POTENTIAL BLOOD BIOLOGICAL MARKERS OF ALZHEIMER'S DISEASE

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POTENTIAL BLOOD BIOLOGICAL MARKERS OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) affects millions of older adults worldwide, and the prevalence and incidence are expected to grow at exponential rates in coming years. Identifying potential targets for intervention to delay onset, slow progression, or effectively treat AD is of extreme public health importance. Better understanding blood biomarkers of AD is one means of identifying such targets. This dissertation investigates potential blood biomarkers of AD, including plasma amyloid beta 42 (Aβ42) and Aβ40, and inflammatory markers interleukin 6 (IL-6), IL-6 soluble receptor (IL-6 sR), and C-reactive protein (CRP).

Measurement of Aβ42 and Aβ40 in cerebrospinal fluid has been used to identify people with AD, and distinguish from other types of dementia. In plasma, the ratio Aβ42/Aβ40 has emerged as a potentially useful biomarker of AD. However, correlates of plasma Aβ40 and Aβ42 are unknown. We investigated the demographic and medical correlates of plasma Aβ40 and Aβ42. We found that in community-dwelling, older adults, plasma Aβ40 and Aβ42 were significantly associated with age, race, sex, education, Apolipoprotein E (APOE) genotype, and serum creatinine.

Due to the inflammatory state associated with AD pathology, and with risk factors of AD, inflammatory markers are potentially useful biomarkers of AD. Previous studies have focused on measuring inflammatory markers at one time point; this has major limitations due to the non-specific nature of inflammatory markers, and the large number of inflammation triggers. We

studied how change in inflammation over time predicts risk of dementia and cognitive decline. In a cohort of oldest old women, we found that high IL-6 sR at two time points, or transitioning to from low to high reduced the risk of developing dementia. Furthermore, in a cohort of older, black and white men and women, we found that extreme variability in CRP over time was associated with increased risk for cognitive decline over 10 years. This association was stronger among women and among those with no APOE e4 allele. We believe these results not only indicate the complexity of the immune system in older adults, but also suggest the role of vascular disease in the development of cognitive decline. New evidence suggesting that vascular disease is also related to Aβ deposition suggests that the relationships we found may hold for vascular dementia and AD. Future research should investigate how all of these markers together relate to cognitive function, as well as to AD and vascular dementia pathologies, such as amyloid deposition and white matter hyperintensities.

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1.0 INTRODUCTION

All cause dementia is classified by the National Institute on Aging and the Alzheimer's Association (NIA-AA) with several criteria (2011).(McKhann et al., 2011) The criteria include cognitive or behavioral symptoms that: 1. interfere with normal function at work or home; 2. Represent a decline from a previous level of functioning or performing; and 3. Are not explained by delirium or another psychiatric disorder.(McKhann et al., 2011) Furthermore, these criteria state that cognitive impairment should be detected or diagnosed through several steps, such as history taking from the patient and a knowledgeable informant or caregiver, and objective assessment of cognitive function either with a "bedside" mental status examination or neuropsychological testing.(McKhann et al., 2011) For a diagnosis of all-cause dementia, the impairment must involve a minimum of two of the following domains: 1. Memory – specifically the ability to acquire and recall new information; 2. Reasoning, consisting of the handling of complex tasks, poor judgment and poor decision making; 3. Visuospatial ability, such as recognition of faces or identifying common objects; 4. Language, including speaking, reading and writing; and 5. Personality, also described as behavior or mood, and encompassing things such as mood swings, increased agitation, apathy, and socially unacceptable behaviors.(McKhann et al., 2011) It should be noted that these criteria are very similar to the newly proposed Diagnostic and Statistical Manual of Mental Disorders (DSM) 5 criteria for a major neurocognitive disorder, which include: a substantial decline in cognitive function from a

previous level in one or more cognitive domains, cognitive deficits severe enough to interfere with independent functioning, and not primarily attributable to delirium or some other psychiatric disorder.(American Psychiatric Association, 2012) The NIA-AA also provides criteria for the Alzheimer's disease (AD) type of dementia, and specify distinct criteria for probable AD, possible AD, and probable or possible AD with evidence of AD pathophysiology.(McKhann et al., 2011) Specifically, the criteria for probable AD include the previously described dementia criteria in addition to: 1. Gradual onset on the order of months to years; 2. An obvious history of worsening cognitive function documented either by observation or report; and 3. Having cognitive deficits that are either amnestic in nature, including impaired learning and memory, or if they are non-amnestic in nature include language deficits such as word finding, visuospatial deficits such as impaired object recognition, and/or executive deficits such as impaired judgment or reasoning.(McKhann et al., 2011) These criteria further state that probable AD should not be diagnosed if there is evidence of cerebrovascular disease.(McKhann et al., 2011) Classification of possible AD is similar, but can include a rapid onset, or be used when there is a lack of information of the patient's history; possible AD can also include a mixed presentation of AD-type symptoms with cerebrovascular disease, dementia with Lewy bodies, or dementia with other medical comorbidities that may have an effect on cognitive function.(McKhann et al., 2011) Finally, for a diagnosis of probable AD with evidence of AD pathophysiological changes, the NIA-AA criteria call for measures of low cerebrospinal fluid (CSF) amyloid beta (Aβ) 42, or positive positron emission tomography (PET) amyloid imaging, or elevated CSF total tau or phosphorylated tau (p-tau).(McKhann et al., 2011) However, it should be noted that the NIA-AA does not call for routine diagnoses to include a check of these biomarkers at this time.(McKhann et al., 2011) The proposed DSM-5 has similar diagnostic

criteria for a neurocognitive disorder due to Alzheimer's disease.(American Psychiatric Association, 2012) DSM-5 criteria states a diagnosis should include evidence of a major or minor neurocognitive disorder, gradual progression or worsening in one or more cognitive domains, an early and prominent feature of impaired memory, and having these symptoms not better accounted for by other conditions, such as cerebrovascular disease, Lewy body disease, Parkinson's disease, fronto-temporal lobar degeneration, or another neurologic or systemic illness.(American Psychiatric Association, 2012) In the brain, Alzheimer's disease is classified primarily by the formation of amyloid plaques and tau neurofibrillary tangles (NFT).(Maarouf et al., 2011) Other pathological changes that occur in the brain include an overall decrease in the number of synapses, neuronal death, loss of myelin sheath on neurons, brain atrophy, and proliferation of astrocytes in the brain.(Maarouf et al., 2011)

Clinical diagnoses of AD often differ across settings. For example, in research settings, a diagnosis may include assessment of medical and family history, determining changes in cognitive function or behavior (from previous visits, or from self or proxy reports), obtaining a caregiver or family report on changes in cognitive functioning or functioning in daily tasks, neuropsychological testing, and a physical and neurological examination.(Alzheimer's Association, 2011) Additionally, sometimes structural or functional magnetic resonance imaging (MRI/fMRI) is used to detect brain atrophy or changes in blood flow, PET scans are conducted for amyloid imaging, or single-photon emission computed tomography (SPECT) is used to distinguish AD from other types of dementia, specifically, dementia with Lewy bodies.(Barber, 2010; Hampel et al., 2010; McKeith et al., 2004) Conversely, in primary care settings, up until 2011, there were no universally recommended or accepted guidelines for systematic screening of dementia, nor a universally recommended tool/assessment for detection or diagnosis.(Fowler et

al., 2012) Several organizations, such as the American Geriatrics Society (AGS) or the American Academy of Neurology (AAN), provide guidelines or steps that a physician may use; it is up to providers, however, to choose to follow such guidelines. Beginning in January 2011, the Centers for Medicare and Medicaid Services began covering the costs of annual wellness visits for Medicare beneficiaries, at which primary care physicians (in order to receive payment for the visit) must focus on preventive care by assessing 9 components or check list items during the visit, including screening for cognitive impairment.(Boustani, Peterson, Hanson, Harris, & Lohr, 2003; Department of Health and Human Services Centers for Medicare and Medicaid Services, 2010) In spite of this, there is still no recommended screening tool; rather it is merely stated that assessment of cognitive function must be performed by direct observation of the patient, while considering information from patient self-report, and concerns raised by family members or caregivers, which has limitations.(Department of Health and Human Services Centers for Medicare and Medicaid Services, 2010) In a study conducted after these wellness visits were mandated, investigators showed that patients and caregivers were both unreliable in accurately reporting cognitive function to a physician, and called for objective screening tests for dementia.(Clionsky & Clionsky, 2011) Furthermore, some feel this new mandate may not be useful until the potential benefits of screening (i.e. an effective treatment) outweigh the potential harms (i.e. depression associated with a diagnosis, loss of driver's license, etc.).(Brayne, Fox, & Boustani, 2007; Fowler et al., 2012) In addition to these concerns, which could very well influence a physician, and his/her willingness to diagnose someone with mild cognitive impairment (MCI) or dementia, there are other factors may also affect diagnosing in a non-specialized setting. For example, physicians often face time constraints in general wellness visits, and other "check-list" items that take priority over examining cognitive status. Barriers to

diagnosis in a non-specialized setting also occur on the patient end, including stigmatizing or fearful beliefs regarding a dementia diagnosis, inability to get to a doctor due to geographic or physical conditions, and lack of awareness of cognitive decline. Other limitations to clinical diagnoses in both specialized and non-specialized settings include a wide range of clinical presentations because of different brain regions affected, difficulty distinguishing between different types of dementia, and cognitive problems that could be induced by other neurological conditions or medications.(Alzheimer's Association, 2011; Castellani, Rolston, & Smith, 2010) Diagnosing dementia may be even further complicated by cognitive reserve which is the term often used to describe when older adults have a high degree of AD-type pathology, but in spite of the pathology have reasonably in-tact cognitive function.(Scarmeas & Stern, 2004; Stern, 2002, 2009) The cognitive reserve hypothesis proposes that some people with a high degree of brain pathology may show no apparent cognitive impairment due to an ability to compensate for pathology or deficits; the ability to compensate is thought to stem from a combination of advantageous lifestyle factors, such as higher education or literacy, and neural factors like greater brain volume or greater brain plasticity.(Scarmeas & Stern, 2004; Stern, 2002) While difficult to quantify, studies have previously used education and literacy as surrogate markers of cognitive reserve.(Bennett et al., 2003; Kemppainen et al., 2008; Rentz et al., 2010; Yaffe, Weston, et al., 2011)

In the literature, AD is often distinguished from other types of dementia, such as vascular dementia, dementia with Lewy bodies, frontotemporal dementia or dementia from multiple causes. However, in reality these distinctions are often cloudy due to a wide-range of methods used for determining cognitive status and dementia diagnosis in research studies; some specialized clinics like the Alzheimer’s Disease Research Centers (ADRCs) may have great

ability to diagnosis, but some epidemiological studies may rely on self-report of previous diagnosis from a physician or on neuropsychological testing. Furthermore, different types of dementia may not have such distinctive boundaries; cerebrovascular disease often contributes to many different types of dementia, including AD.(Gorelick et al., 2011) Finally, while imaging and CSF biomarkers are greatly improving the ability to diagnose dementia and AD, especially in specialized research settings, the only definitive diagnosis of AD is a post-mortem histologic examination of the brain.(Castellani et al., 2010; Knopman et al., 2001) Thus, in many settings, researchers and clinicians are forced to classify people in spite of a clear lack of a biological marker that can reliably and validly diagnose dementia patients, and distinguish AD from other types of dementia.(Castellani et al., 2010)

Mild cognitive impairment (MCI) is increasingly recognized as a state of cognitive impairment somewhere on the continuum between normal cognitive function and dementia.(R.C. Petersen & O'Brien, 2006) While not all people with MCI progress to dementia, it is estimated 5 to 15% of people with MCI will progress to dementia every year.(Ganguli, Dodge, Shen, & DeKosky, 2004; R.C. Petersen et al., 1999) MCI has been widely described according to two criteria.(R. C. Petersen, 2004; Winblad et al., 2004) The Petersen criteria include: 1. memory complaint, ideally reported by subject and informant; 2. Objectively determined impaired cognitive function for age, either determined from a previous measure or compared to norms (specific to age, education, occupation); 3. Relatively in-tact physical function (activities of daily living [ADLs] and instrumental activities of daily living [IADLs]); and 4. Not demented.(R. C. Petersen, 2004) In spite of these criteria, recent studies have suggested that people with MCI may, in fact, be experiencing some mild loss in function.(Weston, Barton, Lesselyong, & Yaffe, 2011) Furthermore, there are several different subtypes, according to the Petersen criteria: 1.

Amnestic – impaired cognition with only memory complaints; 2. Multi-domain amnestic – impaired cognition with memory complaints, plus complaints in at least one other domain (i.e. language, executive function, visual spatial skills); 3. Multi-domain non-amnestic – impaired cognition with multiple domains affected, but memory not being one of them; and 4. Single domain non-amnestic – impaired cognition with only one, non-memory domain affected (i.e. language, executive function, visual spatial skills).(R. C. Petersen, 2004) The Winblad criteria for MCI are: 1. The person being neither normal nor demented; 2. Evidence of cognitive deterioration based on one of several standards – either objective measures over time and/or subjective report of decline by self and/or informant in conjunction with objective cognitive deficits; and 3. Activities of daily living are preserved and complex instrumental functions are either intact or minimally impaired.(Winblad et al., 2004) Similar to the Petersen criteria, there are several different subtypes of MCI, according to Winblad and colleagues: 1. Amnestic; 2. Single non-memory; or 3. Multiple cognitive domains.(Winblad et al., 2004) The Winblad criteria emphasize there is a lot of heterogeneity in both the etiology and progression.(Winblad et al., 2004)

1.1 EPIDEMIOLOGY

1.1.1 Prevalence and Incidence

An estimated 24.3 million older adults worldwide are currently living with dementia, with an estimated incidence of 4.6 million cases each year.(C. P. Ferri et al., 2005) Given these estimates, the predicted prevalence for the year 2020 is 42.3 million cases, and by the year 2040

is 81.1 million cases, unless something is done to slow the progression or delay the onset of dementia.(C. P. Ferri et al., 2005) According to estimates published in 2005, the number of people living with dementia in North America in 2001 was 3.4 million, with 2.9 million cases from the United States; the prevalence is predicted to approach 10 million in North America by 2040.(C. P. Ferri et al., 2005) Similarly, the prevalence in Western Europe in 2001 was nearly 5 million, with projections of 9.9 million by the year 2040.(C. P. Ferri et al., 2005) While studies investigating the prevalence of dementia for individual countries in Asia and Africa have been limited, it has been estimated that Africa had 1.5 million people with dementia, Latin America had 1.8 million, and Asia had 9.9 million in 2001.(C. P. Ferri et al., 2005) Furthermore, in spite of a greater number of dementia studies based in developed countries, it is predicted that it will be the developing regions of the world that experience the greatest proportionate growth of dementia in the coming years.(C. P. Ferri et al., 2005) For example, between 2001 and 2040, developed regions, such as North America and Europe will experience about 100% proportionate increase in dementia cases, compared to an estimated 235% to 393% proportionate increase in Latin America and Africa, and a 314% to 33% proportionate increase in India, China, southeast Asia, and Western-Pacific regions.(C. P. Ferri et al., 2005) These current and expected prevalence estimates and incidence rates are alarmingly high due to an exponential increase in the aging population caused by a combination of increased life expectancy and the occurring demographic transition.(Alzheimer's Association, 2011; Prince & Jackson, 2009)

1.1.2 Public Health Impact

In the United States, dementia is the sixth leading cause of death overall, and the fifth leading cause of death among adults 65 years of age and older.(Alzheimer's Association, 2011)

Worldwide, dementia is thought to cause an average of 7.4 years lived with a disability (YLD) or 11.9% of the total YLDs caused by any chronic disease.(Prince & Jackson, 2009) This is the second largest YLD attributed to chronic disease (second to blindness), and is greater than that caused by stroke, heart disease, diabetes and arthritis.(Prince & Jackson, 2009) Globally, dementia also accounts for 4.1% of Disability Adjusted Life Years (DALY).(Prince & Jackson, 2009) Dementia is thought to cost $315 billion US dollars annually in direct and indirect health care costs, and is the leading cause of dependency, or needing care, among older adults in many countries.(Prince & Jackson, 2009) In addition to the costs incurred by family members or friends who are responsible to the informal care, estimates suggest that 40 to 75% of all dementia caregivers seek later care for significant psychological illness and chronic disease, and have an increased risk for mortality, which contribute to the burden on health care systems.(Alzheimer's Association, 2011; Prince & Jackson, 2009) With the expected increase in dementia over the next 20 years and lack of any effective treatment, we can only expect the public health burden to also increase exponentially.

1.1.3 Risk Factors

One of the biggest risk factors for AD is increasing age.(Alzheimer's Association, 2011; Castellani et al., 2010; Prince & Jackson, 2009) Prevalence and incidence rates both increase rapidly as age increases in adults who are 65 years and older.(Alzheimer's Association, 2011; Castellani et al., 2010) Prevalence is thought to reach as high as 50% in adults who are 85 years of age and older.(Zhu & Sano, 2006) Being Hispanic or African-American are also risk factors for dementia.(Alzheimer's Association, 2011; Castellani et al., 2010) More women than men also have AD; in fact, almost two-thirds of all Americans living with AD are

women.(Alzheimer's Association, 2011) Approximately16% of women aged 71 years have AD, compared with 11% of men.(Alzheimer's Association, 2011) However, this difference is primarily due to the greater life expectancy of women.(Alzheimer's Association, 2011)

Education is another demographic factor that is thought modify the risk of AD; often coupled with education are occupational attainment and cognitive activity. High education and high occupational achievement are widely known to be associated with reduced risk for dementia.(Sattler, Toro, Schonknecht, & Schroder, 2012; Stern et al., 1994) It is thought that higher educational and occupational attainment lead to increased brain size, greater efficiency of neuronal networks, and an overall cognitive reserve.(Scarmeas & Stern, 2004; Stern, 2002; Stern et al., 1994) Staying or being mentally active also appears to be associated with reduced risk of AD.(Sattler et al., 2012) However, this may in fact just be representing a delay in the onset, as it has been suggested that among people with greater cognitive reserve, onset of dementia is delayed, but decline is more rapid once onset occurs.(Hall et al., 2007) Importantly, cognitive activity is a modifiable risk factor, and it has been shown that cognitive training in later life can improve cognitive function. For example, the Advanced Cognitive Training for Independent and Vital Elderly (ACTIVE) study investigated the effects of a 10-week cognitive training in 2,832 older adults, and found sustained improvements in memory, reasoning and processing speed at 2 and 5 years follow-up.(Ball et al., 2002; Willis et al., 2006)

A number of medical conditions are also associated with development of AD. Cardiovascular disease (CVD) risk factors such as hypertension(Kivipelto et al., 2001; Skoog et al., 1997) and high blood cholesterol(Kivipelto et al., 2002; Yaffe, Barrett-Connor, Lin, & Grady, 2002), as well as conditions that affect metabolic regulation such as obesity, diabetes, and impaired glucose tolerance(Akomolafe et al., 2006; Hildreth, Van Pelt, & Schwartz, 2012; Yaffe

et al., 2004) have all been shown to increase the risk of dementia and cognitive decline in older adults. Having hypertension at midlife has not only been associated with a significantly increased risk of developing dementia in prospective studies(Kivipelto et al., 2001) (Kivipelto et al., 2001; Prince & Jackson, 2009), but has also been associated with increased formation of neurofibrillary tangles (NFT) and plaques found at death.(Petrovitch et al., 2000) Late-life hypertension has also been associated with a greater number of amyloid plaques, and increased NFT formation.(Launer, White, Petrovitch, Ross, & Curb, 2001) Furthermore, a 15-year longitudinal study of hypertension in 70 to 85 year olds, showed that high blood pressure within the last 5 to 10 years was associated with increased risk of dementia.(Skoog et al., 1997) Similarly, high total cholesterol levels at both midlife and late life have been associated with increased risk for AD and cognitive decline.(Kivipelto et al., 2002; Solomon, Kivipelto, Wolozin, Zhou, & Whitmer, 2009; Yaffe et al., 2002) Disappointingly, several clinical trials investigating anti-hypertensive and cholesterol lowering medications on AD have largely pointed towards no significant treatment effect in late-life of statins and other anti-hypertensive drugs in reducing the risk of dementia.(Igase, Kohara, & Miki, 2012; McGuinness, Craig, Bullock, & Passmore, 2009) More long-term studies are needed to determine if treatment at midlife reduces the risk of dementia.

Diabetes mellitus and impaired glucose tolerance have also been associated with increased risk of MCI and AD.(Luchsinger et al., 2007; Whitmer, Karter, Yaffe, Quesenberry, & Selby, 2009; Yaffe et al., 2004) For example, in one longitudinal study, diabetes was associated with an increased risk of incident MCI (HR = 1.4, 95% CI: 1.1-1.8).(Luchsinger et al., 2007) In another prospective study of postmenopausal women, there were significant cross-sectional associations between impaired fasting glucose (IFG) and diabetes with poorer performance on

cognitive tests, even after adjustment for age.(Yaffe et al., 2004) Similarly, both IFG and diabetes were associated with greater cognitive decline over 4 years compared to women with normal glucose and with no diabetes, respectively (even after adjustment for age, treatment, race, education and depression).(Yaffe et al., 2004) Furthermore, among older adults with diabetes mellitus, at least one study has documented a dose-response relationship between the number of hypoglycemic episodes and increased risk for dementia.(Whitmer et al., 2009) There are several potential underlying mechanisms between diabetes and AD, including the possibility that insulin degrading enzymes play a role in the processing of Aβ in the body.(Farris et al., 2003) Dietary fat intake may also alter glucose metabolism, and at the same time increase the risk for cardiovascular disease, which through the previously described mechanisms may affect cognitive function.(Devore et al., 2009) Another potential mechanism is advanced glycation end products (AGE), which are created in a body via reactions between glucose and proteins, and increase in diabetes; AGE aggregates with several proteins found in AD pathology, including Aβ, tau proteins, and APOE.(Yaffe, Lindquist, et al., 2011) In spite of observational studies and plausible underlying mechanisms linking diabetes and dementia, results from clinical trials have not been promising. In recently reported results from the Memory in Diabetes, an ancillary study to the Action to Control Cardiovascular Risk in Diabetes study (ACCORD MIND), there was no significant effect of intensive glucose lowering on cognitive function over 24 and 40 months.(Launer et al., 2011) However, the ACCORD MIND study participants may have been too young to see significant effects on cognitive function, as the average age was only 62.5 years; furthermore 40 months may really be too short to see detectable changes in cognitive function.(Launer et al., 2011) Similarly, clinical trials investigating the effects of Rosiglitazone –

a treatment for type 2 diabetes - have found no effect on cognitive function.(Gold et al., 2010; Harrington et al., 2011)

Because of its increasing prevalence, obesity has also been a risk factor of great interest for dementia. One prospective study found that having a mid-life BMI ≥30 was associated with a 3-fold increase (HR=3.10; 95% CI: 2.19, 4.38) of AD in late life, and a BMI of ≥25 and <30 was associated with a 2-fold increase of AD in late life (HR=2.09; 95% CI: 1.69, 2.6) (even after adjustment for age, education, race, sex, hyperlipidemia, hypertension, diabetes, ischemic heart disease and stroke).(Whitmer, Gunderson, Quesenberry, Zhou, & Yaffe, 2007) In another longitudinal study, obesity was associated with an increased risk of AD in females (HR=2.23; 95% CI: 1.09-4.30).(Hayden et al., 2006) Several underlying mechanisms have been proposed for the association between obesity and AD, including increases in inflammation, changes in hormones that affect the brain, and increases in other risk factors of AD (i.e. hypertension).(Profenno, Porsteinsson, & Faraone, 2010) The metabolic syndrome, a collection of cardiovascular and metabolic risk factors, including abdominal obesity, high triglyercides, low HDL cholesterol, IFG and hypertension, has also been shown to increase the risk of AD and dementia.(Yaffe, Weston, Blackwell, & Krueger, 2009) Importantly, it has been suggested that the associated risk of AD resulting from all of these factors together is greater than the additive risk of individual factors.(Yaffe et al., 2009)

Closely related to metabolic and cardiovascular risk factors, physical inactivity has also been widely studied as a potential modifiable risk factor for dementia. For example, in one prospective study, women who reported regular physical activity at any time in their life had a significantly lower risk of cognitive impairment in late life, compared to women who reported no regular physical activity.(Middleton, Barnes, Lui, & Yaffe, 2010) In another longitudinal study

with an average of 21 years of follow-up, physical inactivity was associated with increased risk for AD, and this association was stronger among those with at least one APOE e4 allele.(Kivipelto et al., 2008) In fact, one recent study suggested that reduction of physical inactivity in the US may have one of the largest effects on reducing AD prevalence (when compared to other potential modifiable targets, such as reducing diabetes, mid-life hypertension and obesity, depression, smoking and cognitive inactivity).(Barnes & Yaffe, 2011)

Another behavioral risk factor for AD is cigarette smoking.(Anstey, von Sanden, Salim, & O'Kearney, 2007; Cataldo, Prochaska, & Glantz, 2010) In a meta-analysis of 19 prospective studies of older adults, all with one or more years of follow-up, smokers had an increased risk of AD (RR 1.79; 95% CI: 1.43, 2.23), and increased risk for cognitive decline over time, as measured by the Mini-Mental State Examination (MMSE).(Cataldo et al., 2010) Furthermore, this same study found that when comparing ever smokers versus current smokers, current smokers still had an increased risk of incident AD (RR 1.70; 95% CI: 1.25, 2.31).(Cataldo et al., 2010) Potential underlying mechanisms between smoking and increased risk of AD include increased inflammation in smokers, and increased risk for cardiovascular disease.(Anstey et al., 2007) Smoking is an important risk factor to consider because of its modifiable nature, and it has been estimated that if we could reduce the number of smokers by 10%, nearly 400,000 cases of AD could be prevented worldwide; if the reduction in smokers reached 25%, more than 1 million cases could be prevented.(Barnes & Yaffe, 2011)

Psychiatric conditions such as depression and posttraumatic stress disorder (PTSD) are other important risk factors for dementia. In older adults, depression and depressive symptoms have been associated with an increased risk of cognitive decline, dementia, and mild cognitive impairment (MCI).(Barnes, Alexopoulos, Lopez, Williamson, & Yaffe, 2006; Jorm, 2001)

