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Hanfeng Huang March 20, 2020

Molecular Biomarkers in a Cohort of Middle-Aged African Americans and Caucasians with a Family History of Alzheimer's Disease

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Abstract

Molecular Biomarkers in a Cohort of Middle-Aged African American and Caucasians with a Family History of Alzheimer's Disease

By Hanfeng Huang

Background: African Americans (AAs) have a higher prevalence of Alzheimer's disease (AD) than Caucasians (CCs). AAs are at a higher risk for other chronic health problems that, in combination with genetics, inflammation, and other psychosocial factors, could contribute to the onset of AD. AD neuropathological cascade starts in middle-age, many years before the diagnosis of clinical dementia, making this an optimal target for intervention. Investigating whether molecular biomarkers differ in AAs and CCs may suggest racial differences in biological mechanisms that contribute to AD pathogenesis.

Method: Participants were middle-aged AAs and CCs who are at high risk for AD due to a parent history of AD. Study visits included lumbar puncture for cerebrospinal fluid (CSF) collection, blood draw, and cognitive testing. The primary outcomes were AD biomarkers (amyloid-β, total tau, and hyperphosphorylated tau), markers of inflammation, markers of the renin-angiotensin system, and a novel CSF marker of capillary dysfunction, soluble platelet-derived growth factor receptor-β (sPDGFRβ).

Result: 30 AAs and 50 CCs were enrolled. Participants were middle-aged (59.1 ± 6.8) and well-educated 85% completed college). 83.3% were AAs were female compared with 56.0% of CCs. Systolic blood pressure (126.4 mmHg) suggests an overall healthy sample that did not differ by race. Compared to CCs, AAs had lower levels of CSF T-tau, P-tau, and sPDGFRβ. AAs had higher levels of IL-7, MCP-1, MDC, CRP, and SAP, and lower levels of VCAM-1 in blood, and lower levels MMP-2, IL-7, and VCAM-1 in CSF. After multiple linear regression adjusted for age, gender, race, and education, we found that higher CSF sPDGFRβ was associated with higher CSF T-tau, P-tau, MMP-2, IL-8, TIMP-1, TIMP-2 and VCAM-1 and higher CSF ACE-1 activity was associated with lower plasma IL-9 level and higher CSF IL-7 level.

Conclusion: The results of this study suggest that AAs had lower tau burden than CCs. AAs also had lower levels in three CSF markers that are related to Blood-brain barrier (BBB) dysfunction, MMP-2, VCAM-1, and sPDGFRβ, which point to racial differences in BBB integrity. This, in turn, could lead to race-dependent AD pathological mechanisms.

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1 Introduction

1.1 Alzheimer’s Disease

Alzheimer’s disease (AD) is a chronic progressive neurodegenerative disorder characterized by difficulties with memories, cognitive dysfunction, behavioral disturbances, and challenges with performing daily living activities. One in three seniors dies with AD or another dementia, and AD accounts for 60 to 80 percent of all dementia cases ("2020 Alzheimer's disease facts and figures," 2020). AD is the sixth leading cause of death in the United States, and it kills more than breast cancer and prostate cancer combined ("2020 Alzheimer's disease facts and figures," 2020). An estimated 5.8 million Americans age 65 and older are currently living with AD, and by 2050, this number is projected to increase to nearly 13.8 million ("2020 Alzheimer's disease facts and figures," 2020). In 2020, the total cost of healthcare, long-term care, and hospice services for patients is estimated to be $305 billion, making it one of the costliest conditions for society. By 2050, these costs are projected to increase to more than $1.1 trillion ("2020 Alzheimer's disease facts and figures," 2020).

AD was first described in 1906 by German psychiatrist Alois Alzheimer, but 70 years passed before AD was perceived as one of the most common causes of death and became a significant focus of scientific research ("2018 Alzheimer's disease facts and figures," 2018; Katzman, 1976). AD pathogenesis is complex, involving abnormal amyloid-β (Aβ) metabolism, tau hyperphosphorylation, oxidative stress, reactive glial and microglial changes, and other pathological events (Wang, Gu, Masters, & Wang, 2017). Through many years of basic science and clinical research, it is now widely known that AD pathogenesis begins many years before the symptoms develop. However, there is much to be learned about the biological and physiological changes that lead to the onset of AD symptoms.

In 2011, the National Institute on Aging (NIA)-Alzheimer's Association Workgroups on Diagnostic Guidelines for AD published the revised criteria for the clinical diagnosis of AD. The two significant differences between the new proposed framework and the old criteria published in 1984 are the incorporation of biomarkers and the formalization of different stages of the disease (Jack et al., 2011; McKhann et al., 1984). The term "biomarker" is defined by the World Health Organization (WHO) as "any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease" (World Health & International Programme on Chemical, 2001). The second difference is the expansion of AD into three phases: an asymptomatic preclinical phase, a symptomatic pre-dementia phase (which is also referred to as Mild Cognitive Impairment or MCI), and a dementia phase (Jack et al., 2011). NIA-Alzheimer's Association workgroups also proposed a research-oriented framework defining the preclinical stages of AD (Sperling et al., 2011). The workgroup updated the framework in 2018, where biomarkers were grouped into those of Aβ deposition, pathologic tau, and neurodegeneration, or "AT(N)" (Jack Jr. et al., 2018). Although the long "preclinical" stage gained increased research interest over the past decade, more information is urgently needed concerning the link between early pathological changes and later onset of clinical syndromes. This research will be crucial because not only will it reveal mechanisms about AD pathophysiology, but also the "preclinical" phase serves as a critical window for implementing any potential therapeutic interventions (Sperling et al., 2011).

1.2 Racial Disparities in Alzheimer's Disease

Older African Americans (AAs) are about twice as likely to have AD as older Caucasians (CCs). (Rajan, Weuve, Barnes, Wilson, & Evans, 2019; Steenland, Goldstein, Levey, & Wharton, 2016). There are various reasons for the increased prevalence and incidence rate for

AAs, including genetics, physiological, in particular vascular conditions, socioeconomic disparities (Manly, Schupf, Tang, & Stern, 2005) and psychosocial factors (stress from perceived racism) (Clark, Anderson, Clark, & Williams, 1999) (Barnes & Bennett, 2014). These factors may interact to influence the racial disparities in AD.

Apolipoprotein E (APOE) is a cholesterol transport protein that supports lipid transport and injury repair in the brain (Liu, Kanekiyo, Xu, & Bu, 2013). APOE polymorphic alleles are the primary genetic determinants of AD risk. It is well-established that individuals carrying the ε4 allele are at increased risk of AD compared with those carrying the more common ε3 allele, whereas the ε2 allele decreases AD risk (Liu et al., 2013). The Chicago Health and Aging Project (CHAP) is a cohort of 4917 urban adults where 68% are AA, and 32% are CC. This study showed that AAs have a higher proportion of both APOE ε2 allele (22% vs. 13%) and ε4 allele (33% vs. 24%) than CCs (Rajan et al., 2017). Additionally, genome-wide association data showed that the APOE ε4 allele is associated with an increased risk of AD among AAs (Reitz et al., 2013).

Epidemiological studies have shown that vascular risk factors and diseases, including stroke, hypertension, diabetes, and atherosclerosis are associated with increased risk of AD (Breteler, 2000; Cechetto, Hachinski, & Whitehead, 2008). There are also accumulating evidence that high blood pressure in midlife increases the risk of AD in later life (Kivipelto et al., 2001). AAs have a higher prevalence of these cardiovascular risk factors, and hypertension is, in particular, highly prevalent compared to CCs (Carnethon et al., 2017; Gu, Burt, Paulose-Ram, Yoon, & Gillum, 2008).

Although these risk factors have been a focus of AD research in the past few decades, there is a significant lack of understanding regarding the association between risk factors for

developing AD and the clinical and pathological expression of AD in the AA population. The National Alzheimer's Coordinating Center (NACC) maintains neuropathological data from more than 6000 brains, and only 372 (6%) are non-white. This underrepresentation in autopsy data means biomarker studies could play an essential role in helping us understand whether AD unfolds among AAs in the same way or differently compared to CCs. Many clinical studies, including ours (Wharton et al., 2019), consistently show that biomarkers contribute not only with diagnostically relevant information but also information critical to the preclinical stages. In this study, several panels of biomarkers will be measured and analyzed in middle-aged, pre-symptomatic high-risk AAs and CCs with a parental history of AD. They will each be introduced in the next four sections.

1.3 Established Alzheimer's Disease Biomarkers

The two main neuropathological hallmarks associated with AD are amyloid plaques and neurofibrillary tangles. Amyloid plaques are the extracellular deposition of amyloid-β abundant in the cortex of AD patients, and neurofibrillary tangles are the intracellular accumulation of hyperphosphorylated and misfolded tau proteins (Serrano-Pozo, Frosch, Masliah, & Hyman, 2011). Three CSF biomarkers, namely amyloid-β (Aβ42), total tau (T-tau), and hyperphosphorylated tau (P-tau), reflect the core pathophysiology in AD, and are the most accepted method to diagnose probable AD with high specificity and sensitivity (Humpel, 2011).

