RELATIONSHIPS AMONG *APOE* GENOTYPES, INFLAMMATORY MARKERS, AND RISK FACTORS AMONG AFRICAN AMERICANS WITH ISCHEMIC STROKE

by

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DEDICATION

I would like to dedicate this doctoral dissertation to my parents, Rev. Mr. Eugene S. Wadas and the late Monica N. Wadas. There is no doubt in my mind that without their continued support and counsel I could have completed this process.

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ABSTRACT

African Americans experience a disportionate mortality, morbidity, and disability associated with ischemic stroke. Traditional risk factors offer some explanation for this finding, but novel risk factors have not been explored. *APOE4* genotype, which is more prevalent in African Americans demonstrate a pro-inflammatory phenotype that may result in an exaggerated inflammatory response associated with ischemic stroke, resulting in worse outcomes.

The purpose of this study was to examine relationships among *APOE* genotypes, inflammatory markers (CD11β, platelet leukocyte aggregates, IL-1β, IL-6, IL-8, and tissue necrosis factor alpha), the anti-inflammatory marker, IL-10, and risk factors (hypertension, diabetes type II, obesity, hyperlipidemia, and smoking) in African Americans at 3 days post stroke.

Twenty five patients were enrolled with 12 patients in the *APOE4* group and 13 patients in the non-*APOE4* group. In the *APOE4* group, 75% were male compared to 54% in the non-*APOE4* group. The average age in the *APOE4* group was 56.5 ± 9.0 compared to 66 ± 16.0 years in the non-*APOE4* group. Females in the *APOE4* group were younger with ages comparable to men. All participants had hypertension. Forty two percent of patients in the *APOE4* group had two risk factors and 46% of patients in the non-*APOE4* group had three risk factors. Major findings included 1) there were no statistical difference between inflammatory markers and *APOE* genotypes, and 2) the *APOE4* carrier was not a predictor for overall inflammatory load among African Americans with ischemic stroke. The study was underpowered and small effect sizes were not sufficient to create statistical findings.

This was the first study to examined *APOE* genotypes, inflammatory markers, and risk factors among African Americans with ischemic stroke. More studies are needed to not only investigate novel risk factors, but to also characterize inflammatory and genetic mechanisms with ischemic stroke and their associated outcomes among African Americans. Such studies may lead to primary and secondary prevention of ischemic stroke and reduce the health disparities associated with ischemic stroke among African Americans.

CHAPTER I: STATEMENT OF THE PROBLEM

Stroke is the fourth leading cause of death and the leading cause of long-term disability in the United States (U.S.) (Mozaffarian et al., 2015). Despite national declines in stroke mortality, African Americans (AAs) in the U.S. continue to have high death rates from stroke (Mozaffarian et al., 2015; Kleindorfer et al., 2010; Ovbiagele et al., 2013). In fact, in the U.S. stroke is the second leading cause of death among AAs (Mozaffarian et al., 2015; Ovbiagele et al., 2013). AAs have 2 to 4 times the rate of stroke, stroke recurrence, and stroke-related deaths. The incidence also occurs at an earlier age with a higher overall morbidity and with 1 in 6 survivors having a recurrent stroke within five years (Cushman et al., 2008; Ovbiagele et al., 2013). Thus, AAs are a high risk group even after the initial stroke.

Additionally, from 2005 to 2050 the cost of stroke projected for AAs will exceed 379 billion with the 45 to 64 age group accounting for 67% of the total cost (Brown et al., 2006; Damaerschalk, Hwang, & Leung, 2010; Mozaffarian et al., 2015). AAs have the highest per capita cost for stroke in comparison to other ethnic groups due to stroke occurring at a younger age. Data from the African American Antiplatelet Stroke Prevention Study (AASPS) (Ruland et al., 2006) demonstrated that 48% of AAs were disabled or died following the second stroke, indicating that recurrent stroke had detrimental outcomes for this ethnic group. The excess mortality and morbidity associated with stroke among AAs is a major public health burden and is particularly felt in the southeastern U.S., also known as the stroke belt (Mozaffarian et al., 2015; Ovbiagele et al., 2013). Within the stroke belt, a “buckle” region, comprising North Carolina, South Carolina, and Georgia have even higher stroke mortality rates than the rest of the country.

The overall stroke mortality is 20 per cent higher in the stroke belt and 40 percent higher in the stroke buckle in comparison to other U.S. regions (Howard et al., 2011; Ovbiagele et al., 2013).

Clearly, AAs bear a significant proportion of the stroke burden in the U. S; however, the reason for this has not been fully explored. It is known that AAs demonstrate a disproportionate number of traditional risk factors for stroke as defined by the historical Framingham Study (Wolf, D'Agostino, Belanger, & Kannel, 1991). These risk factors include age, gender, systolic blood pressure, antihypertensive medication use, diabetes mellitus, atrial fibrillation, left ventricular hypertrophy, heart disease, and cigarette smoking. Data from the National Behavioral Risk Factor Surveillance System through 2010 demonstrated that 71.2 % of AAs reported one or more risk factors, suggesting a higher risk profile in comparison to European Americans (EAs) (Cory et al., 2010).

Although the clustering of risk factors is quite common among AAs, it only offers a partial explanation for the rate of mortality and morbidity (Howard et al., 2011; Taylor et al., 2008). In fact, in over 50% of instances, cardiovascular and cerebral ischemic events occur in the absence of traditional risk factors (Cushman et al., 2008; Howard et al., 2011; Howard et al., 2013; Stephens, Bain, & Humphries. 2008). Thus, additional contributing factors that explain excess stroke mortality and morbidity among AAs remain largely unexplained.

Stroke is a complex, heterogeneous, and multifactorial disease of various etiologies involving pathophysiologic and genetic variations. The occlusion of cerebral blood flow results in an ischemic core, which is surrounded by a hypoperfused region known as the penumbra. The penumbra represents the vulnerable region or region at risk that can evolve toward infarction or toward viability (Perera et al., 2006). Several mechanisms have been proposed to explain the

progression of the penumbra to infarction and include the various cytotoxic substances such as free radicals, nitric oxide, excitatory amino acids and calcium and mechanisms such as apoptosis, angiogenesis, and neuroprotection (Deb, Sharma, & Hassan, 2010; Onwuekwe & Eadikaibe, 2012; Woodruff et al., 2011). Additionally, studies from the past decade demonstrate that activation of inflammation, specifically polymorphonuclear neutrophil activation and infiltration, may contribute to the progression of the penumbra to infarction (Deb et al., 2010; Onwuekwe & Ezeala-Akikaibe, 2012; Woodruff et al., 2011). It has been demonstrated that excessive neutrophil adherence to the cerebral vascular microcirculation and subsequent migration to cerebral tissue may contribute to the development of reperfusion injury and neutrophil mediated tissue damage after ischemic stroke (Breckwoldt et al., 2008; Gelderblom et al., 2009; Ritter, Orozco, Coull, McDonagh, & Rosenblum, 2000; Ruehl et al., 2002). Activated neutrophils express surface adhesion molecule CD11β that promotes their adhesion to the microcirculation (Li, Hallden, & Hjemdahl, 2000; Morrison, McKee, & Ritter, 2011; Zhou et al., 2013). Studies indicate neutrophil activation occurs after stroke (Beamer et al., 1998; Caimi et al., 2001; Gelderblom et al., 2009; Morrison et al., 2011; Ritter, Stempel, Coull, & McDonagh, 2005; Welsh et al., 2008). However, there are no studies that have examined neutrophil CD11β levels among AAs with ischemic stroke. Neutrophil activation is mediated in part by cytokines, which include tissue necrosis factor alpha (TNFα), interleukin 1β (IL-1β), interleukin 6 (IL-6), interleukin 8 (IL-8), and interleukin 10 (IL-10). The functions of these inflammatory and anti-inflammatory mediators are essential to host defense and requires a delicate balance of protective versus injurious actions. The response of neutrophils to stimuli is largely determined by their previous or chronic exposure to various agents such as cytokines (Condliffe, Kitchen, &