While the mechanisms are not fully understood, perhaps it has to do with long term exposure to cortisol. For example, it has been shown that among AD patients with a lifetime history of depression, there was increased development of amyloid plaques and neurofibrillary tangles – two hallmarks of AD – in the hippocampus; it was proposed that a sustained history of cortisol exposure may damage the hippocampus, in turn making it more vulnerable to AD pathology.(Rapp et al., 2006) Furthermore, in a prospective cohort study of white and black community-dwelling older adults, those with low plasma Aβ42/Aβ40 and at least one APOE e4 allele had an increased risk for depression over 9 years; this relationship was not observed for those with low Aβ42/Aβ40 and no e4 allele.(A.L. Metti et al., 2012) PTSD is another important risk factor for dementia; it has been estimated that older adults with a history of PTSD are approximately 2 times more likely to develop incident dementia compared to those without PTSD.(Yaffe et al., 2010) Finally, although not a psychiatric disorder, traumatic brain injury (TBI) is another important risk factor for dementia, and because of its frequent occurrence in older adults, should be taken into consideration.(Lye & Shores, 2000; Thompson, McCormick, & Kagan, 2006) TBI accounts for nearly 80,000 emergency department visits each year in adults 65 years and older in the United States, with falls being the major cause of TBI.(Thompson et al., 2006) Both cross-sectional and prospective studies have found an association between TBI and increased risk of AD, with APOE e4 often being an important effect modifier.(Lye & Shores, 2000)

There are also several genetic factors that increase the risk of AD. APOE e4 is the most widely known genetic risk factor for AD.(Alzheimer's Association, 2011) While the underlying mechanisms are not completely clear, APOE is thought to play a role in the processing and clearance of Aβ and in the transport and processing of lipids, both of which could have an effect

on AD.(Alzheimer's Association, 2011) Having one APOE e4 allele is associated with three to five times greater risk of developing AD, and with an increased risk of developing the disease at an earlier age.(Alzheimer's Association, 2011; Ingelsson et al., 2003) Being homozygous for the e4 allele is associated with a 5 to 15-fold increase in the risk of AD.(Ingelsson et al., 2003) Having at least one APOE e4 allele may also increase the risk of progression from MCI to AD.(Elias-Sonnenschein, Viechtbauer, Ramakers, Verhey, & Visser, 2011) Furthermore, genetic mutations in the amyloid-beta precursor protein (APP), and presenilin 1 and 2 (PESN1 and PESN2) have been linked with early onset dementia(Cruts et al., 1998; Goate et al., 1991); recent studies have also provided evidence that rare APP, PESN1 and PESN2 mutations may also increase risk for late onset AD.(Cruchaga et al., 2012)

1.2 BIOMARKERS

Ultimately, the previously reported statistics point to a pressing need to improve a clinician's ability to accurately diagnose AD. Thus, we need to identify reliable biomarkers that can be assessed in a large number of older adults, in cost-effective manner, so that large numbers of older adults can be screened. Identifying biomarkers for AD is important for several other reasons. For example, with no current treatment for AD, it is important to identify those at risk for developing AD, so that the onset of the disease can be delayed and progression slowed. Biomarkers may be able to identify those who are at an increased risk for AD based on a specific profile of protein levels or a biochemical change in the body. If we are able to identify those at risk for developing AD, preventive strategies such as behavior modification (i.e. increasing physical activity, changing diet, increasing cognitive activity, medication management of other

diseases) may be effective in delaying the onset of the disease.(Barnes & Yaffe, 2011) One study suggested that if interventions were able to delay both the onset and progression of dementia by one year, up to 9.2 million cases could be prevented by the year 2050.(Brookmeyer, Johnson, Ziegler-Graham, & Arrighi, 2007) Furthermore, biomarkers may aid in understanding the physiological progression of the disease, or may help us understand which pathways in the body play the biggest role in disease progression.(Anoop, Singh, Jacob, & Maji, 2010; Teunissen, Verwey, Kester, van Uffelen, & Blankenstein, 2010) Better understanding the critical pathways or physiological changes in the body that lead to AD could shed some light on therapeutic strategies for preventing or even curing the disease. Clearly, identifying biomarkers for AD is going to be crucial in coming years.

An ideal biomarker for AD is described as having several characteristics, according to current consensus criteria proposed by the National Institute on Aging (NIA).(Frank et al., 2003; The Ronald and Nancy Reagan Research Institute of the Alzheimer's Association and the National Institute on Aging Working Group, 1998) Specifically, a biomarker for AD would: identify fundamental neuropathology of AD, and be validated in confirmed cases; have a sensitivity greater than or equal to 85% for detecting AD; be able to distinguish AD from other dementias with a specificity of at least 75%; be sensitive to effects of therapy that modified the disease progression; be reliable, reproducible, non-invasive, simple to perform, and inexpensive; and be confirmed by at least two independent studies with published results in peer-reviewed journals.(Frank et al., 2003; The Ronald and Nancy Reagan Research Institute of the Alzheimer's Association and the National Institute on Aging Working Group, 1998) In the current literature, potential biomarkers for AD typically come from several different sources: cerebrospinal fluid, blood plasma and blood serum; and neuroimaging markers, such as amyloid imaging.(Barber,

2010) The focus of my proposed research will be on blood plasma and serum biomarkers, and thus a bulk of this section will focus on these biomarkers. However, I will first briefly highlight the current state of CSF and neuroimaging biomarkers for AD.

1.2.1 Cerebrospinal Fluid

Studies investigating CSF biomarkers for AD have been conducted for at least 30 years.(Baxter, Baldwin, Pomara, & Brinkman, 1984) Unlink other bodily fluids, such as blood plasma or serum, CSF can reflect biochemical changes directly inside the brain because it fills the extracellular space within the brain; this direct contact with the brain has made CSF one of the most commonly investigated sources for biomarkers of AD.(Anoop et al., 2010) The most promising and widely investigated AD biomarkers from CSF have been the amyloid-beta 42 (Aβ42) protein and the tau protein – both total tau and phosphorylated tau (pTau).(Anoop et al., 2010; Blennow, Hampel, Weiner, & Zetterberg, 2010; Teunissen et al., 2010)

It has been shown that concentrations of total tau and pTau in CSF are elevated in patients with AD, when compared to healthy controls.(Andreasen et al., 1999; Formichi, Battisti, Radi, & Federico, 2006; Kurz et al., 1998) Recent studies have also shown that if people are categorized according to level of CSF tau (both total and pTau), those within the highest tertile of total tau and pTau had a faster rate of cognitive decline, less response to cholinesterase inhibitor treatment (which is used to treat symptoms of AD), and a higher mortality rate.(Wallin et al., 2009) One limitation to using CSF tau as a biomarker for AD is that it has low specificity – that is, it is unable to distinguish AD from other types of dementia (i.e. vascular dementia or dementia caused by traumatic brain injury).(Anoop et al., 2010) However, pTau has been shown to be more successful at distinguishing between AD and other dementias.(Formichi et al., 2006)

For example, total tau increases in CSF after ischemic stroke, but pTau does not; pTau also remains low in patients with frontotemporal dementia, but increases in patients with AD.(Hesse et al., 2001; Sjogren et al., 2000)

Similarly, a significant decrease in CSF Aβ42 has been documented in patients with AD, when compared to normal controls.(Formichi et al., 2006) It has been hypothesized that this decrease occurs in the CSF because Aβ42 is accumulating in the brain, and consequently leading to the development of the plaques found in AD patients.(Motter et al., 1995) In fact, in one autopsy study, investigators found that lower Aβ42 CSF levels were correlated with the neuropathological changes in AD, specifically with the development of plaques.(Strozyk, Blennow, White, & Launer, 2003) Other studies have extended the knowledge of the association between Aβ42 and AD by investigating the association in patient populations with less severe cognitive impairment. For example, in patients with MCI, those with the lowest level of CSF Aβ42 were more likely to progress to AD than MCI patients with the highest level.(Kester et al., 2009)

There are some limitations to using CSF Aβ42 as a biomarker of AD. Not all studies have supported the association between low CSF Aβ42 and AD. There have been reports of increased and unchanged levels of Aβ42 in AD patients.(Csernansky, Miller, McKeel, & Morris, 2002; Jensen et al., 1999) These differences could be due to different stages of the disease when Aβ42 was measured, different classification systems used to identify AD patients or differing lab assays to measure Aβ42. They could also be caused generally by different population characteristics which somehow modify the association between Aβ42 and AD. Thus, when using CSF Aβ42 as an indicator for AD, these intricacies and complications should be considered. Several strengths of CSF Aβ42should also be noted: it has been documented as one

of the most sensitive biomarkers for detecting prevalent AD(Shaw et al., 2009; Sunderland et al., 2003); and when used in combination with CSF tau, the result is a higher accuracy than either biomarker alone.(Tapiola et al., 2009)

There are also several limitations to using CSF biomarkers in general. For example, CSF is obtained through a lumbar puncture which is a highly invasive procedure that must be performed by a skilled medical professional.(Anoop et al., 2010) Another weakness of CSF is that the laboratory methods used to quantify biomarkers can be quite expensive.(Anoop et al., 2010) Finally, there have been reported limits on the reproducibility of a diagnosis using CSF because of varying laboratory techniques, and variations in collection, transportation and storage of CSF samples.(Anoop et al., 2010; Teunissen et al., 2010) There are also several strengths to the use of CSF biomarkers. As described earlier, CSF comes into direct contact with the brain, and thus might be the most reflective biomarker of neurochemical changes that occur directly in the brains of AD patients.(Baxter et al., 1984) The relatively high sensitivity and specificity of CSF biomarkers, especially when using Aβ42 and tau together, are another major benefit.(Tapiola et al., 2009)

1.2.2 Neuroimaging

Neuroimaging is useful for identifying pathological features of AD within the brain while the patient is still alive (as opposed to post-mortem autopsies of the brains). For example, imaging can be used to identify plaques, or to note structural changes of the hippocampus or overall neurodegneration.(Barber, 2010) There are four primary types of imaging that are used to detect changes in the brains of AD patients: PET scans, MRI, fMRI, and SPECT.(Barber, 2010; Hampel et al., 2010)

PET scans can be conducted using several different radioactive markers that identify different characteristics of the brain. Two such markers are PiB and amyvid, which are used to detect amyloid plaques in the brain.(Barber, 2010; Klunk et al., 2004) These markers bind to amyloid in the brain, and there is significantly higher retention AD patients, as detected by PET scans.(Barber, 2010; Klunk et al., 2004) Amyloid imaging has been widely validated for identifying amyloid build-up in the brain and for distinguishing AD patients from patients with other types of dementia.(Barber, 2010) Another PET marker known as F-2-fluoro-2-deoxy-D-glucose (FDG) detects changes in glucose metabolism within the brain which is a measure of neuronal activity, and thus in AD patients, retention of the FDG marker is greatly reduced.(Hampel et al., 2010)

One primary weakness of PET scans is that the tracing markers injected into patients are radioactive.(Barber, 2010; Hampel et al., 2010) In addition to the patient being exposed to radioactive material, these radioactive compounds are relatively unstable and have short half-lives, so there is a strict timeline required from the time the hospital or clinic acquires the compound to the time the patient must get scanned.(Barber, 2010; Hampel et al., 2010) Another potential weakness is PET scans require large scanning equipment, time, money, and a hospital or clinical setting where the scanner can be set up with staff to perform the scan and read the images, so PET scans have limited usefulness in population based settings. Identifying reliable and valid blood biomarkers is one potential way to alleviate this weakness because they could identify those at the highest risk for AD or those in later stages of progression who could then be referred for scanning. A strength of PET scans is that they are detecting a pathophysiological change within the brain, and used in combination with other diagnostic tools, such as cognitive tests, they can be very useful.(Barber, 2010; Hampel et al., 2010; Stern, 2009)

Structural MRIs are used to examine the size and integrity of brain structures; in the diagnosis of AD, they are used to assess overall cortical volume loss (atrophy), atrophy of the hippocampus, cortical thickness, and volume of the entorhinal cortex.(Barber, 2010; Hampel et al., 2010) A decrease in hippocampal and entorhinal cortex volume and decreased cortical thickness are all considered biomarkers for AD.(Barber, 2010; Hampel et al., 2010) Volumetric decreases of the hippocampus are the most widely accepted as an indicator for AD and have been well validated.(Barber, 2010; Hampel et al., 2010) Changes in hippocampal volume have also been shown to be reliably predictive of transitioning from MCI to AD.(Barber, 2010) The fMRI detects changes in blood flow in the brain, indicative of neuronal activity, and decreased neuronal activity is a marker of AD.(Greicius, Srivastava, Reiss, & Menon, 2004) Results of fMRI studies have shown that AD patients have significantly less neuronal activity than non-AD patients – both when they are asked to perform some type of mentally taxing activity and when they are just resting; these results have been replicated and well-validated.(Barber, 2010; Greicius et al., 2004; Hampel et al., 2010) Similar to fMRI, functional brain imaging using SPECT can be used to detect cerebral perfusion, and may be useful in distinguishing AD from other types of dementia.(Jagust et al., 2001; McKeith et al., 2004)

Using MRI imaging as an AD biomarker has several limitations, including requiring a lot of time and highly trained staff.(Barber, 2010) Thus, they are difficult to use for diagnostic purposes in a large, population based setting because of limitations on time, staff and money. Furthermore, they may not be useful early on in disease progression because of lack of major or detectable structural change; for this same reason, they are limited as a biomarker in that they may not be able to predict those at high risk. There may also be slight variations in different MRI machines based on calibrations and age of the machine, so if multiple measurements are made on

one person over time to detect a longitudinal change, the same machine would need to be used to increase reliability; this includes differences in magnet strength which is proportional to the resolution of the image.(Barber, 2010) Similar to PET scans, a benefit of MRIs is that they are detecting an actual structural change to the brain. A benefit of MRIs over PET scans is that they are less invasive to the patient because they use a set of magnets to produce an image of the brain, rather than injecting a radioactive substance into the participant. Finally, while direct cost-comparisons of MRI and PET scans in a hospital based setting for dementia were not available, a comparison for these two modalities for epilepsy has shown that MRIs might be slightly more cost-effective than PET scans.(DellaBadia, Bell, Keyes, Matthews, & Glazier, 2002)

1.2.3 Blood Plasma and Serum (see Appendix A)

1.2.3.1 Amyloid Beta

Similar to CSF results, a low level of plasma Aβ42 and a low Aβ42/Aβ40 ratio have been associated with increased risk of developing AD.(Graff-Radford et al., 2007; Lewczuk et al., 2009) Some studies have reported an association between high plasma Aβ40 and increased risk for AD or dementia, although these results have not been consistently found.(Graff-Radford et al., 2007; Lambert et al., 2009; van Oijen, Hofman, Soares, Koudstaal, & Breteler, 2006) In a recent prospective cohort study, we found an association between low plasma Aβ42/Aβ40 and increased cognitive decline over 9 years, and this association was modified by cognitive reserve, such that the association was stronger among those with less education, lower literacy, and with ≥1 APOE e4 allele.(Yaffe, Weston, et al., 2011) These results were similar to those from several prospective studies which reported an association between the Aβ42/Aβ40 ratio and dementia.(Graff-Radford et al., 2007; Lambert et al., 2009; van Oijen et al., 2006) Notably, two

of these studies used the Innogenetics INNO-BIA assay which is one of the newest, and arguably one of the most accurate methods of quantifying plasma Aβ.(Innogenetics, 2007; Lambert et al., 2009; Yaffe, Weston, et al., 2011) However, not all studies have found an association between plasma Aβ40, Aβ42 or Aβ42/Aβ40, and some have even found an association in the opposite direction.(Hansson et al., 2010; Lopez et al., 2008; R Mayeux et al., 1999) For example, in one prospective study of 169 individuals who were dementia free at baseline, among those who developed AD over a 3.5 year time period, baseline plasma Aβ42 was 60% ($p=0.001$) higher compared to those who did not develop AD; there was no statistically significant association with Aβ40.(R Mayeux et al., 1999) Similarly, the Aβ42/Aβ40 ratio was significantly higher at baseline among those who later developed AD ($p=0.04$).(R Mayeux et al., 1999) In other cross-sectional and prospective studies, there have been no reported associations between plasma Aβ42 and the Aβ42/Aβ40 level and AD.(Hansson et al., 2010; Lopez et al., 2008) Finally, a small number of studies have investigated the longitudinal association between plasma Aβ and cognitive decline or dementia, and have found decreasing levels of plasma Aβ42 or Aβ42/Aβ40 over time are associated with greater declines in cognitive function.(R. Mayeux et al., 2003; Okereke, Xia, Selkoe, & Grodstein, 2009; Pomara, Willoughby, Sidtis, & Mehta, 2005; Seppala et al., 2010)

Interpretation of these conflicting results may be due to different laboratory methods, heterogeneous study designs, differences in follow-up time, and small sample sizes in studies.(Graff-Radford et al., 2007; Hansson et al., 2010; Lewczuk et al., 2009; Lopez et al., 2008) Differences may also exist because of different stages of disease progression. Low Aβ42 or low Aβ42/Aβ40 may represent a decrease of plasma Aβ42 with concurrent formation of plaques, but if measured too early in the disease progression, a low level of Aβ42 may not be

detected. This is supported by mice studies which have shown CSF and plasma Aβ decline concurrently as amyloid deposits form in the brain.(Kawarabayashi et al., 2001) In humans, one study reported that plasma Aβ42 decreased as participants age, but Aβ40 remained stable, which also supports this theory.(Graff-Radford et al., 2007) Final supporting evidence comes from longitudinal studies which have found an association between decreasing levels of plasma Aβ42 and greater declines in cognitive function or increased risk in AD.(R. Mayeux et al., 2003; Okereke et al., 2009; Pomara et al., 2005; Seppala et al., 2010)

It is important to note that biological differences in each fragment (Aβ40 and Aβ42) may lead to differing associations with dementia and cognitive decline. Both Aβ40 and Aβ42 are proteolytic fragments of the 40-42 residues that are a product of APP.(Weggen et al., 2001) However, Aβ42 is the longer isoform, is more prone to aggregation, accrues earlier in the plaque formation process of AD because it more fibrillogenic(R Mayeux et al., 1999), and is found in greater quantities in amyloid plaques than Aβ40.(Gravina et al., 1995) Thus, Aβ42 is thought to contribute more to AD pathology than Aβ40 isoforms.

As discussed earlier, cognitive reserve may also have an effect on the association between plasma Aβ, and should be considered when determining the usefulness of potential biomarkers. For example, some individuals with extensive Aβ deposition as measured by autopsy or neuroimaging demonstrate little to no clinical symptoms of AD.(Bennett et al., 2006; Kemppainen et al., 2008; Rentz et al., 2010) Our recent findings provide support for the hypothesis that cognitive reserve modifies the association between plasma Aβ42/Aβ40 and dementia, and are also supported by several other studies.(Bennett et al., 2003; Rentz et al., 2010; Yaffe, Weston, et al., 2011)

There are several possible mechanisms underlying the association between plasma Aβ and dementia. First, plasma Aβ40 and Aβ42 levels could be directly linked to the development of amyloid plaques in the brain, with circulating levels changing as pathological processes of dementia begin. While amyloid proteins are made in the brain and CSF, it is also believed that peripheral Aβ contributes to plaque formation.(Deane, Bell, Sagare, & Zlokovic, 2009) Both Aβ40 and Aβ42 are thought to cross into the brain primarily via the receptor for advanced glycation end products (RAGE), and to be cleared from the brain via low-density lipoprotein receptors.(Deane et al., 2009) Interestingly, a recent Health ABC study found an association between high peripheral advanced glycation end products and increased cognitive decline.(Yaffe, Lindquist, et al., 2011) Other brain changes associated with AD have also been linked with circulating amyloid levels, and could underlie this association. For example, high plasma Aβ40/Aβ42 (consistent with low Aβ42/Aβ40) has been associated with amygdala atrophy, and decreased total brain volume.(Sun et al., 2010) Another potential mechanism through which peripheral amyloid may contribute to dementia is through subclinical cardiovascular disease. There is evidence suggesting plasma Aβ42, a vasoactive protein, is directly related to atherosclerotic risk factors, such as midlife blood pressure, total cholesterol and diabetes.(Blasko et al., 2011; Shah et al., 2012) Similarly, plasma Aβ has been shown to accumulate in microvessels outside of the brain, and in doing so to play an integral role in increasing vasoconstriction, and the development of vascular disease.(Blasko et al., 2011; Shah et al., 2012) Interestingly, while these brain changes are associated with increased risk of AD, they are also associated with increased risk of depression, which in itself is another possible mechanism underlying an association between plasma Aβ and AD.(Sun et al., 2010) A lifetime history of depression has also been associated with formation of amyloid plaques in the

hippocampus.(Rapp et al., 2006) Interestingly, low Aβ42/Aβ40 has also been associated with increased risk for depression among older adults in the Health ABC study with ≥1 APOE e4 allele.(A.L. Metti et al., 2012)

Plasma Aβ42 and Aβ42/Aβ40 are potentially useful biomarkers for several reasons. First, one of the criterion for being a useful AD biomarker is that it should reflect fundamental neuropathology of AD.(The Ronald and Nancy Reagan Research Institute of the Alzheimer's Association and the National Institute on Aging Working Group, 1998) As explained, amyloid plaques are a key pathological component of AD, and there are many underlying mechanisms linking plasma Aβ levels and the formation of plaques. A second key criterion for a useful AD biomarker is that it should be sensitive to the effects of therapy; as most current research on interventions for AD are focused on altering production and clearance of Aβ, plasma measures of these markers are particularly relevant.(Selkoe & Schenk, 2003; The Ronald and Nancy Reagan Research Institute of the Alzheimer's Association and the National Institute on Aging Working Group, 1998) Furthermore, when compared to imaging or obtaining CSF samples, drawing blood to measure plasma Aβ is relatively non-invasive, less expensive, and simpler to perform; all of these qualities lend this biomarker to be useful in large, population-based screenings. More studies are currently needed to investigate the sensitivity, specificity, and reliability of plasma Aβ42 and Aβ42/Aβ40. Furthermore, more longitudinal studies investigating how plasma Aβ40 and Aβ42 fluctuate at multiple stages of disease progression would be useful. Finally, in order to gain a better understanding of how plasma Aβ40 and Aβ42 contribute to the underlying pathophysiology of the brain, more studies are needed to determine how these markers change with other risk factors for dementia, such as demographic and medical characteristics. While it may be difficult to determine the progression of the disease, studies would benefit from having

very rigorous screening to ensure participants are dementia-free, and ideally free of neuropathology at baseline. Furthermore, consistent laboratory methods for quantifying Aβ would strengthen future research.

There are several weaknesses to the current literature investigating plasma Aβ and AD or cognitive decline, including the mixed results. Another weakness is that some studies have reported a large overlap between the distributions of Aβ42 in patients with and without cognitive impairment, so this biomarker may not be very sensitive.(Pomara et al., 2005) Finally, many of the studies conducted up to this point are of relatively small sample sizes, and are primarily cross-sectional in nature; these features make it impossible to interpret causality and generalize the results for a large, diverse population.

The role of Aβ antibodies as a potential biomarker of AD has also been of great interest lately with clinical trials investigating the potential use of Aβ immunization to reduce risk of AD.(Hock et al., 2002; Hock et al., 2003; Nicoll et al., 2003) Investigated first in transgenic mice, immunization of Aβ anti-body was found to significantly reduce amyloid plaque deposition and neuronal death.(Schenk et al., 1999) Following, studies in humans found that in AD patients, compared to normal controls, levels of serum and plasma Aβ antibodies are significantly lower.(Brettschneider et al., 2005; Moir et al., 2005) However, these results have not been consistent, and some studies have found no difference in Aβ antibody level between AD patients and normal controls.(Hyman et al., 2001) While the first clinical trial investigating Aβ immunization to treat patients with AD was terminated early due to inflammation in the CNS, some results from Aβ immunization in people have been published.(Hock et al., 2002; Hock et al., 2003; Moir et al., 2005; Nicoll et al., 2003) For example, one study found that among mildly to moderately demented patients (MMSE of 16-26) who had an immunoresponse

and post-immunization increases in Aβ antibody levels, compared to those who did not respond to the immunization, had decreased cognitive decline (p=0.008) and decreased functional decline (p=0.03) over 1 year.(Hock et al., 2003) Furthermore, only 16% of those who exhibited an immunoresponse progressed to severe dementia, compared to 67% of those who did not (p<0.01).(Hock et al., 2003) There are several potential underlying mechanisms that may explain this association. For example, it has been proposed that Aβ antibodies can cross the blood brain barrier (BBB), and higher levels in the periphery of cognitively normal older adults reflects the ability of Aβ to be cleared from the brain, reducing the risk of amyloid deposition and plaque development.(Moir et al., 2005) Others have hypothesized Aβ antibodies might not cross the BBB, but rather bind to Aβ42 and Aβ40 in the periphery, preventing those fragments from crossing the BBB and depositing in the brain.(Moir et al., 2005)

1.2.3.2 Inflammatory Markers

Several markers of inflammation evaluated in both blood plasma and serum may be potentially useful biomarkers, but results are still unclear. In terms of dementia and AD, the most widely investigated inflammatory markers have been C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-α). Individual studies have found that a high level CRP, IL-6, or TNF-α, is associated with increased risk of AD and cognitive decline.(Gorelick, 2010; Noble et al., 2010; Schneider, Hampel, & Buerger, 2009) In a prospective study of AD patients looking at both CRP and TNF-α with performance on a cognitive test over time, there was a significant baseline and longitudinal association between high TNF-α and poorer performance on the Alzheimer's Disease Assessment Scale–Cognitive subscale (ADAS-COG), but no significant association with CRP.(Holmes et al., 2009) However, while this study was prospective in

nature, less than a year passed between the time of the first and last cognitive assessment which may be too short to detect significant changes in cognitive status.(Holmes et al., 2009) Furthermore, these participants already had dementia at the time of measuring inflammatory markers, so they may reflect a result of the dementia process rather than a predecessor.(Holmes et al., 2009) In a previous Health, Aging and Body Composition (Health ABC) study, among those with the highest serum IL-6 or CRP tertile, 3MS scores were an average of 2 points lower at baseline, compared to those who were in the lowest IL-6 or CRP tertile ($p<0.001$ for both).(Yaffe et al., 2003) Furthermore, those in the highest IL-6 tertile had a 1 point decline over 2 years, compared to those in the lowest tertile ($p=0.01$), and the relationship over time was similar when comparing those in the high versus the low CRP tertile ($p=0.04$).(Yaffe et al., 2003) However, there were no significant differences at baseline or over time between TNF-α and cognitive function.(Yaffe et al., 2003) A strength of this study is that the older adults were highly functioning at baseline with no cognitive impairment, so an association between inflammation and cognitive impairment is more likely to indicate a cause of impairment, rather than a result; another strength is the fairly diverse population with approximately half men and women, and half blacks and whites.(Yaffe et al., 2003) Finally, a large number of potential confounders were assessed. However, this study is limited in that it was conducted when minimal follow-up visits were completed, so only a 2-year decline in cognitive function was assessed. As dementia is a disease that progresses over a long period of time, having more follow-up is critical in assessing the development of the disease; we have proposed a follow-up study which will be discussed later in this document. In the Rotterdam Study, high IL-6 and α1-antichymotrypsin (ACT) were significantly associated with an increased risk for dementia (rate ratio [RR] = 1.28, 95% confidence interval [CI]: 1.06, 1.55 and RR = 1.49, 95% CI: 1.23, 1.81,

respectively), and there was a trend for higher CRP to be associated with increased risk for dementia (RR = 1.12, 95% CI: 0.99, 1.25).(Engelhart et al., 2004b) There are several strengths to this study, including the complete dementia adjudication, and the large number of potential confounders assessed and adjusted for in analyses.(Engelhart et al., 2004b) Furthermore, a large number of potential confounders were assessed in both studies.(Engelhart et al., 2004b) However, similar to the Health ABC study, the Rotterdam study was limited in having only 2 years of follow-up visits completed; as dementia is a disease that progresses over a long period of time, having more follow-up is critical in assessing the development of the disease.