Model of the AD pathological cascade has been proposed to include biomarkers in the preclinical stages of AD from the NIA-Alzheimer's Association workgroups (Jack et al., 2010; Sperling et al., 2011). Secretases cleave Aβ from Amyloid precursor protein (APP) and produces 42 amino acid peptides (Aβ42), which manifests as aggregates in the brain, or amyloid plaques. The toxicity of such aggregates could lead to the cascade of tau hyperphosphorylation, which is

typically bound to microtubules, providing microtubule stability. After being hyperphosphorylated, tau dissociates from microtubules and aggregates into neurofibrillary tangles, which could eventually lead to neuronal dysfunction (Anoop, Singh, Jacob, & Maji, 2010). Tau protein in AD patients can be phosphorylated in almost 39 possible sites, but tau protein phosphorylated at serine 181 is the standard AD biomarker for hyperphosphorylated tau (Sharma & Singh, 2016).

Studies have consistently shown lower levels of Aβ42, and higher levels of T-tau and P-tau in the CSF of AD patients compared to controls (Kaj Blennow, Hampel, Weiner, & Zetterberg, 2010). While controls have an Aβ42 level of 794 ± 20 pg/mL, AD patients have a level of <500 pg/mL. T-tau increases gradually with age from 136 ± 89 pg/mL (21-50 years) to 243 ± 127 pg/mL (51-70 years) to 341 ± 171 (>71 years) in controls. In AD patients, T-tau increases from >450 pg/mL (51-70 years) to >600 (>71 years). Lastly, AD patients have a significant increase in P-tau of >60 pg/mL, compared to 23 ± 2 pg/mL in controls (Humpel, 2011).

Recently, two different studies at different labs have pointed out race differences in T-tau and P-tau. A prospectively recruited cohort of 65 AAs and 70 CCs spanning normal cognition, MCI, and AD at Emory University School of Medicine demonstrated that as a group regardless of diagnosis, AAs (age 69.1 ± 7.4) had lower CSF levels of T-tau and P-tau levels compared to CCs (age 70.8 ± 7.7), whereas Aβ42 levels did not vary by race (Howell et al., 2017). The greatest differences in was found in subjects with normal cognition. Additionally, in this cohort, researchers found that cognitive impairment in AAs is associated with smaller changes in CSF tau markers than CCs (Howell et al., 2017). Following that, researchers at Washington University School of Medicine found similar trends in their Knight Alzheimer's Disease

Research Center cohort of 1255 participants (age 70.8 ± 9.9). Again, mean CSF concentrations of T-tau were significantly lower in AAs than for CCs (293.65 ± 34.61 pg/mL vs. 443.28 ± 18.20 pg/mL), as were mean concentrations of P-tau (53.18 ± 4.91 pg/mL vs. 70.73 ± pg/mL) in both normal cognition and dementia participants (Morris et al., 2019).

1.4 Markers of Inflammation

Chronic stress is considered as a risk factor for AD, and it accelerates AD pathogenesis in human and animal models through increases in inflammatory responses, Aβ accumulation, tau hyperphosphorylation, oxidative stress, and mitochondrial impairment (Machado et al., 2014). It also influences inflammatory markers in both the peripheral and central nervous systems (CNS). Many studies have reported evidence of the pathophysiological role of inflammation in the development of AD. Central and peripheral inflammatory systems are not passive systems activated by amyloid plaques and neurofibrillary tangles. Instead, they play a critical role in the pathogenesis of the disease as do plaques and tangles themselves (Heneka et al., 2015).

However, the detection of inflammatory markers has not yet been established as a valuable method for assessing risk or diagnosis of AD (Olsson et al., 2016). Although previous studies have investigated concentrations of inflammatory markers in the CSF of patients with AD, individual studies of inflammatory markers yielded varying results. In addition to the CNS, systemic immune cells and secreted signaling proteins in the periphery communicate with the brain, and have been associated with neuroinflammation, as well as the neurodegenerative process (Heneka et al., 2015).

In the literature, studies that have compared inflammatory markers in AD patients and healthy controls showed inconsistent, and occasionally, opposite results. A few markers in blood and CSF have been studied as candidate markers for AD. A recent meta-analysis of 170 studies

demonstrated increased CSF inflammatory markers such as interleukins 10 (IL-10), monocyte chemoattractant protein 1 (MCP-1), transforming growth factor β 1 (TGF-β1) and increased peripheral inflammatory markers such as C-reactive protein (CRP), interleukin 6 (IL-6), interleukin 1β (IL-1β) in AD patients compared to healthy controls (Shen et al., 2019). In another study, when peripheral biomarkers are matched with CSF biomarkers from AD patients, IL-6, MCP-1, and other biomarkers were significantly related and indicated that they could be suitable candidate biomarkers for monitoring disease progression, especially the inflammatory processes in AD (Sun et al., 2003).

Researchers have also found that peripheral inflammation is associated with cognition across AD stages. A study found that in a cohort of mild to severe AD patients, a high baseline level of peripheral tumor necrosis factor alpha (TNF-α) was associated with a 4-fold increase in the rate of cognitive decline (Holmes et al., 2009). Another study using a sample of 59 AA and 219 CC healthy participants reported a stronger association between peripheral interleukin-8 and cognitive functioning in AAs than CCs (Goldstein, Zhao, Steenland, & Levey, 2015). Initiated in 2004, the Healthy Aging in Neighborhoods of Diversity across the Life Span (HANDLS) was a longitudinal study of 1467 AAs and 1107 CCs in urban Maryland neighborhoods. The study indicated that there was an association of systematic inflammation and cognitive performance over time and the strongest associations were primarily detected among older (>50 years old) and AA individuals (Beydoun et al., 2018).

Although racial differences have not been explored extensively with specific reference to inflammation in AD, a significant body of literature reports immune differences, especially peripheral inflammatory markers across races in other disorders (Babulal et al., 2019). One study showed that peripheral soluble endothelial markers intercellular adhesion molecule 1 (sICAM-1),

and soluble vascular cell adhesion molecule 1 (sVCAM-1) were reduced in AAs compared CCs (Hwang et al., 1997). Another cross-sectional study of 508 adults with 62% CCs and 38% AAs reported AAs had higher levels of serum CRP and IL-6 than CCs, whereas race differences in IL-10 and TNF-α were not observed (Paalani, Lee, Haddad, & Tonstad, 2011). The systemic inflammatory differences between AAs and CCs could have some implications for differences in AD progression and treatment between races (Babulal et al., 2019).

1.5 Markers of Capillary Dysfunction

The blood-brain barrier (BBB) is a highly selective border of endothelial cells that forms a layer of protection between the extracellular fluid of the CNS and the blood. The barrier is necessary to isolate the brain from potentially harmful substances such as toxins, fluctuations in hormones, ions, certain neurotransmitters, and pathogens. Endothelial cells, astrocyte end-feet, and pericytes, three elements of the brain microvasculature, comprise the BBB. In brain capillaries, the endothelial cells form tight junctions that prevent solute movement between cells. Astrocytic end-feet tightly enclose the vessel wall and appear to play an important role in the induction and maintenance of the tight junction barrier. Pericytes are the least-studied of the three cellular elements of the BBB, but appear to be crucial in angiogenesis, structural integrity, and formation of endothelial tight junctions (Ballabh, Braun, & Nedergaard, 2004). Together, brain capillary endothelial cells form the BBB, which is covered with basement membranes, and is also surrounded by pericytes and astrocyte end-feet (Yamazaki & Kanekiyo, 2017).

Increasing evidence is showing that each of these three elements of the brain microvasculature, namely, endothelial cells, astrocyte end-feet, and pericytes are significantly affected in the presence of AD pathology in both transgenic animal models and human subjects (Yamazaki & Kanekiyo, 2017; Zenaro, Piacentino, & Constantin, 2017). Conversely, growing

animal research evidence shows that BBB breakdown plays a causal role in the neurodegenerative process of AD (Montagne, Zhao, & Zlokovic, 2017). Because some Aβ clearance happens along with these elements, and across the BBB, BBB breakdown may result in exacerbated amyloid plaque accumulation in the brain (Yamazaki & Kanekiyo, 2017).