Chilvers, 1998; Wright, Thomas, Moots, & Edwards, 2013). At certain physiologic levels these agents may enhance or "prime" the neutrophil response to a second activating event, such as ischemic stroke, resulting in an exaggerated inflammatory response. Although information regarding cytokine levels among AAs with stroke is scarce, TNFα and IL-6 is known to be increased in AAs (Wassel et al., 2007).

In addition to neutrophil response, the tissue injury of ischemic stroke triggers an acute phase response resulting in an increase in systemic inflammatory markers, such as fibrinogen and C-reactive protein (CRP). Elevated levels of both markers are associated with stroke mortality or a new vascular event (Di Napoli, Pappa, & Bocola, 2001a; Rothwell et al., 2004; Whiteley et al., 2011). Fibrinogen has been shown to be positively associated with mortality risk with cardiovascular disease and stroke (Ahmadi-Abhari, Luben, Wareham, & Khaw, 2013; Markaki, Franzen, Talani, Loizou, & Kostulas, 2013). Fibrinogen levels are also strongly associated with stroke severity across all studied populations (Di Napoli & Singh, 2009). Studies have demonstrated higher fibrinogen levels in AAs with the highest levels observed in African American (AA) women aged 30 years or older (Albert & Ridker, 2004). Additionally, greater African ancestry among AAs has also been associated with higher fibrinogen levels (Lutsey et al., 2012). Studies investigating fibrinogen levels among AAs with ischemic stroke are scarce and near nonexistent.

In addition, fibrinogen influences leukocyte function. Previously thought to only be a central factor in the hemostatic response, it is now known that fibrinogen also participates in the inflammatory response through cross talk with leukocytes and platelets (Flick, Du, & Degen, 2004). Fibrinogen binds to both neutrophil surface adhesion molecule CD11β and to platelet

surface adhesion molecules, thus, forming a bridge between these blood cell types resulting in potentially injurious platelet leukocyte aggregates (PLAs) (Ritter et al., 2005). These aggregates are increased in the systemic blood after ischemic stroke (Cao, Wang, Zhang, Zeng, & Liu, 2009; Ritter et al., 2005). In vitro studies demonstrated that fibrinogen can significantly alter leukocyte function, which affects cell migration, phagocytosis, and cytokine expression (Rubel et al., 2002; Rubel et al., 2001). Moreover, it has been proposed that neutrophil priming by substances such as cytokines may facilitate fibrinogen and leukocyte interaction (Rubel et al., 2001). PLAs have not been studied among AAs with ischemic stroke.

CRP is rapidly up-regulated by cytokines following ischemic stroke. It is a sensitive indicator of systemic inflammation and is demonstrated to have both diagnostic and prognostic implications for atherosclerosis. Several studies have suggested CRP to be a predictor of first ever stroke, stroke mortality, stroke severity, and recurrent cerebrovascular events (Arenillas et al., 2003; Ghabaee et al., 2014; Idicula, Brogger, Naess, Waje-Andreassen, & Thomassen, 2009; Di Napoli et al., 2001b; Rost et al., 2001). Data from the National Health and Nutrition Examination Survey (NHANES) indicate differences in CRP levels among ethnicities. Additional studies demonstrate differences in CRP levels among gender and ethnicities (Ford, Giles, Mikdad, & Myers, 2004; Ford, 2003; Fox et al., 2008; Khera et al., 2005). Although studies examining AAs with stroke and CRP levels are scarce, it is known that AAs have higher CRP levels than EAs and women have higher CPR levels than men (Khere et al., 2005). Body mass index (BMI) was a significant contributor to CRP levels in both AA women and children (Ford et al., 2004; Ford, 2003; Khere et al., 2005). The Jackson Heart Study, which consisted of a sample of 4,919 AA participants (mean age 55 +/- 13 years, 63% women) found that traditional

risk factors explained 23.8% of CRP's variability with BMI explaining 57.1% of the variability of CRP due to traditional risk factors (Fox et al., 2008). Although the pathological reason for the association of CRP and stroke mortality or a new vascular event is unknown, plausible explanations include: 1) inflammatory processes are linked with cerebrovascular disease and ischemia, and 2) activation of coagulation factors, including fibrinogen may play a role with cerebrovascular ischemia (Di Napoli et al., 2001b).