Several longitudinal studies have reported slightly longer follow-up when investigating the association between inflammation and dementia. In the MacArthur Study of Successful Aging, the association between plasma IL-6 and cognitive function was assessed longitudinally over follow-up periods of 2.5 and 7 years.(Weaver et al., 2002) Those in the high, compared to those in the low IL-6 tertile, were significantly more likely to experience cognitive decline over 2.5 years (OR=2.21; 95% CI: 1.44, 3.42), and over 7 years (OR=1.90; 95% CI: 1.14-3.18).(Weaver et al., 2002) These relationships were only significant when cognitive function was dichotomized, and not when a continuous measure of cognitive function was used.(Weaver et al., 2002) Investigators hypothesize that this could be due to the fact that inflammation is not related to cognitive performance over the full range of cognitive function (i.e. only associated with the worst outcomes, hence the significant dichotomous association).(Weaver et al., 2002) This makes sense because these large changes in cognitive function are also more clinically meaningful.(Weaver et al., 2002) A weakness to this study is that investigators measured inflammation at only one time point, so change in inflammation over time was not assessed.(Weaver et al., 2002) In the Framingham Study, with an average follow-up of 7 years

among participants, investigators reported a significant association between the highest tertile of TNF-α and an increased risk of developing AD (Hazards Ratio [HR] = 2.59, 95% CI: 1.09, 6.12), but not among several other inflammatory markers including IL-1, the IL-1 receptor antagonist, CRP, or IL-6.(Tan et al., 2007) This study was strengthened by having extensive measurement of covariates which were adjusted for in models. However, one weakness is that all of the cytokines, with the exception of CRP, were measured relatively close to the time of outcome adjudication, so again, there may not have been enough time for the development of dementia; while CRP was measured earlier than the other inflammatory markers, investigators found no significant association between CRP and increased risk of AD.(Tan et al., 2007)

In a recent meta-analysis of seven longitudinal studies, pooled hazards ratios were calculated to determine the association between peripheral inflammatory markers and risk of all-cause dementia or AD.(Koyama et al., 2012) With a total of 5,717 pooled participants, investigators reported a significant association between high CRP and increased risk for AD and all-cause dementia (HR=1.21, 95% CI: 1.03, 1.42 and HR=1.45, 95% CI: 1.10, 1.91, respectively).(Koyama et al., 2012) When looking at high IL-6 versus low IL-6, there was a significant increased risk for all-cause dementia (HR=1.32, 95% CI: 1.06, 1.64), but not for AD alone (HR=1.06, 95% CI: 0.83, 1.35).(Koyama et al., 2012) This finding highlights the possibility that inflammatory markers may be contributing to other types of dementia, such as vascular dementia, rather than just AD alone. Several cautionary notes should be made, however, when drawing this conclusion: 1. As stated previously, cerebrovascular disease often contributes to many different types of dementia, including AD(Gorelick et al., 2011); 2. AD is the most commonly diagnosed form of dementia(Castellani et al., 2010); and 3. It is difficult to

interpret from many studies what exact definition of dementia was used, and many causes are often grouped together.

While these results have been promising, some studies have found no association between AD and inflammation, or have found associations in the opposite direction. For example, in a prospective study of approximately 2,000 men, there was no significant association between IL-6 (HR: 0.66; 95% CI: 0.32-1.35) and CRP (HR: 0.79; 95% CI: 0.42-1.44) with risk of dementia.(Gallacher et al., 2010) One weakness to this study is that men were quite young (45-59 years) when inflammatory markers were measured, so the markers may not have been indicative of cognitive status years later when cognitive function was assessed (65 to 84 years of age), and some of these men may have still been too young for the onset of clinically detectable dementia symptoms.(Gallacher et al., 2010) Another cross-sectional study found that high IL-6 and IL-12 were associated with decreased processing speed and executive function, but not with other domains of cognitive function, such as memory, language, or spatial ability.(Troller et al., 2011) Again, this could indicate that inflammation is related to cognitive deficits generally or to all-cause dementia, but maybe not specifically to AD.

The role of inflammation in the oldest old (≥85 years of age) has also been less clear. For example, some studies have found that a higher level of CRP, IL-6 or TNF-α is associated with an increased risk for cognitive decline, but other studies have found no association between inflammation and cognitive decline or dementia.(Kravitz, Corrada, & Kawas, 2009b; Schram et al., 2007; van den Biggelaar et al., 2007) In a prospective study of oldest old adults (all 90 years and older) who had CRP measurements assessed at multiple time points over 4.5 years, there was no association between elevated CRP and all-cause dementia, even after stratifying by APOE e4 and gender.(Kravitz et al., 2009b) Interestingly, at least one study has reported a protective

effect of higher CRP on cognitive function in this age group.(Silverman et al., 2009) Finally, several studies have shown a significant interaction between APOE e4, inflammation, and cognitive function in this age group.(Kravitz et al., 2009b; Schram et al., 2007) With such a mix of results reported, it is very difficult to make any real conclusions about the role of inflammation in the oldest old. This is critical because clinical trials are currently investigating the potential role of anti-inflammatory drugs in AD patients.(ADAPT Research Group, 2007; Aisen et al., 2003; Relkin, 2008; Tsakanikas & Relkin, 2010) If there truly is a different association between inflammation and dementia in the young old, and the oldest old, understanding these differences will be crucial in interpreting results from these trials and in targeting therapeutic strategies to the oldest old.

There have been explanations proposed for why a difference may exist between younger old and oldest old adults. For example, genetic polymorphisms in cytokine genes may play a crucial role.(Dato et al., 2010) In a study of 1,651 Danish older adults born in 1905, visited in 1998 and again in 2008, there were borderline significant associations between single nucleotide polymorphisms (SNPs) of the TNFα, IL-10, IL-18 and IL-15 genes and increased risk for poorer cognitive performance on the MMSE.(Dato et al., 2010) This study was limited by being highly racially and ethnically homogenous, and by assessing cognitive function with only the MMSE.(Dato et al., 2010) Furthermore, an association between inflammatory cytokine gene polymorphisms and cognitive function in the oldest old has not been consistently reported.(Di Bona et al., 2009) Another potential explanation for the difference in the effects of inflammation among the young old versus the oldest old is antagonistic pleiotropy.(Silverman et al., 2009) This idea proposes that the effects of inflammation may be unfavorable at one time point, but favorable at another. A final potential explanation is selective survival where surviving oldest

old adults have some genetic predisposition to be healthier, or more resistant to negative influences such as high inflammation or brain pathology.(Silverman et al., 2009)

As discussed previously, one critical component of a successful biomarker for AD is that the biomarker in question should reflect to the etiologic process of AD.(The Ronald and Nancy Reagan Research Institute of the Alzheimer's Association and the National Institute on Aging Working Group, 1998) Thus, inflammatory markers are intriguing because they may, in fact, be large contributors to the underlying pathology and mechanisms of AD.(Neuroinflammation Working Group et al., 2000; Weuve, Ridker, Cook, Buring, & Grodstein, 2006) It has been hypothesized that inflammation is a precursor to the development of neurofibrillary tangles, amyloid plaques, and neurodegeneration – all hallmarks of AD.(Neuroinflammation Working Group et al., 2000) One weakness to this theory is that in molecular studies, it has been shown that fibrillar Aβ and neurofibrillary tangles directly activate the complement pathway, so inflammation may be a consequence rather than a cause of AD.(Neuroinflammation Working Group et al., 2000) Additionally, IL-6 is intimately involved in other pathophysiological processes of the nervous system, such as demyelination, which could contribute to white matter changes, and neurodegeneration.(Brunello et al., 2000; Neuroinflammation Working Group et al., 2000) If IL-6 production is up-regulated or induced by the general aging process or by other conditions that occur in aging, it is possible that these changes begin the pathological process of dementia. High levels of CRP, IL-6 and TNF-α, have also been associated with decreased total brain volume ($p<0.001$) in a group of approximately 1900 Framingham Offspring.(Jefferson et al., 2007) High serum CRP has also been associated with markers indicative of decreased neuronal function and decreased brain metabolism in middle-aged adults, suggesting it may play a role in cognitive decline or dementia in later life.(Eagan et al., 2012) Finally, in middle-aged

adults, higher levels of IL-6 have been shown to be related to decreased hippocampal volume – a structure critical in the formation of long-term memories, and associated with dementia.(Marsland, Gianaros, Abramowitch, Manuck, & Hariri, 2008)

Another way that inflammation could contribute to the underlying etiologic process of AD is through the development of other risk factors of dementia. For example, elevated IL-6 affects lipid metabolism and triglyceride production, and stimulates the hypothalamic-pituitary-adrenal axis, which is associated with central obesity, hypertension and insulin resistance; all of these factors are associated with increased risk for dementia.(Di Bona et al., 2009; Hildreth et al., 2012; Kivipelto et al., 2001) Higher levels of inflammation are also associated with cardiovascular disease, which is indeed a risk factor for dementia.(Ridker, Rifai, Rose, Buring, & Cook, 2002)

Another possible mechanism linking inflammation and dementia is depression. Depression is a major risk factor for dementia, and a number of studies have shown an association between higher peripheral circulating levels of IL-6, CRP and TNF-α with prevalent depressive symptoms and major depression in older adults.(Penninx et al., 2003; van den Biggelaar et al., 2007) One potential problem is that depression is often both a precursor and a result of dementia, so it is a possibility that results are confounding. In a prospective study of oldest old adults who were dementia and depression free at baseline, the association between circulating levels of CRP, IL-6, TNF-α and interleukin-1 (IL-1β), and development of depressive symptoms and cognitive impairment was investigated with the objective of trying to shed some light on the highly intertwined associations.(van den Biggelaar et al., 2007) Results indicated that depressive symptoms (Geriatric Depress Scale [GDS] scores) attenuated the relationship between MMSE and inflammation to a greater extent than cognitive function attenuated the association

between depressive symptoms and inflammation.(van den Biggelaar et al., 2007) This suggested that inflammation plays a larger role in the development of depressive symptoms.(van den Biggelaar et al., 2007) However, this association was limited to oldest old adults, and as we have previously discussed, there could be something unique about inflammation in this population.

Genetics may also underlie an association between inflammation and AD. For example, APOE e4 was investigated in a cross-sectional study comparing 206 middle-aged offspring with and without parental history of late-onset AD; those with a parental history of AD were significantly more likely to have at least one APOE e4 allele ($p<0.001$), and to have a higher ex-vivo circulating level of IL-1β ($p<0.001$), IL-6 (p-value=0.04) and TNF-α (p-value=0.008).(van Exel et al., 2009) However, when compared to those with no parental history of AD, those with a history were also significantly more likely to have higher systolic blood pressure and higher diastolic blood pressure which could independently affect inflammatory markers.(van Exel et al., 2009) Circulating levels of serum CRP have also been previously associated with the APOE e4 allele, where it was reported that 2% to 5% of the variability in circulating CRP may be due to APOE e4 allele status.(Chasman, Kozlowski, Zee, Kwiatkowski, & Ridker, 2006) Similarly, in a microarray study of immune and inflammation-related genes, investigators showed that there was significant up-regulation of immune or inflammatory-related gene expression in the aging brain.(Cribbs et al., 2012) Furthermore, in a recent genome-wide association study (GWAS) from the Religious Orders Study, investigators identified a variant (rs10808746) associated with the rate of cognitive decline, which influences two genes (PDE7A and MTFR1) that affect oxidative stress and inflammation; this supports the notion that inflammation is an important contributor to the underlying pathways of cognitive decline and dementia.(De Jager et al., 2012)

Similarly, in a GWAS study of cognitively normal older adults, another variant (rs17178006), influencing the MSRB3 gene, which plays a role in regulating oxidative stress, was associated with decreased hippocampal volume.(Bis et al., 2012)

Finally, infection or systemic inflammation may also underlie an association between inflammation and dementia.(Perry, Cunningham, & Holmes, 2007) It has been hypothesized that microglia, or the innate immune cells of the central nervous system, are changed in older adults after a lifetime of insults due to infection and acute inflammatory response.(Perry et al., 2007) This is important because it has been proposed that one way peripheral inflammation affects the brain is by inflammatory mediators in the blood communicating with macrophages, which lack a BBB, and then go on to communicate with microglia, which triggers an inflammatory response in the brain.(Perry et al., 2007) Thus, changes in the microglia associated with aging are quite pernicious because rather than triggering a protective or anti-inflammatory response in the brain, a heightened inflammatory response is initiated in the brain after infection or systemic inflammation occurs, which contributes to more neurodegeneration.(Perry et al., 2007)

Another criterion of a useful biomarker is sensitivity to the effects of therapy that modify the progression of AD.(The Ronald and Nancy Reagan Research Institute of the Alzheimer's Association and the National Institute on Aging Working Group, 1998) However, thus far, clinical trials with non-steroidal anti-inflammatory drugs (NSAIDs) have not been promising.(Fields, Drye, Vaidya, Lyketsos, & Group; Gorelick, 2010; Weggen et al., 2001) For example, in the AD Anti-inflammatory Prevention Trial (ADAPT, n=2528), there was no difference in risk of developing AD in celecoxib or naproxen sodium groups, compared to a placebo group after 3 years; similar results were found in the Alzheimer’s Disease Cooperative Study (ADCS, n=351) which found no difference in cognitive test scores in naproxen or

rofecoxib groups compared to a placebo group.(Fields et al.; Gorelick, 2010) It should be noted that in an extended 2-year follow-up of ADAPT participants, it was concluded that NSAIDs may have pleiotropic effects, such that when given to cognitively normal participants, NSAIDs reduced the risk of AD after 2 to 3 years of administration, but when given to AD participants, NSAIDs had unfavorable effects on disease pathology.(Breitner et al., 2011) However, in a recent comprehensive review of all randomized controlled trials of aspirin, NSAIDs, and steroids in the treatment of AD, investigators found no sufficient evidence that any of these treatments is effective in the treatment of AD.(Juturapatporn, Isaac, McCleery, & Tabet, 2012) It is possible that more studies are needed to follow up participants for longer time periods, as shown with the extended results from the ADAPT trial. It should also be considered that the timing of the anti-inflammatory administration in these trials was not ideal, and that if we had a clearer idea of when inflammation affected AD pathology, new trials administering anti-inflammatory drugs during this critical period would prove effective. Furthermore, it could be that special attention needs to be paid when classifying trial participants and analyzing data because if there is a pleiotropic effect at differing disease stages, this would weaken any reported associations when participants are misclassified. Finally, it is possible that more specific treatments targeted towards particular inflammatory markers that have been shown to have an effect on cognitive function (similar to anti-TNF drugs used in rheumatoid arthritis(Galloway et al., 2011)) would be more beneficial.

One major question that must be addressed is whether peripheral inflammatory markers reflect direct changes in the brain. It has been previously reported that cytokines readily cross the BBB, suggesting that peripheral measures do reflect direct brain changes.(Banks, Plotkin, & Kastin, 1995) Previous studies of mice have linked inflammatory response in the periphery with

inflammation in the brain, especially for IL-6, TNF-α and IL-1β.(Brebner, Hayley, Zacharko, Merali, & Anisman, 2000; Dantzer, 2001) For example, one study of mice investigated the effects of circulating system IL-6 and IL-6 sR levels on the CNS and BBB by comparing wild type with mice, single transgenic mice overexpressing IL-6 or IL-6 sR in the periphery, and double transgenic mice overexpressing both IL-6 and IL-6 sR.(Brunello et al., 2000) Both groups of single transgenic mice were free of neurological impairment, but the double-transgenic mice had considerable neurologic damage and a proliferation of astrocytes in the damaged areas.(Brunello et al., 2000) This suggests that both IL-6 and IL-6 sR are needed to cross the BBB.(Brunello et al., 2000) Previous studies have also shown TNF-α and IL-6 can be transported across the blood brain barrier via saturable systems.(Banks, Kastin, & Broadwell, 1995; Banks, Plotkin, et al., 1995) Ability to cross the BBB may differ by type of inflammatory marker (i.e. cytokines vs. their soluble receptors). In mice, binding of TNF-α to its soluble receptor completely inhibited travel across the BBB, but unbound TNF-α could cross the BBB.(Banks, Plotkin, et al., 1995) Furthermore, the soluble form of the receptor itself could not cross the BBB.(Banks, Plotkin, et al., 1995) Translated to humans, it could mean that having a higher level of soluble receptors circulating may ultimately lead to fewer inflammatory cytokines crossing the BBB because bound cytokines and unbound soluble receptors could not cross.(Banks, Plotkin, et al., 1995) Peripheral inflammatory markers may also set off a cascade of events, for example, up-regulating lipid mediators or communicating with peripheral macrophages, that ultimately leads to an inflammatory response in the brain.(Holmes & Butchart, 2011; Rosano, Marsland, & Gianaros, 2012) It has also been proposed that pro-inflammatory markers increase endothelial permeability, which could increase inflammatory markers crossing the BBB through post-inflammatory dysfunction of the BBB.(Rosano et al.,

2012) Thus, there seems to be evidence supporting that peripheral inflammatory markers can cross the BBB via multiple pathways, but as most studies thus far have been conducted in animals, more work is needed to determine how peripheral inflammatory markers are crossing the BBB in humans.

On the other hand, if inflammatory markers do not cross the BBB, there are other means through which a peripheral inflammatory response may affect inflammation in the brain. As described previously, altered microglia in older adults, which communicate with macrophages after systemic inflammation or injury, may lead to increased inflammatory cytokine production in the brain.(Perry et al., 2007) Another proposed pathway of peripheral inflammatory markers to influence inflammatory response in the brain is via the vagus nerve.(Rosano et al., 2012) Sensory nerve fibers in the vagus nerve, activated in the abdomen and chest by peripheral inflammatory markers, relay information very rapidly to the brain, triggering an inflammatory response in the brain.(Rosano et al., 2012)

An important limitation to understanding the association between inflammation and dementia is that it is not completely clear if the inflammation precedes the symptoms of AD, or if the inflammation is a result.(Neuroinflammation Working Group et al., 2000) Abnormal materials in the periphery are a common cause of inflammation, so it is not strange to consider that plaques and tangles in the brain are a cause of inflammation.(Neuroinflammation Working Group et al., 2000) Aβ and tau neurofibrillary tangles as well as neurodegeneration have all previously been shown to activate the complement pathway.(Neuroinflammation Working Group et al., 2000) Furthermore, the process of aging is associated with chronic, low-grade inflammation which makes it even more difficult to determine if, how and when inflammation may be important in the pathway of AD.(Neuroinflammation Working Group et al., 2000)

Other limitations to understanding the role of inflammatory markers in predicting dementia include relatively new and not well validated or standardized methodology across studies, and highly mixed epidemiological and trial results. Furthermore, many studies investigating these markers only measure inflammation at one time point,(Kravitz et al., 2009b; Schram et al., 2007; Silverman et al., 2009; van den Biggelaar et al., 2007) and it is extremely difficult to conclude if the inflammation measured is the result of some acute event, such as infection or injury, or is an indicator of a consistently elevated level of inflammation. Thus, more studies are needed to assess if change in inflammation over time is associated with dementia. Furthermore, the current gold standard of living biomarkers for distinguishing Alzheimer's disease from other types of dementia are cerebrospinal fluid-derived amyloid beta and tau(Anoop et al., 2010; Teunissen et al., 2010); if considered as useful biomarkers for dementia, peripheral inflammatory markers should be compared against this gold standard, and achieve a good predictive ability, either alone or in combination with other markers. It is unclear if these markers would be as useful at distinguishing AD, or perhaps some other subtype of dementia, but certainly more work is needed in this area.

In spite of these weaknesses, there are important strengths in using peripheral inflammatory markers as potential predictors of dementia. For example, the relative ease of collecting blood, making it a viable option for large, population-based epidemiological studies. Furthermore, methods used to quantify multiple inflammatory markers or inflammation-related genes, such as proteomics or microarray studies are emerging and may strengthen the current research. For example, one study using proteomics found that a panel of approximately 22 serum-derived proteins could be used to identify AD patients from normal controls with a sensitivity of 0.80 and a specificity of 0.91; interestingly a large number of these proteins were,

in fact, inflammatory markers.(O'Bryant et al., 2010) Similar studies have also reported panels of plasma-derived proteins that contain a number of inflammatory proteins and with fairly high accuracy can distinguish AD patients from normal controls.(Johnstone, Milward, Berretta, Moscato, & Alzheimer's Disease Neuroimaging, 2012; Ray et al., 2007) However, one study comparing CSF and plasma proteomes found that the CSF proteome was much better at distinguishing AD patients from normal controls, and in order for the plasma proteome to successfully distinguish these two groups, APOE and age had to be added to the models.(Britschgi et al., 2011)

1.2.4 Summary

Using serum and plasma inflammatory markers as biomarkers of AD has strengths and weaknesses. Weaknesses include relatively new and not well validated methods, highly mixed results in small cross-sectional studies, and the fact that markers taken from the periphery may never come into direct contact with the brain. Furthermore, many studies investigating these markers in both the young old and oldest old have been cross-sectional, or if longitudinal, only measure inflammation at one time point.(Kravitz et al., 2009b; Schram et al., 2007; Silverman et al., 2009; van den Biggelaar et al., 2007) Thus, more studies are needed to assess if change in inflammation over time is associated with dementia. Furthermore, many studies have focused on only one inflammatory marker at a time, rather than assessing the role of multiple markers together.(Kravitz et al., 2009b; Silverman et al., 2009) In spite of these weaknesses, there are important strengths in using plasma or serum which include the relative ease of collecting blood, making it a viable option for large, population-based epidemiological studies. Furthermore, methods used to quantify inflammatory markers in blood serum and plasma are less expensive

than those used to quantify CSF markers, and costs will likely decrease as technology becomes more available. Finally, the peripheral biomarkers discussed today all have biological plausible relationships with AD and with other risk factors for AD.

2.0 SPECIFIC AIMS

Plamsa amyloid beta and inflammatory markers need further investigation and validation as biomarkers of AD. We aim to overcome limitations of previous studies as outlined above, and to advance the knowledge of these proteins as potential biomarkers of AD.

2.1 PLASMA AMYLOID BETA

In spite ongoing research investigating the use of plasma Aβ40 and plasma Aβ42 as potential biomarkers of AD, dementia and cognitive decline, little is known about the general demographic and medical correlates of plasma Aβ40 and Aβ42, and how correlates may impact the relationship between plasma Aβ and cognitive decline. In order to fully understand these proteins as biomarkers, we must understand how the proteins relate to other diseases found in aging, and with demographic correlates, such as age, race and education. By improving our understanding of these relationships, not only will future studies be able to measure and account for all potential confounders, but it will also shed some light on the mechanisms underlying an association between plasma Aβ and cognitive function.

2.1.1 Specific Aim 1

We aim to identify and describe the demographic and medical correlates of plasma Aβ40 and Aβ42 in a population of community-dwelling, non-demented, black and white older adults, age 70-79 at baseline.

2.2 INFLAMMATORY MARKERS

As described previously, numerous studies have investigated the association between inflammatory markers and AD, dementia and cognitive decline due to the biological plausibility of inflammation contributing to the development of dementia via several potential pathways. However, most of these studies have focused on measuring inflammatory markers at only one time point which has major limitations, especially given the non-specific nature and fluctuation of inflammatory markers. Instead, it is likely that inflammation at one time point is not influencing the development of dementia, but rather how steep the slope of change of inflammation is, or how much variability a person has in inflammation over time. Furthermore, the association between inflammation and dementia appears to weaken with advancing age with studies showing inconsistent results in adults who are 85 years of age and older. Better understanding trajectories of change of inflammation, as well as how the strength of these associations change with age will not only improve our understanding of potential mechanisms underlying an association between inflammation and dementia, but also allow us to improve intervention and therapeutic strategies involving anti-inflammatory drugs.

2.2.1 Specific Aim 2

We aim to: 1) determine the association between IL-6, IL-6 sR and tumor necrosis factor soluble receptor 1 (STNF-R1) measured at an initial and an interim visit and subsequent cognitive status among oldest old women from the Study of Osteoporotic Fractures; and 2) to determine the association between change in each inflammatory marker and cognitive status.