However, until very recently, there has been a paucity of biomarkers to investigate this concept in vivo (Zetterberg & Schott, 2019). Using an advanced dynamic contrast-enhanced MRI protocol (DCE-MRI) to quantify regional BBB permeability in vivo, Montagne, et al. showed BBB breakdown in the hippocampus in MCI patients, suggesting that BBB breakdown is an early event that begins in the hippocampus and may contribute to cognitive impairment (Montagne et al., 2015). Histological studies of post-mortem brain tissue revealed significant pericyte loss in AD, as well as a reduction in the platelet-derived growth factor receptor β (PDGFRβ), which is expressed in capillary pericytes (Miners, Kehoe, Love, Zetterberg, & Blennow, 2019). PDGFRβ is shed from injured human pericytes when they are exposed to Aβ (Abhay P. Sagare, Sweeney, Makshanoff, & Zlokovic, 2015). Recently, Nation, et al. developed a novel test of the CSF biomarker of BBB-associated capillary pericyte damage, soluble platelet-derived growth factor receptor-β (sPDGFRβ) (Nation et al., 2019). In cognitively normal individuals or individuals with early cognitive dysfunction, sPDGFRβ closely correlated with DCE-MRI, evidence of BBB dysfunction, and was increased in individuals with more advanced cognitive impairment (Nation et al., 2019).

Another study suggested that sPDGFRβ, the newly developed in vivo marker of pericyte damage, is associated with AD pathology (Miners et al., 2019). CSF sPDGFRβ level was significantly increased in AD cases and correlated positively with T-tau, P-tau in AD cases but

not in age-matched controls. Additionally, CSF sPDGFRβ did not correlate with Aβ42 (Miners et al., 2019).

1.6 Markers of the Renin Angiotensin System

The "classical" renin-angiotensin system (RAS) is best known as a circulating hormonal system that acts on the kidney regulating blood pressure and fluid homeostasis. It is now widely recognized that the RAS not only plays a vital role in the periphery, but it is also expressed separately and modulates several mechanisms in the brain. Renin first converts the inactive angiotensinogen into angiotensin 1 (Ang I), which in turn, is converted by angiotensin-1 converting enzyme (ACE-1) to the active angiotensin II (Ang II). Ang II then binds to its two receptors angiotensin II type 1 receptor (AT1R), and angiotensin II type 2 receptor (AT2R), exerting its hypertensive effects (Patrick G. Kehoe, 2009). However, research over the past two decades has revealed a "regulatory" RAS system which adds additional metabolites, receptors and regulating mechanism to the "classical" RAS system. Angiotensin-converting enzyme-2 (ACE-2) degrades Ang II to angiotensin 1-7 (Ang (1-7)), which subsequently activates its own Mas receptor (MasR). There is emerging evidence suggesting that Ang II to Ang (1-7) conversion process counter-regulates the classical axis of RAS in both the periphery and brain. (Patrick Gavin Kehoe, Wong, Al Mulhim, Palmer, & Miners, 2016)

Numerous components of the RAS have shown to be altered in AD patients, and that dysregulation of the RAS pathway likely contributes to the pathogenesis of AD. Increased ACE-1 activity is found in AD human brain tissues (Miners et al., 2008), and reduced ACE-2 activity is found in AD compared to controls (Patrick Gavin Kehoe et al., 2016). In another cohort study, CSF ACE-1 activity was significantly elevated in AD and positively correlated with ACE2 in AD patients (P. Kehoe, Al Mulhim, Zetterberg, Blennow, & Miners, 2019). The two most

commonly prescribed classes of blood pressure medications for CCs, ACE-1 inhibitor (ACEI) and angiotensin-II type 1 receptor (At1R) blocker (ARB) targeting the classical RAS pathway, have been shown to reduce the incidence, delay cognitive decline and improve cognition in AD (P. Kehoe et al., 2019).

AAs have lower circulating plasma renin levels compared to CCs, and therefore, RAS inhibitors are often not recommended as initial antihypertensive for AAs with hypertension (Williams, Nicholas, Vaziri, & Norris, 2014). Using data from NACC, we reported that individuals receiving antihypertensives at baseline were less likely to convert from MCI to AD and had less cognitive decline than non-users. BBB-crossing RAS-acting medications were associated with even slower cognitive decline. Additionally, AAs receiving RAS-acting medications were associated with more cognitive benefits compared to CCs (Wharton et al., 2015). Currently, we have an ongoing Phase Ib double-blind, randomized, placebo-controlled clinical trial using telmisartan (Micardis), a BBB crossing At1R blocker. Our study sample consists of middle-aged AAs who have a parental history of AD (Wharton et al., 2018). Therefore, it is worthwhile to analyze ACE-1 and ACE-2 activities in serum and CSF in our observational study of AA and CC participants.

1.7 Rationale

In many large-scale studies in the preclinical AD populations, including the Alzheimer's Disease Neuroimaging Initiative (ADNI) (Weiner et al., 2015), Amsterdam Dementia Cohort (van der Flier & Scheltens, 2018), Biomarkers of Cognitive Decline Among Normal Individuals (BIOCARD) (Soldan et al., 2016), Harvard Aging Brain Study (HABS) (Dagley et al., 2017), Mayo Clinic Study of Aging (MCSA) (Roberts et al., 2008), Wisconsin Registry for Alzheimer's Prevention (WRAP) (Johnson et al., 2017), and others, much has been revealed about AD

progression in terms of biomarker changes and cognitive decline. CSF Aβ42 has been shown to reduce longitudinally up to 20 years before the onset of clinical AD symptoms, and CSF tau has been shown to increase around 15 years before symptom onset (Dubois et al., 2016). Importantly, whether these findings from mostly CC cohorts can be applied to other racial minorities remains uncertain.

We believe that in order to lower the incidence and prevalence of AD, it is imperative to study the population most at risk for AD. Hence, enrolling AAs in a study is pressing, as this population is at a higher risk for AD and other chronic health problems, which are independent risk factors for AD. Moreover, middle age is the time of life during which inflammation and sustained vascular complications begin to have a lasting negative impact on the body. It is also the time when AD neuropathology, including plaques and tangle, begins to develop. Therefore, it is an optimal time to stage an intervention to reduce the likelihood of developing AD in later life. In this study, we analyzed baseline molecular biomarkers from a cohort of middle-age AAs and CCs with a parental history of AD and an overrepresentation of the APOE ε4 allele. We investigated whether there are racial differences in these molecular biomarkers, and we also aimed to assess the relationship between markers of capillary dysfunction and the RAS and markers of disease pathology and inflammation, in order to better understand their roles in AD pathogenesis.

2 Methods

2.1 Participants

The Association between Cardiovascular Risk and Preclinical Alzheimer's disease Pathology (ASCEND) study is a two-year observational study of cognitively normal, middle-aged adults at risk for AD. We enrolled middle-aged (45-65 years old) participants who have a biological parent with either autopsy-confirmed or probable AD as defined by NINCDS-ADRDA criteria (McKhann et al., 1984), and verified using the validated Dementia Questionnaire (DQ) (Kawas, Segal, Stewart, Corrada, & Thal, 1994) and medical records when available. Exclusion criteria included a contraindication for lumbar puncture (LP), a history of significant neurologic disease, head trauma, major depression within the last two years, a history of alcohol or substance abuse, diagnosis of AD, MCI or residence in a skilled nursing facility, use of investigational medication, and unwillingness to fast. ASCEND study duration was two years, including three annual visits (baseline, year 1, and year 2), although we are only reporting baseline results in this paper. Participants undergo an LP on Baseline and Year 2, and a blood draw and neuropsychological testing on all three visits.

2.2 CSF and Blood Collection and Analysis

After an 8-hour overnight fast, participants underwent LP to collect CSF for 1) Aβ and tau concentrations, 2) CSF inflammatory markers concentrations, 3) sPDGFRβ concentration, and 4) RAS function measures of ACE activity. CSF samples were collected according to guidelines in the 2014 NIA "Biospecimen Best Practice Guidelines for the Alzheimer's Disease Centers" published by the National Alzheimer's Coordinating Center. Approximately 22 mL of CSF was collected using sterile polypropylene collection tubes. Samples underwent a low-speed spin 2500 rpm for 10 minutes to pellet any cellular debris. They were then aliquoted into 500 µl

polypropylene cryovials, frozen and stored at -80 °C until analysis. Participants also underwent blood draw for 1) APOE genotyping, and 2) inflammatory markers in plasma, and 3) RAS function measures.

CSF AD Biomarkers

CSF Aβ42 concentration was determined using a sandwich ELISA (INNOTEST® β-Amyloid (1-42); Innogenetics, Ghent, Belgium) (Andreasen et al., 1999). CSF T-tau concentration was determined (INNOTEST® hTau Ag; Innogenetics, Ghent, Belgium) (K. Blennow et al., 1995), and CSF P-tau concentration was determined by (INNOTEST® Phospho-Tau (181); Innogenetics, Ghent, Belgium) (Vanmechelen et al., 2000).

