences in neuropsychological performance, the ApoE4 allele has also been associated with structural (Burggren et al., 2008; Cohen et al., 2001; Filippini et al., 2009, 12; Gutiérrez-Galve et al., 2009; Persson et al., 2006; Wishart et al., 2006) and functional (Burggren et al., 2002; Bookheimer et al., 2000; Filippini et al., 2009, 01; Lind et al., 2006; Smith et al., 1999 & 2002) brain differences in cognitively healthy adults. Several studies report structural abnormalities in non-demented E4 carriers that closely resemble those seen in cases of AD, such as lower gray matter density in the medial temporal lobes and prefrontal cortices (Filippini et al., 2009, 12), smaller hippocampal volumes (Burggren et al., 2008; Wishart et al., 2006), and a faster rate of hippocampal volume loss (Cohen et al., 2001). Within hippocampal subregions including the entorhinal cortex and subiculum, E4 carriers demonstrate significantly reduced cortical thickness compared to non-carriers (Burggren et al., 2008). Finally, declines in white matter integrity have been observed in the corpus callosum and medial temporal lobes of

E4 carriers (Wishart et al., 2006), further characteristics commonly observed in cases of AD.

1.5.3. ApoE4 and Functional Brain Changes

Functional neuroimaging studies also point to differences between E4 carriers and non-carriers, but results of these studies are less consistent than structural findings. One study reports that over progressive trials of a learning task involving participants in their 20s, activity among E4 carriers decreased in the bilateral hippocampi and left frontal and temporal regions, whereas activity among E2 carriers increased, leading the authors to conclude that among young adults the E4 allele is associated with more efficient use of neural resources (Lind et al., 2006). Other fMRI studies have similarly demonstrated decreased, "more efficient" task-related activation in middle-aged E4 carriers (Lind et al., 2006; Smith et al., 1999), but numerous reports have indicated just the opposite: that the E4 allele is associated with greater task related activation among healthy adults (Bookheimer et al., 2000; Burggren et al., 2002; Smith et al., 2002). These latter studies are viewed as evidence of "compensatory activation" in the face of degraded neural resources, making it difficult to interpret findings across studies.

One possible way to untangle conflicting fMRI findings is to examine activation patterns observed in AD patients with cognitive impairment and compare these to healthy E4 carriers without cognitive impairment. AD is characterized by a loss of synaptic connections more so than neuronal loss, and the depletion of synapses better correlates with cognitive dysfunction (Palop et al., 2006). This feature of the disease has led to increased interest in changes in functional connectivity between regions rather than activation or deactivation within distinct regions. Many studies examining functional connectivity rely on resting state fMRI because it requires no active participation by the

subject, which can be challenging or impossible for individuals with AD. Resting state fMRI captures spontaneous "background" fluctuations of neuronal activity in the conscious brain in the absence of goal-directed behavior. Under normal physiologic conditions these distributed low frequency fluctuations (DLFFs) are consistent and highly synchronous, forming a pattern of activation known as the default mode network (DMN).

1.5.4. ApoE4 and the Default Mode Network

Anatomically the DMN consists of a primary network involving posterior cingulate/retrosplenial cortex (PCC/Rsp), bilateral inferior parietal lobules (IPL), ventral and dorsal medial prefrontal cortex (V/D MPFC), and bilateral temporal lobes (LTC) (Buckner et al., 2008), see Figure 1.2. The PCC/Rsp provides the primary hub and is densely interconnected with other regions in the network (heavier lines denote greater connectivity). A secondary network consisting of bilateral hippocampi (HC) and parahippocampal cortex (PHC) connects to the first primarily through the PCC/Rsp hub (Buckner et al., 2008). Functionally the DMN is hypothesized to consolidate past information and most likely plays an important role in learning and memory (Buckner & Vincent, 2007). The degree of synchrony between regions of the DMN is used as a measure of functional connectivity, or the temporal correlation of activation in discontiguous regions of the brain. DLFFs within the DMN and other functional networks become more dyssynchronous in the course of normal aging, and as healthy aging progresses through mild cognitive impairment (MCI) and onto AD (O'Sullivan et al., 2001; Zhang et al., 2010). Decreased connectivity also progresses linearly with decreased cognitive performance, and correlates positively with amyloid burden (Hedden et al., 2009).

The emergent pattern found across studies of connectivity within the DMN suggests that disconnection between hippocampi and the rest of the DMN, possibly as the result of increasing amyloid burden, leads to decreased cohesion of the network and a worsening clinical picture (Hedden et al., 2009; O'sullivan et al., 2001; Zhang et al., 2010). Among middle-aged ApoE4 carriers, resting state studies have found differences in DMN activation similar to those seen in AD (Sheline et al., 2010), suggesting that the ApoE4 allele influences changes in functional connectivity within the DMN before the onset of frank cognitive changes. Given that neurodegenerative changes take decades to manifest clinically, structural and functional MRI provide a promising tool for in vivo examination of these changes as they develop over time.

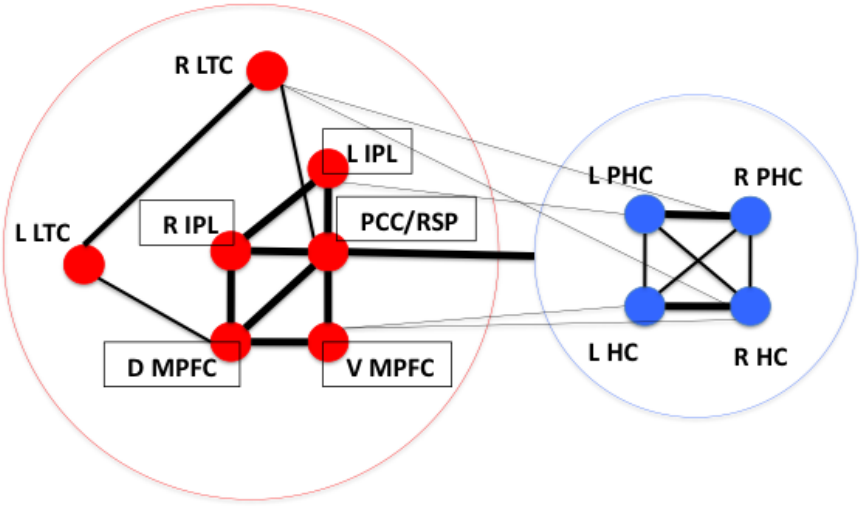

Figure 1.2. Schematic of the Default Mode Network, based on Buckner et al., 2008.

1.6 HERPES SIMPLEX-1: EFFECTS ON COGNITION AND BRAIN IN HEALTHY ADULTS

Typically infection with HSV-1 is only associated with neurological problems in rare instances of HSE. In cases of HSE, lesioning occurs almost exclusively in limbic

and paralimbic regions including the temporal lobes, orbitofrontal cortex, insula, and cingulate gyri (Adams et al., 1973; Damasio et al., 1985; Kennedy et al., 1988). Lesions can be focal or diffuse, but the inflammatory penumbra surrounding them is often quite extensive and causes severe irreversible damage to the structures involved. However, mild cases of HSE have been reported (DeVincenzo & Thorne, 1994; Fodor et al., 1998; Klapper et al., 1984), and some have speculated that chronic subacute HSE is a contributor to the development of AD (Itzhaki et al., 1997).

1.6.1 HSV-1 and Neuropsychological Test Performance

Very few studies have examined neuropsychological changes in otherwise healthy HSV-1-positive adults. Of the few that exist, one found a significant association between HSV-1 infection and overall cognitive performance, with greatest deficits in delayed memory and executive functioning among carriers of the virus (Dickerson et al., 2008). These associations were not found for other human herpes viruses, suggesting that the cognitive impairments observed in this study are specific to HSV-1. Another study examining cognitive performance relative to viral burden (i.e. infection with HSV-1, HSV-2 and/or cytomegalovirus) in elderly adults found that increased viral burden was associated with cognitive impairment in subjects with cardiovascular diseases (Strandberg, T.E., 2003). HSV-1 infection has also been demonstrated as an independent predictor of cognitive deficits in individuals with bipolar disorder (Dickerson et al., 2004) and schizophrenia (Dickerson et al., 2003; Schretlen et al., 2010; Shirts et al., 2008; Yolken et al., 2011).

1.6.2 HSV-1 and Structural Brain Changes

Similarly, very few studies have examined structural brain changes associated with HSV-1 infection. Those that exist focus almost exclusively on cases of HSE and

report gross anatomical changes found at autopsy (Adams et al., 1988; Kennedy et al., 1988). A neuroimaging study employing voxel-based morphometry reported abnormal gray matter density in limbic and paralimbic cortices in patients recovering from HSE (Gitelman et al., 2001), but this may not be translatable to HSV-1-postitive individuals without HSE. Reduced prefrontal gray matter was noted in HSV-1-positive first-episode schizophrenia patients relative to HSV-1-negative patients and healthy controls (Prasad et al., 2006). These differences were not attributable to psychotropic medication exposure (all patients were naïve to medication), illness chronicity, or demographic variables, leading the authors to conclude that HSV-1 infection was a key mediator in the structural differences. To date no functional neuroimaging studies have been performed to look for differences between healthy HSV-1-positive adults and HSV-1-negative controls.

Collectively the few available neuroimaging and neuropsychological studies suggest that HSV-1 is a contributor to structural brain alterations and cognitive deficits in non-elderly adults, especially in conjunction with psychiatric conditions. Arguably HSV-1 infection exacerbates the cognitive decline associated with psychiatric and neurodegenerative conditions because it promotes an inflammatory response in an already compromised neural system.

1.7 The Link Between ApoE4 and HSV-1 in Relation to Brain Vulnerability

Four primary arguments can be made based on the evidence presented thus far. First, the ApoE4 allele, a known genetic risk factor for AD, is linked to brain and neuropsychological differences in carriers versus non-carriers beginning in early adulthood and persisting into old age. Second, HSV-1, a ubiquitous viral pathogen infecting the human nervous system is also linked to neuropsychological and brain differences in carriers versus non-carriers. Third, the overrepresentation of HSV-1 among

E4 carriers suggests that susceptibility to infection and immune response to HSV-1 may be moderated by ApoE allele type. And fourth, HSV-1 may interact with the ApoE4 allele type to increase risk for AD.

Chapter 2: General Summary and Project Overview

2.0 General Summary

The identification of early, modifiable risk factors for cognitive decline presents the most promising opportunity for intervention. To date one of the most robust known risk factors for the most common form of dementia—AD—is the presence of an ApoE4 allele (Lindsay, J. (2002, 12). Risk for sporadic and familial late onset AD increases nearly threefold for each E4 allele an individual carries (Corder et al., 1993; Laws et al., 2003; Slooter et al., 1998). However some E4 carriers do not develop cognitive decline, and many non-E4 carriers do, highlighting the role of environmental variables in the progression to clinical symptoms.

The first specific aim of this study utilizes neuropsychological testing to demonstrate differences in cognitive function among healthy middle-aged adults with and without the ApoE4 allele, who are either positive or negative for HSV-1 infection. Neuropsychological testing has been used previously to demonstrate cognitive variability stemming from genetic difference. Most often these "pencil and paper" tests are employed to differentiate between typical and atypical cognitive performance, such as might exist in the presence of pathology or injury. However, because we expect that all of our participants will be cognitively normal, we will use neuropsychological tests to look for group differences attributable to allele type and viral status.

The second specific aim utilizes magnetic resonance imaging (MRI) to identify structural differences in the brains of healthy middle-aged ApoE4 carriers and non-carriers who are either positive or negative for HSV-1 infection. MRI is an established tool for noninvasive examination of brain structure and function, and has the sensitivity to detect potentially pathological alterations among asymptomatic at-risk individuals. Hence, we believe that MRI provides an ideal method for investigating early brain

changes in vulnerable populations. Moreover, coupling this technology with genotyping cross-validates the predictions independently generated by each of these research tools. Finally, linking genetic vulnerability and viral infection to measurable brain changes in vivo offers invaluable information about the development of cognitive vulnerability, and provides a potential point of intervention before irreversible changes occur.

The third specific aim utilizes functional MRI (fMRI) to identify differences in regional connectivity within the default mode network (DMN) of healthy middle-aged ApoE4 carriers and non-carriers who are either positive or negative for HSV-1 infection. Like the previous two aims this one seeks to provide evidence that HSV-1 infection exacerbates brain changes associated with the possession of the ApoE4 allele, and contributes to the risk for cognitive vulnerability. This technique provides an avenue to examine how differences in brain structure (aim #2) may influence specific aspects of brain function which manifest behaviorally as neuropsychological difference (aim #1). Abnormalities of brain structure and functional connectivity provide a more sensitive measure of change that neuropsychological testing alone.

2.1 SUMMARY OF THE SPECIFIC AIMS

2.1.1 Specific Aim #1

To determine if cognitive vulnerability in middle-aged ApoE4 carriers is exacerbated by HSV-1 infection.

Using a cross-sectional between-subject design, we will compare cognitive performance across a range of neuropsychological tasks designed to test memory, attention, language, and visuospatial abilities in four independent groups of participants: healthy controls negative for both ApoE4 and HSV-1 (E4-/HSV-1-), non-ApoE4 carriers positive for HSV-1 (E4-/HSV-1+), ApoE4 carriers negative for HSV-1 (E4+/HSV-1-),

and ApoE4 carriers positive for HSV-1 (E4+/HSV-1+). Based on previous studies of cognitive performance in ApoE4 carriers (Baxter et al., 2003; Schultz et al., 2007) and HSV-1+ patient populations (Dickerson et al., 2003, 2004, 2008), we hypothesize that E4+/HSV-1+ adults will exhibit lowest cognitive test scores, particularly in the domain of verbal memory, followed by the E4+/HSV-1- group, the E4-/HSV-1+ group and finally, the ApoE4-/HSV-1- group.

2.1.2 Specific Aim #2

To determine if brain vulnerability in terms of midlife cortical thinning in ApoE4 carriers is exacerbated by HSV-1 infection.

To test this aim, participants will undergo structural brain imaging using MRI. Using a cross-sectional between-subject design, we will compare hippocampal volumes and cortical thickness in six bilateral a priori regions of interest: entorhinal, fusiform, middle temporal, parahippocampal, posterior cingulate, and medial and lateral orbitofrontal cortices, between the four groups. These ROIs were chosen because among the regions at risk for cortical thinning in ApoE4 carriers (Burggren et al., 2008; Cohen et al., 2001; Filippini et al., 2009; Gutierrez-Galvea et al., 2009), these regions are the most likely to also demonstrate structural changes associated with HSV-1 infection (Gitelman et al., 2001). Consistent with findings from structural brain studies on patients at genetic risk for Alzheimer's disease (Burggren et al., 2008; Cohen et al., 2001; Filippini et al., 2009; Gutierrez-Galvea et al., 2009; Persson et al., 2006), we hypothesize that the E4+ groups will show reduced hippocampal volumes compared to E4-/HSV-1- controls. Importantly, we expect these differences to be even more pronounced in the E4+/HSV-1+ group, indicating that ApoE4 and HSV-1 act in concert to foster early brain vulnerability.

2.1.3 Specific Aim #3

To determine if functional connectivity alterations of the Default Mode Network (DMN) of ApoE4 carriers are exacerbated by HSV-1 infection.

Participants will undergo functional brain imaging using fMRI. Using a cross-sectional between-subject design, we will compare functional connectivity within the DMN between our four groups. Consistent with functional connectivity findings in patients at genetic risk for Alzheimer's disease (Bookheimer et al., 2000; Petrella et al., 2002; Burggren et al., 2002; Palop et al., 2006), we hypothesize that ApoE4+ groups will show reduced functional connectivity across the DMN, specifically in the medial temporal lobes, midline frontal regions, medial and lateral parietal regions, and the posterior cingulate, compared to the E4-/HSV-1- controls. As above, we expect these differences to be more pronounced in the E4+/HSV-1+ group.

2.2 PROJECT OVERVIEW

2.2.1 Overview and Significance

We propose to test the hypothesis that HSV-1-positive ApoE4 carriers show early brain vulnerability by employing sensitive measures of cognitive and cerebral function. The ApoE4 allele has been robustly associated with increased risk for AD, even after controlling for environmental risk factors (Corder et al., 1993; Kivipelto et al., 2008). HSV-1 has also been shown to have deleterious effects on cognition (Dickerson et al., 2003, 2004 & 2008; Strandberg, T. E. 2003, 12; Schretlen et al., 2010; Shirts et al., 2008; Yolken et al., 2011) and the brain (Adams et al., 1988; Gitelman et al., 2001; Kennedy et al., 1988; Prasad et al., 2006). Evidence that the ApoE4 allele and HSV-1 act synergistically in midlife to promote neurological changes associated with risk for future

cognitive decline will indicate that viral suppression early in the lifespan may be a critical approach to preventing functional loss in older age.

Cognitive decline proceeds linearly from modest changes associated with normal aging through greater deficits associated with MCI, and finally onto profound impairments associated with AD. Similar stepwise changes have been noted for brain structure and for functional connectivity within the DMN (O'Sullivan et al., 2001; Zhang et al., 2010). Evidence suggests that possession of an ApoE4 allele hastens cognitive decline after midlife, and exacerbates structural atrophy and disruptions of connectivity within the DMN (Hedden et al., 2009; Sheline et al., 2010). We hypothesize that the ApoE4 allele and HSV-1 interact to confer greater risk than either factor in isolation, and that cognitive and brain changes will increase in a stepwise fashion relative to risk load. Healthy controls (E4-/HSV-1-) will be considered no risk. HSV-1+ individuals who do not carry an ApoE4 allele (E4-/HSV-1+) will be considered low risk. HSV-1- individuals with an ApoE4 allele (E4+/HSV-1-) will be considered moderate risk since there is more evidence to support the deleterious effects of ApoE4 than HSV-1. Finally, HSV-1+ individuals who also carry an ApoE4 allele (E4+/HSV-1+) will be considered high risk.

2.2.2 Neuropsychological Assessment

Using neuropsychological testing we will examine how the ApoE4 allele, a well-documented risk factor for dementia, and HSV-1, a common pathogen affecting the majority of the adult population, interact to create early brain vulnerability. In particular we will assess global cognitive ability, memory, and executive function because these abilities are subserved by frontal and temporo-parietal brain regions most vulnerable in cases of AD.

2.2.3. Structural Brain Imaging

Using structural neuroimaging we will quantify the volume and thickness of brain regions in HSV-1 positive individuals who either carry the ApoE4 allele or do not, as well as healthy controls. First we will analyze whole-brain, ventricle, and total intracranial volumes, followed by bilateral hippocampal volumes (Cohen et al., 2001). Individual hippocampal volumes will be divided by the total intracranial volume of that subject as a way of correcting for differences in head size. Cortical thickness will be measured in six bilateral a priori regions of interest: entorhinal, fusiform, middle temporal, parahippocampal, posterior cingulate, and medial and lateral orbitofrontal cortices. These ROIs were chosen because among the regions at risk for cortical thinning in ApoE4 carriers (Burggren et al., 2008; Cohen et al., 2001; Filippini et al., 2009; Gutierrez-Galvea et al., 2009; Wishart et al., 2006), these regions are the most likely to also demonstrate structural changes associated with HSV-1 infection (Gitelman et al., 2001).

We propose to test the hypothesis that E4+/HSV-1+ individuals show early brain vulnerability in the form of cortical thinning and grey matter volume reductions not present in healthy controls. Brain volumetry and measurement of cortical thickness provide a sensitive non-invasive means of examining the brain in vivo. Structural changes including reduced hippocampal volumes and cortical thinning would be evidence of early tissue degradation and brain vulnerability. If these changes are more pronounced in the E4+/HSV-1+ group compared to the E4+/HSV-1- group this would be evidence that HSV-1 confers greater brain vulnerability than the ApoE4 allele alone.