2.2.2 Specific Aim 3

We aim to determine the association between the baseline level, slope of change, and variability of CRP and IL-6, measured at a minimum of three time points, and cognitive decline over ten years in the Health, Aging and Body Composition Study.

3.0 PAPER 1: THE DEMOGRAPHIC AND MEDICAL CORRELATES OF PLASMA AB40 AND AB42

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3.1 ABSTRACT

Plasma amyloid beta-42 (Aβ42) and Aβ42/Aβ40 are increasingly recognized as biomarkers for dementia, with low levels indicating increased risk. Little is known about the demographic and medical correlates of plasma Aβ40 or Aβ42. In 997 community-dwelling, non-demented older adults from the Health, Aging and Body Composition Study, we determined the cross-sectional association between a wide range of demographic and medical variables with Aβ40 and Aβ42. In multivariate stepwise linear regression models, Aβ40 was significantly associated with race (β=-14.70, F=22.01, p<0.0001), age (β=1.34, F=6.39, p=0.01), creatinine (β=52.91, F=151.77, p<0.0001), and serum brain-derived neurotrophic factor (BDNF) (β=-0.0004, F=7.34, p=0.007); Aβ42 was significantly associated with race (β=-3.72, F=30.83, p<0.0001), sex (β=1.39, F=4.32, p=0.04), education (β=1.50, F=4.78, p=0.03), Apolipoprotein E (APOE) e4 allele status (β=-2.82, F=16.57, p<0.0001), and creatinine (β=9.32, F=120.09, p<0.0001). These correlates should be considered as potential confounders in future studies investigating plasma Aβ as a biomarker of dementia. Understanding fully how these correlates mediate or modify the association between plasma Aβ and dementia will be a fundamental step in determining the biological pathways through which plasma Aβ40 and Aβ42 are associated with dementia, and in determining their full potential as biomarkers.

3.2 INTRODUCTION

Recent studies have indicated that plasma amyloid beta-40 (Aβ40), Aβ42 and the ratio (Aβ42/Aβ40) may be promising biological markers for cognitive impairment or dementia in older adults. For example, decreased plasma Aβ42 was shown to be associated with increased risk for developing Alzheimer's disease (AD) and increased cognitive decline over time.(Graff-Radford et al., 2007; Lewczuk et al., 2009; Pesaresi et al., 2006; Yaffe, Weston, et al., 2011) Similarly, a prospective longitudinal study found that low plasma Aβ40 was associated with incident AD in older men.(Sundelof et al., 2008) Low Aβ42/Aβ40 has also been associated with increased cognitive decline over time among non-demented elders, and with increased risk for AD.(Graff-Radford et al., 2007; Yaffe, Weston, et al., 2011) These two proteins are of interest because they accumulate in the brain, forming the plaques found in AD patients. Furthermore, these proteins have been well established as biomarkers for AD when measured from cerebrospinal fluid (CSF) samples. For example, CSF Aβ42 declines in patients with incident dementia, and has been documented as one of the most sensitive biomarkers for detecting prevalent AD.(Shaw et al., 2009; Sunderland et al., 2003) Similarly, some studies have shown that low CSF Aβ40 is associated with mild cognitive impairment (MCI) or the early stages of dementia.(Jensen et al., 1999) The possibility of identifying Aβ as a useful biomarker from plasma is clinically important because a simple blood draw is much less invasive and less expensive than the lumbar puncture required to obtain CSF.

In spite of this ongoing research, little is known about the general demographic and medical correlates of plasma Aβ40 and Aβ42, and how correlates may impact the relationship between plasma Aβ and cognitive decline. This information is important so these proteins can be

fully understood as biomarkers, and so future studies investigating the potential use of plasma Aβ40 and Aβ42 as biomarkers for cognitive decline can measure and account for all potential confounders. Furthermore, understanding what factors are related to plasma Aβ40 and Aβ42 may shed some light on the mechanisms underlying an association between plasma Aβ and cognitive function. Importantly, one previous study has investigated correlates of plasma Aβ, but the study population consisted of only 205 cognitively normal controls, while the remaining sample was comprised of patients with MCI (n=348) or AD (n=162).(Toledo et al., 2011) As previous studies have shown that plasma Aβ levels change at different stages of disease progression(Schupf et al., 2008; Seppala et al., 2010), more studies are needed to investigate correlates of these markers in community-dwelling, non-demented older adults. Thus, the objective of this study is to describe the demographic and medical correlates of plasma Aβ40 and Aβ42 in a population of community-dwelling, non-demented, black and white older adults, age 70-79 at baseline.

3.3 METHODS

3.3.1 Study Population

Community-dwelling white and black older adults were enrolled in the ongoing Health Aging and Body Composition (Health ABC) study. This prospective cohort study began in 1997, and adults ranged in age from 70 to 79 years at enrollment, and lived in Memphis, TN or Pittsburgh, PA. Participants were recruited from a random sample of Medicare eligible adults living within the designated zip codes, and were eligible if they reported no difficulties performing activities of daily living, walking a quarter mile, or climbing 10 steps without resting. They also had to be free of life-threatening cancers, and plan to remain within the study area for at least three years.(Yaffe et al., 2007) Plasma Aβ42 and Aβ40 were measured in a random sample of 1000 sex and race stratified participants. Of those, 3 had incomplete plasma amyloid data due to insufficient amounts of stored blood or measurement error; thus our analytic cohort was comprised of 997 participants. All participants included in this analytic cohort were free of cognitive impairment at baseline; consistent with previous literature, cognitive impairment was defined as a Modified Mini-Mental Status Exam (3MS) score <80.(Slinin et al., 2010) Compared to Health ABC participants who did not have plasma Aβ measured, those in this subgroup were more likely to be black, female, and to have lower mean education, but did not differ on other characteristics. This study was approved by the institutional review boards of the University of Pittsburgh and the University of Tennessee, Memphis, and that of the Coordinating Center, the University of California, San Francisco. All participants signed a written informed consent.

3.3.2 Beta Amyloid

Stored plasma obtained at the first Health ABC follow-up visit was used to measure Aβ40 and Aβ42. Plasma was stored at -70° C at Fisher BioServices, Inc. Laboratories and shipped directly to the analytical laboratory. Plasma Aß was measured at the laboratory of Dr. Steven Younkin at the Mayo Clinic using Innogenetics INNO-BIA assays. The detection limit for this assay is 12 pg/ml for Aß40 and 5 pg/ml for Aß42. Mean inter-assay coefficient of variation was 9.9% for Aß40 and 9.3% for Aß42 and mean intra-assay coefficient of variation was 3.5% for Aß40 and 2.3% for Aß42. Consistent with previous literature, tertile cutoffs were used to categorize Aβ40 and Aβ42 into "low" "medium" and "high" groups.

3.3.3 Potential Correlates

At baseline, demographic data including self-reported participant age, race, sex and education were recorded. Literacy was measured at baseline with the Rapid Estimate of Adult Literacy in Medicine (REALM).(Davis et al., 1993) REALM scores were categorized as ≤8th grade and >8th grade, as it has been reported that this cutoff differentiates those who are functionally illiterate versus literate in a healthcare setting.(Osborn et al., 2007; Rothman et al., 2004) Cognitive function at baseline was measured with the Modified Mini-Mental Status Exam (3MS). The 3MS is an assessment of global cognitive function with components for orientation, concentration, language, praxis, and immediate and delayed memory.(Teng & Chui, 1987) Social support scores were based on the average frequency of visits per week with friends, neighbors, and relatives. Scores were dichotomized into two groups: < the median or ≥ the median. Self-

reported information on smoking history was recorded and dichotomized into never versus ever having smoked.

Prevalent disease algorithms based on both self-report and physician diagnoses, recorded medications and laboratory data were used to create comorbidity variables indicating presence of diabetes mellitus, hypertension, stroke or transient ischemic attack (TIA), and myocardial infarction (MI). The Center for Epidemiologic Studies Depression Scale (CES-D) was used to assess depressive symptoms with a score ≥16 consistent with possible depression.(Radloff, 1977) Body mass index (BMI) (kg/m^2) was calculated from direct height and weight measurements at baseline. Physical activity level was determined using a standardized questionnaire designed specifically for the Health ABC study, which has been previously described.(Brach et al., 2004) Total physical activity including household chores, paid work, volunteer work, care giving, stair climbing, non-exercise walking, walking for exercise and other moderate and vigorous exercise activities, were measured in kilocalories per week (kcal/wk).(Brach et al., 2004)

Apolipoprotein E (APOE) e4 allele status was determined using standard Single Nucleotide Polymorphism (SNP) genotyping techniques and dichotomized into having one or more APOE e4 allele versus no allele.(Hixson & Vernier, 1990) Brain-derived neurotrophic factor (BDNF) was measured from frozen serum collected at the first follow-up visit. Assays were performed by R&D Systems' Analytical Testing Service, using their own commercial kit employing an enzyme-linked immunosorbent assay method. The detection limit for this assay is 1250 pg/mL. The mean inter-assay coefficient of variation is 9.0%, and the mean coefficient of variation within assay is 5.0%.

At baseline, low-density lipoprotein cholesterol (LDL) (mg/dl), high-density lipoprotein cholesterol (HDL) (mg/dl), total cholesterol (mg/dl) and triglycerides (mg/dl) were measured

from fasting blood serum. Measures of high sensitivity C-reactive protein (CRP), tumor necrosis factor-α (TNFα), interleukin-6 (IL-6) and creatinine were obtained from frozen serum or plasma collected at baseline after an overnight fast. Samples were frozen at -70°C and were shipped to the Core Laboratory at the University of Vermont.(Minev et al., 2010; Yaffe et al., 2003) Serum CRP was measured in duplicate by enzyme-linked immunosorbent assay on the basis of purified protein and polyclonal anti-CRP antibodies, and assays were standardized according to the World Health Organization First International Reference Standard with a sensitivity of 0.08 µg/ml.(Kalogeropoulos et al., 2010) Plasma IL-6 and TNFα were measured in duplicate by ELISA kits from the R&D Systems (Minneapolis, MN).(Yaffe et al., 2003) The detectable limit for IL-6 was 0.10 pg/ml, and for TNFα was 0.18 pg/ml.(Yaffe et al., 2003) Creatinine was measured with the colorimetric technique on a Johnson & Johnson VITROS 950 Chemistry Analyzer (Johnson & Johnson, New Brunswick, N.J., USA) using the enzymatic method.(Minev et al., 2010)

3.3.4 Statistical Analyses

To describe the distribution of plasma Aβ40 and Aβ42, a variety of descriptive statistics were calculated including assessment of the mean, median, range and standard deviation. The relationship between Aβ40 and Aβ42 was examined using Spearman's correlation coefficient. Fisher's exact, Pearson's chi-square, and t-tests were used, as appropriate, to determine the association between plasma Aβ40 and Aβ42 tertile and baseline participant characteristics. Finally, stepwise multivariate linear regression models were examined for both Aβ40 and Aβ42 to determine which characteristics independently predicted plasma level. Variables that were associated in bivariate analyses ($p<0.10$) with Aβ40 or Aβ42, respectively, were considered for these models. We used a forward stepwise

selection process with variables significant at the p=0.15 entry and exit criteria to determine the final multivariate models. The relative strength of the associations was expressed as an absolute difference in units of change chosen to approximate 1 SD in the distribution for each continuous variable or null category for dichotomous variables, similar to previous studies investigating correlates of markers in older adults.(Cauley et al., 2010; Sheu et al., 2009) The formula used to calculate the absolute difference in rate of change in plasma Aβ per unit change (SD) of the independent variable was β* = unstandardized β x unit change in independent variable. The corresponding 95% confidence intervals (CIs) were calculated using the following formula: β ± (1.96 x SE) x unit change. All statistical analyses were performed using SAS version 9.1 (SAS Institute Inc., Cary, NC).

3.4 RESULTS

Among the 997 community-dwelling older adults, the means (standard deviation) of Aβ40 and Aβ42 were 191.6 pg/ml (50.3) and 33.9 pg/ml (9.6), respectively. The median of Aβ40 was 192.5 pg/ml, and of Aβ42 was 32.8 pg/ml. The Spearman correlation coefficient was 0.51 ($p<0.0001$) for both markers as continuous variables, and 0.42 ($p<0.0001$) for both markers with tertile cutoffs.

Those with low plasma Aβ40 were significantly younger (73.6±3.0 years for low tertile vs. 74.5±2.9 for high tertile, p-value = 0.0001), more likely to have less than a high school education (N(%), 223 (67.2%) vs. 197 (59.5%), p-value = 0.03) to be black (201 (60.4%) vs. 147 (44.1%), p-value <0.0001), to have lower serum creatinine (mg/dl, 1.0±0.2 vs. 1.2±0.5, p-value <0.0001), to have higher HDL cholesterol (mg/dl, 56.6±17.7 vs. 52.9±16.5, p-value=0.02), to have higher BDNF (pg/ml, 23361.6±10648.3 vs. 20944.4±10022.9, p=0.006), and to have lower TNFα (pg/ml, 3.3±2.1 vs. 3.7±1.7, p=0.0007) (Table 3.7.1). There were no differences of Aβ40 by sex, literacy, baseline 3MS scores, social support, physical activity, history of stroke/TIA, diabetes history, myocardial infarction, BMI, depressive symptoms, APOE e4 allele status, C-reactive protein, IL-6, total cholesterol, LDL cholesterol, triglycerides, or smoking history.

Those with low plasma Aβ42 were more likely to be female (196 (59.8%) vs. 183 (55.5), p-value = 0.05), to have less than a high school education (231 (70.6%) vs. 193 (58.8%), p-value = 0.006), to be black (214 (65.2%) vs. 151 (45.8%), p-value <0.0001), to have a history of diabetes (94 (29.8%) vs. 68 (21.3%), p-value = 0.04) to have at least 1 APOE e4 allele (118 (38.3%) vs. 73 (23.1%), p-value=0.0002), to have lower creatinine (mg/dl, 1.0±0.2 1.1±0.6, p-value<0.0001), to have greater social support (179 (54.6%) vs. 154 (46.7%), p-value=0.05), and

to have higher HDL cholesterol (mg/dl, 57.3±17.9 vs. 53.5±16.9, p=0.007) (Table 3.7.2). There were no differences by Aβ42 and age, literacy, baseline 3MS scores, physical activity, history of stroke/TIA, myocardial infarction, BMI, depressive symptoms, C-reactive protein, TNFα, IL-6, BDNF, total cholesterol, LDL cholesterol, triglycerides, or smoking history.

In a stepwise multivariate linear regression model, Aβ40 was significantly associated with age (β=1.34, F=6.39, p=0.01), race (β = -14.70, F=22.01, p<0.0001), creatinine (β = 52.91, F=151.77, p<0.0001), and BDNF (β=-0.0004, F=7.34, p=0.007) (Table 3.7.3). Similarly, Aβ42 was significantly associated with race (β=-3.72, F=30.83, p<0.0001), sex (β=1.39, F=4.32, p=0.04), education (β=1.50, F=4.78, p=0.03), APOE e4 allele status (β=-2.82, F=16.57, p<0.0001), and creatinine (β=9.32, F=120.09, p<0.0001) (Table 3.7.3).

The strength of the associations are expressed as an absolute difference in units of change (1 SD for continuous variables or null category for dichotomous variables) (Table 3.7.4). Age, race, creatinine, and BDNF were all significant correlates of Aβ40; race, sex, education, APOE e4, and creatinine were all significant correlates of Aβ42, although sex and education were borderline (Table 3.7.4).

3.5 DISCUSSION

Our results indicated that plasma Aβ40 and Aβ42 are fairly normally distributed among older adults without dementia. Furthermore, our results suggested that in a community-dwelling sample of white and black older adults, plasma Aβ40 significantly differed by age, race, education, serum creatinine, serum BDNF, HDL cholesterol and the inflammatory marker TNFα. Similarly, plasma Aβ42 was significantly associated with race, sex, education, social support, a history of diabetes, HDL cholesterol, serum creatinine and APOE e4 allele status.

Our results are supported by prior indirect associations between demographic and medical risk factors and AD. For example, we found black race was associated with significantly lower levels of plasma Aβ40 and Aβ42, and being female was significantly associated with a lower level of Aβ42. As it has been shown that African Americans and females have an increased risk of AD, and other studies have shown low Aβ42 is associated with increased risk of dementia, these results seem consistent.(Green et al., 2002; Yaffe, Weston, et al., 2011)

Our results showing an association between diabetes and plasma Aβ42 are in support of earlier studies suggesting that consistently increased insulin levels in the brain may lead to increased Aβ42 deposition and plaque formation, and subsequent decreases in peripheral plasma Aβ42.(Duckworth, Bennett, & Hamel, 1998; Qiu et al., 1998) Our results differ from another longitudinal study that recently found higher total cholesterol and higher LDL cholesterol predicted low plasma Aβ42.(Blasko et al., 2011) Furthermore, this previous study found no association between HDL cholesterol and Aβ42 at baseline or over time, as we did with plasma Aβ40 and Aβ42.(Blasko et al., 2011) Similarly, we found no association between Aβ40 or Aβ42 with LDL, total cholesterol, triglycerides, or history of myocardial infarction or stroke – both of which have high cholesterol as a common risk factor. Finally, the association we found between

both plasma Aβ40 and Aβ42 and serum creatinine – a measure of renal function – is supported by another study which found that in older patients with chronic renal failure, dialysis significantly lowered plasma Aβ42 level; these results indicate a close relationship may exist between renal function and plasma Aβ42.(Rubio, Caramelo, Gil, Lopez, & Garcia de Yebenes, 2006)

It is widely known that the APOE e4 allele is associated with increased risk for AD, and in this study we found older adults with at least one APOE e4 allele were more likely to have low Aβ42; APOE has also been shown to modify the association between Aβ42 and Aβ42/Aβ40 and cognitive decline.(Jarvik, Larson, Goddard, & Wijsman, 1996; Yaffe, Weston, et al., 2011) An interesting finding was the association between higher serum BDNF and low plasma Aβ40. BDNF is thought to have neuroprotective effects and to promote brain plasticity.(Diniz & Teixeira, 2011) BDNF has also been shown to regulate the Amyloid Precursor Protein (APP) which is thought to reduce the production of amyloid peptides (i.e. Aβ40).(Diniz & Teixeira, 2011) Thus, it is possible that higher levels of serum BDNF may, at least in part, regulate plasma Aβ40, resulting in the lower plasma Aβ40 level. There was no relationship found between plasma Aβ42 and serum BDNF. The inflammatory marker TNFα has been shown to be associated with increased risk of cognitive decline over time.(Gorelick, 2010) While we found an association between low TNFα and low plasma Aβ40, which is contrary to what we expected, we believe this could be due to the less specific association between Aβ40 and AD or cognitive decline (versus the relationship between Aβ42 and cognitive function).(Gorelick, 2010)

Importantly, our results are also supported in part by the previous study investigating correlates and predictors of plasma Aβ in a population of cognitive normal, MCI and AD patients.(Toledo et al., 2011) Both studies reported relatively normal distributions of both

plasma Aβ40 and Aβ42, indicating no need to log-transform plasma Aβ40 or Aβ42 when analyzing as continuous variables, although it was reported that the ratio needed log-transformation.(Toledo et al., 2011) Furthermore, both studies found significant associations between higher Aβ40 and Aβ42 and poorer kidney function in t-tests, and found that creatinine significantly predicted both markers.(Toledo et al., 2011) Other similarities were significant associations between age and Aβ42, APOE and Aβ42 and cholesterol and Aβ40.(Toledo et al., 2011) This study did not support our findings in that it found no association between education or race with either plasma marker; in models the most significant predictors of Aβ40 and Aβ42 were creatinine, total proteins and cholesterol, while our results showed different significant predictors of Aβ42 and Aβ40.(Toledo et al., 2011) Interestingly in the other study, age, education nor APOE e4 significantly predicted either marker as we found, and these are known strong risk factors for AD.(Toledo et al., 2011) Finally, the prior study found a much stronger correlation between plasma Aβ40 and Aβ42 (r=0.83 vs. r=0.51).(Toledo et al., 2011) We believe differences are most likely attributable to different populations, as our study was made up of community-dwelling older adults who were all dementia free, compared to a population of primarily MCI and AD patients with only a small proportion of cognitively normal controls.(Toledo et al., 2011) Given these characteristics, the contrasting results could reflect that disease progression largely affects plasma Aβ measurements and correlates. Ultimately, more studies are needed to better understand the fluctuations of plasma Aβ40 and Aβ42 in older adults who age without cognitive decline, with MCI and with AD to better understand these relationships.

If plasma Aβ40 and Aβ42 are indeed useful biomarkers of cognitive decline and dementia, then it is important to fully understand how these biomarkers are influenced by

demographic and medical conditions for several reasons. First of all, at a very basic level, it will be important so that future studies can measure and adjust for all potential confounders when investigating the association of plasma Aβ to ensure the most accurate results are presented. Understanding demographic and medical correlates will also be crucial in considering what factors may regulate plasma Aβ. This will be critical for gaining a better understanding of the mechanisms underlying cognitive decline and dementia. It will also help in identifying possible targets for interventions in reducing risk of or preventing dementia.

This study had several strengths, including the large sample size providing ample analytical power for this study. Measurement of numerous potential confounders allowed us to investigate a large number of demographic and medical characteristics in one sample. Finally, Innogenetics INNO-BIA assays were used for our measurements of Aβ42 and Aβ40, and this method may provide more accurate measurements of Aβ42 and Aβ40 due to its high sensitivity, low variability, and high reproducibility.(Innogenetics, 2007) There are also several weakness that should be taken into consideration when interpreting these results. These adults were all well-functioning and community-dwelling at baseline, so results may not be generalizable to all older adults – for example, nursing home populations. Another potential weakness is that we did not have CSF measurements of Aβ and thus, we could not correlate plasma Aβ to measurements of CSF Aβ. As CSF Aβ has been such a well-established biomarker for dementia, it would have been useful.

This study identified the demographic and medical correlates of plasma Aβ40 and Aβ42. The correlates identified in this paper should be considered in future studies as potential confounders of the association between plasma Aβ and cognitive decline or dementia. Understanding fully how these correlates mediate or modify the association between plasma Aβ

and dementia will be a fundamental step in determining the biological pathways through which plasma Aβ contributes to dementia and cognitive decline. Future studies should continue to investigate exactly how these demographic and medical correlates may influence the relationship between plasma Aβ40 and Aβ42, as it may help us target interventions for preventing and reducing the risk of AD and dementia.

3.6 ACKNOWLEDGEMENTS

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3.7 TABLES

Table 3.7.1 Demographic and Medical Correlates of Plasma Aβ40 among 997 Older Adults

Baseline Characteristics N (%) or Mean (SD)	**Tertile 1 n=333**	**Tertile 2 n=331**	**Tertile 3 N=333**	**p-value**
Age, years	73.6 (3.0)	74.0 (2.8)	74.5 (2.9)	0.0001
Black Race	201 (60.4)	190 (57.4)	147 (44.1)	<0.0001
Female Sex	184 (55.3)	187 (56.5)	179 (53.8)	0.78
Education (≤HS)	223 (67.2)	226 (68.5)	197 (59.5)	0.03
Literacy (REALM, ≤8th grade)	94 (29.9)	85 (27.8)	87 (27.8)	0.79
3MS	91.0 (5.4)	91.2 (5.5)	91.1 (5.2)	0.90
Social Support*	171 (51.4)	165 (49.9)	152 (45.7)	0.26
Body Mass Index	27.7 (5.2)	28.0 (4.8)	27.5 (4.8)	0.35
Physical Activity (kcal per week)	1055.5 (2373.2)	1133.3 (2455.5)	982.4 (1465.0)	0.66
Depressive Symptoms (CES-D≥16)	13 (3.9)	22 (6.7)	11 (3.3)	0.09
Stroke/TIA History	25 (7.5)	33 (10.1)	37 (11.3)	0.25
Diabetes History	74 (23.2)	83 (25.6)	83 (25.9)	0.69
Myocardial Infarction History	25 (7.6)	38 (11.6)	42 (12.8)	0.07
Cholesterol (mg/dl)	202.7 (37.6)	208.4 (41.1)	202.2 (39.3)	0.08
Triglycerides (mg/dl)	126.8 (80.6)	131.1 (100.5)	138.5 (68.0)	0.19
HDL (mg/dl)	56.6 (17.7)	55.1 (16.7)	52.9 (16.5)	0.02
LDL (mg/dl)	121.4 (34.5)	127.2 (35.5)	121.6 (35.4)	0.06
APOE e4 allele (≥1 e4 allele)	96 (30.9)	101 (32.2)	93 (29.5)	0.77
BDNF (pg/ml)	23361.6 (10648.3)	21206.2 (11064.9)	20944.4 (10022.9)	0.006
Creatinine (mg/dl)	1.0 (0.2)	1.0 (0.3)	1.2 (0.5)	<0.0001
C-reactive Protein (µg/mL)	3.0 (3.9)	2.9 (4.0)	3.4 (7.3)	0.42
Tumor Necrosis Factor (pg/ml)	3.3 (2.1)	3.2 (1.3)	3.7 (1.7)	0.0007
IL-6 (pg/ml)	2.3 (1.9)	2.2 (1.7)	2.4 (1.8)	0.28
Smoker (Ever vs. Never)	183 (55.0)	180 (54.4)	180 (54.4)	0.99

*N (%) below the median; variable defined as a combination of friends, neighbors and relatives who visit per week. Aβ40 indicates amyloid beta-40; SD standard deviation; HS high school; REALM Rapid Estimate of Adult Literacy in Medicine; 3MS Modified Mini-Mental Status Exam; CES-D Center for Epidemiologic Studies Depression Scale; TIA transient ischemic attack; HDL high-density lipoprotein cholesterol; LDL low-density lipoprotein cholesterol; APOE Apolipoprotein E; BDNF brain-derived neurotrophic factor; IL-6 interleukin-6.