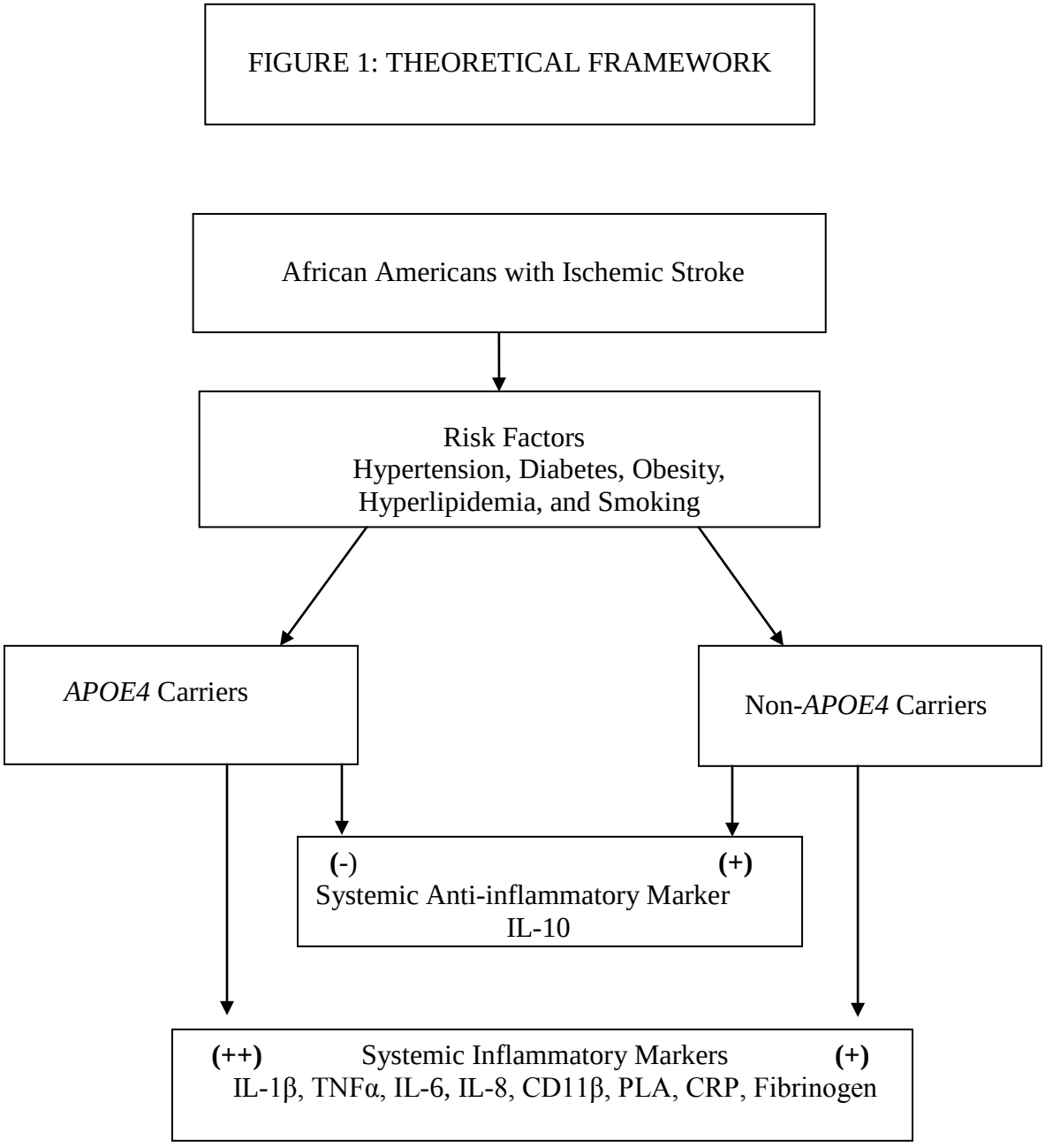
Data also suggest that the regulation of the extent of inflammatory responses after stroke may be under genetic control (Flex et al., 2004).The inflammatory response associated with stroke varies considerably and may be affected by certain genetic polymorphisms (Flex et al., 2004; Rubatuu, Giliberti,& Volpe, 2000). Pro-inflammatory phenotypes may explain the differences in why some individuals may be more susceptible to a greater inflammatory response than others. Interactive effects of this pro-inflammatory phenotype with various risk factors may contribute to a synergistic effect of this phenotype. In widely complex disorders, such as ischemic stroke, interaction between genetic predisposition and modifiable risk factors may affect disease severity and outcome (Flex et al., 2004; Waters & Nicholl, 2005). It has been postulated that individual genetic effects are small and difficult to detect with consistency and reliably without consideration to their interactions (Ioannidis, Trikalinos, & Khoury 2006). Moreover, it has also been proposed that inconsistency in genetic association studies may be due to unrecognized interactions with other genes or environmental factors that could possibly have a substantial impact on outcomes (Marchini, Donnelly, & Cardon, 2005). Despite the potential significance of inflammatory regulation at the level of the gene, little attention has focused on the relationships

among genetic polymorphisms, pro-inflammatory phenotypes, and risk factors with ischemic stroke. The relationship among these variables is *non*existent among AAs with ischemic stroke.

The Apolipoprotein E (*APOE*) is one gene that has been extensively studied in various multifactorial diseases such as Alzheimer's disease (Fekih-Mrissa et al., 2013; Licastro, Porcellini, Caruso, Lio, & Corder, 2007), intracerebral hemorrhage (Biffi et al, 2011; Brouwers, et al., 2012), traumatic brain injury (Jiang et al., 2011; Zhou et al., 2008), Parkinson's disease (Monsell et al., 2014; Pulkes et al., 2011), coronary artery disease (Grammer et al., 2011; Kumar et al., 2012), peripheral vascular disease (Bryson, et al., 2011; Resnick et al., 2000), chronic kidney disease (Chmielewski, Verduijn, & Dekker, 2010; Seshasai, et al., 2012), and diabetes mellitus (Chaudhary, et al., 2012; Xie, et al., 2011). There is also evidence to support *APOE4* gene as modifying immune responses by up regulating pro-inflammatory cytokines (Tsoi, Wong, Liu, & Ho, 2007). The *APOE4* genotype frequency differs among ethnicities within certain groups, such as AAs demonstrating greater *APOE4* carriers (Howard, Gidding, & Liu, 1998). Additionally, there is evidence to suggest interactive effects of *APOE* genotypes (Talmud et al., 2005). The inflammatory responses, in combination with specific *APOE* genotypes and synergistic effects of risk factors, particularly those of chronic inflammatory states that predispose an individual to neutrophil priming may be a mechanism that contributes to exaggerated reperfusion injury and worse outcomes after first stroke. However, the relationships among *APOE* genotypes, inflammatory markers, and risk factors in AAs with ischemic stroke have not been investigated.

Theoretical Framework

The theoretical framework for this dissertation is presented in Figure 1.



AAs are a high risk group for mortality and morbidity associated with ischemic stroke. AAs also demonstrate vascular risk factor clusters that result in a chronic inflammatory state, which may prime neutrophils, leading to an exaggerated inflammatory response associated with ischemic stroke. *APOE4* represents a pro-inflammatory genotype and through interactions with vascular risk factors result in elevated inflammatory markers and a decrease in the anti-inflammatory markers.

Inflammatory processes in the presence of pro-inflammatory genotypes may be one mechanism that contributes to inflammatory mediated tissue injury associated with ischemic stroke. Genetic polymorphisms may influence a pro-inflammatory phenotype. Evidence supports an altered immune response and specifically an imbalance of pro-inflammatory cytokines among *APOE4* carriers. The major immune differences of *APOE4* carriers were demonstrated in microglia morphology, macrophage response, cytokine expression, and systemic inflammation; all of which can alter the overall immune response and disease outcome. These findings characterize *APOE4* carriers as a pro-inflammatory phenotype. However, neither the neutrophil response, particularly the association of neutrophil activation or PLAs with *APOE* carriers, nor mechanisms that may contribute to this exaggerated response, has been investigated. It is possible that priming of neutrophils by cytokines may be one mechanism that may contribute to an exaggerated inflammatory response, thereby contributing to tissue mediated injury associated with reperfusion. No study to date has examined the relationships among *APOE4* carriers and inflammatory responses among AAs with stroke, especially when *APOE4* carriers are more frequent in this population (Howard, Gidding, & Liu, 1998). Moreover, *APOE* genotypes and inflammatory processes that promote an exaggerated neutrophil response among

AAs with ischemic stroke have not been investigated and are not known. It may be possible that *APOE4* carriers with ischemic stroke not only represent a pro-inflammatory phenotype, but also through interactions with risk factors produce a synergistic inflammatory response. Risk factors of chronic inflammatory states, such as hypertension, hyperlipidemia, diabetes, obesity, and smoking are known to exhibit primed neutrophils, reflecting one mechanism that may contribute to the exaggerated inflammatory response. This interaction would ultimately have prognostic implications and worse outcomes following stroke. Characterization of high risk genotypes and their associated inflammatory phenotypes among AAs with ischemic stroke may lead to preventive therapies that could potentially influence clinical outcomes for survivors, especially when primed neutrophils are reversible. Therefore, investigating the relationships among *APOE* genotypes, inflammatory markers, and risk factors among AAs with ischemic stroke is of particular interest.