2.2.4 Functional Connectivity

We will use BOLD fMRI to investigate how the ApoE4 allele and infection with HSV-1 interact to change the brain's "default mode network" (DMN), a network of

distributed brain regions that activate in the absence of a task (Buckner et al., 2007 & 2008, 01). The DMN consists of a number of anatomically distinct core regions commonly vulnerable to AD, including ventral medial prefrontal cortex (vMPFC), posterior cingulate/retrosplenial cortex (PCC/Rsp), inferior parietal lobule (IPL), lateral temporal cortex (LTC), dorsal medial prefrontal cortex (dMPFC), and the hippocampal formation (HF) (Buckner et al., 2008).

We will investigate changes in the activation of DMN regions using functional connectivity analysis. This approach measures the degree of correlation in spontaneous and synchronous fluctuations of DMN regions. Similar to traditional fMRI analysis, functional connectivity analysis uses a voxelwise approach to detect activation. However, instead of the regressor of interest deriving from a pre-determined block design as in the case of task-related fMRI, the regressor of interest in a functional connectivity analysis is empirically derived from a seed region in the same data, reflecting the spontaneous hemodynamic fluctuations linked to that area during a task-free time period. In this way it is possible to correlate a specific node within the DMN to other brain regions and look for changes that may correlate with the neuropsychological profiles and structural changes observed in at-risk groups.

2.3 METHODOLOGICAL OVERVIEW

See Figure 1.3 for an overview of procedures.

This study was approved by the local institutional review committee and was conducted in congruence with the Helsinki Declaration of 1975. Participants were not excluded on the basis of gender, race, ethnicity, or socioeconomic status.

Individual methods will be discussed in the Methods sections of subsequent chapters. Briefly, participants between the ages of 40 and 60 years were recruited from

The Neural Consequences of Metabolic Syndrome study to participate in this study. Participants were included if they were free of overt coronary artery disease, neurological disease (e.g., stroke, Parkinson's disease, clinically significant traumatic brain injury), major psychiatric illness (e.g. schizophrenia, bipolar disorder), diabetes, and substance abuse (i.e., diagnosed abuse and/or previous hospitalization for substance abuse) as assessed by a medical history questionnaire. Exclusion criteria included smoking, impaired global cognitive functioning (FSIQ <85), severe depression (BDI-II total score >28), and MRI contraindications.

Participants underwent neuropsychological and general health assessments, genotyping for ApoE, testing for HSV-1, and structural and functional brain imaging. Assessment visits were completed on separate days.

Participants were grouped based on overall risk as follows: healthy controls (E4-/HSV-1-) were considered no risk. HSV-1+ individuals without an ApoE4 allele (E4-/HSV-1+) were considered low risk. HSV-1- individuals with an ApoE4 allele (E4+/HSV-1-) were considered moderate risk since there is more evidence to support the deleterious effects of ApoE4 compared to HSV-1. Finally, HSV-1+ individuals with an ApoE4 allele (E4+/HSV-1+) were considered high risk.

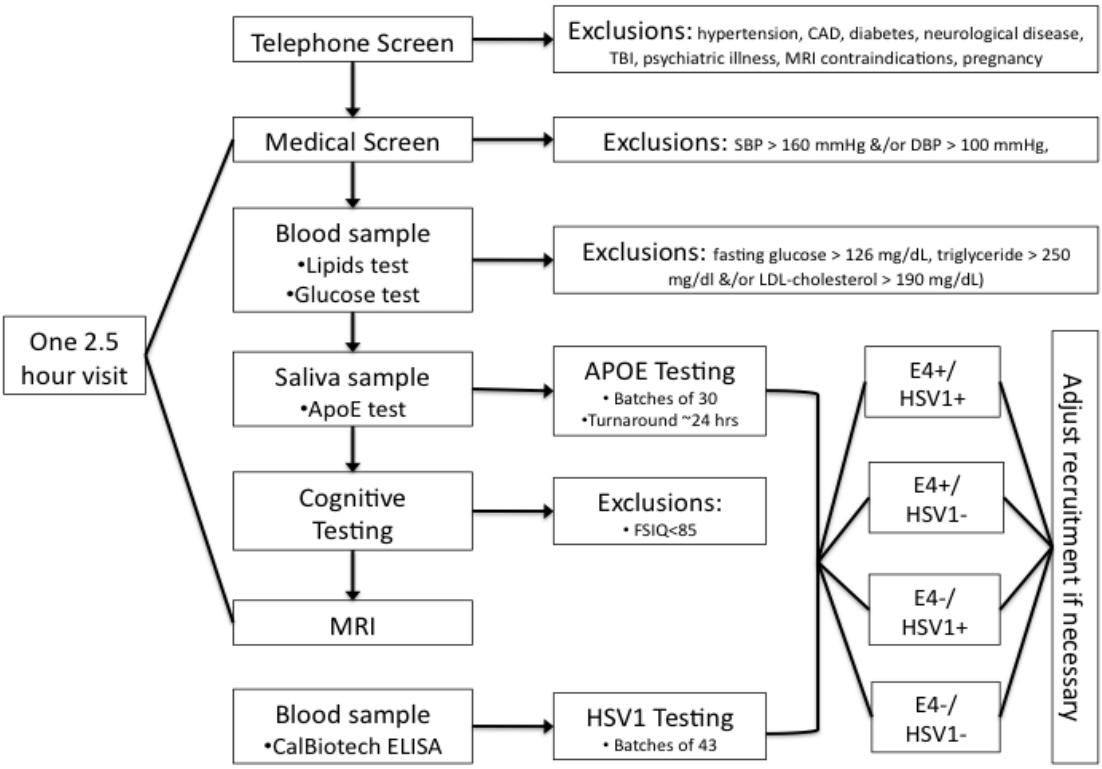

Figure 1.3. Overview of Study Procedures

Chapter 3: Infection with Herpes Simplex-1 is Associated with Reductions in Executive Function Among Healthy Middle Aged Adults with Genetic Risk for Alzheimer's disease

Objective: The ApoE4 allele is a genetic risk factor for sporadic Alzheimer's disease (AD). Herpes Simplex-1 (HSV-1) is a common viral infection, with higher incidence among ApoE4 carriers, which has also been linked to cognitive deficits. The current study sought to determine if cognitive differences were present in healthy middle-aged adults with genetic risk for AD, who were either positive or negative for HSV-1.

Methods: 115 individuals aged 40-60 were grouped by risk for cognitive decline. Healthy controls (N=29) were negative for both HSV-1 and ApoE4; Low Risk (N=52) were HSV-1 positive and ApoE4 negative; Moderate risk (N=14) were ApoE4 positive and HSV-1 negative; and High Risk (N=20) were positive for both HSV-1 and ApoE4. Participants completed a health assessment and a neuropsychological battery assessing FSIQ, executive functioning, and verbal memory.

Results: Group differences were assessed with 2x2 ANOVA. There was a significant interaction between ApoE4 and HSV-1 on Executive Functioning ($p=.01$), with HSV-1 positive ApoE4 carriers performing significantly worse than HSV-1 negative ApoE4 carriers, after controlling for age, education, systolic blood pressure, and FSIQ. No main effects or interactions were observed for verbal memory ($p=0.10$).

Conclusions: While HSV-1 infection did not appear to have a significant impact on performance in ApoE4 non-carriers, HSV-1 positive ApoE4 carriers performed significantly lower on tests of executive functioning than HSV-1 negative ApoE4 carriers. Thus HSV-1 may be an additive risk factor for future cognitive vulnerability in individuals with genetic risk for AD. Further longitudinal research on the effects of HSV-1 suppression on cognition is warranted as a potential means of reducing risk for cognitive decline.

3.0 INTRODUCTION

The ApoE4 allele is the most widely replicated susceptibility gene for sporadic Alzheimer's disease (AD), the most common type of dementia (Laws et al., 2003; Lindsay, J. 2002). However some E4 carriers do not develop cognitive decline, and many non-E4 carriers do, highlighting the role of environmental variables in the progression to clinical symptoms. Unlike genotype, acquired risk factors are potentially modifiable if they are identified prior to the onset of cognitive decline. Early intervention against modifiable risk factors is critical because there are no cures for dementia, and treatment is limited to marginally slowing the progression of symptoms. Herpes simplex-1 virus (HSV-1), a common infection of the peripheral nervous system, may be an example of a modifiable condition that increases cognitive vulnerability, particularly among individuals with genetic risk. This study sought to determine if an interaction exists between HSV-1 infection and ApoE4 genotype with respect to cognitive functioning among cognitively normal middle-aged adults.

HSV-1 is a highly common infection, with a prevalence rate between 60-90% among adults in the U.S. (Kuhlmann et al., 2010; Miller et al., 1998). The virus has the potential to invade the central nervous system (CNS) where it can cause herpes encephalitis (HSE), a devastating infection that targets the same brain regions most commonly affected by AD (Almeida & Lautenschlager, 2005; Dobson & Itzhaki, 1999; Johnson, R., 1964; Stroop et al., 1986). However, HSV-1 may also exist within the CNS in a subacute form, causing inflammatory damage without the life-threatening features of HSE (DeVincenzo & Thorne, 1994; Fodor et al., 1998; Klapper et al., 1984). ApoE4 carriers have greater susceptibility to neurotropic infections including HSV-1, in part because the E4 genotype appears to facilitate viral neuroinvasiveness (Amouyel et al.,

1994; Burgos et al., 2003; Burt et. al., 2008). As a result, risk for cognitive decline may be further increased among ApoE4 carriers who are also infected with HSV-1.

Once HSV-1 breaches the CNS there are three lines of reasoning linking it to increased risk for AD. First, ApoE4 carriers are overrepresented among HSV-1 positive AD patients, suggesting that central HSV-1 infection of ApoE4 carriers confers a higher risk of AD than ApoE4 alone or virus alone (Itzhaki et al., 1997; Itzhaki & Wozniak, 2008). Second, ApoE4 carriers are more likely to exhibit cold sores (Dobson & Itzhaki, 1999; Itzhaki et al., 1997; Itzhaki & Wozniak, 2008), indicating that E4 carriers are less successful at viral suppression, and that HSV-1 in E4 carriers is more likely to result in tissue destruction. Third, HSV-1 DNA is present in the same brain regions most vulnerable to AD: the medial temporal lobes, and temporal and frontal cortices (Dobson & Itzhaki, 1999; Itzhaki et al., 1997; Itzhaki & Wozniak, 2008). The virus has not been found in occipital cortex, which is typically spared in AD (Dobson & Itzhaki, 1999; Itzhaki et al., 1997; Itzhaki & Wozniak, 2008).

To assess for cognitive differences in our sample, participants were grouped according to risk for cognitive decline based on the presence of HSV-1 seropositivity and the ApoE4 allele. They completed a neuropsychological assessment battery measuring executive functioning--planning, problem solving, and reasoning abilities--and verbal memory, domains which show early impairment in ApoE4 carriers and in cases of AD (Flory et al., 2000; Wisdom et al., 2011). We hypothesized that neuropsychological test performance would decline linearly as risk level increased, i.e. the group with both ApoE4 and HSV-1 would perform worse than groups with only one risk factor, which would perform worse than healthy controls. If HSV-1 is identified as an additive risk factor for cognitive vulnerability among middle aged individuals who carry the ApoE4

allele, then viral suppression may be a critical approach to preventing cognitive decline and late life functional loss.

3.1 METHODS

3.1.1 Participants

Adults between the ages of 40 and 60 years were recruited from the community via newspaper and online ads. Participants were included if they were free of overt coronary artery disease, neurological disease (e.g., stroke, Parkinson's disease, clinically significant traumatic brain injury), major psychiatric illness (e.g. schizophrenia, bipolar disorder), diabetes, and substance abuse (i.e., diagnosed abuse and/or previous hospitalization for substance abuse) as assessed by a medical history questionnaire. Exclusion criteria included smoking, impaired global cognitive functioning (FSIQ <85), and severe depression (BDI-II total score >28). 164 participants were recruited into the study and all provided written informed consent. Two participants were removed from the analyses due to impaired global cognitive functioning and two were removed for severe depression. Forty-five participants were removed for failing to complete genotyping and/or health assessment. According to participants' self-report, the ethnic distribution of the sample is as follows: 60.9% Caucasian (N=70), 20.9% Hispanic (N=24), 6.1% African-American (N=7), and 4.3% Other/Did Not Specify (N=5).

3.1.2 Protocol

The study was approved by the local institutional review committee and was conducted in congruence with the Helsinki Declaration of 1975. Participants underwent a neuropsychological assessment and a general health assessment (see below). Assessment visits were completed on separate days.

3.1.3 Health Assessment

Participants were instructed to fast for at least eight hours prior to the first visit. Arterial blood pressure was measured after at least 15 min of rest using a standard oscillometric blood pressure monitor (VP-2000, Colin Medical Instruments, San Antonio, TX). Body mass index (BMI) was calculated as body weight in kilograms divided by the square of the height in meters. Venipuncture of the antecubital vein was performed to obtain approximately 15 cc of blood to measure lipid levels and fasting glucose. Fasting plasma concentrations of glucose, triglyceride, and high and low-density lipoprotein cholesterol (HDL and LDL) were determined using standard enzymatic techniques. A portion of the blood sample was centrifuged and serum was frozen for later HSV-1 testing. Saliva samples were collected for genotyping using commercially available kits (DNA Genotek, Ottawa Canada).

3.1.4 HSV-1 ELISA

Herpes virus was detected using enzyme-linked immunosorbent assay (ELISA) in batches of approximately 40 samples. Samples were assessed for seropositivity to HSV-1 IgG antibodies using the commercially available HSV-1 IgG ELISA kits (Calbiotech, Spring Valley, CA) per manufacturer instructions. Samples were run in duplicate and antibody indices obtained for each subject were averaged. Subjects were considered positive if optical density exceeded 0.9. This assay did not distinguish between HSV type 1 and type 2 owing to the high percentage of shared antigens between the types. However, due to the low incidence of HSV-2 relative to HSV-1 (Xu et al., 2010), we believe that the majority of our positive samples are type 1.

3.1.5 Genotyping

Saliva samples were stored at room temperature prior to analysis. DNA extraction was completed per manufacturer instructions and purified samples were stored at -40 degrees Celsius prior to genotyping. Saliva samples were collected using the Oragene Discover (OGR-500) kit. DNA extraction on 500 uL of saliva was performed using the prepIT·L2P kit from DNAgenotek. PCR was performed using ApoE-Fwd4 (GCT GAT GGA CGA GAC CAT GAA GGA GTT) and ApoE-snapR (GCC CCG GCC TGG TAG ACT GCC A) primers (Ingelsson, et al., 2003). Polymerase chain reaction (PCR) amplification was performed with 10 ng of DNA and 10 pMol primer, using the following amplification protocol: 95 °C for 15 min, 35 cycles of (95 °C 30 sec., 65 °C 30 sec., 72 °C 30 sec.) and hold at 4°C.

ApoE genotype was assessed from PCR amplification and Sanger sequencing (Sanger, F., 1977) at the DNA Sequencing Facility at the University of Texas at Austin, using Variant Reporter Software from Life Technologies (Thermo Fisher Scientific). Individual samples were classified according to allele type (e.g. ApoE3/4 or ApoE3/3) but due to the small sample size we collapsed across E4 carriers (homo- and heterozygous) and compared them to all non-E4 carriers as a single group.

3.1.6 Risk Assessment

Individuals negative for both ApoE4 and HSV-1 were considered healthy controls, and comprised the No Risk group (N=29). HSV-1 positive individuals without an ApoE4 allele were considered Low Risk (N=52). HSV-1 negative individuals with an ApoE4 allele were considered Moderate Risk (N=14) since there is more evidence to support the deleterious effects of ApoE4 than HSV-1. Individuals with both an ApoE4 allele and HSV-1 were considered High Risk (N=20).

3.1.7 Neuropsychological testing

Neuropsychological assessment was conducted using standard clinical measures with established reliability and validity. The test battery was administered and scored by a trained research assistant using standard administration and scoring criteria. To reduce the number of comparisons outcome scores were grouped into two domains: verbal memory and executive functioning. Verbal Memory included: the California Verbal Learning Test II (CVLT-II) immediate recall, delayed recall, and recognition discrimination subscores (Delis et al., 1987); Executive Functioning included: Trail Making Test Parts A and B time (Reitan, R. M., 1958, 12), Stroop Color-Word Interference (Jensen, A. R., & Rohwer, W. D., 1966, 12), Controlled Oral Word Associations Test (COWAT), and Wechsler Adult Intelligence Scale IV (WAIS-IV) Digit Span subtest (Wechsler, D., 2008a). Domain scores were computed by converting raw scores into z-scores and averaging z-scores within each domain. Timed tests were multiplied by -1 so that higher scores indicated better performance. Emotional functioning was also assessed at this time with the Beck Depression Inventory II (BDI-II) and Spielberger State-Trait Anxiety Inventory. Individuals scoring in the clinical range on either measure were eliminated from the analysis.

3.2 STATISTICAL ANALYSES

Group differences in demographic variables were examined with chi-square tests. Group differences in physiological and cognitive/educational variables were examined with one-way analysis of variance (ANOVA) and Tukey-Kramer post hoc analyses. Group differences in cognitive domain scores were independently assessed with two-way analysis of variance (2x2 ANOVA). Age, years of education, full scale IQ (FSIQ), and systolic blood pressure were included in the model as they are known to covary with cognitive functioning and performance on neuropsychological tests (Knopman et al.,

2001; Stern, Y., 2009). All analyses were conducted using IBM SPSS Statistics software (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.).

3.3 RESULTS

3.3.1 Descriptive Statistics

The demographic and physiological characteristics of the study sample are shown in Table 3.1. Gender distribution was comparable across all risk groups, $\chi 2$ (3, N=115) =3.12, p=0.37). Due to high Caucasian enrollment, ethnicity was collapsed across non-whites. There were no significant differences in HSV-1 status between whites and non-whites, $\chi 2$ (1, N=115) =3.63, p=0.06). However there were significantly fewer ApoE4 positive non-whites, $\chi 2$ (1, N=115) =12.09, p=0.00).

A one-way ANOVA was conducted to determine if FSIQ and physiological variables differed between groups with different risk for cognitive decline. Participants were classified into four groups: no risk (n = 29), low risk (n = 52), moderate risk (n = 14) and high risk (n = 20) based on the presence or absence of an ApoE4 allele and HSV1 infection. Data was normally distributed for each group, as assessed by Shapiro-Wilk test ($p > .05$); and there was homogeneity of variances, as assessed by Levene's test of homogeneity of variances ($p = .20$).

Full Scale IQ was significantly lower in the Low Risk group, $F(3, 111) = 6.12$, $p = .001$. Tukey-Kramer post hoc analysis revealed that the decrease in FSIQ from No Risk to Low Risk (-9.87 95% CI (-18.71 to -1.03)) was statistically significant ($p = .022$), as was the decrease from Moderate Risk to Low Risk (-16.82, 95% CI (-28.30 to -5.34), $p = .001$). The High Risk group had significantly higher systolic blood pressure compared to other groups $F(3,111) = 4.22$, $p = .007$. Tukey-Kramer post hoc analysis revealed that the

increase in systolic blood pressure from No Risk to High Risk (13.11 95% CI (1.75 to 24.46)) was statistically significant (p = .017), as was the increase from Low Risk to High Risk (13.66, 95% CI (3.26 to 24.06), p = .005). No other group differences were statistically significant.