Table 3.7.2 Demographic and Medical Correlates of Plasma Aβ42 among 997 Older Adults

Baseline Characteristics N (%) or Mean (SD)	Tertile 1 n=328	Tertile 2 n=339	Tertile 3 n=330	p-value
Age, years	74.0 (3.0)	73.8 (2.9)	74.2 (2.9)	0.17
Black Race	214 (65.2)	173 (51.0)	151 (45.8)	<0.0001
Female Sex	196 (59.8)	171 (50.4)	183 (55.5)	0.05
Education (≤HS)	231 (70.6)	222 (65.7)	193 (58.8)	0.006
Literacy (REALM, ≤8th grade)	95 (30.8)	97 (30.9)	74 (23.8)	0.08
3MS Score	90.7 (5.5)	91.1 (5.3)	91.4 (5.3)	0.30
Social Support*	179 (54.6)	155 (45.7)	154 (46.7)	0.05
Body Mass Index	27.6 (5.2)	27.5 (4.3)	28.1 (5.3)	0.26
Physical Activity (kcal per week)	929.8 (1971.7)	1042.1 (1733.5)	1198.4 (2669.9)	0.27
Depressive Symptoms (CES-D ≥16)	13 (4.0)	12 (3.5)	21 (6.7)	0.17
Stroke/TIA History	28 (8.6)	31 (9.3)	36 (11.0)	0.56
Diabetes History	94 (29.8)	78 (23.7)	68 (21.3)	0.04
Myocardial Infarction History	39 (8.9)	34 (10.2)	42 (12.9)	0.24
Cholesterol (mg/dl)	203.2 (38.0)	205.4 (39.2)	204.6 (41.2)	0.76
Triglycerides (mg/dl)	128.5 (80.9)	127.4 (65.6)	140.8 (101.9)	0.08
HDL (mg/dl)	57.3 (17.9)	53.9 (15.9)	53.5 (16.9)	0.007
LDL (mg/dl)	120.8 (34.7)	125.8 (35.4)	123.5 (35.4)	0.20
APOE e4 allele (≥1 e4 allele)	118 (38.3)	99 (31.3)	73 (23.1)	0.0002
BDNF (pg/ml)	22798.0 (10910.9)	21328.8 (10138.0)	21408.9 (10810.7)	0.14
Creatinine (mg/dl)	1.0 (0.2)	1.0 (0.2)	1.1 (0.6)	<0.0001
C-reactive Protein (μg/mL)	3.2 (6.4)	2.7 (3.4)	3.4 (5.8)	0.16
Tumor Necrosis Factor (pg/ml)	3.3 (2.1)	3.3 (1.4)	3.6 (1.7)	0.07
IL-6 (pg/ml)	2.2 (1.5)	2.4 (2.0)	2.4 (1.9)	0.58
Smoker (Ever vs. Never)	192 (58.5)	184 (54.3)	167 (50.9)	0.15

*N (%) below the median; variable defined as a combination of friends, neighbors and relatives who visit per week. Aβ42 indicates amyloid beta-42; SD standard deviation; HS high school; REALM Rapid Estimate of Adult Literacy in Medicine; 3MS Modified Mini-Mental Status Exam; CES-D Center for Epidemiologic Studies Depression Scale; TIA transient ischemic attack; HDL high-density lipoprotein cholesterol; LDL low-density lipoprotein cholesterol; APOE Apolipoprotein E; BDNF brain-derived neurotrophic factor; IL-6 interleukin-6.

Table 3.7.3 Stepwise Multivariate Linear Regression Associations with Plasma Aβ40 and Aβ42

Variable	Parameter (β)	Standard Error	F-value	p-value
	Aβ40			
Age	1.34	0.53	6.39	0.01
Black race	-14.70	3.13	22.01	<0.0001
Creatinine	52.91	4.29	151.77	<0.0001
Serum BDNF	-0.0004	0.0001	7.34	0.007
	Aβ42			
Black race	-3.72	0.67	30.83	<0.0001
Female sex	1.39	0.67	4.32	0.04
Education	1.50	0.69	4.78	0.03
APOE e4	-2.82	0.69	16.57	<0.0001
Creatinine	9.32	0.85	120.09	<0.0001

Aβ40 indicates amyloid beta-40; Aβ42 amyloid beta-42; BDNF brain-derived neurotrophic factor; APOE Apolipoprotein E.

Table 3.7.4 Multivariate Correlates of the Rate of Change in Plasma Aβ40 and Aβ42

Variable	Unit	Rate of change in plasma Aβ (95% CI) per unit	
		Aβ40	Aβ42
Age	2.91	3.90 (0.88, 6.92)	NA
Race	1	-14.70 (-20.83, -8.57)	-3.72 (-5.03, -2.41)
Sex	1	NA	1.39 (0.08, 2.70)
Education	1	NA	1.50 (0.15, 2.85)
Creatinine	0.85 mg/dl	19.58 (16.47, 22.69)	-2.82 (-4.17, -1.47)
APOE e4	1	NA	3.45 (2.83, 4.07)
BDNF	10631.18 pg/ml	-4.25 (-6.33, -2.17)	NA

Aβ40 indicates amyloid beta-40; Aβ42 amyloid beta-42; CI confidence interval; NA not applicable; APOE Apolipoprotein E; BDNF brain-derived neurotrophic factor.

4.0 PAPER 2: CHANGE IN INFLAMMATORY MARKERS AND COGNITIVE STATUS AMONG OLDEST OLD WOMEN FROM THE STUDY OF OSTEOPOROTIC FRACTURES

4.1 ABSTRACT

Objectives To determine the association between interleukin-6 (IL-6), the IL-6 soluble receptor (IL-6 sR) and tumor necrosis factor soluble receptor 1 (STNF-R1) measured at two time points and subsequent cognitive status among oldest old women, and to determine the association between change in each inflammatory marker and cognitive status.

Design 20-year longitudinal cohort study.

Setting Four clinical sites in the United States.

Participants 905 women from the Study of Osteoporotic Fractures.

Measurements At Year 20, cognitive status was diagnosed as either normal, mild cognitive impairment (MCI), or dementia. Inflammatory markers were measured from stored blood serum at Year 10 and Year 16 in a random sample of women using a DuoSet enzyme-linked immunosorbent assays (ELISAs).

Results Baseline mean participant age was 78.3±2.83 years. Over 10 years, 199 (21.99%) developed MCI, and 145 (16.02%) dementia. There were no significant associations between IL-6 or TNF sR1 measured at Year 10 or Year 16 and cognitive status at Year 20. High IL-6 sR at

Year 16 was significantly associated with decreased risk for dementia (OR=0.54; 95% CI: 0.30, 0.97). Women with high IL-6 sR at both time points (OR = 0.39; 95% CI: 0.17, 0.89) or who had a low level at Year 10 and transitioned to a high level at Year 16 (OR = 0.35; 95% CI: 0.14, 0.88) also had reduced risk of dementia.

Conclusion In this cohort of white, high functioning oldest old women, a consistently high or an increasing level of IL-6 sR is associated with reduced risk of dementia. The effect of inflammation on dementia may differ in younger old and oldest old. Understanding these differences will be crucial in interpreting results from ongoing clinical trials and in targeting therapeutic strategies to the oldest old.

4.2 INTRODUCTION

Both age and inflammation are associated with elevated risks of cognitive impairment, cognitive decline, and Alzheimer's disease (AD).(Engelhart et al., 2004a; Schmidt et al., 2002; Troller et al., 2010; Weaver et al., 2002; Yaffe et al., 2003) Intriguingly, the relationships between inflammatory markers and these disorders appears to weaken with advancing age, with studies showing inconsistent results after age 85.(Jenny et al., 2012; Kravitz, Corrada, & Kawas, 2009a; Kravitz et al., 2009b; Schram et al., 2007; Silverman et al., 2009) This is critically important with a view both to understanding disease mechanisms and to designing appropriate intervention strategies. For example, clinical trials are currently investigating the potential role of anti-inflammatory drugs in AD patients, (Aisen et al., 2003; Breitner et al., 2011; Fields et al.) and if there truly is a different association between inflammation and dementia in the young old, and the oldest old, understanding these differences will be crucial in interpreting results from these trials and in targeting therapeutic strategies to the oldest old. Some limitations to the existing studies of the oldest old are cross-sectional study design, or measuring inflammatory markers at only one time point in longitudinal studies.(Kravitz et al., 2009b; Schram et al., 2007; Silverman et al., 2009) Furthermore, many studies have focused on only one inflammatory marker at a time, rather than assessing the role of multiple markers together.(Kravitz et al., 2009b; Silverman et al., 2009) Thus, more studies are needed to determine the association between inflammation and cognitive status in the oldest old, and to investigate if a change in inflammation over time is associated with cognitive function. The objectives of this study were to determine the association between interleukin-6 (IL-6), the IL-6 soluble receptor (IL-6 sR) and tumor necrosis factor soluble receptor 1 (STNF-R1) measured at an initial and an interim visit and subsequent cognitive status among oldest old women. Because we had inflammation measured at more than

one time point, a second objective was to determine the association between change in each inflammatory marker and cognitive status.

4.3 METHODS

4.3.1 Study Sample

The Study of Osteoporotic Fractures (SOF) is an ongoing prospective cohort study of community-dwelling women, with recruitment of 9,704 mostly Caucasian (99% non-Hispanic white) women, aged 65 years or older occurring between 1986 and 1988 from four regions: Baltimore County, Maryland; Minneapolis, Minnesota; Portland, Oregan; and the Monongahela Valley near Pittsburgh, Pennsylvania. Between 1997 and 1998, a second wave of recruitment occurred to increase African American representation in the sample, and 662 African American women, aged 65 years or older, also from the same study regions, were enrolled. Originally designed to evaluate risk factors for falls and fractures, exclusion criteria included being unable to walk without assistance, and bilateral hip replacement. All women provided written consent, and SOF was approved by the Institutional Review Board (IRB) and each study site.

A random sample of 905 women with measured serum IL-6, IL-6 sR and STNF-R1 at an initial visit (SOF Year 10 – the baseline for the purposes of this study) and with adjudicated cognitive status 10 years later comprised our analytic cohort. These women were also free of cognitive impairment at Year 10; cognitive impairment was defined as a modified Mini-Mental State Examination (mMMSE) score $\leq$22 points).(Middleton et al., 2010) A subset (n=363 for IL-6, n=380 for IL-6 sR, n=393 for STNF-R1) also had these markers measured at an interim exam (Year 16), and were included in longitudinal analyses.

4.3.2 Primary Predictors

Inflammatory markers were measured from blood serum at Year 10 in a random sample of women who came in to the Year 10 clinic visit. Year 16 inflammatory markers, also measured from blood serum, were assessed in a random sample of women from the Minnesota and Pennsylvania sites who participated in the SOF sleep study (had to be willing to wear an actigraphy watch), and had serum collected. IL-6, IL-6 sR and STNF-R1 were measured from stored serum using a DuoSet enzyme-linked immunosorbent assays (ELISAs) from R&D systems. All assays were performed at the University of Maryland Cytokine Core Laboratory (Baltimore, MD). The coefficient of variation for STNF-R1 was 5%, and values ranged from 1250 to 80,000 pg/ml. For IL-6, the coefficient of variation was 8%, with a range of values from 2.3 to 150 pg/ml. Finally, the coefficient of variation for IL-6 sR was 4%, and values ranged from 1,500 to 100,000 pg/ml. To account for potentially non-linear relationships, and because some studies have shown that inflammation is only associated with cognitive function at the extreme ends(Weaver et al., 2002), we decided a priori to create tertile cutoffs for each inflammatory marker in order to compare those with the highest levels to those with the lowest.

4.3.3 Outcomes

The primary outcome for these analyses was adjudicated cognitive status. Cognitive status was adjudicated at SOF Year 20. Study participants who had cognitive test data available from clinic or at home visits at the Year 20 visit were eligible for screening and diagnosis of cognitive impairment. Participants who had only self-administered questionnaire data on cognitive function were excluded. The first step in the adjudication process was identifying those “in need”

of cognitive adjudication. Participants who met any of the following criteria at Year 20 were determined as being "in need": Teng Modified Mini-Mental State Exam (3MS) Score of <88; California Verbal Learning Test (CVLT) Delayed (10 minute) Recall Score <4; Functional Assessment Questionnaire (IQCODE) Score ≥3.6; a self-reported history of dementia diagnosis; or living in a nursing home or personal care home. There were 760 participants who met one or more of these criteria, and had complete Year 20 data, as well as past visit data forwarded to the adjudication committee for cognitive status determination. All adjudication was performed using existing data on age, education, race, previously collected cognitive assessments, functional assessments, including assessments activities of daily living and instrumental activities of daily living, depressive symptoms, lifestyle (i.e. type of residence, living alone), medical history, and medication use. No further exam was performed on the participant, nor any additional data collected.

A panel of clinical experts, including one neurologist, two neuropsychologists, and one geropsychologist, adjudicated cognitive function of the 760 identified as "in need". A diagnosis of dementia was made based on the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria. Furthermore, likely dementia etiology, including vascular dementia, AD, dementia due to multiple etiologies (mixed), or other was determined. MCI was diagnosed using a modified Petersen Criteria.(R. C. Petersen, 2004) Those who did not meet criteria for MCI or dementia were classified as being cognitively normal. Similarly, those who did not meet any of the original five screening criteria were not adjudicated, and were considered cognitively normal. The four different adjudicators were assigned adjudication data packets for review at random, with an equal number of packets assigned to each; the majority of participant data packets were reviewed by only one adjudicator. To check reliability of adjudications, 20

data packets flagged for adjudication were selected at random for review by all four adjudicators (120 pairs of rereads, 6 different pairing of adjudicators). The weighted kappa for adjudicated outcomes was 0.77 (95% CI: 0.71 - 0.84) which indicates substantial strength of agreement between adjudicators.(Landis & Koch, 1977) Furthermore, 20 participants who did not meet any of the criteria for adjudication had their data sent to the adjudication committee, to verify that they would be adjudicated as normal (19 of 20 were adjudicated as normal and 1 of 20 was adjudicated as MCI). In order to further examine the association between inflammatory markers and cognitive function, we also assessed the outcome of change in mMMSE scores from baseline to Year 20.

4.3.4 Other Variables

Data on age, race and education were collected at the SOF baseline visit; age for these analyses was the age at Year 10 because that was the first year inflammation was collected, and serves as our study baseline. Weight and height were measured at study baseline using a balance-beam scale and fixed stadiometer in light indoor clothing; BMI (kg/m^2) was calculated from direct height and weight measurements at our study baseline. Diabetes was defined as self-reported diabetes or taking medication for diabetes. Hypertension was defined as either systolic blood pressure ≥130 mmHg and/or diastolic blood pressure ≥85 mmHg and/or taking medications for high blood pressure. History of stroke and myocardial infarction were self-reported. The 15-item Geriatric Depression Scale (GDS) was used to assess depressive symptoms; a standard cutoff of ≥6 symptoms was used to define depression.(Sheikh & Yesavage, 1986) Apolipoprotein E (APOE) phenotype was determined by isoelectric focusing and immunoblotting(Kamboh, Ferreli, & Kottke, 1988) on most of the women from the Pittsburgh site (n=220, 24.31% of

sample). A categorical variable was created to classify women with ≥1 APOE e4 allele versus no e4 allele. Similar to inflammatory markers, Cystatin-C was measured in a random sample of women who attended the Year 10 clinic visit, and had enough serum to assay Cystatin-C. Concentration of Cystatin-C was determined using a BN100 nephrolometer (Dade Behring Inc., Deerfield, IL). To determine medication use, participants were required to bring in all medications taken daily or almost daily in the 30 days prior to the study visit. Medications were classified according to a computerized coding dictionary, according to brand and generic names. (Pahor et al., 1994)

4.3.5 Statistical Analyses

To examine the association between categorical baseline characteristics of the women, and adjudicated cognitive status, Fisher's exact or Pearson's chi-square tests were used; to examine the association between continuous baseline characteristics and cognitive status, analysis of variance tests (ANOVAs) or Wilcoxon rank sum tests were used, as appropriate. For all analyses, we first included all types of dementia, and then removed non-AD types to see how much results changed. There were no significant changes to the strength or direction of any association, so to keep sample size larger (especially for longitudinal analyses), all types of dementia were included for all analyses. Multinomial logistic regression was used to determine the association between each inflammatory marker at initial and interim visits and subsequent cognitive status (normal, MCI and dementia). Multinomial logistic regression was also used to determine the association between 6-year change in inflammatory marker and subsequent adjudicated cognitive status.

To further examine the association between inflammation and cognitive function, we conducted post-hoc analyses to examine the change in mMMSE score from SOF Year 1 to SOF Year 20. Chi-square tests were used to describe the association between change in mMMSE scores (-1 standard deviation [SD] vs. No change/+1 SD) over 20 years and level of inflammatory marker at Year 10; similar analyses were performed for predictors of Year 16 inflammatory marker and change in inflammatory marker from Year 10 to Year 16. Trajectories of mean mMMSE scores over time by inflammatory marker change group (high-high, high-low, low-high, low-low) were also compared using ANOVAs. Additionally, we formally examined the interaction between baseline mMMSE scores and Year 10 inflammatory marker using a likelihood ratio test. Finally, we also examined the association between the ratio of IL-6sR/IL-6 at Year 10 and adjudicated cognitive status using multinomial logistic regression. All statistical analyses were performed using SAS version 9.1 (SAS Institute Inc., Cary, NC).

4.4 RESULTS

The mean baseline age was 78.3±2.83 years and 88.3 years at the time of cognitive status determination. After 10 years, 199 (21.99%) developed MCI and 145 (16.02%) dementia. Of the 145 with dementia, 116 had AD (80.0%), 18 (12.4%) had vascular dementia, 2 were classified as "other" (1.4%) and 9 (6.2%) were indeterminate. At baseline, compared to those who were cognitively normal, those with dementia or MCI were significantly older ($p<0.0001$) and had less education ($p=0.005$) (Table 4.6.1). There was also a borderline significant association suggesting those with MCI or dementia were more likely to be depressed ($p=0.09$) and those with MCI were more likely to have poorer kidney function (higher Cystatin-C, $p=0.06$) (Table 4.6.3). Finally, although APOE genotype was only available for a small number of participants, those with dementia were significantly more likely to have at least 1 APOE e4 allele ($p=0.03$) (Table 4.6.1). All of these variables except for APOE e4 (due to small sample size) were included as covariates in models. Furthermore, although they did not differ by cognitive status, NSAID use and statin use were included in models because of their effects on inflammation.

There were no significant associations between initial or interim level of IL-6 or STNF-R1 and risk of MCI or dementia in either unadjusted or adjusted models (Table 4.6.2). While there was no significant association between the initial level of IL-6 sR and subsequent cognitive status (Table 4.6.2), those with a high level of IL-6 sR at Year 16 were significantly less likely to be subsequently diagnosed with dementia in unadjusted (OR=0.54; 95% CI: 0.30, 0.97), and adjusted models (Table 4.6.2). When looking at change in inflammatory marker from Year 10 to Year 16, and subsequent cognitive status, there were no significant associations between IL-6 or STNF-R1 and MCI or dementia. Again, however, a high IL-6 sR level at both time points was associated with a reduced risk of a dementia diagnosis in both unadjusted (OR = 0.39; 95% CI:

0.17, 0.89) and adjusted models (Table 4.6.3); this was similar among those who did not have a high IL-6 sR level at the initial, but transitioned to a high level at the interim visit (unadjusted OR = 0.35; 95% CI: 0.14, 0.88) (Table 4.6.3).

When looking at the association between inflammatory marker and 20 year change in mMMSE score, there were once again no significant associations with IL-6 or STNF-R1. However, when compared to those with a low level of IL-6 sR at Year 10, those with a high level were significantly more likely to have a decline of 1 SD or more in mMMSE scores (28.73% vs. 71.27%, p=0.03). While not statistically significantly, there was also a indicating those with a high level of IL-6 sR at both time points were more likely to have a decline of 1 SD or more in mMMSE score over 20 years (Low, Low: 11.56% vs. High, Low: 21.77% vs. Low, High: 20.41%, vs. High, High 46.26%, p=0.14), although the cognitive trajectories of all IL-6 sR change groups over 20 years are strikingly similar (Figure 4.6.1). To further examine this association, we created a scatterplot of baseline mMMSE tertile by 20 year change in mMMSE score, stratified by IL-6 sR level at Year 10 (Figure 4.6.2), and formally tested for an interaction of baseline cognitive test scores with Year 10 IL-6sR level and adjudicated cognitive status using a likelihood ratio test. The likelihood ratio test indicated a significant interaction between baseline mMMSE scores with IL-6sR Year 10 level and adjudicated cognitive status ($\chi^2_{\text{degrees of freedom}=1} = 3.883$, p=0.049). This interaction is further displayed in the scatterplot where those with highest baseline scores and a high level of IL-6 sR were more likely to have a decline of 1 SD or more, but not reach clinically significant cognitive impairment. On the other hand, among those with a high IL-6 sR at Year 10 and low baseline mMMSE scores, there was less decline over time, but more who reached clinically significant cognitive impairment (Figure 4.6.2). Thus, these results do indeed support our original findings. Finally, while it did not reach

statistical significance, there was a borderline significance between the ratio of IL-6sR/IL-6 (OR=0.69; 95% CI: 0.47, 1.02).

4.5 DISCUSSION

In this study of oldest old women, we found that those with a high level of IL-6 sR were significantly less likely to be diagnosed with dementia over 10 years. This was true for women with a high level of IL-6 sR at both time points, and for those who started off with a low level, but increased to a high level over time. Furthermore, upon examining mMMSE test scores, we found that women with a high level of IL-6 sR were more likely to decline 1 SD or more over 20 years, but were less likely to reach a score that indicated clinically significant cognitive impairment (mMMSE $\leq$22 points). Our results are supported by at least one previous study which found that in CSF of AD patients, there were significantly decreased levels of IL-6 sR, when compared to cognitively normal controls.(ADAPT Research Group, 2007) There have been other conflicting results reported on the association between IL-6 sR and AD and cognitive function. For example, several early studies reported an association between lower CSF and serum IL-6 sR level and increased risk for AD(Angelis, Scharf, Mander, Vajda, & Christophidis, 1998; Hampel et al., 1997), and others have reported no significant differences.(Marz et al., 1997)

A particularly interesting finding from these results was the significant protective association between IL-6 sR, and lack of association with IL-6. One explanation for this outcome is the way through which IL-6 and IL-6 sR become functionally available in the body; IL-6 sR regulates IL-6, such that when bound, the normal effect of IL-6 is actually inhibited.(Bagli et al., 2003) Thus, it could be that IL-6 sR is somehow inhibiting negative effects of IL-6.(Neuroinflammation Working Group et al., 2000) To examine this hypothesis statistically, we performed analyses looking at the ratio of IL-6sR/IL-6 at Year 10 and adjudicated cognitive status. If it is indeed true that a high level of IL-6sR is protective because it is inhibiting negative

inflammatory effects of IL-6, then a high ratio of IL-6 sR/IL6 should also be protective. While it does not reach statistical significance, we did find a borderline significance for this association with dementia in the hypothesized direction, indicating that IL-6 sR may be having some small effects on IL-6.

Another interesting finding from these results was that the women with a high level of IL-6 sR were less likely to reach a diagnosis of dementia, but still more likely to decline ≥1 SD over time. These results differ from several previous studies which have reported associations between a high level of inflammation and increased risk for cognitive decline or dementia.(Engelhart et al., 2004a; Eriksson et al., 2011; Holmes et al., 2009; Licastro et al., 2000; Schmidt et al., 2002; Schram et al., 2007; Sundelof et al., 2009; Weaver et al., 2002; Yaffe et al., 2003) There are several potential explanations for this finding. First, it could be that what we are seeing is a healthy participant bias. A relationship between a high level of inflammation and mortality has been previously documented(Alley, Crimmins, Brandeen-Roche, Guarlnik, & Ferrucci, 2007), and perhaps women who were the most sick have died. When comparing women in SOF who were lost to follow-up or died, to those in our analytic cohort, those lost to follow-up were more likely to have a history diabetes ($p=0.002$) and a previous MI ($p<0.0001$); however there was no difference on having a history of stroke ($p=0.33$), and the women in the cohort were more likely to have hypertension ($p=0.0002$).

Another potential explanation is that we are seeing a truly pleiotropic nature of inflammation in older adults, and while a high level is harmful at some time points, but in this age group, it is beneficial. It has been proposed that high inflammation is perhaps be an indicator of a well-functioning immune system in the oldest old, demonstrating the ability for the body to successfully fight off illness, infection, and injury.(Griffin, Michel, Huysman, Logar, &

Vallejo, 2012) Our results may just be highlighting the complexity of immune system abnormalities we see in both normal and pathological aging.

A final explanation could be the idea of cognitive reserve. Briefly, this theory postulates that some people with a high degree of brain pathology may show no apparent cognitive impairment due to an ability to compensate for pathology or deficits; the ability to compensate is thought to stem from a combination of advantageous lifestyle factors, such as higher education or literacy, and neural factors like greater brain volume or greater brain plasticity.(Scarmeas & Stern, 2004; Stern, 2002) Specifically, those with a high IL-6 sR may have a greater cognitive reserve due to a high level of IL-6 sR preventing IL-6 from exerting negative effects on the brain. This is supported by those with a high IL-6 sR having a higher level of cognitive function at baseline (higher mMMSE scores) and a decreased likelihood of being diagnosed dementia.

There are several strengths and weaknesses that should be considered when interpreting the results of this study. One strength is having inflammatory markers measured at multiple points in time. Furthermore, cognitive status was clinically adjudicated which provides more information than individual cognitive assessments, as used in many previous studies. We had data on cognitive test scores over time to strengthen our examination of the association between inflammation and adjudicated cognitive status. Finally, we had data on a large number of potential confounders and covariates collected, so all analyses can be adjusted for these factors. A weakness of this study was having a relatively small sample size, especially in follow-up years. We were also unable to see if these results were modified by APOE e4 genotype due to a small sample size with APOE assessed.

In conclusion, findings suggest a consistently high or an increasing level of IL-6 sR is associated with a subsequent reduced risk of dementia. Furthermore, the women with high IL-6

sR and high baseline mMMSE scores are declining greatly in mMMSE score over 20 years, but not reaching clinically significant impairment, and thus seem to be demonstrating cognitive reserve. This could be due to a healthy survivor effect. Our results may also be highlighting the pleiotropic nature of inflammation, and the complexity of the immune system in older adults. Understanding these differences will be crucial in interpreting results from ongoing clinical trials and in targeting therapeutic strategies to oldest old.