AAs have higher mortality, morbidity, and more severe disability associated with stroke. The economic burden of treating AAs with stroke is significant and affects a younger age group than other ethnic groups. Few studies have examined novel risk factors for stroke. Identification of *non*traditional risk factors, such as systemic inflammatory marker levels and pro- inflammatory genotypes, may lead to prevention of primary and secondary stroke. In addition, identification of these novel risk factors may be used to assess adequacy of therapeutic management strategies after stroke. Ultimately, studies of this kind may reduce mortality, morbidity, and disability from ischemic stroke among AAs. Moreover, this research meets initiatives of advancing the understanding of injury mechanisms associated with diseases and disabilities that contribute to health disparities in cerebrovascular disease.

Statement of Purpose and Specific Aims

The overall purpose of this research was to determine the relationships among *APOE* genotypes, inflammatory and anti-inflammatory markers, and risk factors among AAs with ischemic stroke. Inflammatory markers studied were CD11β, TNFα, IL-1β, IL-6, IL-8, fibrinogen, PLA, and CRP. The anti-inflammatory marker, IL-10 was also studied. Risk factors included those that have demonstrated a chronic inflammatory state (hypertension, diabetes, obesity, hyperlipidemia, and smoking). The specific aims and related hypotheses are:

Specific Aim 1: Determine the *APOE* carriers among AAs after ischemic stroke.

Hypothesis 1. Consistent with the frequency of *APOE4* carriers among the AA population, the frequency of non-*APOE4* carriers will be greater than *APOE4* carriers among AAs with ischemic stroke.

Specific Aim 2: Determine the levels of inflammatory markers and the anti-inflammatory marker between *APOE4* carriers and non-*APOE4* carriers among AAs at 3 days after ischemic stroke.

Hypothesis 2. Inflammatory markers will be increased and the anti-inflammatory marker will be decreased in *APOE4* carriers than with non-*APOE4* carriers among AAs at 3 days after ischemic stroke.

Specific Aim 3*:* Examine *APOE4* carriers as a predictor for a higher inflammatory state (composite scores of inflammatory markers and the anti-inflammatory marker) among AAs at 3 days after ischemic stroke.

Hypothesis 3. *APOE4* carriers will predict a higher inflammatory state (composite scores of inflammatory markers plus the anti-inflammatory marker) as compared to non-*APOE4* carriers among AAs at 3 days after ischemic stroke.

Summary

AAs are a high risk group for mortality and morbidity associated with ischemic stroke. Traditional risk factors offer a partial explanation, but nontraditional risk factors have not been explored. Evidence suggests that inflammatory processes contribute to the progression of ischemic stroke as well as reperfusion injury and neutrophil mediated injury. Genetic polymorphisms, such as *APOE4* carriers characterize a pro-inflammatory phenotype that may result in exaggerated inflammatory responses associated with ischemic stroke. Neutrophil priming may be one mechanism that participates in this response. Interaction of the *APOE4* carriers with risk factors of chronic inflammatory states, which may contribute to neutrophil priming, may also modulate *APOE4*'s effect on the inflammatory response associated with ischemic stroke. Research to date has not investigated inflammatory markers and genetic influences as well as risk factor relationships among AAs with ischemic stroke. This study examined the relationship among *APOE* genotypes, inflammatory markers, and risk factors among AAs with ischemic stroke. The results of this study may lead to primary and secondary prevention for ischemic stroke in this population. Additionally, it may also facilitate knowledge of genetic influences, inflammatory responses, and risk factor relationships associated with injury mechanisms, resulting in health disparities in cerebrovascular disease.

CHAPTER II: LITERATURE REVIEW

This chapter presents a review of literature on ischemic stroke and a) AAs, b) inflammatory markers, c) risk factors, and d) the *APOE* gene.

African Americans and Stroke

Stroke is the fourth leading cause of death and the most significant contributor of disability in the U.S. (Mazoffarian et al., 2015). The World Health Organization defines stroke as the sudden development of focal or global neurological impairment, where symptoms last 24 hours or more or result in death with an apparent vascular cause (WHO, 2014). New and recurrent stroke affects 795,000 individuals in the U.S. annually with 4.7 million stroke survivors. Indirect and direct expenditures for stroke care were $36.5 billion in 2010 with the average lifetime cost of ischemic stroke at $140,048 (Mazoffarian et al., 2015). Ischemic stroke, defined as the interruption of blood flow to the brain, comprises 87% of these insults (Mazoffarian et al., 2015). The stroke burden particularly affects AAs residing in the stroke belt (an area comprised by North Carolina, South Carolina, Georgia, Tennessee, Alabama, Mississippi, Arkansas, and Louisiana) with a stroke death rate 1.5 times above the national average (Howard, et al., 2006; Howard et al., 2013). Stroke mortality is approximately 50% higher among the ages of 45 and 65 years in AAs than EAs in this region (Howard, et al., 2006; Howard, 2013).

Although AAs have a higher stroke-related mortality, the racial differences vary greatly with age (Howard, 2013; Kissela et al., 2004). AAs who are less than 65 years of age have 2 to 5 times the risk of stroke compared to whites of the same age (Howard, et al, 2011; Kissela et al., 2004). This age related disparity decreases and is completely absent by 85 years of age

(Howard, 2013). Stroke risk in AAs is higher in the 35-54 age brackets. Additionally, stroke is more prevalent in men than in women, excluding those 35-44 years of age and in those greater than 85 years of age (Mazoffarian et al., 2015; Goldstein et al., 2011).

In regards to characteristics of stroke severity and outcomes, data from the Get with the Guidelines-Stroke Registry, which was linked with Medicare claims data of over 1900 hospitals in the U.S. were examined. In comparison to EAs, AAs had lower in-hospital mortality rates, were younger, and had more severe strokes as measured by the mean score on the National Institute of Health Stroke Scale (NIHSS) than their EA counterparts (Qian et al., 2013). Even after adjusting for demographics, medical history, socioeconomic status, NIHSS score, and hospital characteristics, the lower risk for short term mortality persisted among AAs with stroke. However, a significant finding of this study was that AAs were more likely to be readmitted within one year of discharge for any cause, including stroke and myocardial infarction causes (Quian et al., 2013).

The mortality differences by AAs may reflect socioeconomic status, greater severity of disease, and poor survival at younger ages (Ayala et al., 2001; Hanchate, Schwamm, Huang, & Hylek, 2013; Schwamm et al., 2010). Other factors that have also been cited include lack of medical care access, lack of health insurance, and lack of knowledge about early warning signs of stroke. Variations in risk factors such as hypertension, diabetes type 2, obesity, and cigarette smoking are also significant (Howard et al., 2011; Howard, 2013). In fact, Howard et al. (2013) found AAs in the U.S. demonstrated the highest prevalence of hypertension in the world, are less likely to have their blood pressure under control, and have a higher impact of elevated blood

pressure levels. For the same 10 mmHg difference in systolic blood pressure, there was a 24% stroke risk increase for AAs in comparison to 8% for whites.