Table 3.1. Demographic and Physiological Characteristics of Study Participants

*Significant at p<.05; **Significant at p<.01; Data are M ± SD

	Healthy Controls HSV-1-/ApoE4- (n=29)	Low Risk HSV-1+ /ApoE4- (n=52)	Moderate Risk ApoE4+ /HSV-1- (n=14)	High Risk ApoE4+ /HSV-1+ (n=20)
Male/Female	16/13	22/30	6/8	6/14
Age (SD)	48.3 (6.2)	50.3 (6.2)	50.4 (5.8)	48.0 (7.1)
Education years	17.3 (3.5)	16.1 (2.3)	17.2 (2.9)	15.4 (2.2)
Full Scale IQ	117.6 (14.2)	107.8 (13.9)	124.6 (9.9)**	113.2 (19.1)
White/Non-white	18 (62.1%)/ 11 (37.9%)	23 (44.2%)/ 29 (55.8%)	13 (92.9%)/ 1 (7.1%)**	16 (80%)/ 4 (20%)**
African American	1 (3.4%)	3 (5.8%)	0	3 (15%)
Asian	1 (3.4%)	4 (7.7%)	0	0
Latino	7 (24.1%)	16 (30.8%)	0	1 (5%)
Other Race	2 (6.9%)	6 (11.5%)	1 (7.1%)	0
Systolic BP (mmHg)	121.1 (14.3)	120.5 (14.3)	123.1 (18.6)	134.2 (13.6)**
Diastolic BP (mmHg)	73.5 (10.0)	72.2 (9.9)	70.4 (13.0)	78.7 (9.2)
Body Mass Index (kg/m2)	30.6 (7.9)	28.6 (6.3)	30.6 (7.7)	31.2 (7.5)
HDL-Cholesterol (mg/dL)	46.2 (14.4)	55.0 (17.0)	49.9 (20.5)	47.74 (16.9)
LDL-Cholesterol (mg/dL)	127.1 (29.8)	122.5 (37.9)	125.3 (40.3)	123.5 (44.0)
Triglyceride (mg/dL)	131.6 (70.5)	108.6 (89.8)	181.3 (162.1)	132.9 (80.8)
Fasting Glucose (mg/dL)	98.4 (18.2)	101.7 (38.7)	95.2 (13.9)	96.2 (12.4)

3.3.2 Risk Level and Executive Domain Score

A 2x2 ANOVA was conducted to determine if Executive Domain Score differed between groups with different risk for cognitive decline. Participants were classified according to risk level, as above. There were no outliers, as assessed by boxplot (Figure 3.1); data were normally distributed for each group, as assessed by Shapiro-Wilk test ($p > .05$); and there was homogeneity of variances, as assessed by Levene's test of homogeneity of variances ($p = .48$). Age, level of education, FSIQ, and systolic blood pressure were entered in the model as covariates. There was a statistically significant interaction between ApoE4 and HSV1 status on Executive Function domain score, $F(3, 107) = 6.58$, $p = .01$, partial $\eta 2 = .058$. Moderate Risk participants had higher Executive Function domain scores ($M = 0.68$, $SE = 0.35$) than participants in other groups, $F(3, 107) = 3.68$, $p = .014$, partial $\eta 2 = .094$ (Figure 3.2).

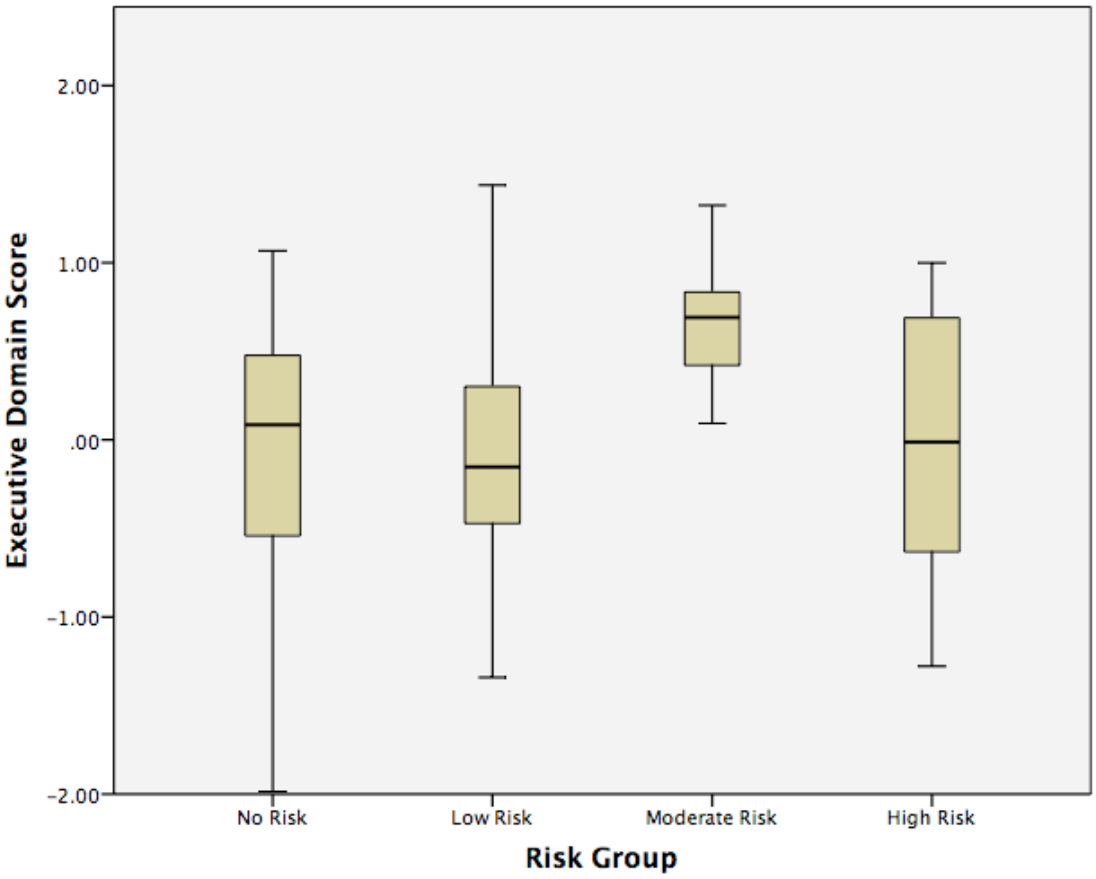

Figure 3.1 Boxplots for Executive Domain Scores Across Groups

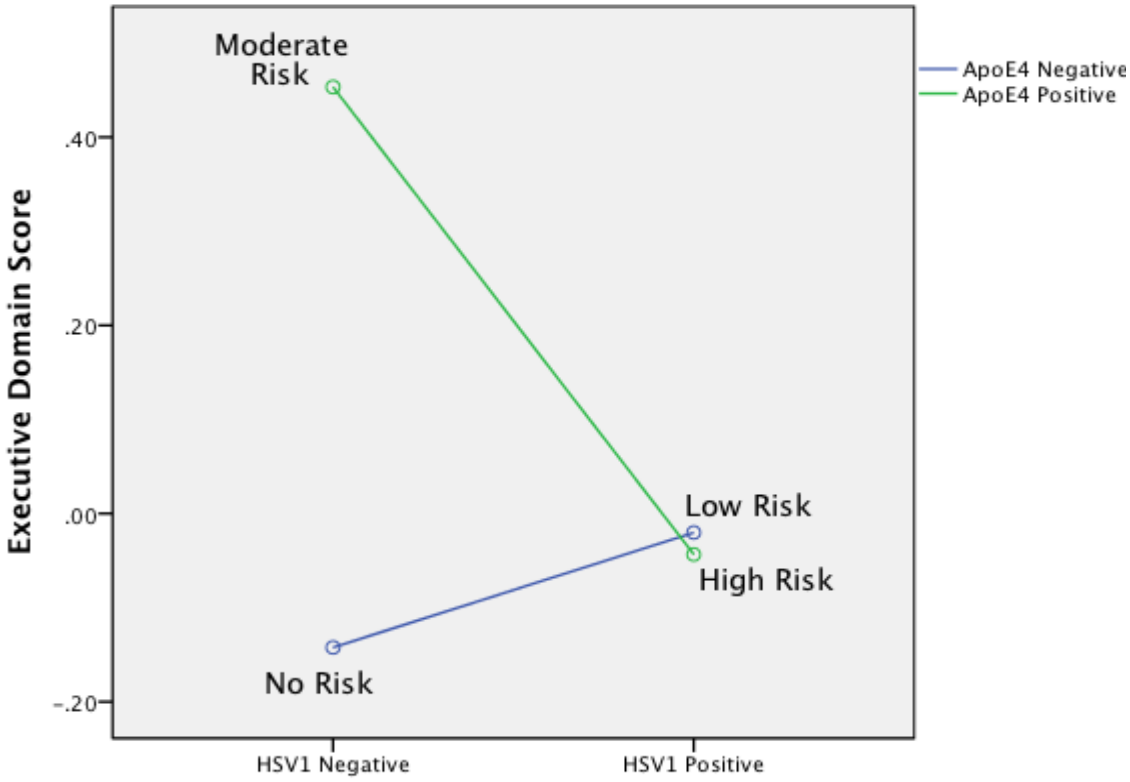

Figure 3.2. Executive Domain Score and Risk Level

3.3.3 Risk Level and Verbal Memory Domain Score

A 2x2 ANOVA was conducted to determine if Verbal Memory Domain Score differed between groups with different risk for cognitive decline. Participants were classified according to risk level, as above. There were no outliers, as assessed by boxplot (Figure 3.3); data were normally distributed for each group, as assessed by Shapiro-Wilk test ($p > .05$); and there was homogeneity of variances, as assessed by Levene's test of homogeneity of variances ($p = .22$). Age, level of education, FSIQ, and systolic blood pressure were entered in the model as covariates. There was no significant interaction of HSV-1 and ApoE4 on Verbal Memory domain score $F(1,107) = 2.06$, $p = .154$, nor were

there any significant main effects of HSV-1, F(1,112) = 2.16, p = .144, or ApoE4, F(1,112) = .165, p = .685, see Figure 3.4.

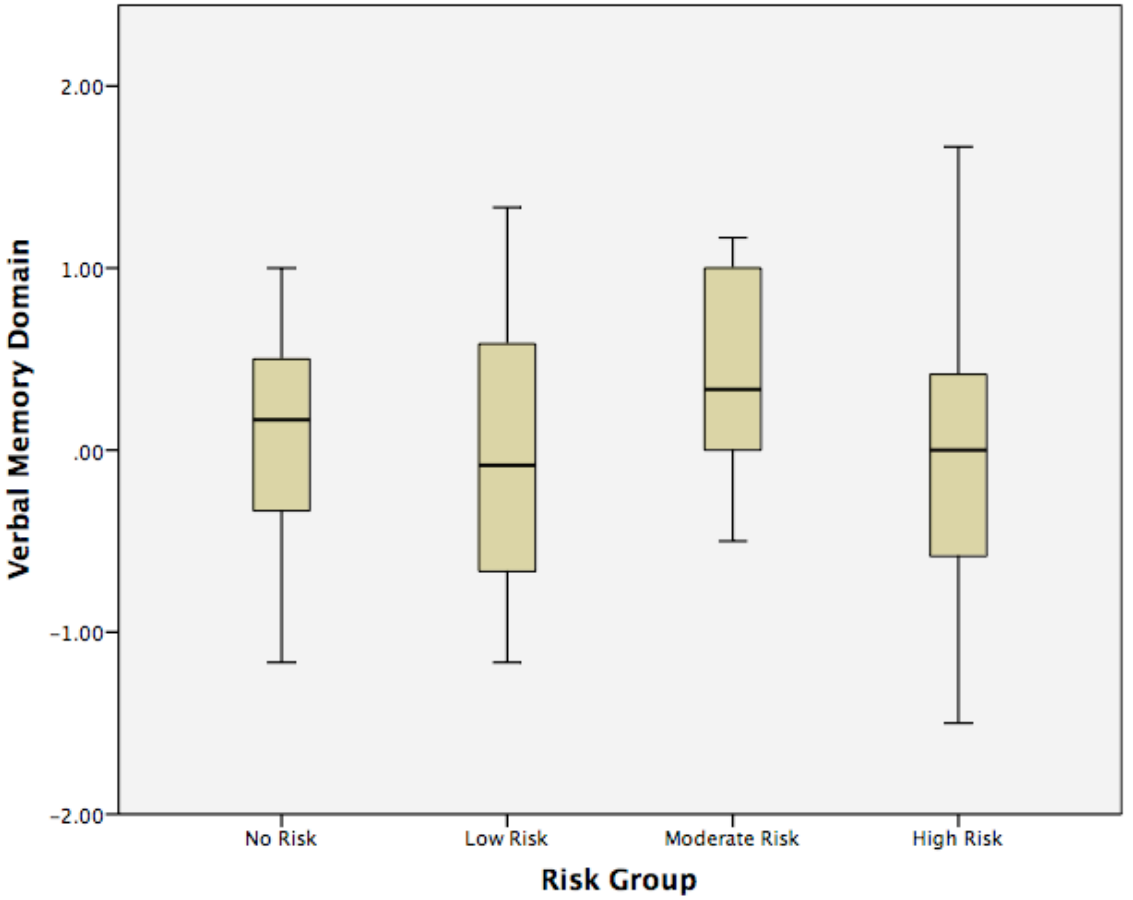

Figure 3.3 Boxplots for Verbal Memory Domain Scores Across Groups

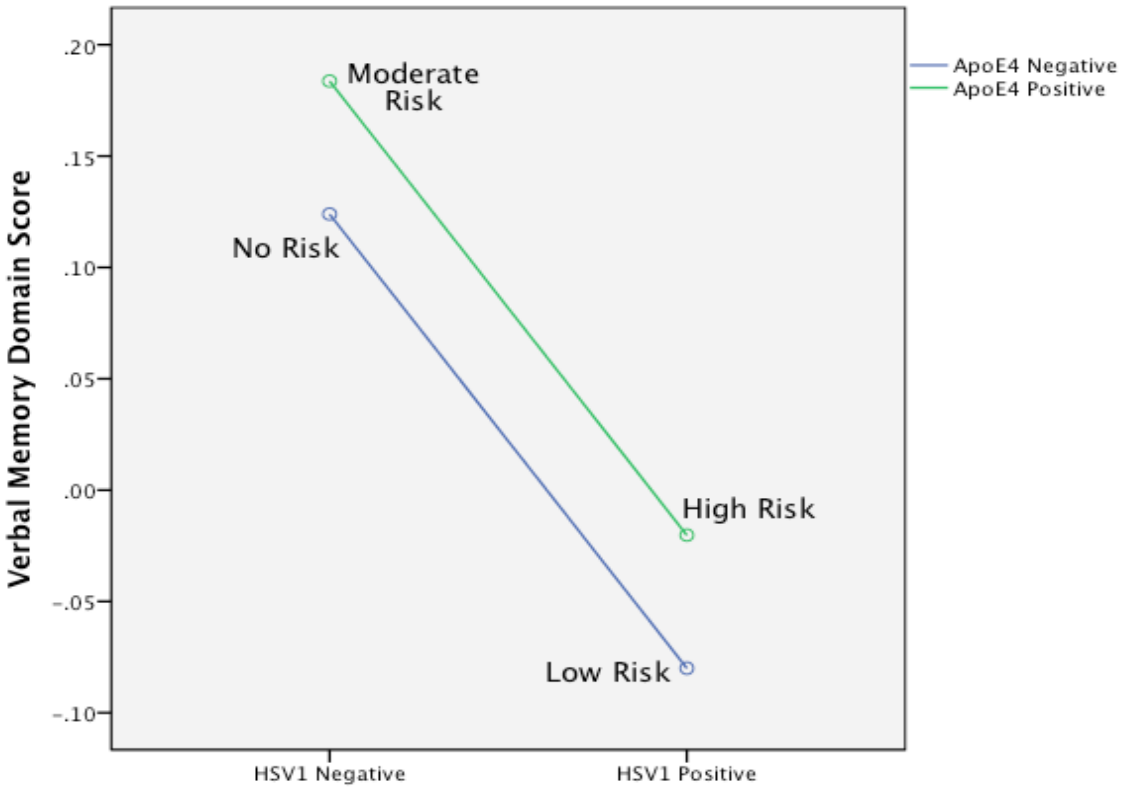

Figure 3.4 Verbal Memory Domain Score & Risk Level

3.4 DISCUSSION

We hypothesized that as cognitive risk increased, performance on measures of executive function and verbal memory would decline. Participants were grouped for risk level according to HSV-1 seropositivity and presence of an ApoE4 allele. After controlling for age, level of education, systolic blood pressure, and FSIQ, we found a significant interaction between ApoE4 and HSV-1 on executive functioning, as measured by Executive Function domain score. Specifically, ApoE4 positive individuals performed significantly worse on measures of executive functioning when they were infected with HSV-1. Contrary to what we hypothesized, there was not a significant interaction of

HSV-1 and ApoE4 on Verbal Memory Domain score, nor were there any significant main effects.

These results replicate prior findings of another group indicating that HSV-1 infection impairs cognitive performance a non-psychiatric adult sample (Dickerson et al., 2008). The current study provides further evidence, using different neuropsychological assessment measures, that the deleterious cognitive impact of HSV-1 can be detected in midlife, well before the onset of clinically significant cognitive change. This study goes beyond others like it by demonstrating that HSV-1 infection significantly affects cognitive performance in middle-aged individuals with genetic risk for AD, and therefore may add to the overall risk for cognitive decline in later life.

Several factors warrant further consideration. First, although better executive functioning among ApoE4 carriers relative to non-carriers was contrary to our expectations, there is evidence that the E4 allele confers cognitive benefits early in life that decline precipitously beginning in the 5th decade of life for heterozygotes and in the 6th decade of life for homozygotes (Baxter et al., 2003; Caselli et al., 2004 & 2009). For example, studies of young E4 carriers (between the ages of 18-30) have shown better performance on mental arithmetic tasks (Puttonen et al., 2003), episodic memory performance (Mondadori et al., 2006), decision-making, prospective memory, and verbal fluency (Marchant et al., 2010) compared to non-carriers. Given that our oldest participants were 60 (N=3, 2.6%), and that the mean age of our E4 carriers was 48.9 (±6.6), it is possible that our sample was too young to detect neuropsychological decline among E4 carriers, and still young enough to detect their neuropsychological advantage.

Second, post hoc analyses revealed that ApoE4 positive individuals had significantly higher FSIQ ($M = 117.9$, $SD = 16.8$) compared to ApoE4 negative individuals ($M = 111.3$, $SD = 14.7$), $F(1,114) = 7.97$, $p = .006$, partial $\eta 2 = .068$, and that

HSV-1 positive individuals had significantly lower FSIQ (M = 109.3, SD = 15.6) compared to HSV-1 negative individuals (M = 119.8, SD = 13.2), $F(1,114) = 8.70$, $p = .004$, partial $\eta 2 = .074$. FSIQ and level of education are both considered measures of cognitive reserve, defined as the brain's ability to resist cognitive decline in the face of damage (Stern, Y., 2009). Despite matched level of education across our groups, higher FSIQ among ApoE4 carriers appears to provide evidence for the group's greater cognitive reserve compared to non-carriers. However, true cognitive reserve would allow ApoE4 carriers to maintain their superior performance despite the addition of risk factors such as HSV-1. That ApoE4 lost their advantage with the addition of HSV-1 in fact suggests that they are lacking in cognitive reserve. Thus it may be that the Moderate Risk group in our sample were especially cognitively robust for reasons--such as demographic variables--unrelated to their ApoE4 status, but that this initial robustness was not enough to sustain their performance in the face of HSV-1 infection.

Third, multiple studies have found that declines in verbal memory are among the earliest neuropsychological changes in ApoE4 carriers and patients in the early stages of AD (Flory et al., 2000; Lehtovirta et al., 1996; Marra et al., 2004; Nilsson et al., 2006), leading us to hypothesize that verbal memory performance would be lower among ApoE4 carriers, especially if they were also positive for HSV-1. In fact we found no significant group differences in verbal memory. However, trends in the data conform to the overall hypothesis that verbal memory is among the first domains to show decline in ApoE4 carriers, and that this decline is exacerbated by HSV-1. The significantly higher performance of ApoE4 carriers compared to non-carriers in executive functioning but not in verbal memory suggests a within-group decline in verbal memory relative to another vulnerable domain. And, when ApoE4 negative participants are removed from the analyses, the main effect of HSV-1 on Verbal Memory domain score becomes significant

between the Moderate and High Risk groups: $F(1,32) = 4.20$, $p = .05$, partial $\eta 2 = .116$. It is likely that with a larger sample size the effects of ApoE4 and HSV-1 on verbal memory would have been statistically significant.