4.6 TABLES AND FIGURES

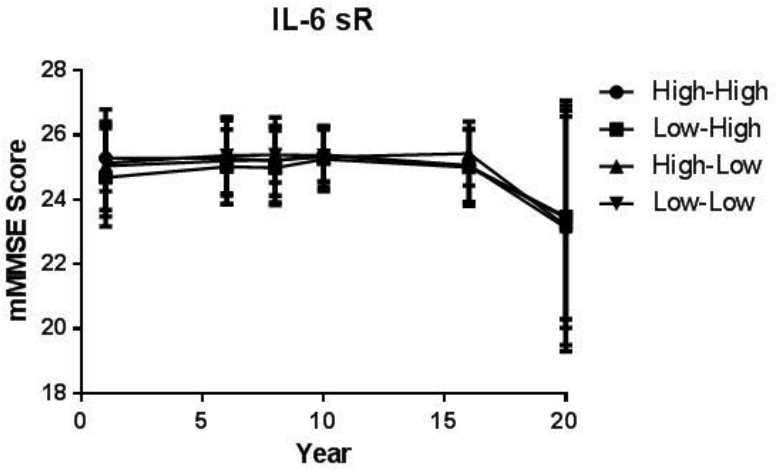

Figure 4.6.1 Cognitive trajectories over 20 years based on mMMSE score, by change in IL-6 sR group.

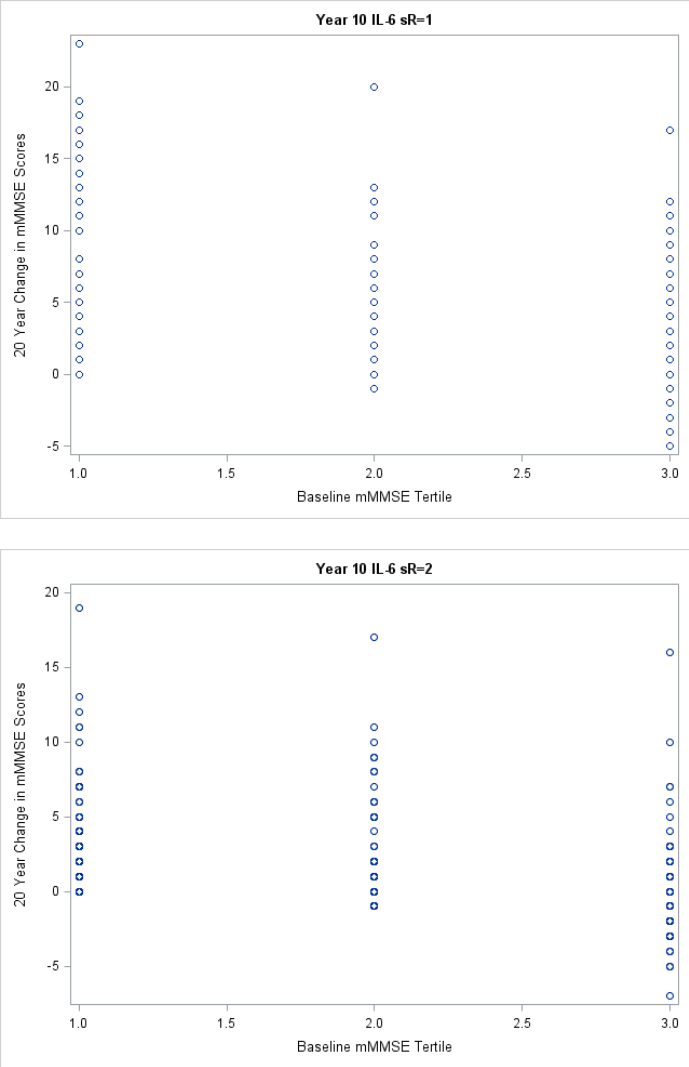

Figure 4.6.2 Scatterplots of baseline mMMSE tertile, by 20-year change in mMMSE score, stratified by year 10 IL-6 sR level. IL-6 sR = 1: High IL-6 sR group; IL-6 sR = 2: Low IL-6 sR group. Greater numbers in 20-year change in mMMSE scores indicate greater decline over 20 years, or a greater loss in cognitive function. Baseline mMMSE tertile was coded such that "1" is indicative of the highest baseline scores, or the highest cognitive performers, and "3" is indicative of the lowest baseline scores.

Table 4.6.1 Baseline characteristics of the 905 oldest old women by cognitive status

Characteristic Mean (SD), Median, or N (%)	Cognitively Normal (n=561)	Mild Cognitive Impairment (n=199)	Dementia (n=145)	p-value
Age	77.83 (2.55)	78.63 (2.75)	79.41 (3.55)	<0.0001
Black Race	2 (0.36%)	2 (1.01%)	0 (0%)	0.34
Education, ≤HS	309 (55.08%)	136 (68.34%)	88 (60.69%)	0.005
Diabetes	19 (3.39%)	5 (2.51%)	7 (4.83%)	0.51
Hypertension	170 (30.30%)	67 (33.67%)	56 (38.62%)	0.15
Stroke	17 (3.03%)	4 (2.01%)	1 (0.69%)	0.24
Myocardial Infarction	8 (1.43%)	1 (0.50%)	1 (0.69%)	0.49
Depression	22 (3.92%)	11 (5.53%)	12 (8.28%)	0.09
Body Mass Index	27.17 (4.30)	27.24 (4.58)	26.92 (5.09)	0.79
APOE e4 allele*	10 (1.78%)	3 (1.51%)	7 (4.83%)	0.03
Cystatin-C (mg/L)**	0.95	0.98	0.95	0.06
Interleukin-6 (pg/ml)**	2.48	2.68	2.98	0.19
Interleukin-6 Soluble Receptor (pg/ml)**	45304.16	45630.41	45140.51	0.79
Tumor Necrosis Factor Soluble Receptor 1 (pg/ml)**	3299.42	3393.24	3280.31	0.32
NSAID use	156 (27.81%)	55 (27.64%)	31 (21.38%)	0.28
Statin use	55 (9.80%)	15 (7.54%)	12 (8.28%)	0.59

*Only on a subset of 220; **Wilcoxon Rank Sum tests used to compare medians rather than means because of slightly skewed distributions.

Table 4.6.2 Adjusted association of inflammation markers at Year 10 or Year 16 and subsequent cognitive status

Inflammation Marker	Odds Ratio (95%CI) Mild Cognitive Impairment	Dementia
	IL-6 Year 10* (n=905)	
Low	Reference	Reference
High	1.19 (0.83, 1.69)	1.32 (0.88, 1.97)
	IL-6 Year 16* (n=360)	
Low	Reference	Reference
High	1.26 (0.72, 2.21)	1.08 (0.56, 2.09)
	IL-6 Soluble Receptor Year 10* (n=905)	
Low	Reference	Reference
High	1.06 (0.75, 1.52)	0.85 (0.57, 1.26)
	IL-6 Soluble Receptor Year 16* (n=377)	
Low	Reference	Reference
High	0.79 (0.46, 1.36)	0.48 (0.25, 0.91)
	TNF Soluble Receptor Year 10* (n=905)	
Low	Reference	Reference
High	1.16 (0.78, 1.70)	1.12 (0.73, 1.73)
	TNF Soluble Receptor Year 16* (n=390)	
Low	Reference	Reference
High	0.79 (0.46, 1.38)	1.06 (0.54, 2.06)

*Models are adjusted for age, education, depression, cystatin-C, statin use and NSAID use.
**Low IL-6 ≤1.87 pg/ml and high IL-6 >1.87 pg/ml; low IL-6 sR ≤37401.36 pg/ml and high IL-6 sR >37401.36; low STNF-R1 <11.98 pg/ml and high STNF-R1 >11.98 pg/ml.

Table 4.6.3 Association of change in inflammation markers over time and Year 20 cognitive status

Inflammation Marker	Odds Ratio (95%CI)	
	Mild Cognitive Impairment	Dementia
	IL-6 Adjusted*	
Low, Low	Reference	Reference
High, Low	1.59 (0.61, 4.16)	3.09 (0.89, 10.66)
Low, High	1.99 (0.80, 4.98)	2.42 (0.69, 8.44)
High, High	1.50 (0.64, 3.47)	2.15 (0.68, 6.79)
	IL-6 Soluble Receptor Adjusted*	
Low, Low	Reference	Reference
High, Low	0.67 (0.28, 1.65)	0.40 (0.15, 1.04)
Low, High	0.50 (0.21, 1.23)	0.23 (0.09, 0.64)
High, High	0.70 (0.31, 1.57)	0.30 (0.13, 0.75)
	TNF Soluble Receptor Adjusted*	
Low, Low	Reference	Reference
High, Low	1.02 (0.43, 2.44)	1.32 (0.44, 4.00)
Low, High	0.75 (0.30, 1.89)	1.30 (0.43, 3.93)
High, High	0.82 (0.39, 1.73)	1.20 (0.46, 3.19)

*Models are adjusted for age, education, depression, cystatin-C, statin use and NSAID use.
**Low IL-6 ≤1.87 pg/ml and high IL-6 >1.87 pg/ml; low IL-6 sR ≤37401.36 pg/ml and high IL-6 sR >37401.36; low STNF-R1 <11.98 pg/ml and high STNF-R1 >11.98 pg/ml.

5.0 PAPER 3: TRAJECTORIES OF INFLAMMATORY MARKERS AND COGNITIVE DECLINE OVER 10 YEARS: FINDINGS FROM THE HEALTH, AGING AND BODY COMPOSITION STUDY

5.1 ABSTRACT

Background: High inflammation has been associated with elevated risks of cognitive decline, and Alzheimer's disease (AD). However, many studies have only investigated inflammation at one or two time points which greatly limits understanding how inflammation affects cognitive function over time. The objective of this study was to examine trajectories of inflammatory markers over time and cognitive decline over 10 years.

Methods Cox proportional hazards models were used to examine the association between interleukin-6 (IL-6) and C-reactive protein (CRP) trajectory patterns and cognitive decline among 1,323 adults, ≥65 years, enrolled in the Health, Aging and Body Composition Study with inflammation measured between three and five time points. Incident cognitive decline was defined as a decline of ≥5 points in the Modified Mini-Mental State Examination (3MS). We tested for a significant interaction by sex, race and apolipoprotein E e4 (APOE) allele presence.

Results Three trajectory components were examined: slope, variability, and baseline IL-6 and CRP levels. There was no significant association between baseline IL-6, slope, or variability and cognitive decline over 10 years. When examined individually, extreme variability in CRP level

was associated with greater incident cognitive decline (HR: 1.54, 95% CI: 1.14-2.09), whereas slope and baseline levels were not. In models adjusted for age, race, education, sex, baseline 3MS, body mass index, diabetes, and serum Cystatin-C, including all three components, extreme variability of CRP remained a significant predictor of cognitive decline (HR 1.57, 95% CI: 1.08-2.29). These associations were significantly modified by sex and APOE e4, such that they were stronger among women (HR=1.82; 95% CI: 1.09, 3.02) and among those with no APOE e4 allele (HR=1.63; 95% CI: 1.06, 2.50)

Conclusions Significant variability in individual trajectories of CRP level was a stronger predictor of cognitive decline than the slope of or an individual level of CRP. This CRP variability may reflect poor control of or greater changes in vascular disease over time which could in turn be associated with cognitive decline.

5.2 INTRODUCTION

The relationship between inflammation and dementia or Alzheimer's disease (AD) has been widely investigated for several reasons. First, inflammatory markers such as interleukin-6 (IL-6) and C-reactive protein have been found the amyloid plaques and neurofibrillary tangles that develop in AD.(Neuroinflammation Working Group et al., 2000) It has also been proposed that inflammatory markers contribute to the etiologic progression of dementia via several pathways, including vascular disease and overall neurodegeneration.(Brunello et al., 2000; Eagan et al., 2012; Ridker et al., 2002) While many studies have found a significant association between elevated CRP and IL-6 measured from one time point, and AD or cognitive decline (Kravitz et al., 2009a; Schmidt et al., 2002; Yaffe et al., 2003), several studies have not supported such an association.(Gallacher et al., 2010; Sundelof et al., 2009; Tan et al., 2007; van Oijen, Witteman, Hofman, Koudstaal, & Breteler, 2005) It has recently been suggested that levels of inflammation over time are highly variable (deGoma et al., 2012). Specifically, CRP has been shown to have considerable intra-individual variability over time, with a minimum of three CRP measurements suggested to accurately determine the association with cardiovascular outcomes.(Koenig et al., 2003) Thus these studies are greatly limited by having inflammation measured at only one time point. Thus, more studies are needed to investigate the association between inflammation measured at multiple time points, and cognitive function.

The objectives of this study were to examine the association between IL-6 and CRP trajectory patterns and incident cognitive decline and impairment over 10 years. A second objective was to determine if these associations were modified by sex or APOE e4. We hypothesized that the slope and variability of IL-6 and CRP trajectories over time would be

stronger predictors of cognitive function than an individual level of either marker, due to intra-individual variability over time. As previous studies have found stronger associations among non-APOE e4 carriers and women, we expected we may find the same.(Eriksson et al., 2011; Kravitz et al., 2009a)

5.3 METHODS

5.3.1 Study Population

Community-dwelling white and black older adults were enrolled in the ongoing Health Aging and Body Composition (Health ABC) study. This prospective cohort study began in 1997, and adults ranged in age from 70 to 79 years at enrollment, and lived in Memphis, TN or Pittsburgh, PA. Participants were recruited from a random sample of Medicare eligible adults living within the designated zip codes, and were eligible if they reported no difficulties performing activities of daily living, walking a quarter mile, or climbing 10 steps without resting. They also had to be free of life-threatening cancers, and plan to remain within the study area for at least three years. Our analytic cohort consisted of 1,323 participants who had CRP and IL-6 measured at a minimum of three time points. All participants included in this analytic cohort were also free of cognitive impairment at baseline; consistent with previous literature, cognitive impairment was defined as a Modified Mini-Mental Status Exam (3MS) score <80.(Slinin et al., 2010) This study was approved by the institutional review boards of the University of Pittsburgh and the University of Tennessee, Memphis, and that of the Coordinating Center, the University of California, San Francisco. All participants signed a written informed consent.

5.3.2 Cognitive Function

Cognitive function was assessed with the Modified Mini-Mental Status Exam (3MS) at baseline (Year 1), and study Years 3, 5, 8 and 10. The 3MS is an assessment of global cognitive function with components for orientation, concentration, language, praxis, and immediate and delayed

memory with scores ranging from 0 to 100 (higher scores indicating higher function).(Teng & Chui, 1987) Consistent with previous studies, we examined two potential outcomes: 1. Incident cognitive decline was defined as the first decline of 5 points or more from baseline, or the equivalent 1 SD of baseline 3MS scores, and 2. Incident cognitive impairment was defined as the first occurrence of a score ≤80 on the 3MS.(Lin et al., 2013; Stweart et al., 2013)

5.3.3 Inflammatory Markers

Measures of high sensitivity CRP and IL-6 were obtained from frozen serum or plasma collected five times throughout the study, at baseline (Year 1), and Years 2, 4, 6 and 8, after an overnight fast. Samples were frozen at -70°C and were shipped to the Core Laboratory at the University of Vermont.(Yaffe et al., 2003) Serum CRP was measured in duplicate by enzyme-linked immunosorbent assay on the basis of purified protein and polyclonal anti-CRP antibodies, and assays were standardized according to the World Health Organization First International Reference Standard with a sensitivity of 0.08 µg/ml.(Kalogeropoulos et al., 2010) Plasma IL-6 was measured in duplicate by ELISA kits from the R&D Systems (Minneapolis, MN).(Yaffe et al., 2003) The detectable limit for IL-6 was 0.10 pg/ml, and for TNFα was 0.18 pg/ml.(Yaffe et al., 2003)

5.3.4 Covariates

At baseline, demographic data including self-reported participant age, race, sex and education were recorded. Prevalent disease algorithms based on both self-report and physician diagnoses, recorded medications and laboratory data were used to create comorbidity variables indicating

presence of diabetes mellitus, hypertension, stroke or transient ischemic attack (TIA), and myocardial infarction (MI). Body mass index (BMI) (kg/m^2) was calculated from direct height and weight measurements at baseline. The Center for Epidemiologic Studies Depression Scale (CES-D) was used to assess depressive symptoms with a score ≥16 consistent with possible depression.(Radloff, 1977) APOE e4 allele status was determined using standard Single Nucleotide Polymorphism (SNP) genotyping techniques and dichotomized into having one or more APOE e4 allele versus no allele.(Hixson & Vernier, 1990) Creatinine and cystatin-C was obtained from frozen serum collected at baseline after an overnight fast. Samples were frozen at -70°C and were shipped to the Core Laboratory at the University of Vermont. An inventory of prescription and over-the-counter medications was recorded at baseline by examining participants' medication bottle(s). Consistent with a previous study using anti-inflammatory medication use as a covariate, we coded medications according to the Iowa Drug Information System (IDIS) code.(Pahor et al., 1994; Yaffe et al., 2003) With use of the IDIS, the daily use of anti-inflammatory drugs (IDIS code 2808), statins (IDIS code 2406), and oral estrogens (with or without progestins) (IDIS code 6816) was compiled.(Pahor et al., 1994; Yaffe et al., 2003)

5.3.5 Statistical Analysis

Pearson's chi-square or analysis of variance (ANOVA) were used to determine the association between baseline characteristics and baseline levels of IL-6 and CRP (≤median versus >median). Because a minimum of three measurements are required to define variability, all 1,323 participants in our analytic cohort had CRP and IL-6 available at three to five time points for analysis of trajectories. To allow for interpretation on a relative scale, and to account for skewed

distributions, CRP and IL-6 levels were log transformed (natural log). We examined the distributions of baseline CRP and IL-6 levels, trajectory slope, and variability around the trajectory. The slope on the log scale can be interpreted as the annual relative change in the original non-transformed scale. The variability around the trajectory was calculated by determining the root mean square error (RMSE) in a model with a linear trajectory which is equivalent to the coefficient of variation (CV). These analytic techniques were based on a previous study examining trajectories of another biological marker with an outcome of mortality.(Cappola et al., 2009)

Cox proportional hazard models were performed to estimate the relative hazard of incident cognitive decline and incident cognitive impairment. There were no statistically significant results for incident cognitive impairment, so only those for incident cognitive decline will be reported. Slope and variability of both IL-6 and CRP were modeled as time-varying covariates; at each measurement of either marker, slope and variability were recalculated using all measures through that date. By updating the slope and variability at each measurement, the hazard ratios (HRs) were estimated with respect to current, not future, exposure status. Relative hazards of incident cognitive decline by standard deviation of baseline ln(IL-6) or ln(CRP) level, and decile of slope and variability were estimated. All models were then adjusted for demographic variables that were selected as covariates a priori, including age, race, education and sex, as well as variables that differed by inflammatory marker at baseline, including BMI, diabetes, APOE e4 allele, and cystatin-C. We also adjusted all models by anti-inflammatory drug use due to the effect this could have on inflammation. Finally, all IL-6 models were adjusted for baseline ln(CRP), and all CRP models were adjusted for baseline ln(IL-6). Interactions of

baseline IL-6 and CRP level, slope, and variability with sex, race and APOE e4 were assessed using likelihood ratio test.

5.4 RESULTS

Participants had a mean age of 73.38 (±2.81) years at baseline, 697 (52.68%) were female, and 388 (29.33%) were black. At baseline, those above the median CRP level, compared to those at or below the median CRP level, were more likely to be black (69.45% vs 49.03%, p<0.001), to be female (59.43% vs. 47.10%, p<0.001), and to have diabetes (25.21% vs. 16.85%, p<0.001), and were less likely to have an APOE e4 allele (24.04% vs. 29.14%, p=0.02) (Table 5.6.1). Those above the median CRP level also had a higher BMI (27.77±4.85 vs. 26.40±4.08, p<0.001), had lower baseline ln(IL-6) (0.50±0.60 vs. 0.92±0.68, p<0.001), had higher Cystatin-C (1.02 mg/L±0.25 vs. 0.99 mg/L±0.21), indicating poorer kidney function (Table 5.6.1). Patterns of baseline characteristics by IL-6 levels were similar, and did not change variables for which we wanted to adjust models by, so they are not reported here. The correlation coefficient of ln(IL-6) and ln(CRP) at baseline was 0.40 (p<0.001), and at Year 8 was 0.36 (p<0.001). The correlation coefficient of ln(CRP) at baseline with ln(CRP) at Year 8 was 0.33 (p<0.001); the correlation coefficient of ln(IL-6) at baseline with ln(IL-6) at Year 8 was 0.42 (p<0.001).

In cox proportional hazards models, there were no significant associations between baseline ln(IL-6) level, slope or variability and cognitive decline (Table 5.6.2). There were also no significant associations between baseline ln(CRP) level or slope and cognitive decline (Table 5.6.3). However, those with extreme variability of CRP compared to those with minimal variability had an increased risk of cognitive decline in 3 models: one with variability only (HR=1.54; 95% CI: 1.14, 2.09); one with slope and variability (HR=1.51; 95% CI: 1.06, 2.14); and one with baseline ln(CRP), slope, and variability (HR=1.54; 95% CI: 1.07, 2.21) (Table 5.6.3). These associations remained significant after adjustment for age, race, sex, education,

BMI, diabetes, APOE e4, cystatin-C, anti-inflammatory drug use and baseline ln(IL-6) (Table 5.6.3).

There was a significant interaction of baseline ln(CRP) level, slope and variability with APOE (χ^2=372.949, 2 degrees of freedom, p<0.001), and with sex (χ^2=52.256, 2 degrees of freedom, p<0.001), but not with race. The relationship between the CRP trajectory pattern, specifically the variability component of the trajectory pattern, was stronger among women, and among those with no APOE e4 allele. In a model with baseline ln(CRP), slope and variability, women with extreme variability compared to those with minimal variability had an increased risk of cognitive decline (HR=1.82; 95% CI: 1.09, 3.02), but the same was not true for men (HR=1.32; 95% CI: 0.82, 2.19) (Table 5.6.4). This remained true after adjustment for age, race, education, BMI, diabetes, APOE e4, cystatin-C, anti-inflammatory drug use and baseline ln(IL-6) (women HR=1.96, 95% CI: 1.17, 3.27 and men HR=1.25; 95% CI: 0.72, 2.17) (Table 5.6.4). Similarly, in a model with baseline ln(CRP), slope and variability, those with no APOE e4 allele and extreme CRP variability had an increased risk of cognitive decline (HR=1.63; 95% CI: 1.06, 2.50), but the same was not true for those with ≥1 APOE e4 allele (HR=1.37; 95% CI: 0.63, 2.97) (Table 5.6.5). This remained true after adjustment for age, race, sex, education, BMI, diabetes, Cystatin-C, anti-inflammatory drug use and baseline ln(IL-6) (no APOE e4 allele HR= 1.69; 95% CI: 1.10, 2.60 and ≥1 APOE e4 allele HR=1.39; 95% CI: 0.62, 3.11) (Table 5.6.5).

5.5 DISCUSSION

To our knowledge, this is the first prospective cohort study to look at the association between trajectories of inflammation measured from three to five time points and cognitive decline. We found no significant associations between trajectory patterns of IL-6 and cognitive decline. However, those with extreme variability in CRP over time had an increased risk of cognitive decline over 10 years. This association remained significant even after adjustment for multiple covariates and confounders, including age, race, education, sex, education, APOE e4, BMI, diabetes, cystatin-C, anti-inflammatory drug use, and baseline IL-6. Furthermore, this association was stronger among women and among those with no APOE e4 allele.

Our results complement previous studies reporting a significant association between elevated CRP measured from one time point, and AD or cognitive decline.(Kravitz et al., 2009a; Schmidt et al., 2002; Yaffe et al., 2003) Similarly, one study which had CRP measured at 2 time points found that doubling of CRP was significantly associated with greater decline in the 3MS over 9 years.(Jenny et al., 2012) However, it has recently been found that there may be high intra-individual variability in CRP levels over time, so our interpretation of the results from these previous studies is limited.(deGoma et al., 2012; Koenig et al., 2003) Thus, our study contributed uniquely to the literature by not only looking at elevated levels of CRP at baseline, but by examining individuals' slope and variability of CRP trajectories over time. Our finding that variability of CRP over time was a significant predictor of cognitive decline points to the need to have inflammation and cognitive function measured at multiple time points when assessing such an association, and supports previous literature finding similar results with cardiovascular outcomes.(deGoma et al., 2012; Koenig et al., 2003)

One potential mechanism underlying an association between CRP variability and increased cognitive decline over time is greater overall morbidity, especially in terms of vascular and metabolic disease. Elevated levels of CRP have been associated with a variety of vascular and metabolic disease states, including obesity, cardiovascular disease, atherosclerosis and diabetes.(deGoma et al., 2012; C. Ferri et al., 2007; Wang et al., 2013) Other evidence supporting such a pathway is an indication that CRP is more stable, and lacks intra-individual variability in the absence of disease.(Kluft & de Maat, 2001) Thus, what we may be measuring with variability in CRP is greater morbidity over time, and perhaps conditions that are more poorly controlled by medication and behavior interventions. This could have clinical implications because it could mean that measuring CRP over time and detecting greater variability could be more useful than obtaining a simple medical history from patients where questions are answered with a simple yes/no. The previous associations between CRP and increased risk for vascular disease, combined with our findings could also suggest that CRP variability over time is associated with cognitive decline may be due to vascular changes in the brain, rather than with AD-specific pathology.