Plasma and CSF Inflammatory Markers

Several panels of biomarkers were measured in plasma: 1) cytokines and chemokines, including interleukin 7 (IL-7), interleukin 8 (IL-8), interleukin 9 (IL-9), interleukin 10 (IL-10), monocyte chemoattractant protein 1 (MCP-1), macrophage derived protein (MDC), transforming growth factor alpha (TGF-α), tumor necrosis factor alpha (TNF-α), and Interferon gamma (Interferon-γ) (Interferon-γ levels were only measured in plasma, and as they were not consistently detectable, a downstream marker interferon gamma-induced protein 10 (IP-10) was used as a surrogate); 2) endothelial markers, including soluble intercellular adhesion molecule 1 (sICAM-1) and soluble vascular cell adhesion molecule 1 (sVCAM-1); 3) C-reactive protein (CRP), and serum amyloid protein (SAP).

In addition to all the inflammatory markers mentioned above that were measured in plasma, several markers of matrix metalloproteinases were also measured in CSF. matrix metalloproteinase 1 (MMP-1), matrix metalloproteinase 2 (MMP-2), and matrix

metalloproteinase 9 (MMP-9), tissue inhibitor of metalloproteinase 1 (TIMP-1), tissue inhibitor of metalloproteinase 2 (TIMP-2).

Cytokines and chemokines were measured using the MILLIPLEX® MAP Human Cytokine/Chemokine Magnetic Bead Panel (HCYTOMAG-60K; Merck-Millipore, Burlington, MA, USA). Endothelial markers levels were measured using MILLIPLEX® MAP Human Neurodegenerative Magnetic Bead Panel 3 (HNDG3MAG-36K; Merck-Millipore, Burlington, MA, USA). CRP and SAP were measured using MILLIPLEX® MAP Human Cardiovascular Disease Magnetic Bead Panel 3 (HCVD3MAG-67K; Merck-Millipore, Burlington, MA, USA). MMP-1, MMP-2, and MMP-9 were measured using MILLIPLEX® MAP Human MMP Magnetic Bead Panel 2 (HMMP2MAG-55K; Merck-Millipore, Burlington, MA, USA). TIMP-1 and TIMP-2 were measured using MILLIPLEX® MAP Human TIMP Magnetic Bead Panel 1 (HTMP1MAG-54K; Merck-Millipore, Burlington, MA, USA). All kits were run on the Luminex 200 platform.

Assays were conducted following the manufacturer's protocol except for a few modifications. For CSF cytokines, 100 µl sample was loaded. For TIMP-1 and TIMP-2, 25 µl of 1:75 diluted sample was loaded.

CSF sPDGFRβ

CSF sPDGFRβ level was determined using a commercially available sandwich ELISA (Invitrogen Catalog # EHPDGFRB; ThermoFisher Scientific, Loughborough, UK) following the manufacturer's protocol as previously described (Miners et al., 2019).

Serum and CSF ACE Fluorogenic Activity Assay

ACE-1 activity was measured in CSF using an ACE-1 specific FRET peptide substrate (Abz-FRK(Dnp)-P; Biomol International, Exeter, UK) (Miners et al., 2008) and ACE-2 activity

was measured using the ACE-2 specific FRET substrate ((Mca-APK)(Dnp); Enzo Life Sciences, Exeter, UK) (Patrick Gavin Kehoe et al., 2016) as previously described.

APOE Genotyping

Venous blood was collected into Ethylenediaminetetraacetic acid (EDTA) anticoagulated tubes. DNA was extracted from the buffy coat, and APOE genotypes were determined by real-time polymerase chain reaction using TaqMan® SNP Genotyping Assays (Applied Biosystems Inc.) unique for each APOE single nucleotide polymorphism, rs429358 (Assay ID C 3084793 20) and rs7412 (Assay ID C 904973 10), according to the manufacturer's protocol.

2.3 Cognitive Testing

A battery of approximately 1-hour cognitive testing provided assessment of several cognitive domains, including verbal memory, executive function, visuospatial cognition. Tests were selectively chosen to be used in cognitively normal but high-risk participants for AD. Testing includes Montreal Cognitive Assessment (MoCA) (Nasreddine et al., 2005), Trail Marking Test (Bowie & Harvey, 2006), Forwards and Backwards Digit Span Memory Test (Wechsler, 1981), Mental Rotation Test (Vandenberg & Kuse, 1978), Benson Complex Figure Recall (Corwin & Bylsma, 1993), Buschke Memory Test (Buschke, 1973), and Multilingual Naming Test (MINT) (Ivanova, Salmon, & Gollan, 2013).

2.4 Statistical Analysis

Demographic variables were summarized using descriptive statistics. Differences in categorical variables between AAs and CCs were analyzed by a chi-square test. Data normality of continuous variables was assessed by histogram and Shapiro-Wilk test. Differences for continuous variables between AAs and CCs were compared using a two-sample t-test for normally distributed data and Mann-Whitney U test for variables with non-normal distribution.

Multiple linear regressions were used to investigate associations between ACE activity, CSF sPDGFRβ level, and core AD biomarkers, and markers of inflammation. To control for confounding factors, demographic variables, including age, gender, race, and education were included in all regression models.

All statistical analyses were performed by SAS® ver. 9.4 (SAS Institute, Cary, NC, USA).

3 Results

In our ASCEND cohort, 82 middle-aged participants were enrolled. One participant was lost to follow-up, and one withdrew from the study. Of the 80 remaining participants, 30 were AA, and 50 were CC. The mean age was 60.1 ± 7.8, and 83.3% were female among AAs. The mean age was 58.5 ± 6.1, and 56.0% were female among CCs. Both AAs and CCs were well-educated (85% completed college), but the income was significantly higher in CCs than AAs. Mean systolic blood pressure was 127.6 ± 13.3 mmHg for AAs and 125.1 ± 12.3 mmHg for CCs, suggesting an overall healthy sample that did not differ by race. For AAs, 48.3% were APOE ε4 positive, whereas, for CCs, 50.0% were APOE ε4 positive, consistent with prior studies of AD family history (Johnson et al., 2017; Morris et al., 2019) (Table 1).

AAs performed more poorly than CCs on all cognitive tests, and significantly on MoCA ($p=0.0051$), Trails B ($p=0.0239$), Buschke Delay ($p=0.0218$), and MINT ($p=0.0017$) (Table 2). These specific tests measure global cognition, executive function, verbal memory, and language, respectively.

On average, participants of both races were within the normal range of core AD biomarkers. AAs had lower levels of T-tau ($p=0.0036$) and P-tau ($p=0.0055$) compared to CCs (Table 3 and Figure 1).

AAs had higher levels of 10 of 13 inflammatory biomarkers in plasma, with significantly higher values of IL-7 ($p=0.0249$), MCP-1 ($p=0.0011$), MDC ($p=0.0046$), CRP ($p=0.0008$) and SAP ($p=0.0419$). On the other hand, CCs have higher levels of 9 of 15 stress biomarkers in CSF, with significantly higher values of MMP-2 ($p=0.0256$), IL-7 ($p=0.0046$), and VCAM-1 ($p=0.0056$) (Table 4).

There were no differences between AAs and CCs among ACE-1 and ACE-2 activity in both serum and CSF. AAs had a significantly lower level of CSF sPDGFRβ level (p=0.0055) (Table 5 and Figure 2).

Linear regression analyses adjusted for age, gender, race, and education showed that higher CSF sPDGFRβ level was associated with higher level of T-tau (p=0.0012) and higher level of P-tau in CSF (p=0.0010) (Table 6 and Figure 3). Of the inflammatory biomarkers, higher CSF sPDGFRβ level was associated with higher CSF MMP-2 (p=0.0028), IL-9 (p=0.0313), TIMP-1 (p=0.0023), TIMP-2 (p=0.0432), and VCAM-1 (p=0.0013) (Table 7 and Figure 4). ACE-1 and ACE2 in both serum and CSF were not associated with the core AD biomarkers. Higher CSF ACE-1 activity was associated with lower plasma IL-9 level (p=0.0377) and higher CSF IL-7 level (p=0.0101) (Table 8 and Figure 5).

4 Discussion

This is the third study to show reduced T-tau and P-tau, but not Aβ42, in AAs compared to CCs. It was first shown in two cohorts of older adults in both normal cognition and impaired cognition groups (Howell et al., 2017; Morris et al., 2019). This is the first study to show changes in a middle-aged, healthy cohort at risk for AD by virtue of parental history. These findings have several clinical implications. Diagnostically, lower levels of tau in AAs may lead to underdiagnosis of AD for AAs when T-tau and P-tau are used as diagnostic biomarkers from CSF. For clinical research proposes, investigators and pharmaceutical companies developing tau-targeting therapeutics should note that there is the possibility of different genetic, physiological, and environmental factors contributing to different patterns of tau accumulation or clearance mechanism between AAs and CCs. Therefore, when tau-based therapeutics are tested in a mainly CC study population, the results may not be generalizable to other races, especially AAs.