Finally, previous findings by other studies have indicated that HSV-1 detrimentally affects cognitive functioning (for example see Dickerson et al., 2003; Dickerson et al., 2004; Schretlen et al., 2010; Shirts et al., 2008; Strandberg et al. 2003; Yolken et al., 2011). These studies did not assess genetic risk for cognitive decline and focused primarily on clinical populations, but they still may provide one reason why HSV-1 positive individuals in our study (both with and without the ApoE4 allele) trended lower in their domain scores, and had lower FSIQs, than HSV-1 negative individuals. However it is also important to note that demographic factors linked to rates of HSV-1 infection also correlate with academic achievement (Fatahzedeh & Schwartz, 2007). Our study adds to the existing body of research by demonstrating that neuropsychological changes associated with HSV-1 infection are detectable in midlife in an otherwise healthy sample.

This study has several limitations that should be considered when interpreting the results. First, the largely Caucasian sample precludes generalization of the results to other ethnic groups. This is especially relevant when considering that HSV-1 incidence varies based on demographic variables and tends to be higher among non-whites and individuals with lower socioeconomic status (Fatahzedeh & Schwartz, 2007). Additionally, our sample had a high level of educational achievement ($M = 16.2$ years), and higher than average FSIQ ($M = 113.2$), two measures of cognitive reserve that may have obscured some cognitive deficits. Future studies should examine a more representative sample in terms of ethnicity, educational attainment, and FSIQ. We also did not differentiate between subtypes (type 1 versus type 2) of HSV. Since HSV-1 is more prevalent than

HSV-2 (Malkin, J. 2002, 12) it is likely that most subjects were infected with HSV-1. In addition, central nervous system infection (e.g. HSE) caused by HSV-2 is very rare in adults, therefore it is unlikely that HSV-2 would cause cognitive changes. Further research is required to assess the impact of HSV subtype on risk for AD.

Additionally, future research should attempt to look at structural brain differences in non-psychiatric HSV-1 carriers with genetic risk for AD. Neuroimaging methodologies such as magnetic resonance imaging of frontal and/or temporal lobe regions may enable detection of early pathogenesis due to HSV-1. Finally, longitudinal studies will be necessary to confirm the impact of HSV-1 status on individual cognitive trajectories. The identification of HSV-1 as a contributor to neuropsychological deficits in midlife suggests that suppression of HSV-1 early in life may present an important target for early intervention against future cognitive decline, especially in ApoE4 carriers, or other individuals with genetic risk for AD.

Chapter 4: ApoE4 is Associated with Reduced Bilateral Hippocampal Volumes in Healthy Middle Aged Subjects

Objective: ApoE4 is a genetic risk factor for Alzheimer's disease (AD). Herpes Simplex-1 (HSV-1), a common viral infection, with higher incidence among ApoE4 carriers, is also linked to cognitive deficits. The current study sought to determine if hippocampal volumes and cortical thickness in six, bilateral regions of interest (ROIs) differed among ApoE4 carriers and non-carriers who were also either positive or negative for HSV-1.

Methods: 111 individuals aged 40-60 were grouped according to risk for cognitive decline based on ApoE4 and HSV-1 status. Participants completed a general health assessment, including assay of HSV-1 seropositivity, and underwent structural neuroimaging. Cortical thickness and hippocampal volumes were measured using Freesurfer software. Age, level of education, full scale IQ, systolic blood pressure, and total intracranial volume were included in the model as covariates.

Results: Group differences were assessed with 2x2 ANOVA. There were no significant interactions of ApoE4 and HSV-1 on cortical thickness or hippocampal volume. However there were significant main effects of ApoE4 on left ($p = .01$) and right ($p = .05$) hippocampal volume.

Conclusions: ApoE4 carriers had significantly smaller bilateral hippocampal volumes regardless of HSV-1 status. This replicates previous findings that ApoE4 is a risk factor for structural brain vulnerability in otherwise healthy middle-aged individuals.

4.0 INTRODUCTION

Figures reported by The Alzheimer's Disease Association's (ADA) in 2013 indicate that during that year an estimated 5.2 million Americans were living with Alzheimer's disease (AD), the most common form of dementia. Current annual estimated costs associated with dementia exceed $400 billion, and incidence is expected to more than double by 2050. There is no cure for dementia and current treatments offer only modest slowing of symptom progression. As the population in the US continues to age, the rising rate of AD and other dementias will place increasing social and economic burdens on the population at large. Thus, determining successful methods of prevention and intervention are fast becoming critical healthcare issues facing the 21st century.

To date the most promising protection for brain and cognitive health is the identification of modifiable risk factors in midlife, well before the onset of cognitive decline. One way to approach this is to examine the interplay of genetic and environmental risk factors. The Apolipoprotein E4 (ApoE4) allele is the most robustly replicated genetic risk factor for sporadic AD, which represents approximately 75% of all dementia cases (Corder et al., 1993; Laws et al., 2003; Slooter et al., 1998). However approximately half of E4 carriers do not develop cognitive decline, and many non-E4 carriers do, highlighting the contribution of acquired risk factors. One such risk factor is herpes simplex-1 (HSV-1), a common viral infection that can damage the same brain regions affected in AD (Itzhaki et al., 1997), and cause cognitive declines that may increase the likelihood that an infected person develops AD (Burgos et al., 2006; Letenneur et al., 2008). Further evidence suggests that HSV-1 infection compounds genetic risk from ApoE4 and increases risk for AD (Dobson & Itzhaki, 1999; Itzhaki et al., 1997; Itzhaki & Wozniak, 2008).

Measurable cognitive decline is preceded by declines in brain health by years if not decades. Premorbid physical changes in brain are identifiable only with neuroimaging, which can detect early structural signs of neurological abnormality. While many studies have noted deleterious structural changes associated with the ApoE4 allele (Burggren et al., 2008; Filippini et al., 2009; Wishart et al., 2006), very few have examined structural anomalies associated with non-encephalitic HSV-1 infection, except in clinical populations (e.g. Gitelman et al., 2001; Prasad et al., 2007). Despite evidence that HSV-1 interacts with ApoE4 to promote declining brain health, to date no studies have examined the effects of both factors in the brains of cognitively normal middle-aged adults for whom mitigating intervention is still possible.

The current study examined the interaction between ApoE4 and HSV-1 on brain structure in cognitively normal adults. 111 individuals aged 40-60 underwent structural magnetic resonance imaging (MRI) to obtain bilateral hippocampal volumes and cortical thickness in six bilateral a priori regions of interest: entorhinal, fusiform, middle temporal, parahippocampal, posterior cingulate, and medial and lateral orbitofrontal cortices. These regions were chosen because previous studies suggested they were most likely to exhibit pathological changes associated with ApoE4 and HSV-1. We sought to determine if HSV-1 seropositivity conferred additional risk for structural changes in regions of interest in carriers of the ApoE4 allele. We hypothesized that ApoE4 carriers positive for HSV-1 would show early brain vulnerability in the form of cortical thinning and hippocampal volume reductions, and that vulnerability would be greater among this group than among those with a single risk factor (ApoE or HSV-1 alone), or among healthy controls. If evidence of early tissue degradation and brain vulnerability is more pronounced among ApoE4 carriers infected with HSV-1, this would provide evidence that HSV-1 confers greater brain vulnerability than the ApoE4 allele alone. Moreover,

early identification of these risk factors affords time for mitigating intervention, such as viral suppression.

4.1 METHODS

4.1.1 Participants

Adults between the ages of 40 and 60 years were recruited from the community via newspaper and online ads. Participants were included if they were free of overt coronary artery disease, neurological disease (e.g., stroke, Parkinson's disease, clinically significant traumatic brain injury), major psychiatric illness (e.g. schizophrenia, bipolar disorder), diabetes, and substance abuse (i.e., diagnosed abuse and/or previous hospitalization for substance abuse) as assessed by a medical history questionnaire. Exclusion criteria included smoking, impaired global cognitive functioning (FSIQ <85), severe depression (BDI-II total score >28), and MRI contraindications. 172 participants were recruited into the study and all provided written informed consent. 59 participants were removed from the analyses for failure to complete one or more portions of the assessment (e.g. HSV-1 testing, ApoE4 testing, or neuroimaging); one person was removed for extreme outlier status in left hemisphere medial orbitofrontal thickness and another for extreme outlier status in right hemisphere medial orbitofrontal thickness (observed value > 3 standard deviations from the group mean). According to participants' self-report, the ethnic distribution of the sample is as follows: 59.5% Caucasian (N=66), 22.5% Hispanic (N=25), 6.3% African-American (N=7), and 11.7% Other/Did Not Specify (N=13).

4.1.2 Protocol

The study was approved by the local institutional review committee and was conducted in congruence with the Helsinki Declaration of 1975. Participants underwent a

general health assessment and structural brain imaging (see below). Assessment visits were completed on separate days.

4.1.3 Health Assessment

Participants were instructed to fast for at least eight hours prior to the first visit. Arterial blood pressure was measured after at least 15 min of rest using a standard oscillometric blood pressure monitor (VP-2000, Colin Medical Instruments, San Antonio, TX). Body mass index (BMI) was calculated as body weight in kilograms divided by the square of the height in meters. Venipuncture of the antecubital vein was performed to obtain approximately 15 cc of blood to measure lipid levels and fasting glucose. Fasting plasma concentrations of glucose, triglyceride, and high and low-density lipoprotein cholesterol (HDL and LDL) were determined using standard enzymatic techniques. A portion of the blood sample was centrifuged and serum was frozen for later HSV-1 testing. Saliva samples were collected for genotyping using commercially available kits (DNA Genotek, Ottawa Canada).

4.1.4 HSV-1 ELISA

Herpes virus was detected using enzyme-linked immunosorbent assay (ELISA) in batches of approximately 40 samples. Samples were assessed for seropositivity to HSV-1 IgG antibodies using the commercially available HSV-1 IgG ELISA kits (Calbiotech, Spring Valley, CA) per manufacturer instructions. Samples were run in duplicate and antibody indices obtained for each subject were averaged. Subjects were considered positive for HSV-1 if optical density was greater or equal to 0.9. This assay did not distinguish between HSV type 1 and type 2 owing to the high percentage of shared antigens between the types. However, due to the low incidence of HSV-2 relative to HSV-1 (Xu et al., 2010), we believe that the majority of our positive samples are type 1.

4.1.5 Genotyping

Saliva samples were stored at room temperature prior to analysis. DNA extraction was completed per manufacturer instructions and purified samples were stored at -40 degrees Celsius prior to genotyping. Saliva samples were collected using the Oragene Discover (OGR-500) kit. DNA extraction on 500 uL of saliva was performed using the prepIT·L2P kit from DNAgenotek. PCR was performed using ApoE-Fwd4 (GCT GAT GGA CGA GAC CAT GAA GGA GTT) and ApoE-snapR (GCC CCG GCC TGG TAG ACT GCC A) primers (Ingelsson, et al., 2003). Polymerase chain reaction (PCR) amplification was performed with 10 ng of DNA and 10 pMol primer, using the following amplification protocol: 95 °C for 15 min, 35 cycles of (95 °C 30 sec., 65 °C 30 sec., 72 °C 30 sec.) and hold at 4°C.

ApoE genotype was assessed from PCR amplification and Sanger sequencing (Sanger, F., 1977) at the DNA Sequencing Facility at the University of Texas at Austin, using Variant Reporter Software from Life Technologies (Thermo Fisher Scientific). Individual samples were classified according to allele type (e.g. ApoE3/4 or ApoE3/3) but due to the small sample size we collapsed across E4 carriers (homo- and heterozygous) and compared them to all non-E4 carriers as a single group.

4.1.6 Risk Assessment

Individuals negative for both ApoE4 and HSV-1 were considered healthy controls, and comprised the No Risk group (N=27). HSV-1 positive individuals without an ApoE4 allele were considered Low Risk (N=51). HSV-1 negative individuals with an ApoE4 allele were considered Moderate Risk (N=12) since there is more evidence to support the deleterious effects of ApoE4 than HSV-1. Individuals with both an ApoE4 allele and HSV-1 were considered High Risk (N=19).

4.1.7 Structural Image Acquisition

T1-weighted anatomical scans of the entire brain in the sagittal plane were collected using a high-resolution Magnetization Prepared Rapid Gradient Echo (MP-RAGE) sequence (256 x 256 matrix, FOV=24 x 24 cm2, 1 mm slice thickness, 0 gap).

4.1.8 Segmentation and Generation of Cortical ROIs

Segmentation of brain structures from T1-weighted anatomical scans and estimation of structure volumes was performed using freely available FreeSurfer software: (http://surfer.nmr.mgh.harvard.edu/). Surface-based reconstruction was achieved using representations of the gray/white matter and gray matter/CSF boundaries (Fischl et al., 2004), resulting in measures of cortical thickness. Surface models were generated and cortical thickness was determined by calculating the distance between gray and white matter boundaries (Fischl et al., 2002, 12). After intensity normalization for a tissue type, skull-stripping, and white matter labeling, volume based analyses were generated utilizing a probabilistic atlas approach that automatically assigns a neuroanatomical label to each voxel in an MR volume (Dale, A., 1999, 12). These techniques are comparable in accuracy and reliability to manual labeling (Fischl, B., 2004, 12). Volumetric measurements are automatically calculated by counting the number of voxels within the labeled region.

Structural ROIs were generated from the automatic segmentation of cortical structures for the whole brain. Cortical thickness was measured in six bilateral a priori regions of interest: entorhinal, fusiform, middle temporal, parahippocampal, posterior cingulate, and medial and lateral orbitofrontal cortices. All data were visually inspected for quality assurance prior to analyses and were analyzed using the latest public FreeSurfer software version (5.1.0).

4.1.9 Segmentation and Generation of Hippocampal Volumes

Hippocampal volumes were generated from the automatic segmentation of subcortical structures for the whole brain. Whole-brain, ventricle, and total intracranial volumes, were analyzed. Total intracranial volume was used as a covariate to correct for differences in head size. Right and left hippocampal volumes were estimated separately for each structure.

4.2 STATISTICAL ANALYSES

Group differences in demographic variables were examined with chi-square tests. Group differences in physiological and cognitive/educational variables were examined with one-way analysis of variance (ANOVA) and Tukey-Kramer post hoc analyses. To limit multiple comparisons, multivariate analysis of variance (MANOVA) was used to model the interaction between ApoE4 and HSV-1, as well as any main effects, on cortical thickness in six bilateral a priori ROIs. Left and right hemispheres were modeled independently, with all six ROIs entered as dependent variables. The interaction and main effects of ApoE4 and HSV-1 on bilateral hippocampal volumes were modeled using 2x2 analysis of variance (ANOVA). Age, years of education, and Full Scale IQ (FSIQ) were included as covariates because they contribute to active cognitive reserve (Stern, Y., 2009). Systolic blood pressure was also included as a covariate because of its influence on total brain and hippocampal volume (Korf et al., 2004; Wiseman et al., 2004). Finally total intracranial volume was included as a covariate to control for differences in head size. All analyses were conducted using IBM SPSS Statistics software (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.).

4.3 RESULTS

4.3.1 Descriptive Statistics

The demographic and physiological characteristics of the study sample are shown in Table 4.1. Gender distribution was comparable across groups, $\chi 2$ (3, N=111) =3.03, p=0.39). Due to high Caucasian enrollment, race was collapsed across non-whites. There were no significant differences in HSV-1 status between whites and non-whites, $\chi 2$ (1, N=111) =2.10, p=0.15). However there were significantly fewer ApoE4 positive non-whites, $\chi 2$ (1, N=111) =8.01, p=0.00).

One-way ANOVA was used to determine if cognitive/educational and physiological variables differed between groups with different risk for cognitive decline. Participants were classified into four groups: No Risk (n = 29), Low Risk (n = 51), Moderate risk (n = 11) and High Risk (n = 19) based on the presence or absence of an ApoE4 allele and HSV1 infection. Data was normally distributed for each group, as assessed by Shapiro-Wilk test ($p > .05$); and there was homogeneity of variances, as assessed by Levene's test of homogeneity of variances ($p = .51$). Years of education was significantly higher among the No Risk group $F(3,107) = 2.87$, $p = .04$, and FSIQ was significantly higher among the Moderate Risk group, $F(3,107) = 3.47$, $p = .02$. Tukey-Kramer post hoc analysis revealed that the decrease in FSIQ from Moderate to Low Risk (-13.33 95% CI (-25.17 to -1.50)) was statistically significant ($p = .021$), however no pairwise group comparisons were significantly different for years of education. The High Risk group had significantly higher systolic blood pressure compared to other groups $F(3,107) = 3.16$, $p = .03$. Tukey-Kramer post hoc analysis revealed that the increase in systolic blood pressure from No Risk to High Risk (11.69 95% CI (0.247 to 22.94)) was statistically significant ($p = .022$). No other group differences were statistically significant.

Table 4.1. Demographic and Physiological Characteristics of Study Participants

*Significant at p<.05; **Significant at p<.01; Data are M ± SD

	Healthy Controls HSV-1-/ApoE4- (n=29)	Low Risk HSV-1+ /ApoE4- (n=51)	Moderate Risk ApoE4+ /HSV-1- (n=11)	High Risk ApoE4+ /HSV-1+ (n=19)
Male/Female	17/12	21/30	4/7	6/13
Age (SD)	47.7 (6.2)	50.0 (6.2)	49.0 (6.3)	49.3 (7.1)
Education years	17.4 (2.7)**	16.0 (2.4)	16.3 (1.9)	15.6 (2.4)
Full Scale IQ	115.9 (12.6)	109.0 (14.4)	121.4 (13.2)*	113.1 (18.7)
White/Non-white	18 (62.1%)/ 11 (37.9%)	23 (45.1%)/ 28 (54.9%)	10 (83.3%)/ 2 (16.7%)**	15 (78.9%)/ 4 (21.1%)**
African American	1 (3.4%)	3 (5.9%)	0	3 (15%)
Asian	1 (3.4%)	3 (5.9%)	0	0
Latino	7 (24.1%)	16 (31.4%)	1 (8.3%)	1 (8.3%)
Other Race	2 (6.9%)	6 (11.8%)	1 (8.3%)	1 (8.3%)
Systolic BP (mmHg)	121.4 (14.1)	121.1 (14.0)	122.6 (16.8)	133.7 (14.8)*
Diastolic BP (mmHg)	73.0 (9.2)	72.4 (9.9)	71.2 (12.0)	77.7 (8.4)
Body Mass Index (kg/m2)	31.5 (7.8)	29.1 (6.5)	30.1 (7.7)	31.0 (7.3)
HDL-Cholesterol (mg/dL)	50.7 (14.0)	57.9 (17.6)	55.0 (25.9)	57.6 (21.2)
LDL-Cholesterol (mg/dL)	118.7 (30.6)	116.9 (35.5)	118.1 (38.1)	118.9 (25.2)
Triglyceride (mg/dL)	138.5 (59.6)	122.8 (83.0)	205.8 (161.0)	148.5 (74.3)
Fasting Glucose (mg/dL)	119.8 (45.4)	135.3 (54.7)	148.9 (74.3)	113.5 (38.0)

4.3.2 Risk Level & Cortical Thickness

Cortical thickness was measured in 6 bilateral a priori regions of interest: entorhinal, fusiform, medial and lateral orbitofrontal, middle temporal, parahippocampal, and posterior cingulate cortices. Shapiro-Wilk tests of normality were nonsignificant for all regions except for left hemisphere medial orbitofrontal cortex. This was due to an extreme outlier (mean < 3 standard deviations from the group mean) so this individual was removed. Multivariate analysis of variance (MANOVA) indicated no statistically significant effect of HSV-1 status on the combined left hemisphere, $F(7, 97) = 3.15$ $p = .95$; Wilks' $\Lambda = .978$; partial $\eta 2 = .022$, or right hemisphere ROIs $F(7, 97) = 3.40$, $p = 0.93$; Wilks' $\Lambda = .976$; partial $\eta 2 = .024$. Nor was there a statistically significant effect of ApoE4 status on the combined left hemisphere, $F(7, 97) = 1.14$ $p = .35$; Wilks' $\Lambda = .924$; partial $\eta 2 = .076$, or right hemisphere ROIs $F(7, 97) = .820$, $p = 0.57$; Wilks' $\Lambda = .944$; partial $\eta 2 = .056$. However ApoE4 positive individuals had significantly thinner left medial temporal cortex, $F(8, 101) = 3.51$, $p = .05$, partial $\eta 2 = .036$.