We found the association between CRP variability and increased risk for cognitive decline was stronger among those with no APOE e4 allele. This could be due to the etiology of the cognitive decline. It is widely known that those with at least one APOE e4 allele are at an increased risk for AD(Ingelsson et al., 2003), so perhaps we are measuring cognitive decline related to vascular changes rather than AD pathology. Our study also found the association between CRP variability and increased cognitive decline was stronger among women. While men are known to have a greater burden of vascular disease earlier on in life, this gap decreases with age, and once over the age of 80 years, women actually have a greater burden of

cardiovascular disease.(Roger et al., 2012) Perhaps, given the age of these participants (approximately 73 years at baseline and 83 years when cognitive function was last assessed), this is due to and increased vascular disease burden among women.(Roger et al., 2012)

This study had several strengths, including the measurement of inflammatory markers at 3 to 5 time points. Furthermore, we had a relatively large sample size providing ample analytical power for this study. Measurement of numerous comorbidities and demographic data also allowed us to investigate a large number potential covariates and confounders. There are also several weakness that should be taken into consideration when interpreting these results. These adults were all well-functioning and community-dwelling at baseline, so results may not be generalizable to all older adults – for example, nursing home populations. We were also limited to examining the inflammatory markers CRP and IL-6 due to what was measured, but other inflammatory markers have previously been related to cognitive function, and may provide more information.

We found that high CRP variability over time was significantly associated with increased risk for cognitive decline over 10 years, and that variability measured from 3 to 5 time points was a stronger predictor than individual levels of CRP or the slope of CRP over time. Theses associations were stronger among women and those with no APOE e4 allele. We believe CRP variability may reflect a greater burden of vascular and metabolic disease, and perhaps signify conditions that are more poorly controlled. Future studies should investigate if CRP variability is related to AD- and vascular dementia-specific pathologies, such as amyloid deposition in the brain and white matter hyperintensities to allow a better understanding of how CRP variability may be influencing cognitive function.

5.6 TABLES AND FIGURES

Table 5.6.1 Association between baseline characteristics and baseline CRP level

Baseline Characteristic Mean (SD) or N (%)	<= Median CRP (N=724)	>Median CRP (N=599)	p-value
Age	73.47 (2.77)	73.28 (2.86)	0.23
Black Race	355 (49.03%)	416 (69.45%)	<0.0001
Education (≤High School)	321 (44.34%)	300 (50.08%)	0.11
Female Sex	341 (47.10%)	356 (59.43%)	<0.0001
Body Mass Index	26.40 (4.08)	27.77 (4.85)	<0.0001
Diabetes	122 (16.85%)	151 (25.21%)	0.0002
Hypertension	511 (70.58%)	436 (72.79%)	0.38
Stroke/TIA	58 (8.01%)	44 (7.35%)	0.65
Myocardial Infarction	70 (9.67%)	53 (8.85%)	0.61
Baseline 3MS Score	93.89 (3.91)	93.72 (3.74)	0.44
APOE e4 Allele	211 (29.14%)	144 (24.04%)	0.02
Serum Creatinine	1.02 (0.24)	1.00 (0.23)	0.21
Cystatin-C	0.99 (0.21)	1.02 (0.25)	0.01
Anit-Inflammatory Drug Use	409 (56.49%)	322 (53.76%)	0.34
CES-D Score	4.47 (5.25)	4.37 (4.66)	0.71
Baseline IL-6	0.92 (0.68)	0.50 (0.60)	<0.001

Table 5.6.2 Association between IL-6 and cognitive decline over 10 years

Unadjusted Model	Measure	HR (95% CI)
Baseline only	Baseline	1.03 (0.93, 1.14)
Slope only	Slope	1.01 (0.72, 1.42)
Variability only	Minimal Variability	Reference
	Moderate Variability	1.11 (0.88, 1.41)
	Extreme Variability	0.84 (0.58, 1.20)
Slope + Variability	Slope	1.04 (0.73, 1.48)
	Minimal Variability	Reference
	Moderate Variability	1.11 (0.87, 1.41)
	Extreme Variability	0.83 (0.57, 1.21)
Baseline + Slope + Variability	Baseline	1.05 (0.93, 1.19)
	Slope	0.97 (0.65, 4.44)
	Minimal Variability	Reference
	Moderate Variability	1.08 (0.84, 1.39)
	Extreme Variability	0.81 (0.55, 1.18)
Adjusted Model*	**Measure**	**HR (95% CI)**
Baseline only	Baseline	1.06 (0.93, 1.20)
Slope only	Slope	0.99 (0.69, 1.43)
Variability only	Minimal Variability	Reference
	Moderate Variability	1.13 (0.87, 1.46)
	Extreme Variability	0.87 (0.59, 1.29)
Slope + Variability	Slope	1.01 (0.69, 1.47)
	Minimal Variability	Reference
	Moderate Variability	1.13 (0.87, 1.47)
	Extreme Variability	0.87 (0.58, 1.30)
Baseline + Slope + Variability	Baseline	1.09 (0.94, 1.26)
	Slope	0.90 (0.59, 1.38)
	Minimal Variability	Reference
	Moderate Variability	1.10 (0.84, 1.44)
	Extreme Variability	0.84 (0.55, 1.26)

*Adjusted for age, race, sex, education, BMI, diabetes, APOE e4, Cystatin-C, anti-inflammatory drug use and baseline ln(CRP).

Table 5.6.3 Association between CRP and cognitive decline over 10 years

Unadjusted Model	**Measure**	**HR (95% CI)**
Baseline only	Baseline	1.02 (0.92, 1.14)
Slope only	Slope	1.32 (0.97, 1.78)
Variability only	Minimal Variability	Reference
	Moderate Variability	1.06 (0.82, 1.37)
	Extreme Variability	1.54 (1.14, 2.09)
Slope + Variability	Slope	1.05 (0.72, 1.53)
	Minimal Variability	Reference
	Moderate Variability	1.06 (0.81, 1.37)
	Extreme Variability	1.51 (1.06, 2.14)
Baseline + Slope + Variability	Baseline	1.02 (0.92, 1.14)
	Slope	0.97 (0.65, 1.44)
	Minimal Variability	Reference
	Moderate Variability	1.05 (0.80, 1.38)
	Extreme Variability	1.54 (1.07, 2.21)
Adjusted Model*	**Measure**	**HR (95% CI)**
Baseline only	Baseline	0.99 (0.87, 1.13)
Slope only	Slope	1.20 (0.87, 1.67)
Variability only	Minimal Variability	Reference
	Moderate Variability	1.01 (0.77, 1.33)
	Extreme Variability	1.48 (1.07, 2.05)
Slope + Variability	Slope	0.95 (0.63, 1.43)
	Minimal Variability	Reference
	Moderate Variability	1.02 (0.77, 1.35)
	Extreme Variability	1.51 (1.04, 2.19)
Baseline + Slope + Variability	Baseline	0.97 (0.85, 1.11)
	Slope	0.85 (0.56, 1.31)
	Minimal Variability	Reference
	Moderate Variability	1.01 (0.75, 1.35)
	Extreme Variability	1.57 (1.08, 2.29)

*Adjusted for age, race, sex, education, BMI, diabetes, APOE e4, Cystatin-C, anti-inflammatory drug use and baseline ln(IL-6).

Table 5.6.4 Association between CRP and cognitive decline over 10 years by sex.

		HR (95% CI)	
Unadjusted Model	**Measure**	**Male HR**	**Female HR**
Baseline only	Baseline	1.05 (0.91, 1.23)	1.01 (0.86, 1.17)
Slope only	Slope	1.52 (0.99, 2.35)	1.18 (0.77, 1.81)
Variability only	Minimal Variability	Reference	Reference
	Moderate Variability	0.96 (0.67, 1.39)	1.16 (0.82, 1.66)
	Extreme Variability	1.59 (1.05, 2.43)	1.48 (0.95, 2.31)
Slope + Variability	Slope	1.46 (0.87, 2.44)	0.77 (0.44, 1.35)
	Minimal Variability	Reference	Reference
	Moderate Variability	0.91 (0.63, 1.33)	1.21 (0.84, 1.73)
	Extreme Variability	1.36 (0.84, 2.21)	1.67 (1.01, 2.78)
Baseline + Slope + Variability	Baseline	1.04 (0.89, 1.21)	1.01 (0.87, 1.19)
	Slope	1.48 (0.87, 2.53)	0.65 (0.36, 1.17)
	Minimal Variability	Reference	Reference
	Moderate Variability	0.91 (0.62, 1.35)	1.19 (0.82, 1.74)
	Extreme Variability	1.32 (0.80, 2.19)	1.82 (1.09, 3.02)
Adjusted Model*	**Measure**	**Male**	**Female HR**
Baseline only	Baseline	1.03 (0.85, 1.25)	0.93 (0.78, 1.12)
Slope only	Slope	1.36 (0.85, 2.17)	1.08 (0.68, 1.70)
Variability only	Minimal Variability	Reference	Reference
	Moderate Variability	0.96 (0.65, 1.41)	1.09 (0.74, 1.59)
	Extreme Variability	1.45 (0.90, 2.33)	1.47 (0.93, 2.33)
Slope + Variability	Slope	1.40 (0.81, 2.43)	0.66 (0.36, 1.21)
	Minimal Variability	Reference	Reference
	Moderate Variability	0.90 (0.61, 1.35)	1.15 (0.78, 1.70)
	Extreme Variability	1.26 (0.73, 2.15)	1.77 (1.06, 2.97)
Baseline + Slope + Variability	Baseline	1.00 (0.82, 1.21)	0.93 (0.78, 1.13)
	Slope	1.40 (0.79, 2.49)	0.53 (0.28, 1.01)
	Minimal Variability	Reference	Reference
	Moderate Variability	0.88 (0.58, 1.35)	1.15 (0.77, 1.72)
	Extreme Variability	1.25 (0.72, 2.17)	1.96 (1.17, 3.27)

*Adjusted for age, race, education, BMI, diabetes, APOE e4, Cystatin-C, anti-inflammatory drug use and baseline ln(IL-6).

Table 5.6.5 Association between CRP and cognitive decline over 10 years, by APOE e4 allele.

		HR (95% CI)	
Unadjusted Model	**Measure**	**≥1 APOE e4 allele**	**No APOE e4 allele**
Baseline only	Baseline	1.01 (0.83, 1.23)	1.02 (0.90, 1.17)
Slope only	Slope	1.40 (0.75, 2.60)	1.15 (0.79, 1.67)
Variability only	Minimal Variability	Reference	Reference
	Moderate Variability	1.02 (0.63, 1.65)	1.02 (0.74, 1.40)
	Extreme Variability	1.35 (0.70, 2.60)	1.51 (1.05, 2.18)
Slope + Variability	Slope	1.21 (0.57, 2.60)	0.87 (0.54, 1.39)
	Minimal Variability	Reference	Reference
	Moderate Variability	1.00 (0.61, 1.63)	1.04 (0.75, 1.44)
	Extreme Variability	1.22 (0.57, 2.64)	1.61 (1.06, 2.45)
Baseline + Slope + Variability	Baseline	1.03 (0.84, 1.25)	1.01 (0.89, 1.16)
	Slope	1.03 (0.46, 2.30)	0.80 (0.49, 1.32)
	Minimal Variability	Reference	Reference
	Moderate Variability	1.03 (0.62, 1.70)	1.00 (0.71, 1.41)
	Extreme Variability	1.37 (0.63, 2.97)	1.63 (1.06, 2.50)
Adjusted Model*	**Measure**	**≥1 APOE e4 allele**	**No APOE e4 allele**
Baseline only	Baseline	0.86 (0.67, 1.11)	1.04 (0.89, 1.22)
Slope only	Slope	1.36 (0.71, 2.63)	1.17 (0.80, 1.71)
Variability only	Minimal Variability	Reference	Reference
	Moderate Variability	1.08 (0.65, 1.77)	1.01 (0.73, 1.40)
	Extreme Variability	1.32 (0.65, 2.77)	1.58 (1.09, 2.30)
Slope + Variability	Slope	1.18 (0.53, 2.63)	0.88 (0.55, 1.41)
	Minimal Variability	Reference	Reference
	Moderate Variability	1.05 (0.63, 1.75)	1.03 (0.74, 1.44)
	Extreme Variability	1.22 (0.54, 2.73)	1.67 (1.09, 2.54)
Baseline + Slope + Variability	Baseline	0.86 (0.67, 1.11)	1.02 (0.87, 1.19)
	Slope	1.04 (0.45, 2.42)	0.81 (0.49, 1.34)
	Minimal Variability	Reference	Reference
	Moderate Variability	1.06 (0.63, 1.79)	1.00 (0.70, 1.42)
	Extreme Variability	1.39 (0.62, 3.11)	1.69 (1.10, 2.60)

*Adjusted for age, race, sex, education, BMI, diabetes, Cystatin-C, anti-inflammatory drug use and baseline ln(IL-6).

6.0 DISCUSSION

6.1 SUMMARY, CONCLUSIONS AND FUTURE RESEARCH

The primary aim of these papers was to investigate the potential role of plasma Aβ40, Aβ42 and serum- and plasma-derived inflammatory as biological markers of AD. We found plasma Aβ0 and Aβ42 were significantly associated with age, race, sex, education, APOE e4 genotype, and creatinine. We further found that there were borderline significant associations with plasma Aβ and myocardial infarction, total cholesterol, and markers of inflammation. Finally, we found that changes in inflammatory markers over time had stronger associations with cognitive outcomes than measuring these markers at only one time point. Having extreme variability in CRP may increase the risk of cognitive decline. Finally, the association between high inflammation and dementia may be pleiotropic, appearing to be protective in the oldest old. This could be due to survival bias or to the complexity of the immune system in advanced age. While the results do not provide sufficient evidence for the use of these proteins as biomarkers of AD, they do highlight some potential underlying mechanisms linking these proteins to the development of dementia, and provide insight for future directions of this research.

Taken together, these findings highlight the importance of vascular mechanisms in the development of AD and dementia. Our findings that plasma Aβ40 and Aβ42 have borderline significant associations with total, HDL and LDL cholesterol, myocardial infarction, and markers

of inflammation add to a growing body of literature linking these proteins to vascular disease.(A. L. Metti et al., 2012) Recent studies have suggested that Aβ40 and Aβ42 are vasoactive proteins, and are directly related to vascular risk factors, such as midlife blood pressure, total cholesterol and diabetes.(Blasko et al., 2011; Shah et al., 2012) Importantly, plasma Aβ40 and Aβ42 have also been found to accumulate in microvessels outside of the brain, and contribute to vasoconstriction of vessels and to the development of vascular disease.(Iadecola, Park, & Capone, 2009; Smith & Greenberg, 2009) Plasma Aβ40 has also emerged as a potential biomarker of white matter hyperintensities among people with AD and MCI, indicating that it may not only be a marker of amyloid development, but also of microvascular damage in older adults.(Gurol et al., 2006) Most recently, measures of vascular disease, such as elevated resting blood pressure, brachial-ankle pulse wave velocity, and mean arterial pressure were shown to be related to amyloid deposition in the brains of non-demented older adults suggesting the relationship between vascular disease and amyloid deposition may be bi-directional.(Hughes et al.)

Inflammation is also related to vascular disease. Specifically, inflammation is known as both a risk factor and consequence of vascular disease, and vascular disease risk factors such as obesity and diabetes.(C. Ferri et al., 2007; Wang et al., 2013) Subclinical measures of vascular disease, such as carotid intima-media thickness have also been shown to be risk factors for dementia and cognitive decline.(Wendell, Zonderman, Metter, Najjar, & Waldstein, 2009; Zhong et al., 2012) Our finding that high CRP variability is associated with increased risk of cognitive decline suggests a potential vascular mechanism. Elevated levels of CRP have been associated with a variety of vascular and metabolic disease states, including obesity, cardiovascular disease, atherosclerosis and diabetes.(deGoma et al., 2012; C. Ferri et al., 2007; Wang et al., 2013)

Furthermore, CRP has been shown to be less variable among adults who are disease-free (Kluft & de Maat, 2001), suggesting that with CRP variability, we may be assessing greater morbidity over time. Our finding that plasma Aβ40 and Aβ42 had borderline significant associations with inflammatory markers also points to the potentially complex interrelationship between inflammatory markers and plasma Aβ.(A. L. Metti et al., 2012) Due to limitations in sample size with measurements of both inflammation and plasma Aβ, we were unable to investigate the association between all of these markers together and cognitive decline, but this is a proposed direction of future research.

Other potential underlying mechanisms, such as diabetes and kidney function were also highlighted through our research. Beginning with diabetes, we found that both plasma Aβ40 and Aβ42 were significantly associated with a history of diabetes.(A. L. Metti et al., 2012) We also found in our inflammatory analyses that a history of diabetes was significantly related to our predictors IL-6, IL-6 sR, and CRP. Furthermore, diabetes has also been shown to be a risk factor for cognitive decline and mild cognitive impairment.(Luchsinger et al., 2007; Yaffe et al., 2004) Interestingly, hyperinsulinemia has also been associated with concurrent increases in CSF inflammatory markers and Aβ42, although studies have not investigated similar relationships with plasma or serum biomarkers.(Fishel et al., 2005) We also found that kidney function, measured with serum creatinine was significantly associated with plasma Aβ40 and Aβ42.(A. L. Metti et al., 2012) In our inflammatory analyses, cystatin-C, another measure of kidney function was significantly related to CRP and IL-6 level. Previous studies have shown that poorer kidney function, as measured by cystatin-C, creatinine, or estimated glomerular filtration rate, is related to increased risk for cognitive decline and dementia.(Bugnicourt, Godefroy, Chillon, Choukroun, & Massy, 2013; Kurella et al., 2005) When considering the biology of the brain and kidneys,

there are several similarities that should not be ignored: 1. They are both passively perfused at a high volume throughout the cardiac cycle; 2. Both the brain and kidneys have arterioles that are upstream of vasodilation, exposing these arterioles to high pulsatile pressure; and 3. Both organs are low-resistance end-organs, exposed to high-volume blood flow under constantly changing flow conditions, and are thus both vulnerable to vascular damage.(Barinas-Mitchell, 2013; Bugnicourt et al., 2013) Of course, given these similarities relating the brain and kidney back to vascular damage, and considering the fact that diabetes is also a risk factor for vascular disease, once again, we circle back to the idea that vascular disease may also be a potential underlying mechanism.

Thus, future studies should investigate the role of inflammatory markers and plasma Aβ, and determine if vascular disease, diabetes, kidney disease or a combination of all three are a potential mechanism underlying an association between inflammation, amyloid and cognitive decline. More work is also needed to determine the reproducibility, validity and reliability of these markers in predicting cognitive decline or dementia, but before that can be done, standardization of laboratory methods across studies will need to be a primary goal. Due the heterogeneity and complexity of dementia, and the vascular implications discussed above, studies should investigate how these biomarkers are related to specific types of dementia pathology – for example white matter hyperintensities and amyloid plaques. Additionally, future research should investigate how these markers fluctuate over time with the development of these pathologies, and in terms of these comorbid conditions. Finally, in the future, it would be ideal if studies could use multiple-array techniques or proteomics to allow for the investigation of multiple biological markers at one time, rather than just measuring these markers individually, as they are likely highly interrelated.

6.2 PUBLIC HEALTH IMPLICATIONS

An estimated 24.3 million older adults worldwide are currently living with dementia, with an estimated incidence of 4.6 million cases each year.(C. P. Ferri et al., 2005) In the United States, dementia is already the sixth leading cause of death overall, and the fifth leading cause of death among adults 65 years of age and older.(Alzheimer's Association, 2011) Furthermore, the predicted prevalence for the year 2020 is 42.3 million cases, and by the year 2040 is 81.1 million cases, unless something is done to slow the progression or delay the onset of dementia.(C. P. Ferri et al., 2005) With the expected increase in dementia over the next 20 years and lack of any effective treatment, we can only expect the public health burden to also increase exponentially, and as such, there is a pressing need to improve the ability to accurately diagnose AD. Identifying reliable markers that can be assessed in a large number of older adults, in a cost-effective manner, so that large numbers of older adults can be screened, such as the blood-derived biomarkers discussed in these papers, is one potential way of improving diagnosis. Gaining a better understanding of these biomarkers is also important because it will continue to shed light on those who are at an increased risk for the disease, and potential targets for treatment or behavioral interventions. As discussed previously, vascular disease, kidney disease and diabetes all seem to be underlying mechanisms highlighted in our work that may potentially be a link between these markers and cognitive function. Thus, perhaps primary or secondary prevention of these conditions may be potentially useful in either delaying the onset of dementia or preventing the occurrence altogether. In fact, one study showed that in the United States, if we could reduce diabetes prevalence by 10%, we could prevent nearly 17,000 cases of AD; a similar reduction in midlife hypertension, midlife obesity, physical inactivity or smoking – all risk factors for vascular disease – could prevent nearly 40000, 36000, 90000 and 50000 cases of AD,

respectively.(Barnes & Yaffe, 2011) If all efforts were combined, this could lead to more than 200,000 cases of AD prevented in the United States alone. Finally, it is important to remember that we have no current treatment for AD. Thus, by further examining these complex interrelationships between biomarkers, comorbidities and dementia, we can hopefully shed some light on therapeutic strategies for preventing or curing this disease. For example, drugs that effectively prevent or treat inflammation, vascular disease, kidney disease or diabetes may be potentially useful.

APPENDIX A

A.1 SUPPLEMENTARY TABLE

Table A.1.1 A summary of current, promising plasma and serum biomarkers of Alzheimer's disease.

Biomarker/Reference	Source	Outcome	Association	Potential Mechanism	Sample Characteristics	Adjusted Covariates
Aβ42				Amyloid pathway/deposition		
Fukumoto, et al., 2003. *Arch Neurol.*	Plasma	AD, MCI, cognitively normal	No association between continuous level of plasma Aβ42 and diagnosis of AD or MCI, compared to normal controls (52.4 ±17.5 pmol/L and 47.8±15.0 pmol/L vs. 48.9±17.2, respectively)		N=371; Memory clinic setting; overall μ_{age} not reported; μ_{age} =76.0 for AD and 69.4 for normal controls	Age, sex, APOE; family history, medication use
Graff-Radford, et al., 2007. *Arch Neurol*.	Plasma	Risk of developing MCI or AD over time (3 to 7 years)	No association between q-uartile of Aβ42 and risk of developing MCI or AD Q1:Q4 RR=1.18 (0.50, 2.75)		N=563; μ_{age} =78.0 years; all white	Age, APOE
Lopez, et al., 2008. *Neurology*.	Plasma	Incident dementia	No association between continuous Aβ42 and development of incident dementia 4 to 5 years later; OR: 1.46 (0.96, 2.22)		N= 274; Cardiovascular Health Study Cognition Study; μ_{age}=74.0 years at	Age, 3MS scores, APOE, cystatin-C, MRI identified infarcts

Table A.1.1 Continued

				baseline	
		Incident MCI	No association; OR=1.34 (0.90, 1.99)		
		Cross-sectional with AD diagnosis	No association; OR=1.30 (0.87, 1.94)		
Mayeux, et al., 2003. *Neurology*.	Plasma	Incident AD	Those in the highest quartile, compared to the low had increased risk of developing AD; RR: 2.5 (1.3, 4.8)	N=530; community-dwelling; μ_{age} for those who developed dementia=79.3 years at baseline vs. 75.5 years for those who didn't; white, Hispanic, and African-American (overall percentages not reported)	Age, education, APOE, BMI
Mehta, et al., 2000. *Arch Neurology*.	Plasma	Cognitive status	No significant association between continuous level and cognitive status (no specific measure of association or p-value reported).	N=78 with AD and 75 controls; 2 university medical centers (New York and Finland); 53% women	Not reported
Pomara, et al., 2005. Am *J Geriatr Psychiatry*.	Plasma	Cognitive decline (MMSE) over ~4.2 years	Higher initial Aβ42 (continuous measure) (Spearman's r=-0.35, p=0.05) and greater reductions were associated with greater declines on the MMSE (Spearman's r=0.41, p=0.02)	N=34; μ_{age}=65.4 years at baseline; 56% women	None

Table A.1.1 Continued

Schupf, et al., 2008. *Proc Natl Acad Sci*.	Plasma	Incident AD over ~4.6 years Change in plasma Aβ with risk of AD	Those in the highest quartile were significantly more likely to develop AD, compared to lowest quartile; HR=3.4 (1.6, 7.6) Compared to those with increasing levels, those with decreasing levels had a higher risk of AD; OR=2.8 (1.6, 5.1)		N=1,125; Washington Heights-Inwood Columbia Aging Project; >50% women (overall percentage not reported); white, Hispanic and African American (specific percentages not reported)	Age, sex, ethnicity, education, BMI, APOE
Van Oijen, et al., 2006. *Lancet Neurol*.	Plasma	Incident AD over a mean of 8.6 years	No association between high quartile compared to low; HR: 1.32 (0.90, 1.96)		N=1756; Rotterdam Study; μ_{age}=68.6 years at baseline; 61% women	Age, sex, APOE, creatinine, total cholesterol, HDL cholesterol, BMI
Yaffe, et al., 2011. *JAMA*.	Plasma	9-year change in 3MS	Low tertile: -6.96 (-5.53, -8.03) vs. High tertile: -4.30 (-3.03, -5.47); p=0.007		N=977; Health ABC; μ_{age}=74.0 years at baseline; 54% black; 55% female	Age, race, education, diabetes, smoking, APOE, baseline 3MS scores
Aβ42/Aβ40				Amyloid pathway/deposition		
Graff-Radford, et al., 2007. *Arch Neurol*.	Plasma	Risk of developing MCI or AD over time (3 to 7 years)	Q1:Q4 RR=3.08 (1.12, 8.30)		N=563; μ_{age} =78.0 years; all white	Age, APOE
Lopez, et al., 2008. *Neurol*.	Plasma	Incident dementia	No association between continuous Aβ42/Aβ40 and development of incident dementia 4 to 5 years later OR: 1.37 (0.91, 2.06)		N= 274; Cardiovascular Health Study Cognition Study; μ_{age}=74.0 years at	Age, 3MS scores, APOE, cystatin-C, MRI identified infarcts