Moreover, AAs display a cluster of risk factors, especially in women. In the NHANES III 1988-1994 AA adults revealed the highest prevalence of three or more risk factors, resulting in a prevalence of 1 in 5 adults (Watkins, 2004). Differences in conventional stroke risk factors offer a partial explanation for the disparity, but not completely. A particular noteworthy finding is that approximately half of the black-to-white disparity with stroke occurs in the absence of conventional risk factors (Albert & Ridker, 2004; Ferdinand, 2006; Howard et al., 2011; Stephens, Bain, & Humphries, 2008; Taylor et al., 2008). Stroke reoccurrence is also a concern among this population. The AAASPS revealed that stroke recurrence during the first year following stroke was 10.9% (Ruland et al., 2006). Among the participants who suffered stroke reoccurrence, 48% of AAs developed disability or died following the event. Although hypertension in this study was one of the most significant contributors to second stroke, other *non*- traditional indicators were not explored. Similar to primary stroke, there remains a lack of research regarding *non*-traditional factors contributing to secondary stroke in AAs.

Inflammatory Markers and Ischemic Stroke

Neutrophil Priming and Activation

Neutrophils exist in the blood stream in one of three functional states: quiescent (resting state), primed, or activated. When quiescent (in a resting state) neutrophils encounter a sub-maximal stimulus, they are “primed” to produce an augmented or exaggerated response to a second activating stimulus. Priming refers to amplification of an inflammatory response to a second activating stimulus following exposure to a priming agent (Condliffe et al., 1998;

Hurtado-Nedelec, Makni-Maaleij, Gougerot-Pocidalo, Dang, & El-Benna, 2012). Priming agents can include various cytokines such as IL-8, IL-6), and TNFα. (Condliffe et al., 1998; Hurtado-Nedelec et al, 2012). The response of neutrophils to various pro-inflammatory stimuli is primarily determined by their previous exposure to agents. The other major concepts associated with priming includes 1) these agents to do not elicit the effector function on their own and 2) to be effective the agent must be presented to the cell for a variable period before the cell is exposed to the activating stimulus (Condliffe et al., 1998; Hurtado-Nedelec et al, 2012). Neutrophil priming and activation have been hypothesized to play a significant role in neutrophil-endothelial cell interaction and also plays a key role in the neutrophil response at the injury site. Neutrophil priming and activation may also represent the common denominator in various clinical states, such as accelerated atherosclerosis (Baetta & Corsini, 2010), hyperlipidemia (Mazor et al., 2008), chronic kidney disease (Sela et al., 2005); type 2 diabetes (Shurtz-Swirski et al., 2001), hypertension (Kristal et al., 1998), and cigarette smokers (Sela et al., 2002).

Evidence also suggests that neutrophil expression is influenced by multiple mechanisms, including 1) dose of stimuli, 2) phase of neutrophil maturation, 3) age, genetic factors, and physical conditions of the host, and 4) time of measurement (Pillay et al., 2007). Neutrophil adhesion molecule expression profiles, therefore, may provide information regarding clinical risk assessment and may very well serve as a *non*traditional marker of cardiovascular disease and their complications (Jacobi, Sela, Cohen, Chezar, & Kristal, 2006; Baetta & Corsini, 2010; Mazor et al., 2008; Pillay et al., 2007).

Priming modulates the adhesiveness and migratory effects of neutrophils, contributing to neutrophil tissue mediated injury. It should also be noted it was previously thought that neutrophil priming was irreversible. But this premise was challenged and refuted by a study which demonstrated that primed neutrophils by platelet activating factor was fully reversible (Kitchen, Rossi, Condliffe, Haslett, & Chilvers, 1996). The ability of neutrophils to undergo a complete cycle of priming, de-priming, and re-priming presents an opportunity to examine strategies that may control neutrophil behavior and evaluate potential mechanisms for limiting neutrophil mediated tissue damage or "cell rescue" at the site of injury. Leukocyte activation is one mechanism that has not been evaluated in terms of priming by cytokines and ApoE4 carriers among AAs with ischemic stroke.

The neutrophil-endothelium interaction is a high affinity interaction mediated by adhesion molecules, specifically the integrins (Granger & Kubes, 1994). Integrins are a family of heterodimeric proteins consisting of two different subunits, namely an α subunit and ß subunit that are integral to the process of cell adhesion. Of this family, the $ß_2$ have been the most investigated (Wang et al., 2007). The $ß_2$ integrins consist of three distinct subunits (CD11α, CD11b, and CD11c) that are bound to a common ß subunit (CD18). Neutrophils express all three CD11 subunits and the contribution to adherence of each sub-unit may vary, depending on the stimulus.

Upon encountering a maximal first stimulus, or a second stimulus following priming, the neutrophil proceeds to full activation, releasing reactive oxygen species, granule contents, and inflammatory mediators to the surrounding tissues. The primary effects of leukocyte activation include: 1) inducing inflammation of the endothelium causing endothelial cell damage, 2)

increasing adhesiveness, permeability, and expression of procoagulant activity, and 3) secreting various pro-inflammatory mediators and affecting other leukocyte cells, thereby affecting the inflammatory response (Falke, Elneihoum, & Ohlsson, 2000). The three steps of rolling, adhesion, and transendothelial migration of leukocytes initiate tissue injury, often seen in ischemic-reperfusion injury associated with ischemic stroke (Wang et al., 2007).

Neutrophil Activation and Stroke

Neutrophils play a fundamental role in the inflammatory response associated with cerebral ischemia and reperfusion injury. Leukocyte activation leads to neutrophil adherence to the endothelium with resulting leukocyte accumulation and infiltration. If reperfusion is established, the additional neutrophils from the injury site are carried to the site of tissue ischemia (Schofield, Woodruff, Halai, Wu, & Cooper, 2013). After adhesion to the microvasculature, the neutrophils pass through the blood brain barrier. This response leads to further brain tissue injury and release of pro-inflammatory mediators (Barone & Zeuerstein, 1999; Schofield et al., 2013). Secondary cerebral injury of potentially salvageable tissue within the prenumbra surrounding the infarct core may result (Wang et al., 2007). Peripheral leukocytes entering the brain further amplify this inflammatory process, which ultimately leads to further brain damage. These inflammatory responses are particularly pronounced during reperfusion leading to massive release of reactive oxygen species and leukocytes into the brain (Wang et al., 2007; Schofield et al., 2013). At the same time, pro-inflammatory cytokines directly act on endothelial cells, which can result in increased blood brain barrier permeability (Barone & Feuerstein, 1999; Schofield et al., 2013). Studies that support neutrophil infiltration in ischemic injury were demonstrated by experimental animal models involving middle cerebral artery occlusion (MCAo) and reperfusion (MCAo-R)

in rats and mice as well as human studies involving various methodologies. In both animal models and humans, neutrophil accumulation is observed in the “reactive zone”, described as a rim of tissue located around the infarcted core with peak accumulation occurring at 48-72 hours (Yilmaz & Granger, 2008).