It is unlikely that the reduced T-tau and P-tau levels in AAs is solely because AAs have less neurodegeneration in the brain compared to CCs. This group is not only in general at higher risk for AD, but also showed poorer performances on all the cognitive tests in our study. Admittedly, the fact that cognitive tests have cultural biases that favor CCs could have contributed to the differences in performance (Brickman, Cabo, & Manly, 2006). Recent CSF data from our cohort and two other cohorts have posed a new question to investigators. Does AD pathology manifest differently in AAs and CCs? While it is too early to tell the answer to this question, we looked at whether several other biomarkers derived from CSF and blood differ between AAs and CCs and how inflammation, RAS, and capillary dysfunction could explain racial differences in CSF tau concentration.

Our results of inflammatory markers suggest AAs have significantly higher biological indices of inflammation in blood than CCs, in 10 of 13 peripheral markers. Levels of IL-7, MCP-1, MDC, CRP, and SAP were significantly elevated in AAs.

IL-7 is a member of the γ chain family of cytokines that include IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21, and these cytokines signal via a common γc receptor. Further signaling cascade will regulate the homeostasis of T cells, B cells, and natural killer cells of the immune system (Nguyen, Mendelsohn, & Larrick, 2017). MCP-1 is a member of the human CC chemokine family, which are chemoattractant cytokines that mediate inflammatory responses and regulate immune cell trafficking. It has been identified as a critical pro-aging immune factor involved in cellular senescence (cessation of cell division) (Bettcher et al., 2019). MDC is also a member of the CC chemokine family and has been shown to act as a chemoattractant for monocytes and activated T cells (Columba-Cabezas et al., 2002).

CRP and SAP belong to the pentraxin family of calcium-dependent ligand-binding plasma proteins. CRP is a well-known trace protein that markedly increases after acute injury, infection, or other inflammatory stimuli. A meta-analysis of 10 studies shows no significant difference in the level of CRP between AD patients and healthy controls, where studies selected showed both elevated and decreased CRP in AD patients (Gong et al., 2016). SAP binds a variety of ligands, including Aβ, and regulates the immune system by inhibiting the differentiation of fibrocytes and promoting the formation of macrophages (Pilling & Gomer, 2018). Although the mechanism of how these peripheral inflammatory markers connect to AD pathology is unknown, most of their systemic actions have been shown as proinflammatory, indicating that AAs have a higher level of peripheral inflammation than CCs in our middle-aged, healthy cohort at risk for AD.

Of note, this discrepancy between races in plasma is reversed in CSF inflammatory markers, such that CCs have higher levels of 9 of 15 biomarkers in CSF. Levels of MMP-2 and IL-7, and VCAM-1 were significantly lower in AAs.

Matrix metalloproteases (MMPs) are a family of enzymes able to catabolize the extracellular matrix (ECM), which is critical for neural plasticity and normal BBB functions (Bjerke et al., 2011). MMP-2 and MMP-9 belong to a specific group of the MMP family called gelatinases. The effects of gelatinases in the CNS are complex (Rosenberg, 2009). Gelatinases exert beneficial effects in neurogenesis, angiogenesis, remyelination, and regeneration of axons, as well as adverse effects such as BBB disruption (Rosenberg, 2009). Gelatinases are expressed in neurons and secreted by astrocytes and microglia. Their function is regulated by tissue inhibitors of matrix metalloproteinases (TIMPs). Studies have shown that in addition to ECM, MMPs also degrade Aβ both in vitro and in transgenic mice (Bjerke et al., 2011; Hernandez-Guillamon et al., 2015). Therefore, it is assumed that they have a protective role in Aβ pathology. However, based on results from animal studies, it has also been proposed that MMPs digest tight junctions and basement membrane proteins, and therefore disturbs BBB integrity (Rempe, Hartz, & Bauer, 2016). One autopsy study showed that the expression of MMP-2 and MMP-9 was elevated in areas of brain hemorrhages, and MMP-2 reactivity was found in Aβ-damaged vessels (Hernandez-Guillamon et al., 2012). One biomarker study showed that MMP-2 level was significantly decreased in CSF samples with significantly reduced Aβ42 levels, suggesting that low MMP-2 could impede the clearance of Aβ, which eventually leads to amyloid plaque deposition (Mlekusch & Humpel, 2009). More data about the interaction between MMPs, TIMPs, with Aβ and tau pathology in human subjects is needed to support that

lower MMP-2 concentration level in AAs could potentially put them at higher risk of developing plaques and tangles.

In the CNS, oligodendrocytes produce IL-7. Although data on CSF IL-7 is limited, several publications have shown reduced levels of CSF IL-7 in AD patients compared to controls (Fagan & Perrin, 2012). However, it should be noted that IL-7 and other cytokines have large inter-site variabilities and consistent measurements are difficult to be reproduced across different research laboratories, cohorts, and assay platforms (Gangishetti et al., 2018). In our cohort, the level of plasma IL-7 was significantly higher in AAs compared to CCs, and this was inverted in CSF. This supports the supposition that peripheral inflammation does not necessarily map onto inflammation in the CNS. Beyond this, there is a lack of research in the literature to explain why the average IL-7's level is reversed.

We also report here that AAs have lower VCAM-1 in both the periphery, which is in line with previous data (Hwang et al., 1997) and the CNS. VCAM-1 and ICAM-1 are cell adhesion molecules that are typically expressed by endothelial cells and upregulated under inflammatory conditions (Rosenberg, 2009). A recent CSF biomarker study showed evidence that neuroinflammation and cerebrovascular dysfunction occur in the early preclinical phase of AD and contribute to AD pathogenesis. Although earlier studies that have measured ICAM-1 and VCAM-1 in CSF have produced mixed results, a recent study using 508 cognitively unimpaired elderly, 256 MCI patients and 57 AD patients from the Swedish Biomarkers for Identifying Neurodegenerative Disorders Early and Reliably (BIOFINDER) cohort showed elevated CSF levels of ICAM-1 and VCAM-1 in AD patients during the preclinical, MCI and dementia stages of AD. This study also identified that these ICAM-1 and VCAM-1 were correlated with Aβ, tau, cortical thinning, and later cognitive decline in cognitively unimpaired elderly controls

(Janelidze et al., 2018). In the periphery, studies have also shown that blood levels of VCAM-1 and ICAM-1 are increased in AD (Ewers, Mielke, & Hampel, 2010). Because MMP-2 and VCAM-1 are both involved in endothelial activation and BBB permeability, these results from markers of neuroinflammation in our cohort show that there is a possibility that BBB dysfunction is racially different in our cohort of healthy individuals at risk for AD.

Using the novel CSF biomarker of pericyte damage, sPDGFRβ, which in turn reflects BBB breakdown, we have shown that AAs have a lower level of sPDGFRβ than CCs. Although there have only been two studies that reported pericyte injury and BBB leakiness in AD using this novel biomarker (Miners et al., 2019; Nation et al., 2019), sPDGFRβ, along with MMP-2 and VCAM-1, supports these findings in our cohort, AAs have less BBB disruption than CCs. However, we cannot rule out the explanation that AAs have innately lower levels of these biomarkers in their CNS, and this may not necessarily reflect their BBB integrity and function. Additionally, we showed that in our healthy middle-aged cohort with a family history of AD, higher levels of sPDGFRβ is significantly correlated with higher levels of T-tau and P-tau, but not Aβ42, which is in line with the previous finding relating tau in older (age 76.2 ± 6.11) AD patients (Miners et al., 2019). We have also found positive associations of sPDGFRβ with MMP-2, TIMP-1, TIMP-2, and VCAM-1, which would be predicted, as these markers of neuroinflammation (MMPS, TIMPs, and VCAM-1) are all involved in endothelial activation, basement membrane remodeling, and are all intricately related in the process of maintaining normal BBB functions (Rempe et al., 2016).

The reduced CSF levels of T-tau and P-tau in AAs may be related to less BBB breakdown. Likely, reduced tau in the CSF is partially caused by less BBB breakdown in AAs. However, the causality in this is uncertain, as in the literature of in vivo and animal studies, both

tau pathology leading to BBB breakdown and conversely, BBB breakdown leading to tau pathology have been shown (Zenaro et al., 2017). Using a tau transgenic mouse model, one study showed that tau alone could initiate the breakdown of the BBB, as measured using T cell and red blood cell infiltration and the Evans blue (EB) dye. Additionally, Blair, et al. showed that BBB in mice could recover integrity as tau levels are reduced using doxycycline (Blair et al., 2015). In reverse, several animal studies indicated that BBB dysfunction leads to the development of tau pathology. When transgenic animals susceptible to AD were challenged by adding pericyte loss (A. P. Sagare et al., 2013), and other vascular hits at the BBB such as diminished glucose transporter 1 (GLUT1) and low density lipoprotein receptor-related protein 1 (LRP1) expression, these events contributed to the development of neurodegenerative changes including accumulation of Aβ, tau hyperphosphorylation, and neuronal loss (Montagne et al., 2017). Additionally, one study that investigated whether tau proteins can cross the BBB showed that several key forms of tau proteins, tau-441, tau-410, truncated tau 151-391, and truncated tau 121-227 crossed the BBB readily and bidirectionally (Banks et al., 2017). Taken together, these studies have established the role of BBB breakdown and dysfunction in the development of AD's amyloid and tau pathology (Montagne et al., 2017). In our study, we suspect that the reduced tau in CSF in AAs is connected to their lesser extent of BBB dysfunction.