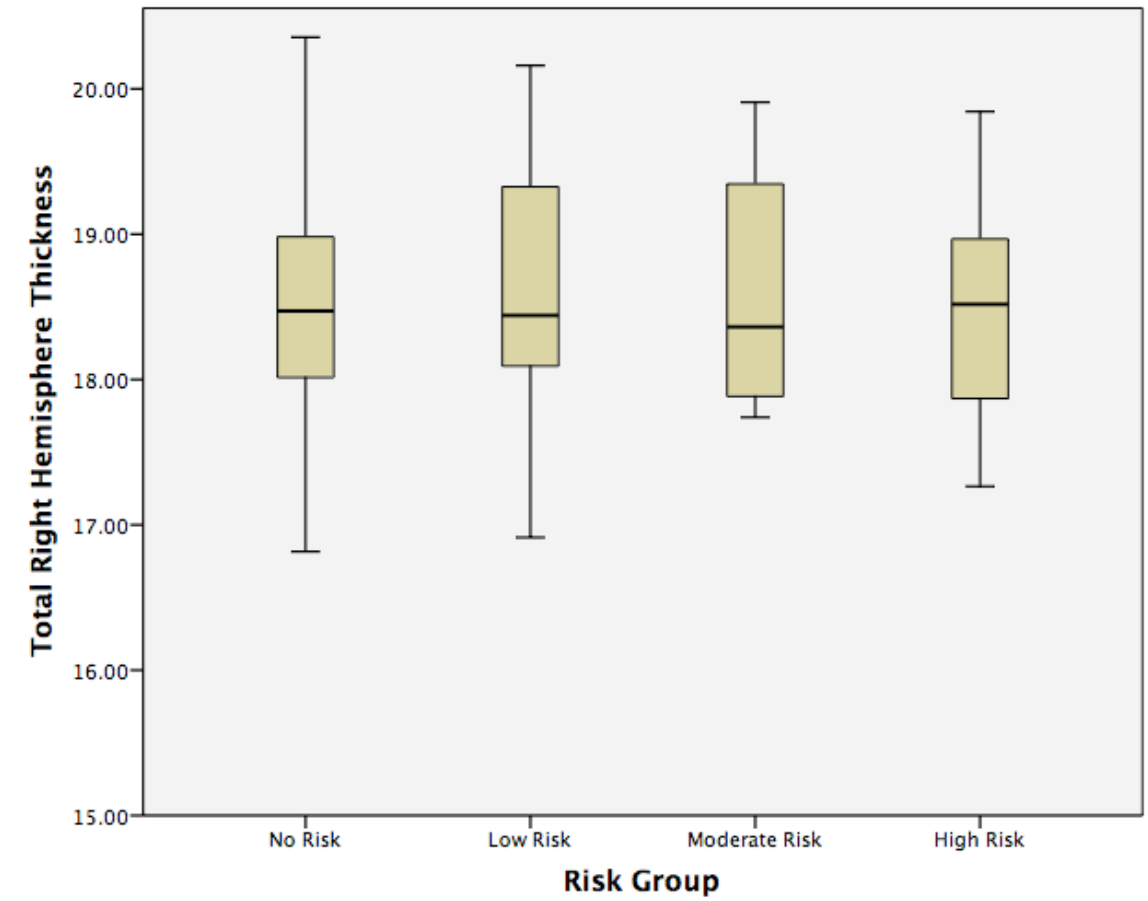

Figure 4.1. Boxplots of Right Hemisphere Cortical Thickness by Group

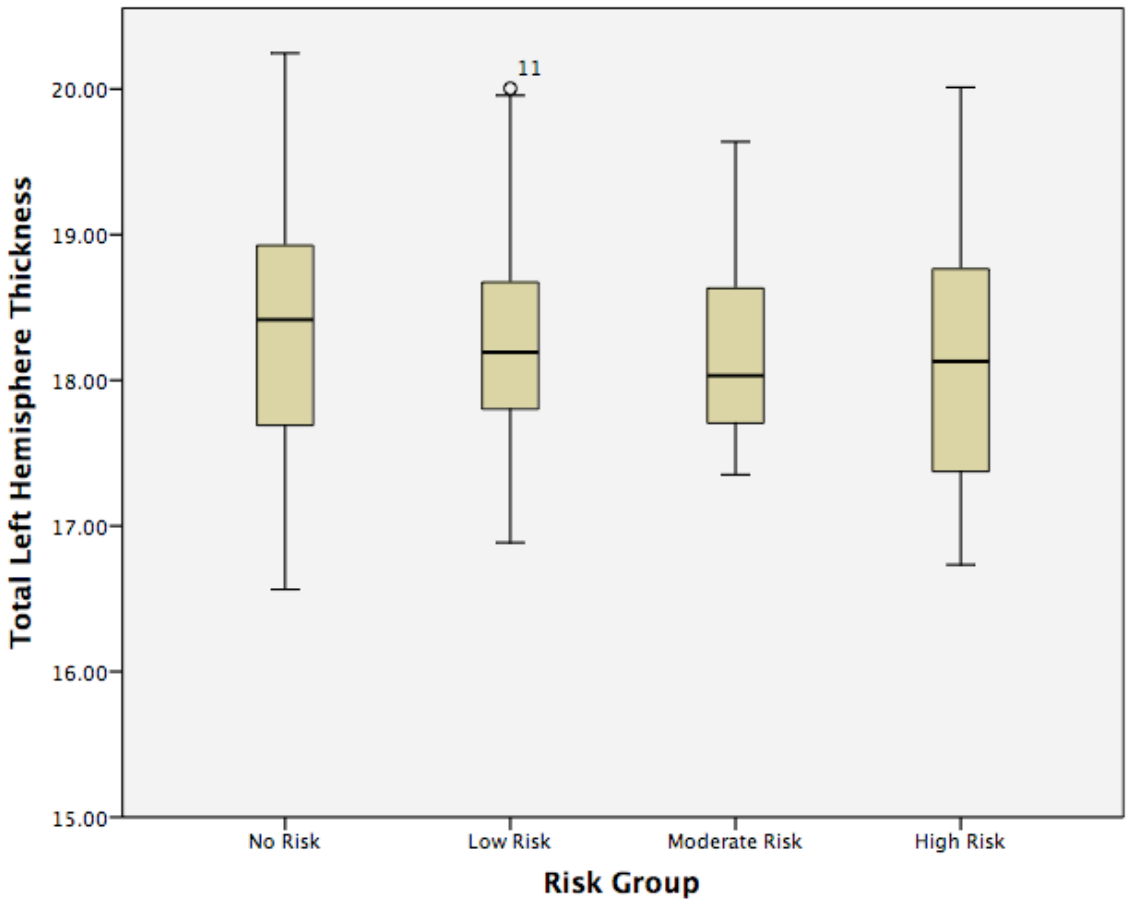

Figure 4.2. Boxplots of Left Hemisphere Cortical Thickness by Group

4.3.3 Risk Level and Hippocampal Volumes

A 2x2 ANOVA was conducted to determine if hippocampal volume differed between groups with different risk for cognitive decline. Participants were classified according to risk level, as above. For left hippocampal volume there were no outliers, as assessed by boxplot (Figure 3.3). There were three outliers for right hippocampal volume, but not exceeded two standard deviations, so these data points were maintained in the set.

Data were normally distributed for each group, as assessed by Shapiro-Wilk test ($p > .05$); and there was homogeneity of variances, as assessed by Levene's test of homogeneity of variances ($p = .14$). Age, level of education, FSIQ, systolic blood pressure, and total intracranial volume were entered in the model as covariates.

There were no significant interactions of ApoE4 and HSV-1 on either right hippocampal volume, $F(8, 101) = .010$, $p = .922$, or left hippocampal volume, $F(8, 101) = .896$, $p = .346$. However there were significant main effects of ApoE4 on bilateral hippocampal volumes. Right hippocampal volume was significantly smaller among the ApoE4 positive group ($M = 3903.5$, $SD = 412.3$) compared to the ApoE4 negative group ($M = 3999.1$, $SD = 385.0$), $F(1, 102) = 4.10$, $p = .05$, partial $\eta 2 = .040$. Similarly, left hippocampal volume was significantly smaller among the ApoE4 positive group ($M = 3717.7$, $SD = 464.8$) compared to the ApoE4 negative group ($M = 3861.0$, $SD = 387.5$), $F(1, 102) = 7.04$, $p = .01$, partial $\eta 2 = .065$, see Figures 4.1 and 4.2.

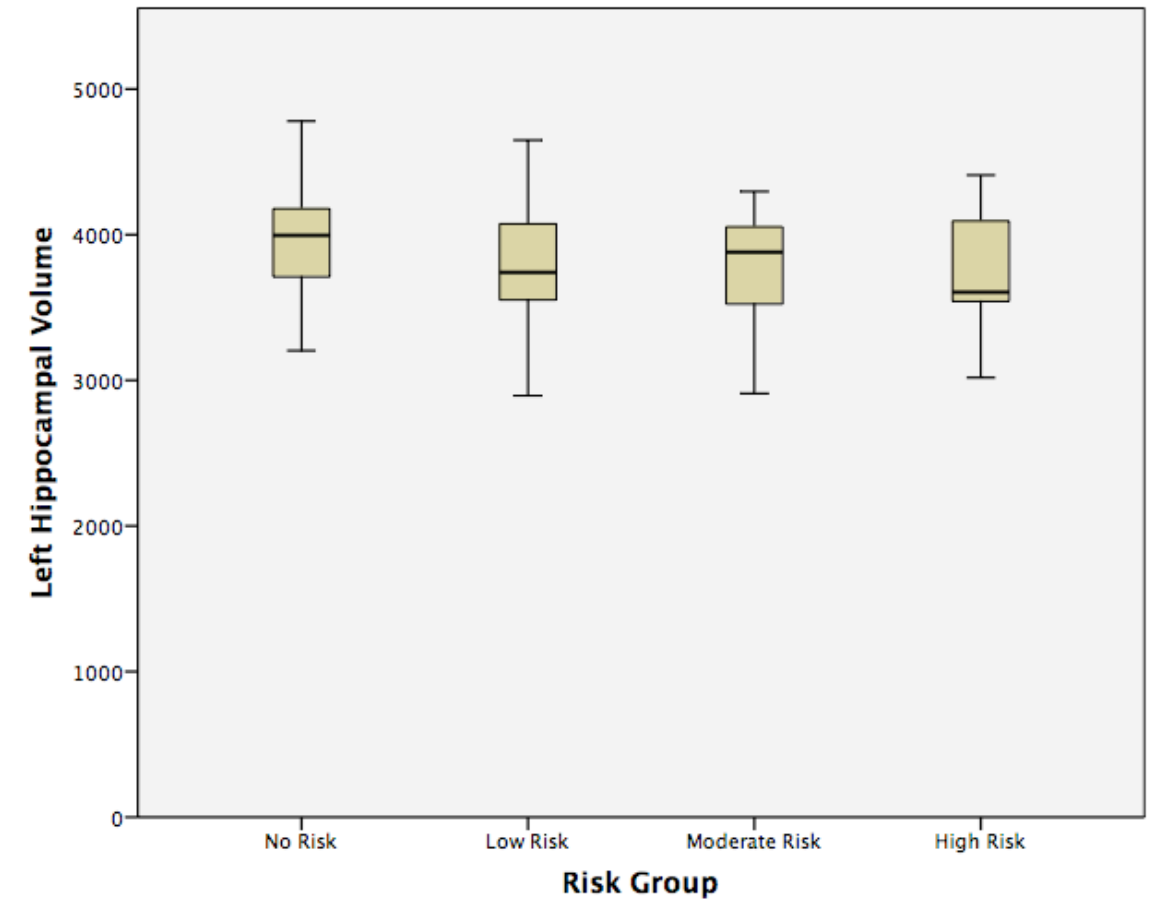

Figure 4.3. Boxplots of Left Hippocampal Volume by Group

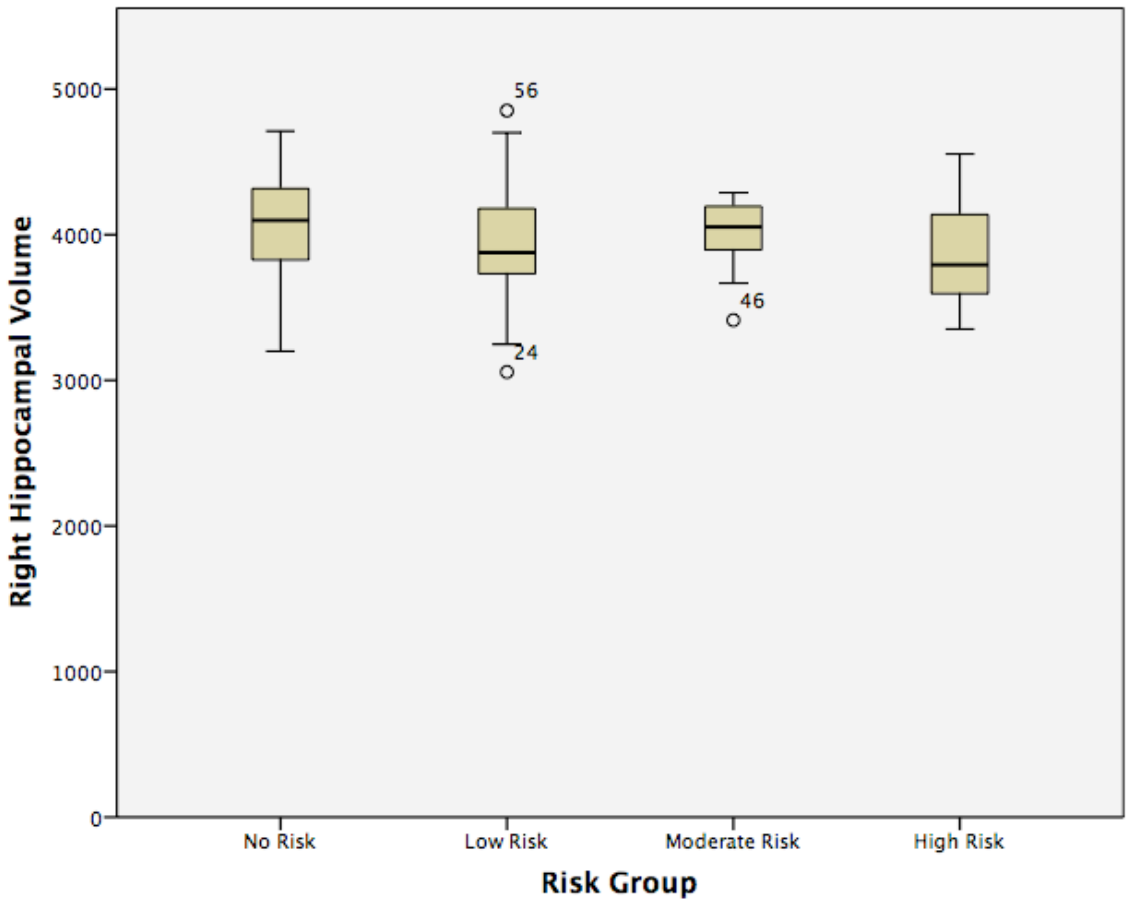

Figure 4.4. Boxplots of Right Hippocampal Volume by Group

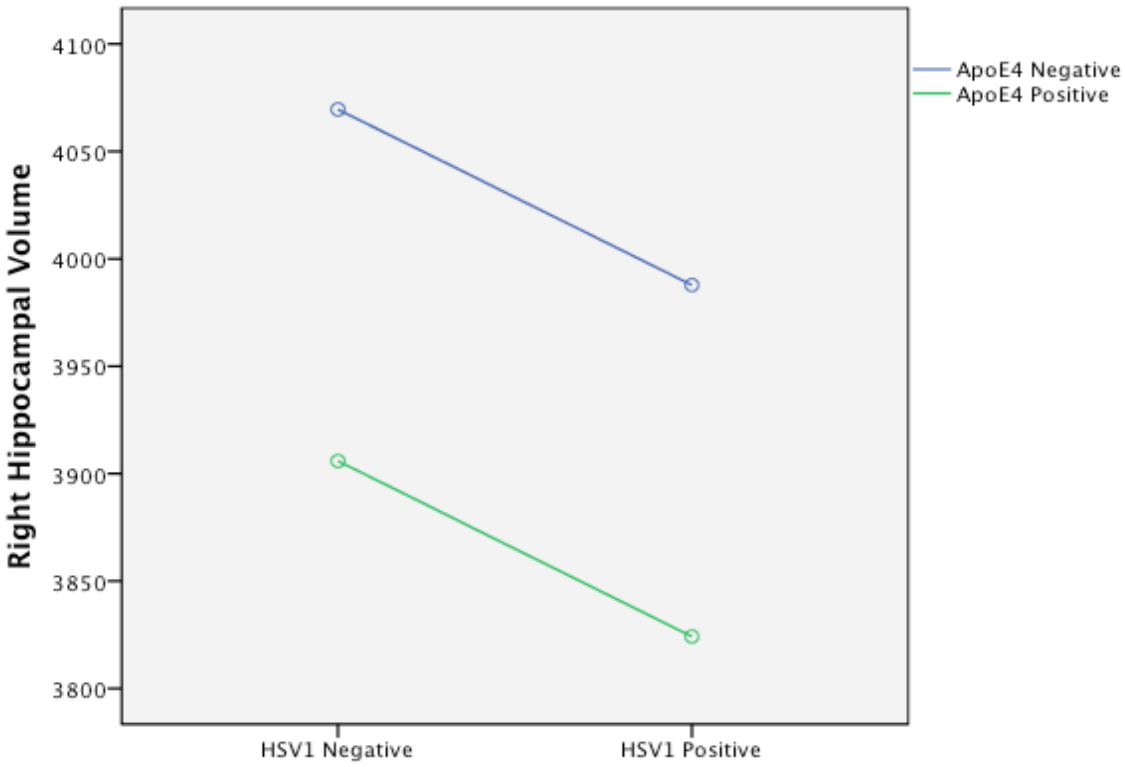

Figure 4.5: The Main Effect of ApoE4 on Right Hippocampal Volume

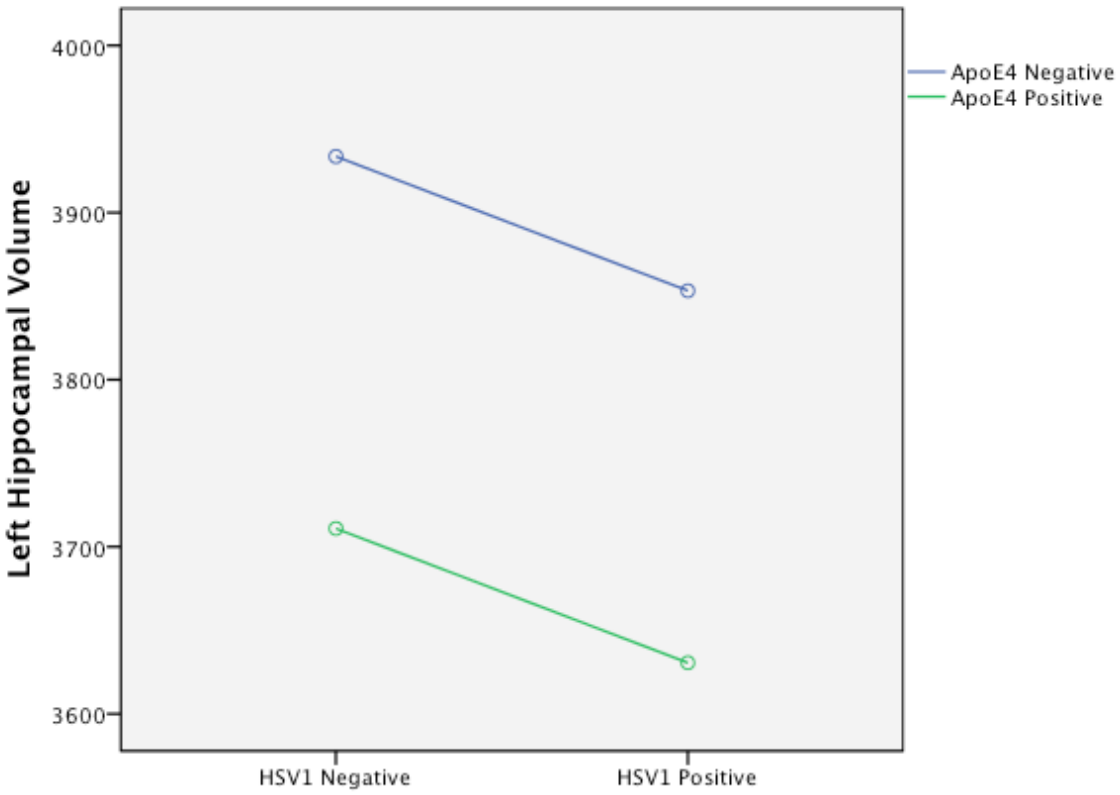

Figure 4.6. The Main Effect of ApoE4 on Left Hippocampal Volume

4.4. DISCUSSION

We predicted that ApoE4 carriers who tested positive for HSV-1 would exhibit greater hippocampal volume loss and cortical thinning in ROIs compared to other risk groups and controls, indicating that HSV-1 infection exacerbates the deleterious effects of ApoE4 on brain health. This hypothesis was based on prior studies indicating that ApoE4 carriers have smaller hippocampal volumes and regional cortical thinning compared to non-carriers (Burggren et al., 2008; Cohen et al., 2001; Filippini et al., 2009, 12; Gutiérrez-Galve et al., 2009; Plassman et al., 1997; Wishart et al., 2006), and that HSV-1 infection is linked to structural brain changes among clinical populations (Gitelman et al., 2001; Prasad et al., 2007). We measured bilateral hippocampal volumes and cortical thickness in six bilateral a priori regions of interest chosen for their shared vulnerability to both ApoE4 and HSV-1. To our knowledge this is the first study to examine the interaction of ApoE4 and HSV-1 on structural brain integrity in cognitively normal middle-aged adults.