Animal Models of Neutrophil Activation and Stroke

Evidence of early accumulation of neutrophils in ischemic brain damage was demonstrated by various animal model studies (Clark et al., 1993; Hallenbeck et al., 1986; Morrison et al., 2011; Ritter et al., 2000; Zhang, Chopp, Chen, & Garcia, 1994). Clark et al. (1993) studied the development and resolution of ischemic lesion by middle carotid artery occlusion in spontaneous hypertensive rats. Initial brain lesion changes corresponded with histologic changes. Neutrophil infiltration into infarcted tissues with increased number corresponded with cerebral infarct. This study was able to define the critical cellular events and characterize the evolution of infarction. Hallenbeck et al. (1986) investigated neutrophil accumulation follow ischemia in the canine model. This study also found that neutrophil accumulation was found to occur in the acute phase of ischemic brain injury. Zhang et al. (1994) investigated the temporal profile of ischemic tissue damage, neutrophil response, and vascular occlusion after permanent and transient middle carotid artery occlusion in the rat. Findings revealed significant differences between the neutrophil infiltration pattern depending on whether the middle carotid artery occlusion was transient or permanent, but the difference in brain size lesion disappeared after 48 hours following the onset of ischemia. Ritter et al. (2000) utilizing the filament method of stroke and fluorescence microscopy found significant increase in leukocyte rolling and adhesion in venules and a reduced blood shear rate in the microcirculation during one hour of reperfusion.

Leukocytes may interact with blood vessels by activating and damaging the vessel and surrounding brain cells, thus, resulting in an exaggerated inflammatory response to reperfusion. In a more recent study, Morrison et al., (2011) characterized systemic leukocyte response and neutrophil CD11β expression at 15 minutes and 24-hours post-reperfusion in a mouse model of ischemic stroke. The investigators found that systemic neutrophil activation began as early as 15 minutes and remained evident 24 hours after the initiation of reperfusion

Neutrophil Activation and Human Studies.

Clinical studies have also demonstrated neutrophils accumulate in human cerebral infarction and that this accumulation correlates with the severity of stroke and poor neurological outcomes after ischemic stroke (Akopov, Simonian, & Grigorian, 1996; Buck et al., 2008; Pozzilli et al., 1985; Price et al., 2004). Pozzilli et al. (1985) investigated circulating white blood cells in patients with cerebral infarct by in vitro labeling with radioisotope. The cells were then re-injected to study the inflammatory process during stroke by gamma camera imaging. Seven patients were studied and findings revealed increased radioactivity in the infarcted hemisphere, indicating active migration and tracking of labeled leukocytes in cerebral infarct. In a similar study (Akopov et al., 1996) the dynamics of polymorphonuclear leukocyte accumulation in patients with cerebral infarction and neurological outcome and brain lesion were investigated. Polymorphonueclear leukocyte accumulation was studied by using in-labeled leukocyte technique. Volume of brain infarct was evaluated by computerized tomography scan. The dynamics of leukocyte accumulation, neurological outcome, and infarct volume was evaluated at 3-6, 6-12, and 12-24 hours and 6-9, 28, and 90 days after stroke onset. Findings revealed progressive increase in polymorphonuclear leukocyte accumulation, which remained elevated up

to 6-9 days. In patients with severe polymorphonuclear leukocyte accumulation, neurological outcome was worse and lesion volume larger than in patients with less polymorphonuclear leukocyte accumulation. These findings suggest that polymorphonuclear leukocyte accumulation correlated with the severity of brain tissue damage and poor neurological outcome. Price et al. (2004) studied patterns and temporal profile of cerebral neutrophil recruitment to area of acute ischemic stroke and correlated this with neurological status and outcome in 15 patients. Findings revealed neutrophils were demonstrated in areas of the ischemic brain within 24 hours of symptom onset. Neutrophil accumulation increases over time. In addition, neutrophil accumulation may be associated with either the magnitude or rate of infarct growth. Buck et al. (2008) studied 173 patients with ischemic stroke and found higher peripheral leukocyte and neutrophil counts were associated with larger infarct volumes measured by magnetic resonance imaging. These results were consistent with previous studies that reported a relationship between lesion size and elevated systemic inflammatory markers, such as white blood cell count, and CRP (Audebert et al., 2004; Christensen & Boysen, 2004).

Cytokines and Stroke

Cytokines are glycoproteins that are involved in many physiological processes including the regulation of immune and inflammatory processes. Cytokines are particularly important in cell signaling and once released, affect the behavior of other cells, and sometimes, the releasing cell itself. Cytokines act by autocrine signaling (a cell secretes a hormone or chemical messenger that binds to the autocrine receptors of the same cell, leading to changes in the cell) or paracrine signaling (a form of cell-cell communication in which a cell produces a signal to induce changes in nearby cells, thus altering the behavior or differentiation of those cells). Cytokines may

include: interleukins, interferons, lymphokines, and growth factors. Some cytokines are chemical switches that turn certain immune cell types on and off. Another group of cytokines chemically attract specific cell types and are called chemokines. Certain cytokines represent a group of inflammatory mediators that are up-regulated during ischemic stroke and contribute to the inflammatory process following stroke. These inflammatory mediators may either exacerbate or alleviate inflammatory damage to ischemic brain tissue. Several studies have reported prominent changes in cytokine expression following stroke (Beamer et al., 1998; Barone & Feuerstein, 1999; Emsley et al., 2003; Lindsberg & Grau, 2003). The most important pro-inflammatory cytokines, TNFα, IL-1B, IL-6, and IL-8 have all been shown to be increased following ischemic stroke (Perera et al., 2006). Moreover, it appears that these pro-inflammatory cytokines drive the inflammatory process and may aggravate inflammation (Perera et al., 2006). The response of target cell to a given cytokine is determined by the specific receptor and the nature of the link between the receptor and the target cell (Zheng & Yenari, 2004). These inflammatory mediators are elevated within hours of stroke and have been found in the cerebrospinal fluid within 24 hours following stroke (Emsley et al., 2003; Zaremba, Skrobanksi, & Losy, 2001). Elevations of these inflammatory mediators have been demonstrated in brain tissue, providing support for the role of inflammation in the stroke process (Zaremba et al., 2001; Emsley et al., 2003; Tarkowski et al., 1995, Intiso et al., 2004; Perini et al., 2001). The following discussion summarizes the role of these pro-inflammatory cytokines and stroke.

Interleukin 1β (IL-1β)

IL-1β is a pro-inflammatory cytokine expressed in the ischemic brain within 30 minutes after ischemic-reperfusion injury (Lakhan, Kirchgessner, & Hofer, 2009). Activated IL-1β can be

found in microglia as the major source as well as other immune cells (Shichita et al., 2012). IL-1β is considered to be a neurotoxic mediator that directly induces neuronal cell death and enhances cytokine expression. This role is based on several studies that consistently found that when IL-1β function is lost, such as with knockout mice, reduced infarct size resulted (Galea & Brough, 2013; Touzani et al., 2002). A meta-analysis of animal model studies also revealed that IL-1β antagonist markedly reduced infarct volume by 38.2% (Banwell, Sena, & Macleod, 2009). Additionally, chronic release of IL-1β is associated with increased expression of adhesion molecules and blood-brain barrier permeability, further promoting leukocyte infiltration (Shaftel et al., 2007).