The reasonable next step would be to continue to investigate measures of BBB integrity in our cohort. The two most established ways to measure BBB in vivo are using neuroimaging to measure microbleeds and CSF/serum albumin ratio. Damage to blood vessels leads to BBB breakdown, which in the brain, is manifested as cerebral microbleeds (microhemorrhages), which are frequently seen in AD and MCI individuals (Sweeney, Sagare, & Zlokovic, 2018). T2-weighted and susceptibility-weighted imaging (SWI) MRI are used to measure microbleeds and

involve radiologists to count each microhemorrhages manually. Therefore, it may lead to substantial variability across different research labs. The albumin concentration is about 200 times lower in CSF compared to serum (Tibbling, Link, & Öhman, 1977). A meta-analysis showed elevated CSF/serum albumin ratio in AD. However, the effect size was small (Olsson et al., 2016). Therefore, in a cohort of middle-aged, healthy individuals, slight BBB impairment would be challenging to be detected and measured accurately. Ideally, post-mortem tissue analysis for brain capillary leakages, pericyte, endothelial degeneration, and cellular infiltration will provide more information on BBB disruption (Sweeney et al., 2018)

Finally, in the literature of in vitro and animal studies, there have been contradicting reports concerning the role of ACE in AD pathology (Gebre, Altaye, Atey, Tuem, & Berhe, 2018). In vitro studies have generally shown the role of ACE in the degradation of Aβ peptides, and as a result, reducing deposition and accumulation of amyloid plaque. Moreover, Bernstein, et al. crossed $ACE^{10/10}$ mice, which overexpress ACE in myelomonocytes, with AD^{+} transgenic model mice. Results showed that $AD^{+}ACE^{10/10}$ mice had a reduced level of brain Aβ peptides, and reduced plaque burden (Bernstein et al., 2014). Additionally, the administration of Ramipril (an ACEI) increased Aβ peptides in $AD^{+}ACE^{10/10}$ mice (Bernstein et al., 2014). However, other animal studies showed contradicting results. For example, another ACEI, Captopril, reduced Aβ peptides in animal models of AD (AbdAlla, Langer, Fu, & Quitterer, 2013). Several other studies showed beneficial effects in reducing AD signs and symptoms in transgenic AD models animals (Gebre et al., 2018). In human studies, although only a few studies have looked at the link between RAS and AD, ACEIs and ARBs have consistently shown beneficial effects in reducing and slowing cognitive decline associated with AD (Gebre et al., 2018). In our cross-sectional data, we did not find a correlation between ACE-1 and ACE-2 activities in serum and CSF and

established AD markers. When correlating CSF ACE-1 activity with markers of inflammation, we showed that CSF ACE-1 activity is negatively correlated with plasma IL-7 and positively correlated with CSF IL-9 levels, indicating that a potential link between cytokines and ACE.

In conclusion, in our cohort of middle-aged, healthy AAs and CCs with a family history of AD, we showed racial differences in several biomarkers that have been used in current AD research, including CSF T-tau, P-tau, cytokines, and markers of BBB dysfunction. Because of our sample size, and the lack of a good measure of BBB dysfunction in human subjects, these results are yet preliminary. Our cohort consists mostly of people from the southern United States, where there is a large proportion of AAs in the general population, and it is unknown how well these results could be generalized to AAs from other parts of North America or native Africans. It is also unclear on the exact causes, including genetic, physiological, socioeconomic, and psychological factors, and consequences of these racial differences between the two races. There is an urgent need for a larger, national-level, longitudinal cohort that is racially diverse in order to achieve these goals.

5 Tables and Figures

Table 1: Demographic Characteristics

	African Americans (n=30)	**Caucasians (n=50)**	**p Value**
Age	60.1 ± 7.8	58.5 ± 6.1	0.30
Gender (% Female)	83.3%	56.0%	**0.0123**
Education	10.7% High School/GED 39.3% College Graduate 50.0% Postgraduate	18.0% High School/GED 38.0% College Graduate 44.0% Postgraduate	0.68
Income	10.7% ⩽$19,000 17.9% $20-39,000 28.6% $40-59,000 17.9% $60-79,000 25.0% ⩾$80,000	 12.0% $20-39,000 4.0% $40-59,000 18.0% $60-79,000 66.0% ⩾$80,000	**0.0005**
Systolic Blood Pressure (mmHg)	127.6 ± 13.3	125.1 ± 12.3	0.42
Diastolic Blood Pressure (mmHg)	77.3 ± 7.0	77.3 ± 9.0	0.99
APOE ε4 Status	48.3%	50.0%	0.88

P-Values marked in bold indicate values or proportions significantly differed between the two races ($p<0.05$).

Table 2: Cognitive Testing

	African Americans (n=30)	Caucasians (n=50)	p Value
MoCA	25.0 (24.0 – 27.0)	27.0 (25.0 – 29.0)	**0.0051**
Trails B	81.0 (69.0 – 101)	70.0 (56.0 – 82.0)	**0.0239**
Forwards Digit Span	6.5 (5.5 – 7.0)	7.0 (6.0 – 8.0)	0.22
Backwards Digit Span	4.0 (3.0 – 5.0)	5.0 (4.0 – 6.0)	0.10
Mental Rotation	17.5 (15.0 – 12.0)	18.5 (13.0 – 21.0)	0.50
Benson Delay	12.0 (10.0 – 14.0)	12.0 (10.0 – 13.0)	0.28
Buschke Delay	6.0 (2.0 – 8.0)	7.0 (5.0 – 9.0)	**0.0218**
MINT	29.0 (28.0 -31.0)	31.0 (30.0 – 32.0)	**0.0017**

Reported values are median (interquartile range).

P-Values marked in bold indicate values significantly differed between the two races ($p<0.05$).

Table 3: Core AD Biomarkers

	African Americans (n=30)	Caucasians (n=50)	p Value
Amyloid-β (pg/mL)			
MSD Triplex Aβ38	2029.2 ± 672.9	2466.3 ± 760.3	**0.0268**
MSD Triplex Aβ40	5020.9 ± 1312.7	5750.0 ± 1618.2	0.07
MSD Triplex Aβ42	413.1 ± 107.5	419.9 ± 150.0	0.85
ELISA Aβ42	722.0 ± 164.2	703.7 ± 197.3	0.71
Tau (pg/mL)			
T-tau	199.0 (166.0 – 244.0)	297.0 (228.0 – 423.0)	**0.0036**
P-tau	37.0 (34.0 – 42.0)	48.0 (37.0 – 64.0)	**0.0055**

T-tau and P-tau levels were non-parametric and necessitated use of Wilcoxon test. Reported values for these variables are median (interquartile range).

P-Values marked in bold indicate values significantly differed between the two races ($p<0.05$).

Table 4: Markers of Inflammation

	African Americans (n=30)	Caucasians (n=50)	p Value
Plasma Inflammatory Markers (pg/mL)			
IL-7	6.1 (3.9 – 9.7)	4.7 (2.8 – 6.2)	**0.0249**
IL-8	6.2 (4.4 – 17.1)	9.9 (5.2 – 31.6)	0.10
IL-9	1.7 (1.1 – 3.2)	1.3 (0.7 – 2.6)	0.28
IL-10	8.5 (6.8 – 14.1)	10.5 (7.7 – 14.3)	0.53
MCP-1	213 (186 – 259)	164 (138 – 203)	**0.0011**
MDC	1071 (876 – 1441)	854 (689 – 1152)	**0.0046**
TGF-α	2.3 (1.3 – 4.4)	1.6 (0.9 – 3.9)	0.46
TNF-α	7.8 (5.4 – 10.1)	5.5 (4.7 – 7.6)	0.10
Interferon-γ	12.6 (7.7 – 20.4)	10.3 (4.5 – 19.7)	0.63
ICAM-1	532 (430 – 631)	516 (462 – 633)	0.82
VCAM-1	3442 (3045 – 4002)	3930 (3482 – 4782)	**0.0051**
CRP, µg/mL	13.3 (5.1 – 20.5)	3.3 (1.5 – 6.9)	**0.0008**
SAP, µg/mL	12.0 (10.1 – 13.2)	9.9 (8.3 – 12.5)	**0.0419**
CSF Inflammatory Markers (pg/mL)			
MMP-1	5.7 (4.1 – 7.7)	6.6 (4.9 – 8.6)	0.22
MMP-2	17645 (14621 – 21356)	20657 (17766 – 23490)	**0.0256**
MMP-9	12.8 (7.4 – 24.5)	11.6 (5.9 – 18.1)	0.34
IL-7	1.3 (0.8 – 1.6)	1.7 (1.3 – 2.4)	**0.0046**
IL-8	71.3 (63.4 – 90.6)	69.4 (57.3 – 88.2)	0.37
IL-9	3.2 (1.5 – 4.0)	3.9 (2.3 – 5.3)	0.13
IL-10	6.1 (3.9 – 8.0)	5.4 (4.1 – 6.7)	0.55
MCP-1	5579 (5505 – 5931)	5590 (5375 -5838)	0.06
MDC	88.3 (67.4 – 188)	99.5 (69.2 – 144)	0.97
TGF-α	8.9 (7.3 – 9.8)	9.1 (7.1 – 9.5)	0.70
TNF-α	1.2 (1.0 – 1.9)	1.1 (0.6 – 1.5)	0.28
TIMP-1	35.5 (31.1 – 42.1)	36.2 (31.3 – 42.1)	0.70
TIMP-2	37.9 (33.5 – 45.3)	41.4 (36.8 – 47.2)	0.12
ICAM-1	301 (188 – 402)	243 (183 – 337)	0.34
VCAM-1	19.8 (13.6 – 23.8)	26.0 (18.8 – 34.0)	**0.0056**

Reported values are median (interquartile range).