Contrary to what we hypothesized, there were no statistically significant interactions of ApoE4 and HSV-1 on cortical thickness in either right or left hemisphere ROIs, nor were there any significant main effects. However a main effect of ApoE4 trended towards significance in left medial temporal cortex and left lateral orbitofrontal cortex. Additionally, contrary to expectation, there were no significant interactions of ApoE4 and HSV-1 on hippocampal volumes. However ApoE4 carriers had significantly smaller hippocampal volumes bilaterally compared to non-carriers.

Although the failure to obtain a significant interaction of ApoE4 and HSV-1 on structural brain health was disappointing, we nevertheless confirmed findings from other studies that the ApoE4 allele is linked to reduced hippocampal volume in cognitively healthy middle-aged adults, suggesting both that ApoE4 carriers demonstrate structural

brain vulnerability that is detectable before the onset of cognitive decline, and that the hippocampus is uniquely vulnerable, compared to other brain regions, in individuals with genetic risk for AD (Burggren et al., 2008; Plassman et al., 1997; Wishart et al., 2006).

The causative role ApoE4 plays in smaller hippocampal volume remains incompletely understood, but it is consistent over the lifespan. Studies have shown that ApoE4 positive children and young adults have smaller hippocampi than non-carriers (Alexopoulos et al., 2011; Shaw et al., 2007), as do non-demented middle-aged (Plassman et al., 1997), and older adults (Burggren et al., 2008; Cohen et al., 2001). Further evidence suggests that the ApoE4 allele accelerates hippocampal volume loss in older adults (Jak et al., 2007). The notion that ApoE4 hastens brain aging among people over 60 is further supported by neuropsychological evidence that E4 carriers undergo a swifter transition from cognitive vulnerability to frank decline and impairment than non-carriers (Baxter et al., 2003; Caselli et al., 2004 & 2009; Hedden et al., 2004; Minati et al., 2009).

The fact that E4 carriers begin with smaller hippocampi may leave them with lower passive cognitive reserve--the theory that larger brains are better able to compensate for structural insult (Stern, Y., 2009)--than non-carriers, causing them to decline more quickly once a threshold of risk factors is crossed in later life (Hedden et al., 2004; Minati et al., 2009). Notably ApoE4 is also associated with a pro-inflammatory immune profile that may be a source of cumulative brain insult over the lifespan. Upregulation of pro-inflammatory markers is believed to contribute to the higher rates of cardiovascular disease among E4 carriers (Frautschy et al., 1998; Jofre-Monseny et al., 2011; McGreer & McGreer, 2001), as well as poorer cognitive outcomes following certain infections (Amouyel et al., 1994; Burgos et al., 2003; Burt et. al., 2008), and following brain injury (Friedman et al., 1999; Teasdale et al., 1997). In addition to

exhibiting a stronger and more aggressive inflammatory response than other allele types, ApoE4 carriers are also less efficient at clearing neurotoxic products, including beta-amyloid deposits, which further potentiates inflammation (Frautschy et al., 1998; McGreer & McGreer, 2001). Lower passive cognitive reserve plus higher inflammation may leave ApoE4 carriers cognitively disadvantaged as additional peripheral risk factors add up over the course of a lifetime.

Smaller hippocampi also suggest microstructural differences among E4 carriers not present in other allele carriers. For example, transgenic ApoE4 mice show gray matter differences, such as lower dendritic spine density in certain cortical layers, not observed in ApoE3 mice (Dumanis et al., 2009). Alterations in dendritic spine density affect synaptic function, plasticity, and connectivity between brain regions, (Penzes et al., 2011). Moreover, beginning in childhood ApoE4 carriers exhibit reduced and/or compromised white matter not observed in other allele types (O'Dwyer et al., 2012; Persson et al., 2006). Evidence that ApoE4 is disadvantageous to structural brain integrity at the level of synapses, myelination, and tractography has led to the view that AD pathology collectively may reflect underlying problems of connectivity between brain regions, rather than merely localized dysfunction. Indeed, other disorders known for severe information processing deficits, such as Autism Spectrum Disorder and schizophrenia, are also associated with impairments in neuronal connectivity and plasticity (Penzes et al., 2011).

It appears that our study was able to detect innate differences in passive cognitive reserve between ApoE4 carriers and non-carriers, but failed to detect any exacerbation of this condition stemming from a chronic inflammation attributable to HSV-1 infection. One reason for this might be that the duration of infection with HSV-1 matters; i.e. perhaps longer term infections, beginning in childhood, are necessary to bring about

significant structural changes in the brain. We did not determine infection duration in our sample, nor did we test for active versus latent infection. Among older adults at least one study has found that active infection (measured by the presence of immunoglobulin M antibodies), increases longitudinal risk for AD, but that latent infection (measured by the presence of immunoglobulin G antibodies) does not (Letenneur et al., 2008). Another possibility is that HSV-1 does not penetrate the CNS except in rare cases of encephalitis. This is unlikely given the number of human and animal studies that have found evidence that HSV-1 is able to cross the BBB and enter the CNS (Itzhaki et al., 1992; Jamieson et al., 1992; Rock, 1983; Schlitt et al., 1986; Wozniak et al., 2002). Finally, it is possible, as some have suggested (Jamieson et al., 1992; Wozniak et al., 2005), that HSV-1 is only able to cross the BBB and penetrate the CNS in older age when immune function is less robust, but not in midlife when the immune system is still functioning normally.

This study has several limitations that should be considered when interpreting the results. First, the largely Caucasian sample precludes generalization of the results to other ethnic groups. Additionally, our sample had a high level of educational achievement (M = 16.4 years), and higher than average FSIQ (M = 113.0), two measures of active cognitive reserve that may influence structural brain integrity. Future studies should examine a more representative sample in terms of ethnicity, educational attainment, and FSIQ. We also did not differentiate between subtypes (type 1 versus type 2) of HSV. Since HSV-1 is more prevalent than HSV-2 (Malkin, J. 2002, 12; Xu et al., 2010) it is likely that most subjects were infected with HSV-1. Encephalitic herpes infection caused by HSV-2 is also very rare in adults, therefore it is unlikely that HSV-2 would be linked to observable changes in the CNS.

Additionally, future research should attempt to look at functional brain differences in non-psychiatric HSV-1 carriers with genetic risk for AD. Specifically,

neuroimaging methodologies for determining connectivity between brain regions may be useful for measuring early changes in ApoE4 carriers that are abetted by HSV-1 infection. Finally, longitudinal studies will be necessary to fully disconfirm (or confirm) the role of HSV-1 on the structural brain integrity of ApoE4 carriers. The identification of HSV-1 as a contributor to premorbid brain changes in individuals at risk for AD would indicate that suppression of HSV-1 early in life may present an important target for early intervention against future cognitive decline.

Chapter 5: The Effects of the APOE4 Allele and Herpes Simplex 1 Infection in Resting State Network Connectivity Among Healthy Middle Aged Adults

Objective: The ApoE4 allele is associated with reduced functional connectivity within the default mode network (DMN) and an increased risk for Alzheimer's disease (AD), even after controlling for environmental risk factors. Herpes Simplex-1 (HSV-1), is a highly prevalent neurotropic viral infection also associated with brain changes in the same regions affected by AD. ApoE4 carriers may be at increased risk for HSV-1 infection, and together the two risk factors may pose a higher risk of cognitive decline than either factor in isolation. The current study sought to determine if functional disconnection within the DMN was present in healthy middle-aged HSV-1 positive ApoE4 carriers compared to HSV-1- ApoE4 carriers and healthy controls.

Methods: 98 individuals aged 40-60 were grouped according to risk for abnormal functional connectivity based on ApoE4 and HSV-1 status. Participants completed a general health assessment, including assay of HSV-1 seropositivity, and underwent functional neuroimaging. Functional connectivity was calculated using FSL software. Group differences in connectivity within the primary and secondary DMN were further modeled using MANOVA. Age, full scale IQ, and systolic blood pressure were included in the model as covariates.

Results: No significant group interactions or main effects were found.

Conclusions: A different methodological approach to data analysis may have yielded more informative results.

5.0 INTRODUCTION

Dementia is a devastating condition that limits human functioning and independence by causing memory impairment, dysexecutive syndrome, and behavioral changes. Alzheimer's disease (AD) currently affects over 5.2 million Americans. As the population continues to age this number is expected to more than double by 2050. Annual costs associated with dementia (including unpaid care) are estimated to exceed $419 billion in the United States alone (all statistics reported from the 2013 Alzheimer's disease facts and figures, 2013, 12). There are no cures for dementia and current treatments offer only modest slowing of symptom progression. Increasing prevalence of the disease and the massive social and economic costs it poses have prompted extensive research on early intervention strategies aimed at modifiable risk factors.

The Apolipoprotein E4 (ApoE4) allele is the most robustly replicated genetic risk factor for sporadic AD, which represents approximately 75% of all dementia cases (Corder et al., 1993; Laws et al., 2003; Slooter et al., 1998). However approximately half of E4 carriers do not develop cognitive decline, and many non-E4 carriers do, highlighting the contribution of acquired risk factors. Unlike genetic risk factors, which cannot be changed, acquired risk factors are often modifiable, and offer opportunities to intervene an individual's overall risk for cognitive decline. A shared feature of nearly all modifiable risk factors is their capacity to induce chronic inflammation. Many chronic pro-inflammatory conditions, such as hypertension, diabetes, and certain infections, are linked to increased risk for neurodegenerative disease, especially as their negative effects accumulate over the lifespan (Fuller et al., 2010; Holmes et al., 2009; Strachan et al., 2008). For example, there is evidence that genetic risk for AD is compounded by the inflammatory effects of herpes simplex-1 (HSV-1), a common neurotropic viral infection

that causes inflammation in the same brain structures affected in AD (Itzhaki et al., 1997).

Changes in the brain resulting from inflammatory processes precede observable cognitive decline by decades, leaving ample time for intervention provided that pathological changes are identified early enough. To this end, functional magnetic resonance imaging (fMRI) presents the most promising tool for identifying in vivo premorbid changes in brain activity among cognitively normal individuals at risk for dementia. fMRI measures the brain's hemodynamic response (HR), the delivery of oxygenated blood to brain regions involved in a particular task. Changes in regional HR are well documented in AD (Gron et al., 2002; Grossman et al., 2003; Kato et al., 2001; Lustig C., 2003; Saykin et al., 1999) but also in normal aging (Ajmania et al., 2000; Huettel, Singerman, & McCarthy, 2001; O'Sullivan et al., 2001), making it difficult to dissociate pathological from normal change in fMRI studies of regional activation. Unlike normal aging however, neurodegenerative conditions are associated with reduced synaptic plasticity and corresponding reductions in connectivity between brain regions, hence analysis of functional connectivity presents itself as a useful way of identifying individuals at risk for cognitive decline (Palop, Chin, & Mucke, 2006).

Functional connectivity is defined as correlations in distributed low frequency fluctuations (DLFFs) across spatially remote brain regions (Buchel, C., 1997). Research on disruptions of functional connectivity has focused on the default mode network (DMN), a distributed network of brain regions that activate in the absence of a task. The DMN consists of a "hub"—the cingulate/retrosplenial cortex (PCC/Rsp)—and a number of anatomically distinct regions commonly vulnerable to AD, including ventral medial prefrontal cortex (vMPFC), posterior cingulate/retrosplenial cortex (PCC/Rsp), inferior parietal lobule (IPL), lateral temporal cortex (LTC), dorsal medial prefrontal cortex

(dMPFC), parahippocampal cortex (PHC), and the hippocampal formation (HC) (Buckner et al., 2008), see Figure 5.1. The functional connectivity the DMN is measured as correlations (or anticorrelations) between the PCC/Rsp and other distributed regions of the DMN. Greater correlation between DLFFs in the PCC/Rsp and other DMN regions represents increased synchronization between these regions, or high functional connectivity, whereas reduced correlation between DLFFs in the PCC/Rsp and other DMN regions represents the opposite. Among middle-aged ApoE4 carriers, studies have found differences in DMN activation similar to those seen in AD (Bookheimer et al., 2000; Burggren, et al., 2002; Filippini et al., 2009; Sheline et al., 2010), suggesting that the ApoE4 allele influences changes in functional connectivity within the DMN before the onset of frank cognitive changes.

To date there have been no studies examining changes in functional connectivity among cognitively healthy HSV-1 positive individuals. However, if HSV-1 infection represents an additive risk for cognitive decline in those with elevated genetic risk, examination of DMN function in infected individuals could provide insight into the interaction between genetic vulnerability and acquired risk, and could bolster the rationale that viral suppression is an important neuroprotective intervention.

The present study utilized resting-state fMRI to investigate the interaction of ApoE4 and HSV-1 on DMN connectivity. We predicted that as risk for cognitive decline increases, measured in terms of ApoE4 status and HSV-1 seropositivity, there will be greater changes in connectivity, measured as the correlation (or anticorrelation) of DLFFs between regions of the DMN. More specifically, we expected to see reduced correlation (increased dyssyncrhony) between the bilateral parahippocampi gyri and hippocampal formation and the DMN hub, the PCC/Rsp. This hypothesis is based on previous studies indicating that the hippocampi are more structurally vulnerable in ApoE4 carriers, and to

HSV-1 infection (Burggren et al., 2008; Cohen et al., 2001; Filippini et al., 2009, 12; Gitelman et al., 2001; Gutiérrez-Galve et al., 2009; Plassman et al., 1997; Prasad et al., 2007; Wishart et al., 2006).

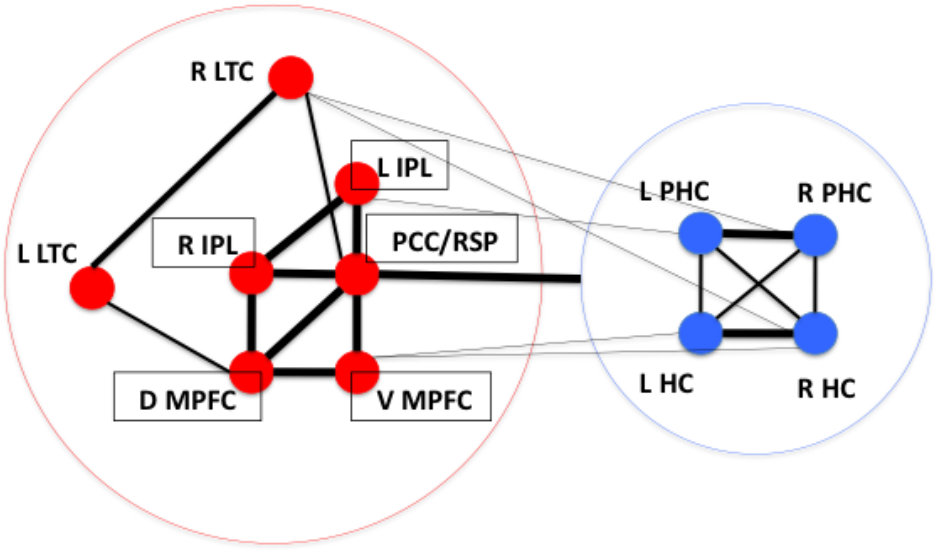

Figure 5.1: Schematic of the Default Mode Network, adapted from Buckner et al., (2008). Heavier lines denote greater connectivity

5.1 METHODS

5.1.1 Participants

Adults between the ages of 40 and 60 years were recruited from the community via newspaper and online ads. Participants were included if they were free of overt coronary artery disease, neurological disease (e.g., stroke, Parkinson's disease, clinically significant traumatic brain injury), major psychiatric illness (e.g. schizophrenia, bipolar disorder), diabetes, and substance abuse (i.e., diagnosed abuse and/or previous hospitalization for substance abuse) as assessed by a medical history questionnaire. Exclusion criteria included smoking, impaired global cognitive functioning (FSIQ <85), severe depression (BDI-II total score >28), and MRI contraindications. 162 participants

were recruited into the study and all provided written informed consent. 61 participants were removed from the analyses for failure to complete one or more portions of the assessment (e.g. HSV-1 testing, ApoE4 testing, or neuroimaging). According to participants' self-report, the ethnic distribution of the sample is as follows: 64.3% Caucasian (N=63), 18.4% Hispanic (N=18), 6.1% African-American (N=6), and 7.1% Other/Did Not Specify (N=7).

5.1.2 Protocol

The study was approved by the local institutional review committee and was conducted in congruence with the Helsinki Declaration of 1975. Participants underwent a general health assessment and structural brain imaging (see below). Assessment visits were completed on separate days.

5.1.3 Health Assessment

Participants were instructed to fast for at least eight hours prior to the first visit. Arterial blood pressure was measured after at least 15 min of rest using a standard oscillometric blood pressure monitor (VP-2000, Colin Medical Instruments, San Antonio, TX). Body mass index (BMI) was calculated as body weight in kilograms divided by the square of the height in meters. Venipuncture of the antecubital vein was performed to obtain approximately 15 cc of blood to measure lipid levels and fasting glucose. Fasting plasma concentrations of glucose, triglyceride, and high and low-density lipoprotein cholesterol (HDL and LDL) were determined using standard enzymatic techniques. A portion of the blood sample was centrifuged and serum was frozen for later HSV-1 testing. Saliva samples were collected for genotyping using commercially available kits (DNA Genotek, Ottawa Canada).