Interleukin 6 (IL-6)

IL-6 is also a pro-inflammatory cytokine that has been well studied in the setting of ischemic stroke. IL-6 shares some of the properties of IL-1 in response to inflammation. In rats that were subjected to middle cerebral artery occlusion, IL-6 expression was demonstrated as early as three hours with peaked levels at 12 hours, which persisted for 24 hours (Wang, Yeu, Young, Barone, & Feuerstein, 1995). Human studies demonstrate a pro-inflammatory role of IL-6. Evidence also suggests that serum concentration of IL-6 demonstrate the strongest independent predictive value for in-hospital mortality associated with ischemic stroke (Rallidis et al., 2005). Peripheral blood levels are detectable within a few hours of ischemic stroke and are higher in individuals with stroke (Tarkowski et al., 2001; Orion et al., 2008). Cerebrospinal fluid has also demonstrated higher levels of IL-6 and IL-1 after stroke. Higher cerebrospinal fluid and serum levels of IL-6 correlated with larger infarct size and poorer clinical outcome, which was demonstrated by computed tomography and magnetic resonance imaging (Tarkowski et al., 2001; Smith et al.,

2004). Increasing evidence also suggests that IL-6 has anti-inflammatory effects and it remains unclear if IL-6 is a cytokine with proinflammatory, anti-inflammatory, or both functions (Gruol & Nelson, 1997; Sotgui et al., 2006).

Interleukin 8 (IL-8)

IL-8 is one of the best known cytokines and is known as chemokines, which has potent neutrophil chemotactic and activating activity (Baggioloini, Moser, & Clark-Lewis, 1994). They are produced by various cell types, including neutrophils and its release is stimulated by other cytokines such as IL-1 as well as injury such as ischemia or hypoxia. Data from both animal and human studies demonstrate IL-8's ability to function as a neutrophil chemoattractrant. It induces shape change, chemotaxis, and release of granule contents, up regulates adhesion proteins, and produces respiratory burst in the neutrophil (Baggioloini et al., 1994). Data suggest that in animal models of cerebral ischemia and reperfusion, IL-8 is induced on cerebral endothelium and infiltrating leukocytes and anti-IL-8 antibodies can reduce the infarct size, presumably by reducing the accumulation of leukocytes in the ischemic brain (Yamasaki et al., 1995a; Yamaskaki et al., 1997; Matsumoto et al., 1997a; Matsumoto et al., 1997b). Domac and Misirli (2008) investigated the role of neutrophils and IL-8 in ischemic stroke infarct evolution and course of the disease. They studied 76 patients and 28 control subjects and obtained serum levels within 24 hours of the stroke. In comparison between patients and control groups, there was a statistically significant difference between IL-8 and neutrophil levels. IL-8 levels were associated with the extent of the lesion, but no difference was found with prognosis. There was also no difference found according to age, gender, and etiology

Interleukin 10 (IL-10)

IL-10 is an anti-inflammatory cytokine, which is known to suppress cytokine expression and activation (Hans & Yenari, 2003). IL-10 is produced in the central nervous system as well as by several inflammatory cells such as macrophages and monocytes. IL-10 has been shown to be upregulated in experimental stroke (Zhai, Futrell, & Chen, 1997). In previous studies, subjects with IL-10 levels have an increased risk for stroke (Van Excel et al., 2002). There are also studies that demonstrate anti-inflammatory and neuroprotective role of IL-10 in acute ischemic stroke (Baird, 2006; Protti, Gaqliardi, Forte, & Sprovieri, 2013). In addition, animal and human studies demonstrated low IL-10 were decreased following stroke (Nayak et al., 1998; Protti et al., 2013; Spera, Ellison, Feurerstein, & Barone, 1998). Nayak et al. (2009) examined IL-2 and IL-10 and prognostic usefulness in 17 stroke patients within 24 hours of the onset of symptoms and then at 24, 48, 72, and 144 hours after admission. IL-10 levels were decreased in stroke patients who improved at 24 hours followed by significant increases at 72 hours and 144 hours. IL-10 was found to also correlate with stroke outcome.

Tissue Necrosis Factor Alpha (TNF*α*)

TNFα is probably the most extensively studied cytokine in experimental stroke (Lambertsen, Biber, & Finsen, 2012). TNFα is produced upon stimulation by monocytes, macrophages, T and B lymphocytes, neutrophils and mast cells. It is an immune mediator that regulates the inflammatory response, regulates growth and cellular differentiation, and activates blood coagulation (Lambertsen et al., 2012; Um, Jeong, Park, Hong, & Kim, 2005). Data which examined the temporal profile of TNFα demonstrated the up-regulation of TNFα closely parallels IL-1 and IL-6 within the first few hours of ischemic stroke (Lambertsen et al., 2012;

Yamaski et al., 1995a). Evidence also demonstrated cerebrospinal fluid levels of TNFα were markedly elevated within 24 hours of ischemic stroke and that serum and cerebrospinal fluid TNFα levels positively correlated with the volume of brain infarct (Lambertsen et al., 2012; Zaremba, Skrobanki, & Losy, 2001). TNFα induces adhesion molecule expression within the cerebral microvasculature, thus facilitating neutrophil accumulation and migration in the microvasculature (Lambertsen et al., 2012; Feuerstein, Liu, & Barone, 1994). There is also evidence to suggest that TNFα may participate in transformation hemorrhage or the disruption of the blood brain barrier (Huang et al., 2006).

Fibrinogen

Fibrinogen is a soluble plasma glycoprotein that is synthesized in the liver by hepatocytes and megokarocytes. Fibrinogen serves as the principle protein for blood clotting (Andrews & Berdnt, 2004). Elevated fibrinogen levels are strongly associated with ischemic stroke as well as recurrent stroke (Rothwell et al., 2004). A meta-analysis of six observational studies demonstrated that fibrinogen levels were a powerful independent predictor of cardiovascular disease and stroke (Ernst & Resch, 1993). Increased fibrinogen levels are also associated with poor functional outcomes at 90 days following stroke (Del Zoppo et al., 2009; Beamer et al., 1998). Fibrinogen levels peak at three days post stroke. Fibrinogen has been demonstrated to participate in inflammatory processes by modifying leukocyte function. A study that compared gene targeted mice producing normal fibrinogen with mice producing mutant fibrinogen found that mutant fibrinogen severely compromised inflammatory response in vivo (Flick et al., 2004). This finding demonstrated that fibrinogen is physiologically relevant for CD11/CD18

interaction, ultimately affecting leukocyte function and having relevance in regulating inflammatory processes.

Platelet Leukocyte Aggregates (PLA)

Fibrinogen serves as the bridge between neutrophils and platelets. PLAs form through this interaction and can increase the immune and hemostatic function of both cell types and worsen reperfusion injury associated with ischemic stroke (Ritter et al., 2005). Studies demostrate that PLA is increased in individuals with ischemic stroke (Cao et al., 2009; Schmalbach et al., 2015). Schmalback et al.(2015) investigated whether PLA correlated with clinical features (clincal and radiological parameters, laboratory parameters, and genetic parameters) of ischemic stroke and the influence of genetic factors on PLA. The investigators recruited 79 patients with acute ischemic stroke and 151 control subjects without vascular disease from a German center. Findings revealed that PLA correlated weakly with stroke severity, but not with thrombus length or stroke etiology. PLA also correlated with stroke, age, and gender. PLA was independent of other vascular risk factors. Thus, PLA can serve as a sensitive biomarker to platelet activation for ischemic stroke (Cao et al., 2009).