P-Values marked in bold indicate values significantly differed between the two races ($p<0.05$).

Table 5: ACE Activity and sPDGFRβ Level

	African Americans (n=30)	**Caucasians (n=50)**	**p Value**
Serum ACE-1 Activity (r.f.u.)	23415.3 ± 9956.8	24151.6 ± 11753.0	0.79
Serum ACE-2 Activity (r.f.u.)	939.5 (660.0 - 1742)	1355 (1011 - 2040)	0.09
CSF ACE-1 Activity (r.f.u.)	5326 ± 1053	5753 ± 1769	0.22
CSF ACE-2 Activity (r.f.u.)	2219 (2109 - 2478)	2230 (2005 - 2369)	0.37
CSF sPDGFRβ (pg/mL)	375 ± 152	499 ± 166	**0.0055**

ACE 1 and ACE 2 activities were expressed as relative fluorescence units (r.f.u.). Serum ACE 1 and CSF ACE 1 activities are mean ± standard deviation. Serum ACE 2 and CSF ACE 2 activities were not normally distributed and reported value is median (interquartile range).

P-Values marked in bold indicate values significantly differed between the two races ($p<0.05$).

Table 6: Adjusted Correlation between CSF sPDGFRβ and Core AD Biomarkers

CSF sPDGFRβ		
	B (SE)	p
ELISA Aβ42	0.81 (1.13)	0.4759
T-tau	4.78 (1.40)	**0.0012**
P-tau	38.51 (11.04)	**0.0010**

Adjusted for Age, Gender, Race, and Education. Unstandardized coefficient (B), standard error (SE), and p Value are reported.

Table 7: Adjusted Correlation between CSF sPDGFRβ and CSF Inflammatory Markers

CSF sPDGFRβ		
	B (SE)	p
MMP-1	1.69 (3.79)	0.6576
MMP-2	0.01 (0.00)	**0.0028**
MMP-9	-1.26 (1.92)	0.5120
IL-7	-10.90 (30.49)	0.7220
IL-8	2.01 (0.91)	**0.0313**
IL-9	14.92 (10.44)	0.1587
IL-10	-0.89 (8.79)	0.9198
MCP-1	0.04 (0.04)	0.3931
MDC	-0.15 (0.32)	0.6428
TGF-α	16.13 (9.67)	0.1009
TNF-α	2.24 (27.76)	0.9361
TIMP-1	8.85 (2.78)	**0.0023**
TIMP-2	6.31 (3.05)	**0.0432**
ICAM-1	0.164(0.13)	0.2126
VCAM-1	6.73 (2.00)	**0.0013**

Adjusted for Age, Gender, Race, and Education. Unstandardized coefficient (B), standard error (SE), and p Value are reported.

Table 8: Adjusted Correlation between CSF ACE-1 and Inflammatory Markers

CSF ACE-1 Activity		
Plasma Inflammatory Markers		
	B (SE)	p
IL-7	43.11 (61.61)	0.4870
IL-8	-7.29 (7.58)	0.3403
IL-9	-70.00 (32.85)	**0.0377**
IL-10	-2.55 (23.58)	0.9143
MCP-1	-5.19 (2.83)	0.0717
MDC	0.07 (0.55)	0.9012
TGF-α	-8.90 (13.72)	0.5192
TNF-α	-8.45 (35.87)	0.8147
Interferon-γ	-1.87 (1.79)	0.3015
ICAM-1	-1.14 (0.70)	0.1069
VCAM-1	-0.10 (0.09)	0.2761
CRP	8.98 (16.13)	0.5798
SAP	-66.46 (64.56)	0.3077
CSF Inflammatory Markers		
MMP-1	12.40 (36.96)	0.7385
MMP-2	0.01 (0.05)	0.8795
MMP-9	11.35 (18.63)	0.5448
IL-7	731.42 (274.77)	**0.0101**
IL-8	6.44 (9.14)	0.4843
IL-9	-40.10 (104.30)	0.7021
IL-10	4.26 (85.53)	0.9605
MCP-1	0.29 (0.41)	0.4834
MDC	3.66 (3.08)	0.2392
TGF-α	57.72 (95.36)	0.5473
TNF-α	3.78 (267.76)	0.9888
TIMP-1	-10.78 (29.57)	0.7166
TIMP-2	-18.07 (30.82)	0.5598
ICAM-1	0.05 (1.29)	0.9677
VCAM-1	29.95 (21.08)	0.1608

Adjusted for Age, Gender, Race, and Education. Unstandardized coefficient (B), standard error (SE), and p Value are reported.