5.1.4 HSV-1 ELISA

Herpes virus was detected using enzyme-linked immunosorbent assay (ELISA) in batches of approximately 40 samples. Samples were assessed for seropositivity to HSV-1 IgG antibodies using the commercially available HSV-1 IgG ELISA kits (Calbiotech, Spring Valley, CA) per manufacturer instructions. Samples were run in duplicate and antibody indices obtained for each subject were averaged. Subjects were considered positive if optical density exceeded 0.9. This assay did not distinguish between HSV type 1 and type 2 owing to the high percentage of shared antigens between the types. However, due to the low incidence of HSV-2 relative to HSV-1 (Xu et al., 2010), we believe that the majority of our positive samples are type 1.

5.1.5 Genotyping

Saliva samples were stored at room temperature prior to analysis. DNA extraction was completed per manufacturer instructions and purified samples were stored at -40 degrees Celsius prior to genotyping. Saliva samples were collected using the Oragene Discover (OGR-500) kit. DNA extraction on 500 uL of saliva was performed using the prepIT·L2P kit from DNAgenotek. PCR was performed using ApoE-Fwd4 (GCT GAT GGA CGA GAC CAT GAA GGA GTT) and ApoE-snapR (GCC CCG GCC TGG TAG ACT GCC A) primers (Ingelsson, et al., 2003). Polymerase chain reaction (PCR) amplification was performed with 10 ng of DNA and 10 pMol primer, using the following amplification protocol: 95 °C for 15 min, 35 cycles of (95 °C 30 sec., 65 °C 30 sec., 72 °C 30 sec.) and hold at 4°C.

ApoE genotype was assessed from PCR amplification and Sanger sequencing (Sanger, F., 1977) at the DNA Sequencing Facility at the University of Texas at Austin, using Variant Reporter Software from Life Technologies (Thermo Fisher Scientific). Individual samples were classified according to allele type (e.g. ApoE3/4 or ApoE3/3) but due to the small sample size we collapsed across E4 carriers (homo- and heterozygous) and compared them to all non-E4 carriers as a single group.

5.1.6 Risk Assessment

Individuals negative for both ApoE4 and HSV-1 were considered healthy controls, and comprised the No Risk group (N=27). HSV-1 positive individuals without an ApoE4 allele were considered Low Risk (N=45). HSV-1 negative individuals with an ApoE4 allele were considered Moderate Risk (N=11) since there is more evidence to support the deleterious effects of ApoE4 than HSV-1. Individuals with both an ApoE4 allele and HSV-1 were considered High Risk (N=15).

5.1.7 Structural Image Acquisition

T1-weighted high-resolution anatomical scans of the entire brain in the sagittal plane were collected using a Magnetization Prepared Rapid Gradient Echo (MP-RAGE) sequence (256 x 256 matrix, FOV=24 x 24 cm2, 1 mm slice thickness, no slice gap).

5.1.8 Functional Image Acquisition

Functional imaging was performed using a whole brain echo-planar imaging (EPI) sequence (TR=3000 ms, TE=30 ms, FOV=24 x 24 cm2, 64 x 64 matrix, 42 axial

slices, 3 mm slice thickness, 0.3 mm gap). Resting State Functional MRI acquisition was collected over 5 minutes while participants rested quietly and watched a fixation cross.

5.1.9 Pre-processing

After conversion from DICOM to NiFTI format and reorientation, the first three volumes of each functional run were trimmed to account for the scanner reaching equilibrium due to progressive saturation. Each functional run was then skull-stripped using FSL's BET utility and rigid body motion correction parameters were calculated and saved using FSL's MCFLIRT utility. Each high-resolution anatomical image was skull-stripped and segmented using FSL's FLIRT utility.

Prior to connectivity analysis, nuisance trends were removed from the data by running a regression analysis in FSL's FEAT program on each functional time-series. Regressors included the six motion parameters calculated during rigid body motion correction and one regressor corresponding to the mean time series of cerebrospinal fluid (CSF) in that functional run, derived from a mask of tissue classified as CSF during segmentation of each subject's high-resolution anatomical scan. The temporal derivative of each of these parameters was also included as regressors. This nuisance model also applied intensity normalization, a high-pass filter (100s), and a 5 mm FWHM Gaussian smoothing kernel. The residuals of this nuisance model contained the resting state activation without the confound of nuisance trends, and were carried forward for statistical analysis.

Prior to a subject-wise correlation analysis, each functional time series of residuals was scrubbed to remove spurious, spatially structured nonlinear artifact arising from head motion. Head movement, or framewise displacement, was calculated as the sum of the absolute values of the differentiated realignment estimates (by backwards differences) at every timepoint; volumes exceeding a framewise displacement of 0.25 were excluded, (Power et al., 2014). For each subject, a Pearson's correlation was computed between the time-series of that subject's posterior cingulate cortex (pCC) and each voxel in the brain. The pCC was chosen because it has been identified as a central component of the DMN (Buckner et al., 2008; Greicius et al., 2008, 12) and has been successfully used as a seed region to define the DMN (e.g., O'Sullivan et al., 2001; Zhang et al., 2010). A Fisher's Z transform was applied to the resulting map of correlation coefficients to improve the normality of the values.

5.2.0 Group analysis

An ROI analysis was carried using regions defined by the WFU Pickatlas version 2.4 (Maldjian et al., 2003). Regions of interest included bilateral fusiform gyri, bilateral hippocampi, bilateral parahippocampal gyri, middle orbito-frontal cortex, bilateral inferior parietal lobules, bilateral middle temporal gyri, bilateral superior frontal gyri bilateral, precentral gyri, bilateral frontal operculum, and bilateral insula, see Table 5.1.

The mean correlation coefficient for the ROIs were extracted from each subject's first level ("correlation model") whole-brain map of correlation coefficients.

Table 5.1: Anatomical ROIs based on the WFU-Pick Atlas

Common Name	WFU-Pickatlas Label
Bilateral fusiform gyri (FG)	55, 56
Bilateral hippocampi (HC)	37, 38
Bilateral parahippocampal gyri (PHC)	39, 40
Middle orbito-frontal cortex (MOFC)	9, 10
Bilateral inferior parietal lobules (IPL)	61, 62
Bilateral middle temporal gyri (MTG)	85, 86
Bilateral superior frontal gyri (SFG)	3, 4
Bilateral precentral gyri (PCG)	39, 40
Bilateral frontal operculum (FO)	11, 12
Bilateral insula (INS)	29, 30

5.2.1 Connectivity Analysis

To reduce multiple comparisons, the interaction of ApoE4 and HSV-1 on connectivity (i.e. mean correlation coefficients for each ROI of each subject, excluding HC/PHC) was analyzed using multivariate analysis of variance (MANOVA) for each hemisphere, in SPSS (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.). The interaction of ApoE4 and HSV-1 on HC/PHC connectivity was modeled separately because the HC/PHC is considered part of a secondary network with connectivity to the primary DMN. Age, full scale IQ, and systolic blood pressure were included in all models as covariates.

5.3 RESULTS

5.3.1 Descriptive Statistics

The demographic and physiological characteristics of the study sample are shown in Table 5.2. Gender distribution was comparable across groups, $\chi 2$ (1, N=98) =0.267, p=0.36. There were no significant differences in HSV-1 status between whites and non-whites, $\chi 2$ (1, N=98) = .927, p=0.34). However there were significantly fewer ApoE4 positive non-whites, $\chi 2$ (1, N=98) =6.37, p=0.01).

One-way ANOVA was used to determine if cognitive/educational and physiological variables differed between groups with different risk for cognitive decline. Participants were classified into four groups: No Risk (n = 27), Low Risk (n = 45), Moderate risk (n = 11) and High Risk (n = 14) based on the presence or absence of an ApoE4 allele and HSV1 infection. Data was normally distributed for each group, as assessed by Shapiro-Wilk test ($p > .05$); and there was homogeneity of variances, as assessed by Levene's test of homogeneity of variances ($p = .55$).

Full scale IQ (FSIQ) was significantly higher among the Moderate Risk group $F(3,94) = 4.32$, $p = .00$. Tukey-Kramer post hoc analysis revealed that the decrease in FSIQ from Moderate to Low Risk (-15.67 95% CI (-28.14 to -3.12)) was statistically significant ($p = .008$). Systolic blood pressure was significantly higher among the High Risk group, $F(3,94) = 3.64$, $p = .02$. Tukey-Kramer post hoc analysis revealed that the increase in systolic blood pressure from No Risk to High Risk (13.97 95% CI (1.56 to 26.38)) was statistically significant ($p = .021$), as was the increase in systolic blood pressure from Low to High Risk (13.42 95% CI (1.90 to 24.91, $p = .015$). No other group differences were statistically significant.

Table 5.2 Demographic and Physiological Characteristics

*Significant at p<.05; **Significant at p<.01; Data are M ± SD

	Healthy Controls HSV-1-/ApoE4- (n=27)	Low Risk HSV-1+/ ApoE4- (n=45)	Moderate Risk ApoE4+ /HSV-1- (n=11)	High Risk ApoE4+ /HSV-1+ (n=15)
Male/Female	14/13	18/27	7/4	3/12
Age (SD)	48.4 (6.2)	49.8 (6.2)	47.6 (6.7)	49.7 (6.8)
Education years	17.7 (3.4)	16.2 (2.5)	16.3 (2.0)	16.2 (2.4)
Full Scale IQ	117.4 (13.7)	109.5 (14.0)	125.2 (12.8)**	114.8 (16.4)
White/Non-white	18(66.7%)/ 9 (33.3%)	23(51.1%)/ 22 (48.9%)	9 (81.8%)/ 2 (18.2%)**	13 (86.7%)/ 2 (13.3%)**
African American	1 (4.0%)	3 (6.7%)	0	2 (13.3%)
Asian	1 (4.0%)	3 (6.7%)	0	0
Latino	5 (19.0%)	12 (26.7%)	1 (9.1%)	0
Other Race	2 (7.4%)	4 (8.9%)	1 (9.1%)	0
Systolic BP (mmHg)	121.3 (15.7)	121.8 (13.7)	126.5 (15.3)	135.3 (15.6)**
Diastolic BP (mmHg)	73.3 (10.6)	73.3 (10.2)	74.1 (11.7)	76.9 (9.4)
Body Mass Index (kg/m2)	30.6 (7.9)	30.5 (8.1)	31.2 (7.1)	30.7 (7.7)
HDL-Cholesterol (mg/dL)	47.5 (14.0)	55.8 (16.2)	45.5 (20.6)	48.4 (17.5)
LDL-Cholesterol (mg/dL)	122.5 (30.5)	122.6 (38.5)	142.4 (37.7)	123.2 (28.5)
Triglyceride (mg/dL)	127.7 (64.0)	100.7 (43.9)	204.2 (171.7)	135.6 (85.7)
Fasting Glucose (mg/dL)	97.6 (17.8)	94.8 (17.5)	113.5 (38.5	93.5 (11.3)

5.3.2 Risk Level & Connectivity Between the PCC/Rsp & Hemispheric DMN ROIs

After controlling for age, FSIQ, and systolic blood pressure, there was no statistically significant interaction of ApoE4 and HSV-1 on the combined left hemisphere ROIs $F(4, 88) = .188$ $p = .94$; Wilks' $\Lambda = .992$; partial $\eta 2 = .008$, or right hemisphere ROIs $F(4, 88) = .929$, $p = 0.45$; Wilks' $\Lambda = .959$; partial $\eta 2 = .041$, nor were there any significant main effects.

5.3.3 Risk Level & Connectivity Between the PCC/Rsp & HC/PHC Formation

After controlling for age, FSIQ, and systolic blood pressure, there was not a statistically significant interaction of ApoE4 and HSV-1 on left HC/PHC connectivity $F(1, 91) = 0.603$, $p = .44$, or right HC/PHC connectivity, $F(1, 91) = .318$, $p = .57$, nor were there any significant main effects of either factor.

5.4 DISCUSSION

We predicted that increased risk for cognitive decline, as defined by ApoE4 status and HSV-1 seropositivity, would be associated with lower correlation in DLFFs between the DMN hub, the posterior cingulate/retrosplenial cortex (PCC/Rsp), and other DMN ROIs. In particular we believed that bilateral hippocampal formation and parahippocampi gyri (HC/PHC) would show the greatest dyssynchrony from the PCC/Rsp. This hypothesis was based on previous studies, including our own work, indicating that the hippocampi are more structurally vulnerable in ApoE4 carriers, and to HSV-1 infection (Burggren et al., 2008; Cohen et al., 2001; Filippini et al., 2009, 12; Gitelman et al., 2001; Gutiérrez-Galve et al., 2009; Plassman et al., 1997; Prasad et al., 2007; Wishart et al., 2006). Despite evidence that HSV-1 infection exacerbates the genetic risk for AD conferred by ApoE4, to date no studies have examined the interaction of the two factors on DMN connectivity.

Contrary to what we hypothesized, after controlling for age, FSIQ, and systolic blood pressure, there were no statistically significant interactions or main effects of ApoE4 and HSV-1 on connectivity to the PCC/Rsp within primary DMN regions (lateral parietal, medial prefrontal, superior frontal, and inferior temporal cortices), or between bilateral HC/PHC connectivity to the PCC/Rsp.

Anatomically the DMN consists of a primary network involving posterior cingulate/retrosplenial cortex (PCC/Rsp), bilateral inferior parietal lobules (IPL), ventral and dorsal medial prefrontal cortex (V/D MPFC), and bilateral temporal lobes (LTC) (Buckner et al., 2008). The PCC/Rsp provides the primary hub and is densely interconnected with other regions in the network. A secondary network consisting of bilateral hippocampi (HC) and parahippocampal cortex (PHC) connects to the first primarily through the PCC/Rsp hub (Buckner et al., 2008). Connectivity between regions is measured as correlations of distributed low frequency fluctuations (DLFFs) of neuronal activity (Damoiseaux et al., 2008). Under normal physiologic conditions DLFFs across the DMN are consistent and highly synchronous but become dyssynchronous in the course of normal aging, and as healthy aging progresses through mild cognitive impairment (MCI) and onto AD (O'Sullivan et al., 2001; Zhang et al., 2010).

Our findings do not concur with others indicating that ApoE4 carriers are subject to vulnerabilities of brain connectivity not observed in non-carriers (O'Sullivan et al., 2001; Zhang et al., 2010). Specifically, we expected to see greater dyssynchrony, measured as reduced correlation, between the PCC/Rsp and other regions of the DMN, particularly the HC/PHC. Reduced correlation of DLFFs between the PCC/Rsp and the secondary HC/PHC network has been linked to memory impairment and increased beta amyloid deposition, a pathognomic feature of AD that is higher among ApoE4 carriers (Hedden et al., 2009). HSV-1 is known to co-occur in amyloid plaques where it may

further upregulate inflammatory response to β-amyloid (Kammerman et al., 2006). An emergent pattern found across studies of the DMN suggests that increasing amyloid burden fosters reduced correlation between the secondary HC/PHC network and the larger DMN, leading to lowered network cohesion and a worsening clinical picture (Hedden et al., 2009; O'sullivan et al., 2001; Zhang et al., 2010). The brain's immune response to non-self antigens such as virus and amyloid plaques results in the expression of cytokines and activated microglia, and it is this neuroinflammation that likely mediates the relationship between most risk factors and alterations in correlated DLFFs (Frautschy et al., 1998; Mcgeer, P., 2001; Mcgeer & Mcgeer, 1995; Wersching et al., 2010).

These findings formed the theoretical basis of our hypotheses and our methods for testing them. While we cannot be certain why we did not find support for what we hypothesized, one possibility is that the use of atlas-based ROIs resulted in poor overlap with regions of activation within our sample. Future directions could involve the use of graph theory to better capture local relationships within the DMN, as well as distal relationships between different anatomical sectors of the network, (Bullmore, E., & Sporns, O., 2009, 12; Wijk et al., 2010). Finally, longitudinal studies will be necessary to fully disconfirm the role of HSV-1 on the structural brain integrity of ApoE4 carriers. The identification of HSV-1 as a contributor to premorbid brain changes in individuals at risk for AD would indicate that suppression of HSV-1 early in life may present an important target for early intervention against future cognitive decline.

Chapter 6: General Discussion

6.0 Introduction

The identification of early, modifiable risk factors for cognitive decline presents the most promising opportunity for intervention, as current treatments offer only modest slowing of symptom progression. To date the most robustly replicated genetic risk factor for the most common form of dementia, Alzheimer's disease (AD), is the Apolipoprotein E4 (ApoE4) allele. Risk for sporadic AD increases nearly threefold for each E4 allele an individual carries (Corder et al., 1993). However some E4 carriers do not develop cognitive decline, and many non-E4 carriers do, highlighting the role of environmental variables in the progression to clinical symptoms. This study focused on herpes simplex-1 (HSV-1), a common neurotropic viral infection with affinity for the same brain structures affected in AD, as an acquired risk factor that may compound the genetic risk associated with ApoE4. Using a cross-sectional between-subject design we examined the interaction between the ApoE4 allele and HSV-1 in cognitively normal middle-aged adults using neuropsychological testing and structural and functional neuroimaging.

6.1 Review of Specific Aims and Findings

6.1.1 Specific Aim #1

Our first specific aim sought to determine if cognitive vulnerability in middle-aged ApoE4 carriers was exacerbated by HSV-1 infection. We compared cognitive performance in executive functioning, measured by Executive Domain score, and verbal memory, measured by Verbal Memory domain score. Domain scores represented the normalized, aggregated results of neuropsychological tests in each domain. Participants were grouped according to their risk for cognitive decline based on possession of an ApoE4 allele and infection with HSV-1. Based on previous studies of cognitive

performance in ApoE4 carriers (Baxter et al., 2003; Schultz et al., 2007) and HSV-1 positive clinical populations (Dickerson et al., 2003, 2004, 2008), we hypothesized that as cognitive risk increased, domain scores would decline, with greater declines in verbal memory compared to executive functioning.

6.1.2 Results of Specific Aim #1

After controlling for age, level of education, systolic blood pressure, and FSIQ, we found a significant interaction between ApoE4 and HSV-1 on Executive Function domain score. Specifically, ApoE4 positive individuals performed significantly worse on measures of executive functioning when they were infected with HSV-1. Contrary to what we hypothesized, there was not a significant interaction of HSV-1 and ApoE4 on Verbal Memory Domain score, nor were there any significant main effects. Post hoc analyses revealed that ApoE4 positive individuals had significantly higher FSIQ compared to ApoE4 negative individuals, and that HSV-1 positive individuals had significantly lower FSIQ (M = 109.3, SD = 15.6) compared to HSV-1 negative individuals.

6.1.3 Specific Aim #2

Our second specific aim sought to determine if brain vulnerability, defined as smaller hippocampal volumes and cortical thinning, was greater in ApoE4 carriers who were also infected with HSV-1. We compared hippocampal volumes and cortical thickness in six bilateral a priori regions of interest: entorhinal, fusiform, middle temporal, parahippocampal, posterior cingulate, and medial and lateral orbitofrontal cortices, between the four groups. These ROIs were chosen because they shared the most overlap between regions affected by AD and regions likely to be affected by HSV-1 (Burggren et al., 2008; Cohen et al., 2001; Filippini et al., 2009; Gitelman et al., 2001;

Gutierrez-Galvea et al., 2009). Participants were grouped according to their risk for cognitive decline based on possession of an ApoE4 allele and infection with HSV-1. We hypothesized that as risk increased, hippocampal volume and cortical thickness would decrease.

6.1.4 Results of Specific Aim #2

After controlling for age, level of education, systolic blood pressure, FSIQ and total intracranial volume, we found that ApoE4 carriers had significantly smaller hippocampal volumes bilaterally compared to non-carriers. There were no significant interactions of ApoE4 and HSV-1 on hippocampal volume, nor were there any significant main effects of HSV-1. There were also no statistically significant interactions or main effects of ApoE4 and HSV-1 on cortical thickness in either right or left hemisphere ROIs. The main effect of ApoE4 trended towards significance in left medial temporal cortex and left lateral orbitofrontal cortex.