Table A.1.1 Continued

		Incident MCI	No association; OR=1.06 (0.74, 1.54)		baseline	
		Cross-sectional with AD diagnosis	No association; OR=1.19 (0.81, 1.75)			
Schupf, et al., 2008. *Proc Natl Acad Sci*.	Plasma	Incident AD over ~4.6 years	No association; Q4:Q1 HR=0.9 (0.5, 1.7)		N=1,125; Washington Heights-Inwood Columbia Aging Project; >50% women (overall percentage not reported); white, Hispanic and African American (specific percentages not reported)	Age, sex, ethnicity, education, BMI, APOE
		Change in plasma Aβ with incident AD	Compared to those with increasing levels, those with decreasing levels had a higher risk of AD; OR=3.6 (1.1, 12.1)			
Van Oijen, et al., 2006. *Lancet Neurol*.	Plasma	Incident AD over a mean of 8.6 years	Those in the high quartile, compared to those in the low quartile have a reduced risk of AD; HR: 0.53 (0.35, 0.76)		N=1756; Rotterdam Study; μ_{age}=68.6 years at baseline; 61% women	Age, sex, APOE, creatinine, total cholesterol, HDL cholesterol, BMI
Yaffe, et al., 2011. *JAMA*.	Plasma	9-year change in 3MS	Low tertile: -6.38 (-5.15, -7.61) vs. High tertile: -3.44 (-2.21, -4.67); p=0.02 *Modified by low cognitive reserve (education and literacy) and by APOE e4 allele status		N=977; Health ABC; μ_{age}=74.0 years at baseline; 54% black and 55% female	Age, race, edu, diabetes, smoking, APOE e4, baseline 3MS scores
Aβ Antibodies				Amyloid pathway/deposition		

Table A.1.1 Continued

Brettschneider, et al., 2005. *Biol Psychiatry*.	Serum	AD vs. normal control	Aβ42 autoantibodies were significantly reduced in AD patients, compared to normal controls (3.1±3.28 vs. 5.88±4.54, p=0.001), even after age-matching (p=0.01)	N=; University clinic, Germany; μ_{age}=70 years at baseline for AD and 55 at baseline for controls; 61% female	Age, APOE, MMSE score
Hock, et al., 2003. *Neuron*.	Serum	Cognitive and functional decline after active Aβ42 immunization	Patients who generated antibodies (as measured in serum) after immunization had significantly lower rates of cognitive decline (p=0.008) and functional decline (activities of daily living; p=0.03) compared to those who didn't. After 1 year, 67% of those who didn't develop antibodies progressed to severe dementia (MMSE <14) vs. only 16% of patients who developed antibodies (p<0.01)	N=28 (19 with antibodies and 9 controls); mildly to moderately demented (MMSE 16-26) at baseline	Age, sex, medication (including acetyl-cholinesterase inhibitors), head trauma, and APOE
Hyman, et al., 2001. *Ann Neurol*.	Plasma	Risk of developing AD over 7 years	There were low detectable levels of Aβ antibodies in only half of the samples. Aβ antibodies were not significantly correlated with risk of developing AD (p=0.85)	N=365 (82 with AD, 271 healthy controls); sub-cohort from prospective study of Medicare-eligible in Manhattan, NY; μ_{age}=76 years; >60% women; African American,	Sex, age, ethnicity, APOE

Table A.1.1 Continued

					Hispanic and white (exact percentages not given)	
Moir, et al., 2002. *J Biol Chem.*	Plasma	Cross-sectional comparison of Aβ antibody levels in AD patients vs. normal controls	Plasma immunoreactivity to Aβ was significantly decreased in AD patients compared to normal controls (p=0.02)		N=118 (59 AD and 59 normal control); memory clinic setting (Boston);	None reported
IL-6				Inflammation		
Engelhart, et al., 2004. *Arch Neurol*.	Plasma	Risk of AD over 1 year	High IL-6 was significantly associated with increased risk for AD (Rate ratio per standard deviation increase in IL-6 =1.28 (1.06-1.55)		N=727; μ_{age}=71.7 years at baseline; 53% female; Rotterdam Study subgroup	Age, sex, education, smoking, BMI, diabetes, anti-inflammatory medication use, atherosclerosis
Eriksson, et al., 2011	Serum	Prevalent and incident all-cause dementia and AD	When compared to controls, those with all-cause dementia and AD had significantly elevated levels of IL-6. There was no significant association between serum IL-6 and incident all-cause dementia or AD.		N=3,937; μ_{age}=77.7 years for controls; 75.3 years for dementia cases	APOE e4, BMI, smoking, blood pressure, education, diabetes, coronary heart disease, stroke
Gallacher, et al., 2010. *Arterioscler Thromb Vasc Biol.*	Plasma	Risk of dementia over 20 years	There was no significant association between continuous baseline IL-6 and risk of dementia (HR: 0.66; 95% CI: 0.32-1.35)		N=865; all men; 65-84 years of age at cognitive status determination, but 45 to 59 when inflammatory markers measured)	Age, social class, systolic blood pressure, BMI, smoking, total cholesterol, alcohol use

Table A.1.1 Continued

Licastro, et al., 2000. *J Neuroimm.*	Plasma	Cross-sectional comparison of level in AD patients and normal controls	Levels of IL-6 were increased in patients with AD compared to normal controls ($p<0.001$)	N=196 (145 AD, 51 controls); hospital setting, neurology department, Italy; μ_{age}=78 years for AD and 75 years for controls; 62% female	None stated
Ravaglia, et al., 2007. *Neurobiol Aging*.	Serum	Incident all-cause dementia, AD, and vascular dementia	There were no significant associations between IL-6 and incident all-cause dementia, AD, or vascular dementia. However, when considered in combination with CRP, high levels of both were significantly associated with an increased risk of vascular dementia (HR=2.56, 95% CI: 1.21, 5.50).	N=804; μ_{age}=73.6 years at baseline	Age, sex, education, APOE e4, stroke, cardiovascular disease, physical activity, BMI, homocysteine, creatinine, folate, Vitamin B12
Schram, et al., 2007. *J Am Geriatr Soc*.	Plasma	Cognitive function (test scores) and decline (over 4.5 years in the Rotterdam Study and 5 years in the Leiden 85+ Study)	Rotterdam Study: Higher IL-6 (per SD) was associated cross-sectionally with lower MMSE scores, poorer global cognitive function and poorer executive function ($p=0.03$, $p=0.009$, and $p=0.008$). There was no significant association between IL-6 and decline over time. Leiden 85+ Study: There was no cross-sectional association between continuous IL-6 and cognitive	N=3,874 from the Rotterdam Study; μ_{age}=72 years N=491 from the Leiden 85+ Study;	Age, sex, education, BMI, diabetes, cardiovascular disease

Table A.1.1 Continued

			function (p>0.2 for all). Those with higher IL-6 had greater decline over time on delayed recall tasks (p=0.03). Associations were modified by APOE e4 allele, such that they were stronger among those with ≥1 allele.		
Sundelof, et al., 2009. *J Alzheimers Dis*.	Serum	Risk of all-cause dementia, AD, and non-AD dementia over 11.3 years	Age 70 Cohort: Those with a high level of serum IL-6 had a significantly greater risk of all-cause dementia (HR=1.45, 95% CI: 1.06, 2.07) and non-AD dementia (HR=2.21, 95% CI: 1.23, 3.95), but there was no association with AD. Age 77 Cohort: There were no significant associations between IL-6 and all cause dementia, AD, or non-AD dementia.	Age 70 cohort N=1,062; μ_{age}=71.0 years Age 77 cohort; N=749; μ_{age}=77.5 years	Age, APOE e4, diabetes, NSAID treatment, aspirin treatment, smoking status, BMI, hypertension, serum cholesterol, stroke, education
Tan, et al., 2007. *Neurology*.	Serum	Risk of developing AD	There was no significant association between serum IL-6 and risk of developing AD.	N=691; μ_{age}=79 years; Cohort from the Framingham Study	Age, sex, APOE e4, stroke, education, homocysteine levels, smoking history, BMI, anti-inflammatory drug use
Weaver, et al., 2002. *Neurology*.	Plasma	Baseline cognitive function and	There was trend-level significance for those in the highest tertile, compared to	N=779; MacArthur Study of Successful Aging; about half	Age, race, sex, education, alcohol use, activity level,

Table A.1.1 Continued

		cognitive decline over 2.5 and 7 years *Measured with 5 tasks assessing verbal memory, naming, recall, language, and spatial ability, with a combined score	those in the lowest tertile, to have significantly worse cognitive function at baseline (OR=1.46; 95% CI: 0.97, 2.20) After 2.5 years, those in the highest tertile were significantly more likely to experience cognitive decline than those in the low tertile (OR=2.03; 95%CI: 1.30, 3.19) After 7 years, those in the highest tertile were significantly more likely to experience cognitive decline than those in the low tertile (OR=1.90; 95% CI: 1.14, 3.18)	men; majority white (exact percentages for full sample not given)	BMI, history of cancer, diabetes, fasting blood glucose levels
Yaffe, et al., 2003. *Neurology*.	Plasma	Cognitive function (3MS), change in 3MS over time, and clinically significant cognitive decline (>5 point decline over 2 years)	Those in the highest IL-6 tertile had significantly worse scores on the 3MS, compared to those in the lowest tertile (88.9 points vs. 90.6 points, $p<0.001$) Those in the highest IL-6 tertile experience significantly greater cognitive decline over 2 years (-2.0 points vs. -1.2 points, p=0.01) IL-6 was not significantly	N=3,031; Health ABC participants; μ_{age}=74 years at baseline; 41% African American	Age, education, race, sex, smoking, alcohol use, BMI, self-reported health, depression, MI, diabetes, hypertension, stroke, NSAID use, and estrogen use (for women)

Table A.1.1 Continued

			associated with clinically significant cognitive decline after adjustment (OR=1.23, 95% CI: 0.96, 1.59)			
CRP				Inflammation		
Engelhart, et al., 2004. *Arch Neurol*.	Plasma	Risk of AD over 1 year	High CRP was not associated with increased risk for AD (Rate ratio per standard deviation increase in CRP =1.12 (0.99,1.25)		N=727; μ_{age}=71.7 years at baseline; 53% female; Rotterdam Study subgroup	Age, sex, education, smoking, BMI, diabetes, anti-inflammatory medication use, atherosclerosis
Eriksson, et al., 2011	Serum	Prevalent and incident all-cause dementia and AD	There was no significant association between serum CRP and prevalent or incident all-cause dementia or AD.		N=3,937; μ_{age}=77.7 years for controls; μ_{age}=75.3 years for dementia cases	Apolipoprotein E (APOE) e4, BMI, smoking, blood pressure, education, diabetes, coronary heart disease, and stroke
Gallacher, et al., 2010. *Arterioscler Thromb Vasc Biol.*	Plasma	Risk of dementia over 20 years	There was no significant association between continuous baseline CRP and risk of dementia (HR: 0.79; 95% CI: 0.42-1.44)		N=865; all men; 65-84 years of age at cognitive status determination, but 45 to 59 when inflammatory markers measured)	Age, social class, systolic blood pressure, BMI, smoking, total cholesterol, alcohol use
Holmes, et al., 2009. *Neurology*.	Serum	Cognitive performance on the ADAS-COG, and change in ADAS-COG	At baseline, there was no significant difference in ADAS-COG scores between those in the lowest CRP quartile compared to those in the highest quartile (p=0.1)		N=300; community-dwelling from United Kingdom; all had mild to severe AD	Not stated

Table A.1.1 Continued

		over 1 year	There was no significant association between worsening in ADAS-COG scores and CRP quartile (p=0.03)		
Kravitz, et al., 2009. *Alzheimers Dement.*	Serum	Prevalent all cause-dementia	Compared to undetectable CRP levels (reference group), those with detectable CRP levels and elevated CRP levels were significantly more likely to have all cause dementia (OR=3.0, 95% CI: 1.2, 7.3; OR=5.0, 95% CI: 1.9, 12.9, respectively)	N=305; μ_{age}=94.3 years; Leiden 90+ Study; Netherlands; 62% women	Age, sex
Kravitz, et al., 2009. *J Am Geriatr Soc*.	Serum	Risk of developing dementia over time (> 1 year)	There was no significant association between CRP tertile and risk of developing dementia (HR=1.2, 95% CI: 0.6-2.1), even among those with ≥1 APOE e4 allele (HR=4.5; 95% CI: 0.9, 23.3)	N=227; μ_{age}=93.9 years; Leiden 90+ Study; Netherlands; 62% women	Age, sex, education, APOE, hypertension, coronary artery disease, congestive heart failure and transient ischemic attack
Ravaglia, et al., 2007. *Neurobiol Aging*.	Serum	Incident all-cause dementia, AD, and vascular dementia	There were no significant associations between CRP and all-cause dementia, AD, or vascular dementia. However, when considered in combination with IL-6, high levels of both were significantly associated with an increased risk of vascular	N=804; μ_{age}=73.6 years at baseline	Age, sex, education, APOE e4, stroke, cardiovascular disease, physical activity, BMI, plasma total homocysteine, serum

Table A.1.1 Continued

			dementia (HR=2.56, 95% CI: 1.21, 5.50).		creatinine, serum folate, and serum Vitamin B12
Schmidt, et al., 2002. *Ann Neurol*.	Serum	All-cause dementia, AD, AD with cardiovascular disease (CVD), and vascular dementia	Those with a high level of CRP were significantly more likely to have all-cause dementia (HR=2.8, 95% CI: 1.6,5.1), AD with CVD (HR=4.7, 95% CI: 1.2, 17.9), and vascular dementia: (HR=5.1, 95% CI: 1.8, 14.8). However, there was no significant association between CRP and AD **(**HR=2.2, 95% CI: 0.9, 5.1). *All reported hazard ratios are comparing the highest quartile of CRP with the lowest quartile reference group.	N=1,050; μ_{age} not reported for entire cohort; Cohort from the Honolulu-Asia Aging Study	Education, midlife smoking status, midlife average cholesterol, midlife blood pressure, age, years of follow-up, APOE e4, BMI
Schram, et al., 2007. *J Am Geriatr Soc*.	Plasma	Cognitive function (test scores) and decline (over 4.5 years in the Rotterdam Study and 5 years in the Leiden 85+ Study)	Rotterdam Study: Higher CRP (per SD) was associated cross-sectionally with poorer global cognitive function and executive function ($p<0.001$ for both). There was no significant association between CRP and decline over time. Leiden 85+ Study: There was	N=3,874 from the Rotterdam Study; μ_{age}=72 years N=491 from the Leiden 85+ Study;	Age, sex, education, BMI, diabetes, cardiovascular disease

Table A.1.1 Continued

			no cross-sectional association between continuous CRP and cognitive function ($p>0.2$ for all). There was no significant association between CRP and decline over time.		
Silverman, et al., 2009. *Res Letters*.	Plasma	Performance (scores) on a neuropsych test battery	Those in the lowest CRP tertile had significantly worse memory scores than those in the other 2 tertiles ($p<0.0001$)	N=176; Bronx VA Medical Center; μ_{age}=85 years; 36% women	Age, sex, education, APOE
Sundelof, et al., 2009. *J Alzheimers Dis*.	Serum	Risk of all-cause dementia, AD, and non-AD dementia over 11.3 years	There were no significant associations between CRP and all-cause dementia, AD or non-AD dementia for either cohort.	Age 70 Cohort: N=1,062;μ_{age} =71.0 years at baseline Age 77 Cohort: N=749; μ_{age} =77.5 years at baseline	Age, APOE e4, diabetes, non-steroidal anti-inflammatory drug (NSAID) use, aspirin treatment, smoking status, BMI, hypertension, serum cholesterol, stroke, education
Tan, et al., 2007. *Neurology*.	Serum	Risk of developing AD	There was no significant association between serum CRP and risk of developing AD.	N=691; μ_{age} =79 years; Cohort from the Framingham Study	Age, sex, APOE e4, stroke, education, homocysteine levels, smoking history, BMI, anti-inflammatory drug use
Yaffe, et al., 2003. *Neurology*.	Serum	Cognitive function (3MS) and clinically	Those in the highest CRP tertile had significantly worse scores on the 3MS, compared	N=3,031; Health ABC participants; μ_{age}=74 years at	Age, education, race, sex, smoking, alcohol

Table A.1.1 Continued

		significant cognitive decline (>5 point decline over 2 years)	to those in the lowest tertile (88.7 points vs. 90.4 points, p<0.001) Those in the highest CRP tertile experience significantly greater cognitive decline over 2 years (-1.7 points vs. -1.1 points, p=0.05) CRP was not significantly associated with clinically significant cognitive decline after adjustment (OR=1.24, 95% CI: 0.96, 1.63)		baseline; 41% African American	use, BMI, self-reported health, depression, MI, diabetes, hypertension, stroke, NSAID use, and estrogen use (for women)
TNF-α				Inflammation		
Holmes, et al., 2009. *Neurology*.	Serum	Cognitive performance on the ADAS-COG, and change in ADAS-COG over 1 year	At baseline, those in the lowest TNF-α quartile had significantly better ADAS-COG scores, compared to those in the highest quartile (p=0.02) Worsening in ADAS-COG scores was significantly greater among those in the highest serum TNF-α quartile, compared to those in the low quartile (p=0.02)		N=300; community-dwelling from United Kingdom; all had mild to severe AD	Not stated
Yaffe, et al., 2003. *Neurology*.	Plasma	Cognitive function (3MS) and clinically significant cognitive decline (>5	There was no significant association between baseline TNF-α tertile and 3MS scores (high 89.5 points vs. low 89.4 points, p=0.66)		N=3,031; Health ABC participants; μ_{age}=74 years at baseline; 41% African American	Age, education, race, sex, smoking, alcohol use, BMI, self-reported health, depression, MI,

Table A.1.1 Continued

		point decline over 2 years)	There was no significant association between TNF tertile and cognitive decline over 2 years (high -1.4 points vs. low -1.3 points, p=0.75) IL-6 was not significantly associated with clinically significant cognitive decline (OR=1.23, 95% CI: 0.95, 1.63)		diabetes, hypertension, stroke, NSAID use, and estrogen use (for women)
α1-antichymotrypsin (ACT)			Inflammation		
DeKosky, et al., 2003. *Ann Neurol*.	Plasma	Cross-sectional comparison of level in AD patients and normal controls	Plasma ACT was significantly higher in AD patients vs. normal controls (541.34±8.13 mg/L vs. 500.58±13.78 mg/L, p=0.01) Plasma ACT level was significantly related to dementia severity (several cognitive test scores) There was no association between plasma ACT and other types of dementia	N=561 (359 AD, 44 other dementia, 113 normal controls); all white; University of Pittsburgh Alzheimer's Disease Research Center (ADRC)	Sex, education, APOE, ACT genotype
Engelhart, et al., 2004. *Arch Neurol*.	Plasma	Risk of AD over 1 year	High ACT was significantly associated with increased risk for AD (Rate ratio per standard deviation increase in ACT =1.49 (1.23-1.81)	N=727; μ_{age}=71.7 years at baseline; 53% female; Rotterdam Study subgroup	Age, sex, education, smoking, BMI, diabetes, anti-inflammatory medication use, atherosclerosis

Table A.1.1 Continued

Licastro, et al., 2000. *J Neuroimm.*	Plasma	Cross-sectional comparison of level in AD patients and normal controls	Levels of ACT were increased in patients with AD compared to normal controls ($p<0.001$)	N=196 (145 AD, 51 controls); hospital setting, neurology department, Italy; μ_{age}=78 years for AD and 75 years for controls; 62% female	None stated

*All biomarkers selected to be summarized here were published in manuscripts during or after the year 2000, included humans (not animal models) and were selected based on two comprehensive reviews of the most promising blood and serum biomarkers (Schneider, Frank).

APPENDIX B

B.1 SUPPLEMENTARY FIGURE

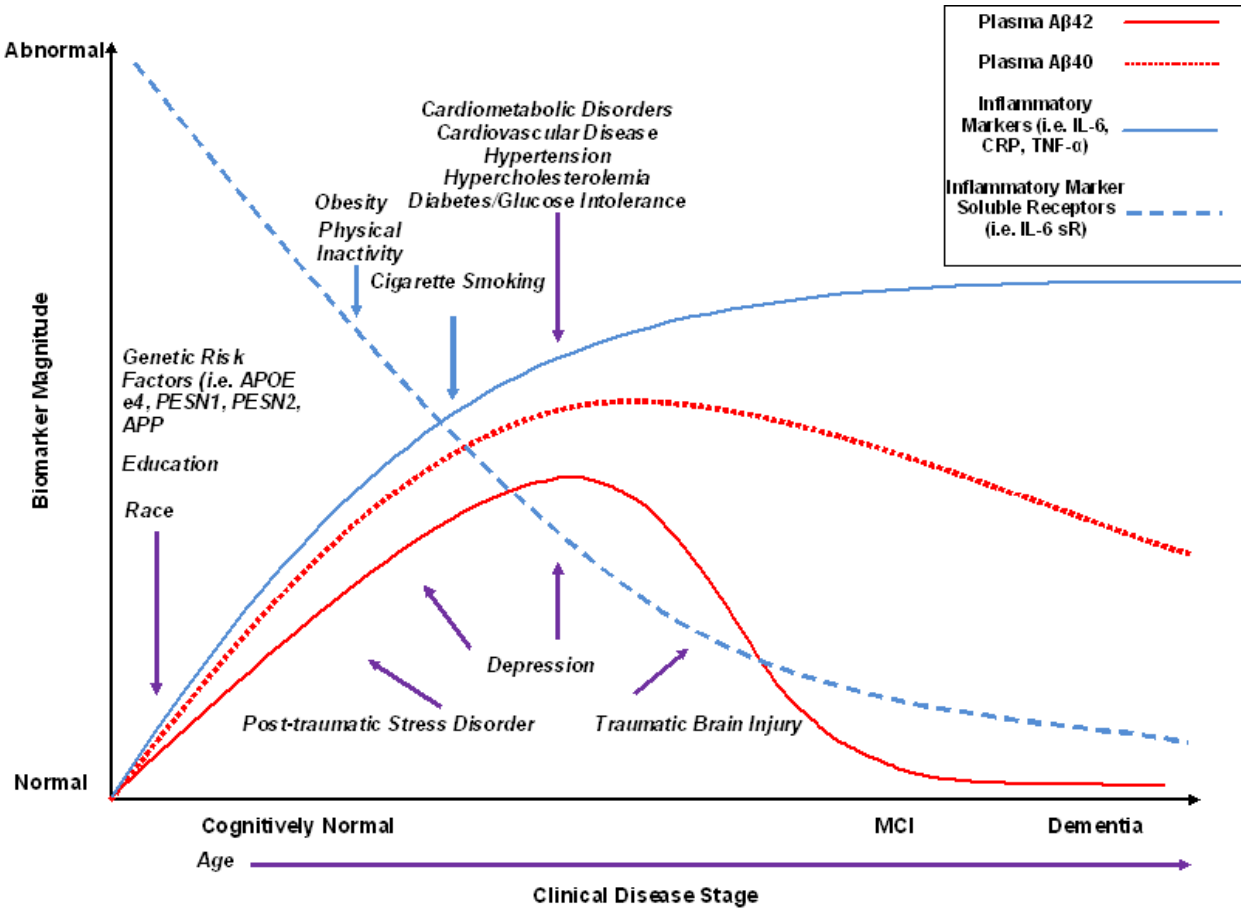

Figure B.1.1 Proposed association between established risk factors of AD and potential blood biological markers.

B.1.1 Figure legend.

In Figure B.1.1. a purple arrow indicates risk factors that have a proposed effect on both plasma Aβ and inflammatory markers; a blue arrow indicates risk factors that have a proposed effect on inflammatory markers only. Following is a proposed description of the association between these risk factors and biomarkers.

Plasma Aβ42 increases with risk factors for AD (i.e. increased age, low education, black race, female sex, APOE e4 genotype).(Graff-Radford et al., 2007; A. L. Metti et al., 2012). It has been proposed that levels of plasma Aβ42 increase to a certain point before beginning to selectively aggregate in the form of Aβ plaques 10 to 15 years before the clinically detectable onset of dementia.(Graff-Radford et al., 2007) Because Aβ42 is more fibrillogenic, and is more likely to form amyloid plaques, following the increase, Aβ42 may begin to decrease to a greater extent among those who are at an increased risk for AD, as it begins to aggregate and form amyloid plaques.(Gravina et al., 1995; R Mayeux et al., 1999) Furthermore, cardiometabolic risk factors are related to plasma Aβ42: 1) Those with low Aβ42 are more likely to have a history of diabetes;(A. L. Metti et al., 2012) 2) Aβ42 enhances vasoconstriction, and is thus associated with increased blood pressure (BP), increased vascular pathology, and microvessel dysfunction;(Shah et al., 2012) 3) There is an interaction between midlife BP and plasma Aβ42 such that those with low Aβ42 are at an even greater risk for developing AD as BP increases;(Shah et al., 2012) 4) Low plasma Aβ42 measured in life has been associated with greater cerebral amyloid angiopathy at autopsy;(Shah et al., 2012) 5) Higher levels of total cholesterol and LDL cholesterol are associated with lower plasma Aβ42;(Blasko et al., 2010) and 6) Higher BMI is associated with lower Aβ42.(Blasko et al., 2010) Depression has also been associated with low plasma Aβ42,

and low Aβ42/Aβ40.(A.L. Metti et al., 2012) TBI has been associated with, at least short term, increases in CSF Aβ42 – which has been shown to be highly correlated with plasma Aβ42.(Lye & Shores, 2000)

Plasma Aβ40 also increases with increased risk factors for AD/MCI (i.e. increased age, lower education, greater number of family members with a history of dementia, APOE e4 allele status).(Graff-Radford et al., 2007) However, Aβ40 is less likely to accumulate in the form of amyloid plaques, and therefore will likely not decrease as much with the development of MCI/dementia.(Gravina et al., 1995; R Mayeux et al., 1999) Aβ40 has been shown to have some associations, albeit weaker than those found with Aβ42, with cardiometabolic factors.(Blasko et al., 2011; A. L. Metti et al., 2012; Shaw et al., 2009)

Inflammatory markers increase with age and with increased cardiometabolic disorders, and cigarette smoking.(Chung, Kim, Kim, & Yu, 2001; Pearson et al., 2003; Yasue et al., 2006) High levels of inflammation also lead to increased permeability of the BBB, and a greater inflammatory response within the brain (via several different pathways), which all leads to an increased risk of AD and dementia.(Rosano et al., 2012) Soluble receptors may be higher among those with normal cognitive function, and decreased in those with MCI/dementia. While the mechanisms of this decrease still remain unclear, it be due to injury, infection, cardiometabolic disorders, genetic disorders, or naturally with age.(Banks, Kastin, et al., 1995; Banks, Plotkin, et al., 1995; Griffin et al., 2012; Holmes & Butchart, 2011)

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