Figure 1: AAs had lower T-tau and P-tau levels compared to CCs

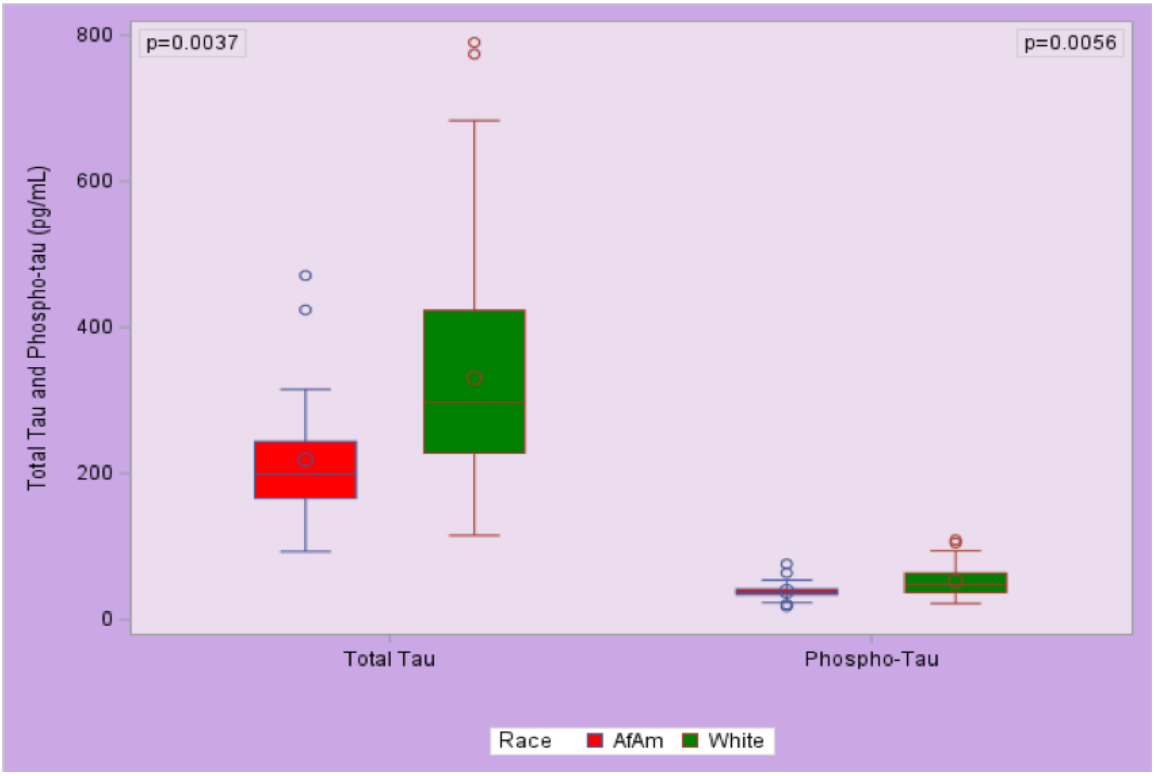


Figure 2: AAs had lower CSF sPDGFRβ level compared to CCs

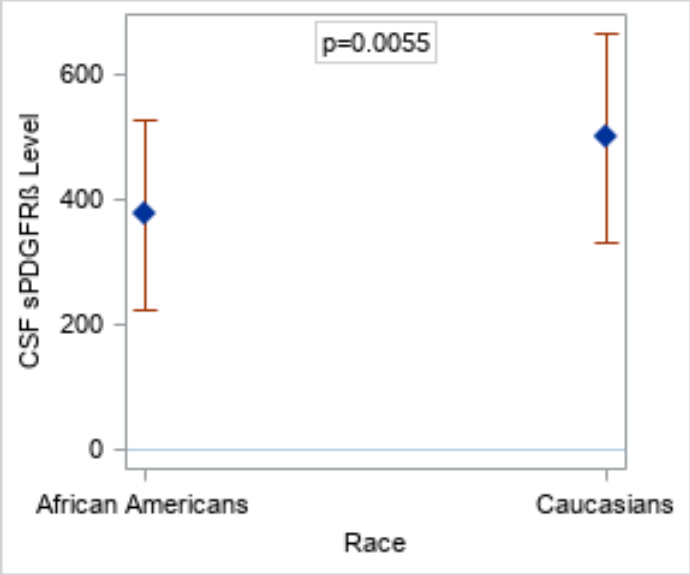


Figure 3: Relationships between CSF sPDGFRβ and CSF Core AD Biomarkers

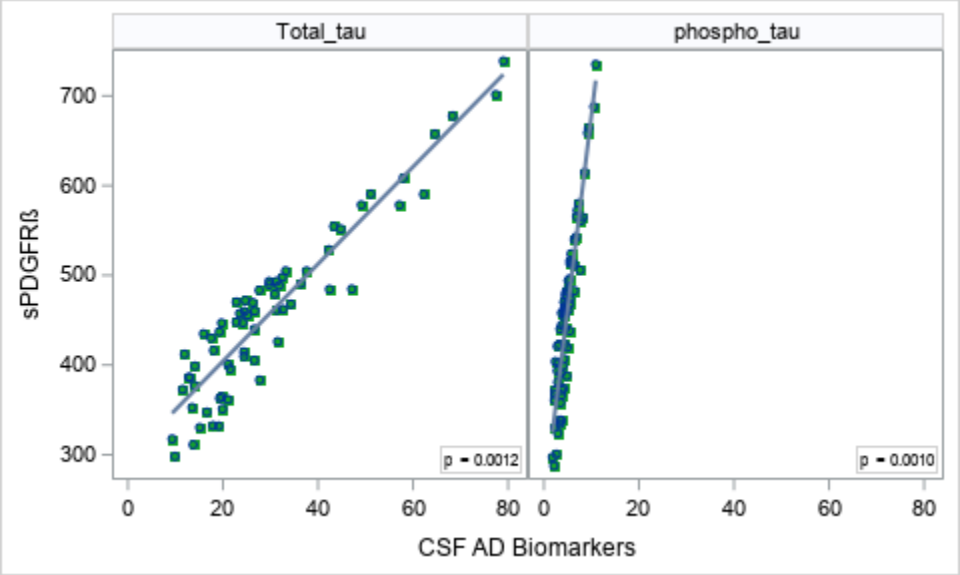


Figure 4: Relationships between CSF sPDGFRβ and CSF Inflammatory Markers

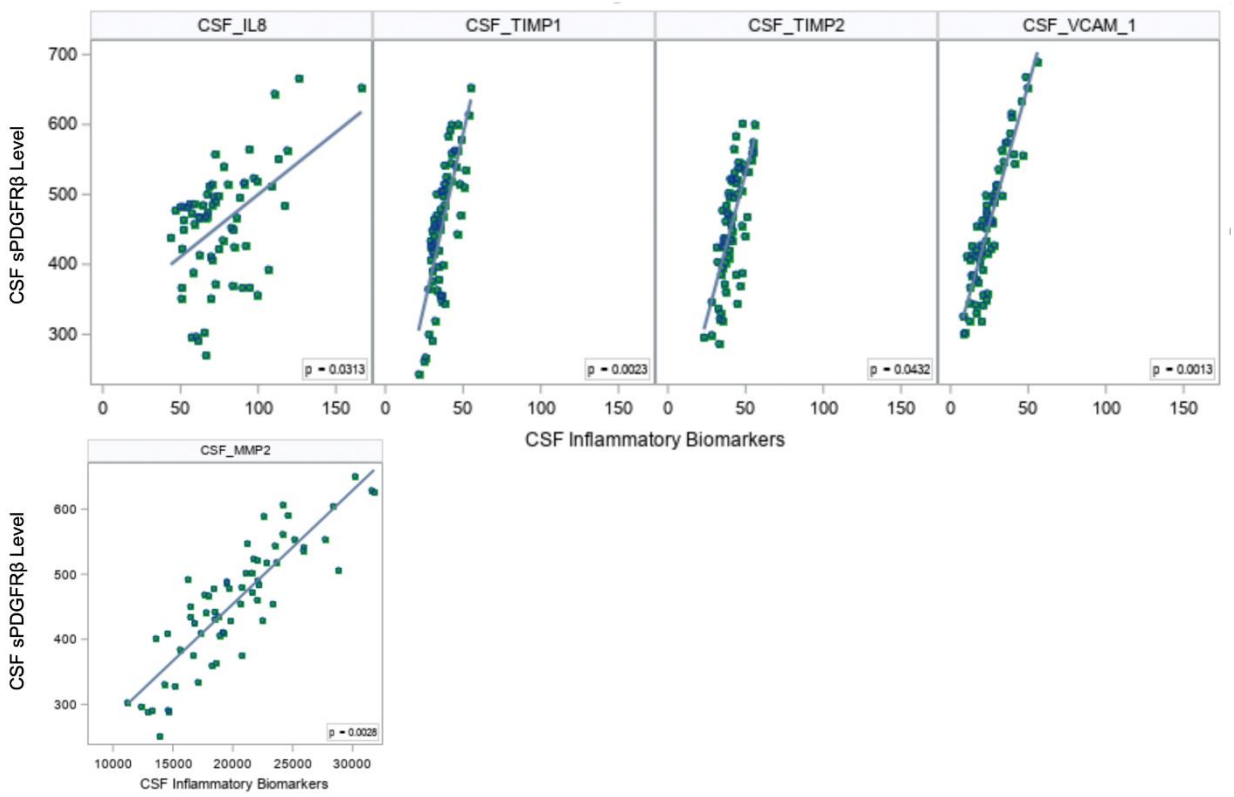
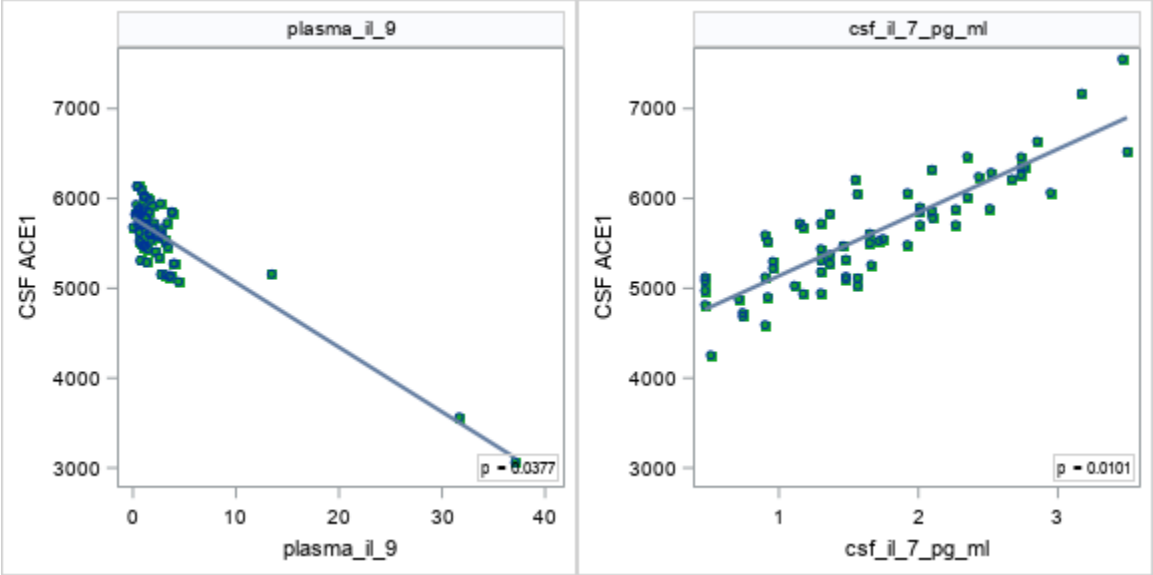


Figure 5: Relationships between CSF ACE-1 Activity and Inflammatory Markers



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