6.1.5 Specific Aim #3

Our third specific aim sought to determine if aberrations in functional brain connectivity within the Default Mode Network (DMN) were greater in ApoE4 carriers who were also infected with HSV-1. We compared functional connectivity, measured as correlations between the PCC/Rsp (the DMN hub) and other DMN ROIs, between our four groups. Once again participants were grouped according to their risk for cognitive decline based on possession of an ApoE4 allele and infection with HSV-1. We hypothesized that as risk increased, correlated fluctuations of activity between the PCC/Rsp and other regions of the DMN would show greater alterations (in the form of either increased synchrony or dyssynchrony) compared to healthy controls. In particular,

we expected to see reduced correlation between distributed low frequency fluctuations (DLFFs) in the bilateral para/hippocampi and other regions of the DMN.

6.1.6 Results of Specific Aim #3

After controlling for age, FSIQ, and systolic blood pressure, we found no statistically significant interactions of ApoE4 and HSV-1 on primary DMN regions or on the secondary para/hippocampal segment of the DMN.

6.2 Summary of All Findings

Collectively we found that ApoE4 and HSV-1 interact to reduce executive functioning, and that they are each associated with differences in FSIQ (ApoE4 with higher IQ, HSV-1 with lower IQ). We also replicated findings from other studies by showing that ApoE4 is associated with smaller hippocampal volumes bilaterally. Unfortunately we did not find any significant effects of either ApoE4 or HSV-1 on functional connectivity between the PCC/Rsp hub of the DMN and other components of the network.

6.3 Interpretation of All Findings And Significance

At the start of this study we posed the following question: does HSV-1 infection increase risk for cognitive, structural, or functional brain changes in ApoE4 carriers, who already possess increased genetic vulnerability for declines in brain health associated with AD? Posing the question this way allowed us to take an increasingly fine-grained approach to answering it. This was important because our sample was middle-aged and cognitively normal, meaning that evidence of cognitive vulnerability was likely to be subtle, if detectable at all. We chose to examine a middle-aged sample for two reasons. First, and primarily, the identification of modifiable risk factors in middle age allows time for risk-mitigating intervention. Once clinical signs of cognitive decline emerge

there are no cures, and little in the way of treatment. Second, even though risk for cognitive decline begins in childhood, by middle-age enough risk factors have accumulated that it is possible to begin to tease them apart, or observe their interactions, and from this infer patterns of risk based on individual factors. Healthy individuals younger than middle-aged are unlikely to demonstrate enough definitive risk factors to make a well informed statement about their risk for developing dementia. Although our results did not always support our hypotheses, several significant findings nevertheless emerged.

First, we found that HSV-1 infection impaired executive functioning among ApoE4 carriers but not non-carriers. Executive function reflects the brain's ability to direct attention and inhibit responses; it is important to judgment, abstract reasoning, and decision making, all of which become impaired in more advanced cases of dementia. We also found that ApoE4 carriers had significantly higher FSIQs than non-carriers, suggesting greater active cognitive reserve in this group. Active cognitive reserve refers to acquired characteristics, e.g. higher FSIQ and educational attainment, that have been identified as protective because they allow for greater compensation and better preserved functioning following brain insults (see Stern, Y., 2009 for a comprehensive review of cognitive reserve). Passive reserve, on the other hand, refers to brain structure: larger, more densely interconnected brain are thought better able to compensate for insults than smaller, more sparsely interconnected brains.

Initially it appeared as though ApoE4 carriers had higher active cognitive reserve. This corresponded with other studies suggesting that the allele is associated with cognitive advantage early in life, followed by a more precipitous decline than non-carriers around age 60 (Baxter et al., 2003; Caselli et al., 2004 & 2009; Marchant et al., 2010; Mondadori et al., 2006; Puttonen et al., 2003). However, if this were true then

ApoE4 carriers should have been able to sustain their performance on tasks of executive function even if they contracted HSV-1. In fact, higher FSIQ appeared advantageous unless an ApoE4 carrier was also infected with HSV-1, at which point they lost their cognitive edge and executive function plummeted. This is aligned with other literature showing that even young ApoE4 carriers have worse outcomes following brain injury compared to non-carriers, indicating they are less able to compensate for their deficits (Friedman et al., 1999; Teasdale et al., 1997). Interestingly, we also found that HSV-1 carriers had significantly lower FSIQs than people without the virus, regardless of genetic status. Thus it may be that HSV-1 infection is enough to reduce a measure of global cognition in the majority of people. However, given the skewed demographics of our study it is difficult to make a clear inference from the data about this.

Among ApoE4 carriers we observed the same trend in verbal memory performance. Although it did not reach statistical significance, the verbal memory performance of HSV-1 positive ApoE4 carriers was worse than non-carriers ($p = .10$). Moreover, the significantly higher performance of ApoE4 carriers compared to non-carriers in executive functioning but not in verbal memory suggests a within-group decline in verbal memory relative to another vulnerable domain. It is possible that our sample was too young (Mean age = 48.9, ±6.6), to detect neuropsychological decline among E4 carriers, and still young enough to detect their neuropsychological advantage.

In summary, our neuropsychological findings support the notion that even with a substantial protective factor such as higher FSIQ, ApoE4 carriers are nonetheless susceptible to reduced performance on tasks of executive functioning if they become infected with HSV-1. With a larger sample it is likely that these findings would be replicated for verbal memory tasks. Our study adds to the existing body of research by

demonstrating that neuropsychological changes associated with HSV-1 infection are detectable in midlife in an otherwise healthy sample.

Secondly, using structural neuroimaging we found that ApoE4 carriers had significantly smaller hippocampal volumes bilaterally compared to non-carriers. This confirmed findings from other studies that the ApoE4 allele is linked to reduced hippocampal volume in cognitively healthy middle-aged adults, suggesting both that ApoE4 carriers demonstrate structural brain vulnerability detectable before the onset of cognitive decline, and that the hippocampus is uniquely vulnerable, compared to other brain regions, in individuals with genetic risk for AD (Burggren et al., 2008; Plassman et al., 1997; Wishart et al., 2006). Smaller hippocampal volumes are found consistently among ApoE4 carriers of all ages (Alexopoulos et al., 2011; Burggren et al., 2008; Cohen et al., 2001; Plassman et al., 1997; Shaw et al., 2007), suggesting that this group possesses lower passive cognitive reserve throughout the lifespan, a finding supported by our neuropsychological data. We also found borderline significant main effects of ApoE4 in left medial temporal cortex ($p = .06$) and left lateral orbitofrontal cortex ($p = .07$).

In addition to lower passive cognitive reserve, the ApoE4 allele is also associated with a pro-inflammatory immune profile posited as a source of cumulative brain insult (Frautschy et al., 1998; McGreer & McGreer, 2001). This unfortunate combination indicates that ApoE4 carriers are doubly disadvantaged in terms of long-term brain health. Susceptibility to the negative effects of inflammation was one of our motivations for choosing to look at the influence of pro-inflammatory HSV-1 on the structural integrity of ApoE4 brains. Although our study was able to detect differences in passive cognitive reserve, we failed to find any exacerbation of this condition related to HSV-1 infection. However, much of the damage attributable to inflammation occurs at a microscopic level not appreciable with structural imaging techniques (Dumanis et al.,

2009; O'Dwyer et al., 2012; Penzes et al., 2011; Persson et al., 2006). Moreover, AD is characterized by greater synaptic than neuronal loss, which is likely not visible as gross anatomical changes in middle-age. However, synaptic loss correlates with reduced functional connectivity between brain regions (Palop, Chin, & Mucke, 2006), which can be visualized using functional neuroimaging.

Thus, in our final analysis we approached our question from a functional vantage. Contrary to what we hypothesized, after controlling for age, FSIQ, and systolic blood pressure, there were no statistically significant interactions or main effects of ApoE4 and HSV-1 on DMN connectivity, nor between the DMN hub and the secondary para/hippocampal network. We were disappointed at these results but believe they stem more from methodological issues (e.g. the use of atlas-defined ROIs resulted in poor overlap with areas of obtained activation), and that the application of different analysis techniques may yet salvage some interesting results.

This study has several limitations that should be considered when interpreting the results. First, the largely Caucasian sample precludes generalization of the results to other ethnic groups. This is especially relevant when considering that HSV-1 incidence varies based on demographic variables and tends to be higher among non-whites and individuals with lower socioeconomic status (Fatahzedeh & Schwartz, 2007). Additionally, our sample had a high level of educational achievement and higher than average FSIQ, two measures of cognitive reserve that may have obscured some cognitive deficits. Future studies should examine a more representative sample in terms of ethnicity, educational attainment, and FSIQ. We also did not differentiate between subtypes (type 1 versus type 2) of HSV. Since HSV-1 is more prevalent than HSV-2 (Malkin, J. 2002, 12) it is likely that most subjects were infected with HSV-1. In addition, central nervous system infection (e.g. HSE) caused by HSV-2 is very rare in adults, therefore it is unlikely that

HSV-2 would cause cognitive changes. Further research is required to assess the impact of HSV subtype on risk for AD. Finally, longitudinal studies will be necessary to fully elucidate the role of HSV-1 on the structural and functional brain integrity of ApoE4 carriers. The positive identification of HSV-1 as a contributor to premorbid brain changes in individuals at risk for AD would indicate that suppression of HSV-1 early in life may present an important target for early intervention against future cognitive decline.

Appendices

Appendix A: Telephone Screening Form

Name:
Address:
Phone/Email:
Age: Height: Weight: BMI :
Medical Screening Questions
Have you ever been diagnosed with and/or treated for the following medical conditions:
Yes No Heart arrhythmia/ Heart murmur? – If yes, reject
Yes No Cardiac arrest/Heart attack? – If yes, reject
Yes No Coronary artery disease? – yes, reject
Yes No BP_____/_____ Do you have high blood pressure?
Yes No Diabetes? If yes: type 1 or type 2 ?
Yes No Kidney problems? – If yes, reject
Yes No Thyroid problems? – If yes, reject
Yes No Liver problems? – If yes, reject
Yes No Head injury including loss of consciousness >5min? – If yes, reject
Yes No Stroke? – If yes, reject
Yes No Epilepsy? – If yes, reject
Yes No Alcohol or drug abuse? – If yes, reject
Yes No Emotional problems, "such as anxiety, depression, ADHD…" – reject if: ADHD, Bipolar, OCD, personality disorders; if anxiety or depression – ok if not current
MRI Safety Screening Questions
Please indicate if you have any of the following:
Yes No Electronic implant or device
Yes No Magnetically-activated implant or device
Yes No Cardiac pacemaker
Yes No Any type of prosthesis
Yes No Any metallic fragment or foreign body
Yes No Surgical staples, clips, or metallic sutures
Yes No Joint replacement (hip, knee, etc.)
Yes No Bone/joint pin, screw, nail, wire, plate, etc.
Yes No Dentures or partial plates
Yes No Tattoo or permanent makeup
Yes No Body piercing jewelry – if removable, okay
Yes No Breathing disorder
Yes No Motion disorder or tremors
Yes No Claustrophobia
Yes No Other _____
Current Medications:

Subject approved: YES NO – if no, please shred immediately
Interviewer: _____ Date _____

Appendix B: Medical History Questionnaire

Participant #_____

Welcome to the Neural Consequences of Metabolic Syndrome Study

Thank you very much for your interest in our study! This is to confirm your study visit, which is scheduled for at am/pm. Your appointment will last approximately 2-3 hours.

Directions are provided in this mailing.

If you wear hearing aids or eyeglasses, please bring them with you to the appointment.

Please complete the enclosed questionnaire to the best of your ability and bring it with you to your appointment. The following questions are designed to provide us with information concerning your medical history.

Please answer the questions honestly and completely. All information will be kept confidential.

If it is necessary for you to change your appointment, please call The UT Clinical Neuroscience Laboratory at (512) 471-7926 at least 48 hours in advance.

Thank you for your cooperation, and we look forward to seeing you.

Please answer each question as honestly and accurately as possible, and bring this completed questionnaire with you to your appointment. Your answers will be kept strictly confidential.

Today's Date:_____/_____/_____

General Information
Age: Date of Birth: / / Sex (please circle): Male Female
Relationship Status (please circle):
Married Domestic Partnership Single Divorced Separated Widowed
Ethnicity (please circle): Hispanic/Latino Non-Hispanic/Non-Latino Unknown/Other
Race (please circle): American Indian Asian Pacific Islander African American
Caucasian More Than One Race Unknown/Other

Current Height:_____ Current Weight:_____
Women: Are you post-menopausal?_____
Mailing Address:_____
_____
Primary Telephone Number:_____ (please circle: home work cell)
Alternate Telephone Number:_____ (please circle: home work cell)

Emergency Contact Information
Name of closest relative or friend (for emergency contact):

Relationship to you: Telephone:

Physician Information
Primary Care Physician Name: _____ Telephone:_____
Primary Care Physician Address:_____
_____
Cardiologist Name:_____ Telephone:_____
Cardiologist Address:_____
_____

IMPORTANT: Do you experience adverse reactions to nitroglycerin?
(please circle) Yes No Unsure

Family History
Has someone in your immediate family (that is, your biological mother, father, brother, sister, or any of your children) experienced any of the following problems: heart attack, bypass, angioplasty/stent placement, dementia, hypertension BEFORE age 60?
YES or NO
If so, please indicate below all family members with problems. Also, be sure to indicate the age at which their heart problem began:

Educational Background
Primary/First Language: Secondary Language:
Years of Education: Highest Academic Degree:
Occupation:
(if retired, please indicate your occupation before retirement)
If you were not born in the USA, please indicate what year you moved to the USA_____

Current Medications
Please list current medications and dosages, use the back of the page if necessary:

Medication Type, Name of Medication, and Dose Last time taken?
Heart medicine
Blood pressure medicine
Cholesterol medicine
Thromboembolic disease medicine
Hypercoaguability medicine
Steroids
Hormones/HRT
Birth Control
Medicine for breathing/lungs
Insulin
Other medicine for diabetes
Arthritis medicine
Medicine for depression
Medicine for anxiety
Thyroid medicine

Medicine for ulcers
Allergy medicine
Pain killers (prescription or over-the-counter)
Dietary supplements (herbs, vitamins, etc)
Other (please specify)

Medical Problems
Have you ever had any of the following general medical problems?

Please Indicate Year of Onset for YES answers:

Angina (Chest pain) YES or NO
Arrhythmia YES or NO
Arthritis YES or NO What type?
Asthma YES or NO How is it treated?
Atrial fibrillation YES or NO
Autoimmune Disease YES or NO What type?
Blood Clots YES or NO Where? (e.g. lungs, legs)
Blood pressure YES or NO Was it too high or too low?
Cardiac Arrest/Heart Attack/Heart Failure YES or NO
Coronary Artery Disease YES or NO
Angioplasty/Bypass Surgery/Heart Valve Surgery YES or NO
Cancer YES or NO What type (e.g., prostate, colon)?
Cushing's Syndrome YES or NO
Diabetes YES or NO Circle: Type I (child onset) or Type II (adult onset)
Circle: Controlled by diet, oral medication, or insulin
Glaucoma YES or NO
Hepatitis YES or NO Circle: Hepatitis A, B, or C
High Cholesterol YES or NO
Kidney Problems YES or NO What type?
Liver Problems YES or NO What type?
Thyroid Problems YES or NO Hypothyroidism (underactive) or hyperthyroidism (overactive)
Viral Infection YES or NO What type? Date of last infection:

Have you ever had any of the following neurological problems?
Please Indicate Year of Onset for YES answers:
Head Injury YES or NO Was there a loss of consciousness?
For how long?
Brain hemorrhage YES or NO Please explain:
Stroke YES or NO Please explain:
Transient Ischemic Attack (TIA or Mini- Stroke) YES or NO Please explain:
Brain infection/meningitis YES or NO
Multiple Sclerosis YES or NO
Parkinson's disease YES or NO

Have you ever had any of the following additional problems?
Please Indicate Year of Onset for YES answers:

Anxiety YES or NO Have you undergone treatment?
Depression YES or NO Have you undergone treatment?
Schizophrenia YES or NO Have you undergone treatment?
Other Psychiatric Illness YES or NO Please specify:
Have you undergone treatment?
Alcohol/Drug Abuse YES or NO Have you undergone treatment?
Smoking YES or NO How many packs per day: If you quit, please indicate when:

Diet Information
In an average day do you (Please Circle):
Add salt to your cooking? YES or NO
Add salt at the table? YES or NO
Add salt before you have tasted your food? YES or NO
Try to reduce the amount of salt you use because of health reasons? YES or NO
Eat 3 or more fruits and vegetables? Note: fruit juice counts as only 1 serving, no matter how much you drink. (Please do not count potatoes as vegetables). YES or NO
Use whole milk or milk products instead of lowfat milk products? YES or NO
Eat at least 1 meal or snack containing fried foods (deep fried foods, chips, French fries)? YES or NO
Eat at least one meal containing red meat (beef, pork, or lamb)? YES or NO
Consume 2 or more sugar containing soft drinks (e.g. soda) or fruit juice? YES or NO
Consume 2 or more servings of sweets (e.g. desserts, candy, cookies, pastries, Pop Tarts, ice cream)? YES or NO
Consume pre-prepared foods (e.g. canned soups, frozen dinners)? YES or NO
Cook for yourself? YES or NO
Drink 2 or more alcoholic beverages? YES or NO
Skip breakfast? YES or NO

Exercise Information
These questions are about your physical activity in the last 7 days. These can be activities you do at work, at home or in your yard, to get from place to place, and in your spare time for recreation, exercise, or sport.
Vigorous activities refer to activities that take hard physical effort and make you breathe much harder that normal.
Moderate activities refer to activities that take moderate physical effort and make you breathe somewhat harder that normal.

1. During the last 7 days, on how many days did you do vigorous physical activities like heavy lifting, digging, aerobics, or fast bicycling?

_____ days per week

No vigorous physical activities: Skip to question 3

2. How much time did you usually spend doing vigorous physical activities on one of those days?

_____ hours _____ minutes per day

None

3. During the last 7 days, on how many days did you do moderate physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Think only about those physical activities you did for at least 10 minutes at a time. Do not include walking.

_____ days per week

No moderate physical activities: Skip to question 5

4. How much time did you usually spend doing moderate physical activities on one of those days?

_____ hours _____ minutes per day

5. During the last 7 days, on how many days did you walk for at least 10 minutes at a time? This includes at work and at home, walking to travel from place to place, and any other walking that you might do solely for recreation, sport, exercise, or leisure.

_____ days per week

No walking Skip to question 7

6. How much time did you usually spend walking on one of those days?

_____ hours _____ minutes per day

7. During the last 7 days, how much time did you spend sitting on a week day? Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

_____ hours _____ minutes per day

Sleep Information: Sleep Scale from the Medical Outcomes Study

1. How long did it usually take for you to fall asleep during the past 4 weeks? (Circle One)

0-15 minutes……..…..1

16-30 minutes……..2

31-45 minutes……..3

46-60 minutes……..4

More than 60 minutes……..5

2. On the average, how many hours did you sleep each night during the past 4 weeks?

Write in number of hours per night:

How often during the past 4 weeks did you… (Circle One Number On Each Line)
All of The Time = 1
Most of the Time = 2
A Good Bit of the Time = 3
Some of the Time = 4
A Little of the Time = 5
None of the Time = 6

3. Feel that your sleep was not quiet (moving restlessly, feeling tense, speaking, etc., while sleeping)?
1 2 3 4 5 6

4. Get enough sleep to feel rested upon waking in the morning?
1 2 3 4 5 6

5. Awaken short of breath or with a headache?
1 2 3 4 5 6

6. Feel drowsy or sleepy during the day?
1 2 3 4 5 6

7. Have trouble falling asleep?
1 2 3 4 5 6

8. Awaken during your sleep time and have trouble falling asleep again?
1 2 3 4 5 6

9. Have trouble staying awake during the day?
1 2 3 4 5 6

10. snore during your sleep?
1 2 3 4 5 6

11. Take naps (5 minutes or longer) during the day?
1 2 3 4 5 6

12. Get the amount of sleep you needed?
1 2 3 4 5 6

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