C-Reactive Protein (CRP)

C - reactive protein (CRP) is a protein produced by the liver and fat cells (adipocytes). It is a member of the pentraxin family of proteins and is an acute phase protein due to its expression during acute inflammatory processes. CRP was first discovered in the 1930s by William Tillett and Thomas Francis (Ridker, 2009). Over the next several decades, numerous clinical trials have validated high sensitivity methods for CRP measurements (hs-CRP), which led to its reproducibility and adoption as a global risk biomarker for cardiovascular disease, resulting in

endorsement in early 2003 by the American Heart Association and Center for Disease Control (Ridker, 2009). Hs-CRP has been confirmed as an independent predictor of cardiovascular events in over 30 diverse cohorts (Ridker, 2009). Moreover, analysis from the NHANES data (Ford & Giles, 2000), the Framingham cohort (Rost et al., 2001), and the Women's Health Study (Everett, Kurth, Buring, & Ridker, 2006) have confirmed hs-CRP as an independent predictor for stroke. Several studies support elevated CRP levels in patients with ischemic stroke (Beamer et al., 1998; Muir et al., 1999; Di Napoli et al., 2001b). Variations in CRP levels have been noted with two distinct patterns. Each of these patterns have varying prognostic implications and include: 1) a benign pattern characterized by either a persistently normal or decreasing values through the stroke hospitalization and 2) an adverse pattern characterized by persistently elevated or increasing values during the stroke hospitalization (Di Napoli et al., 2001b). CRP levels generally peak at three days post stroke. Persistently elevated CRP levels may represent chronic inflammatory processes or cerebral ischemia extension (Muir et al, 1999; Shoamanesh et al., 2015). Evidence from animal models and human studies also indicate that inflammatory mechanisms contribute to further injury after cerebral ischemic, specifically secondary neuronal injury (Bova, Bornstein, & Korczyn, 1996; Grau et al., 1995; Grau, 1997; Zheng, Huang, & Yu, 2014). Thus, elevated CRP levels are linked to not only the cerebral ischemia processes, but also a persistent inflammatory response found among stroke survivors.

Studies Examining Inflammatory Markers after Stroke

Although fewer human than animal studies have been conducted, there is evidence to support leukocyte activation persists following stroke. Elneihoum et al. (1996) investigated whether plasma markers of systemic activation were elevated in acute ischemic stroke. Plasma levels of

neutrophil granules such as neutrophil gelatinase-associated lipocalin, neutrophil proteinase 4 TNF, and soluable TNF receptor protein-1 p55 was measured in 120 Europeans with acute ischemic stroke on day 1-3 following stroke, 48 patients with transient ischemic attack, and in 35 age and sex matched controls. Findings demonstrated that systemic markers for neutrophil activation were higher in patients with acute ischemic stroke than in healthy controls. These findings were consistent with a study performed by Beamer et al. (1998) who examined the time course of acute phase markers elevation after ischemic stroke and how these markers related to stroke risk factors, stroke mechanism, and subsequent vascular events. Measurements of fibrinogen, CRP, white blood cell count, polymorphonuclear leukocytes, and IL-6 and IL-1 receptor antagonist were measured at stroke onset and at 6 weeks, 6 months, and 1 year after enrollment or until a vascular event occurred in 136 acute ischemic stroke patients, 76 with comparable risk factors for stroke, and 48 age matched controls. Acute phase markers decline gradually after stroke, but fibrinogen remained elevated and was associated with increased risk for recurrent vascular events. Another noteworthy finding included that white blood cell count and polymorphonuclear leukocytes levels were chronically elevated in patients with stroke risk factors and in stroke survivors, suggesting persistent leukocyte activation. These findings were similar to a more recent study which investigated the association of inflammatory cytokines and risk for recurrent stroke in 591 stroke subjects. Findings demonstrated IL-6, TNFα, CRP, and fibrinogen levels were associated with risk for recurrent stroke and were independent of traditional stroke risk markers (Welsh et al., 2008). In a study specifically examining the neutrophil, Caimi et al. (2001) investigated the neutrophil profile (CD11a, CD11b, CD11c, CD18) in 19 patients with acute ischemic stroke using indirect immunofluroscence and flow

cytometry at baseline, during in vitro activation, and prolonged at 5 minutes and 15 minutes with chemotactic agents. These results were compared to a control group of 24 healthy subjects who were free of major risk factors. Results revealed that the stroke group at baseline evidenced an increase in CD11c and CD18 and a decrease in CD11b. However, after activation, the controls had an increase in all CD markers, whereas in the stroke group only CD11b and CD18 were activated. Falke, Elneihoum, and Ohlsson (2000) investigated the association of systemic leukocyte activation and cardiovascular mortality after stroke. Plasma markers of neutrophil gelatinase-associated lipocalin, neutrophil proteinase 4 TNF, and soluable TNF receptor protein-1 p55 were measured in 144 European patients (90 with stroke and 54 with transient ischemic attack 1-3 days after cerebral ischemia. Follow-up over a 4 year period found that 29% of the original sample died of cardiovascular causes. Patients who evidenced higher leukocyte activation had a higher four year cardiovascular mortality rate than those without evidence of leukocyte activation. Together, these findings support leukocyte activation as an important marker of inflammation, and markers of activation that could help identify high risk groups for adverse events following stroke.

Inflammatory Markers in African Americans

Evidence supporting inflammatory processes associated with ischemic stroke in AAs is scarce. There are a few studies that have investigated leukocyte activation in the setting of sickle cell disease (Lard, Mul, de Haas, Roos, & Duits, 1999). Additionally, studies that have examined cytokine levels in AAs have been in the area of obstetrics and associated outcomes (Velez et al. 2008; M*enon* 2008). Of the inflammatory markers, CRP has been the most extensively studied and has been found to correlate with BMI (Albert & Ridker, 2004). AAs have lower white blood

cell counts in comparison to EAs. Higher fibrinogen level and greater fibrolytic activity has also been found in AAs (Albert & Ridker, 2004).

The Atherosclerosis Risk in Communities (ARIC) Study enrolled 729 participants of which 19% were AAs. The study examined the relationship between vascular adhesion molecules, such as vascular cell adhesion molecule, E selectin, and intracellular adhesion molecule, ICAM-1, and carotid arteriosclerosis, and coronary heart disease incidence. Significantly higher levels of E selectin were observed in AAs participants with incident coronary heart disease and carotid arteriosclerosis, whereas ICAM-1 levels were elevated in all participants (Shih-Jen et al., 1997). One study examined biomarkers of inflammation: CRP and interleukin genes, and plasma CRP and IL-6 levels and presence of MRI defined white matter lesions and brain infarcts (Fornage et al, 2008). Fourteen percent of the sample was AAs. Findings demonstrated plasma IL-6 levels and CRP were associated with the presence of white matter lesions and brain infarcts in both races. In summary, studies investigating inflammatory processes among AAs with ischemic stroke are scarce. Studies examining genetic polymorphism and their influence on inflammatory responses with ischemic stroke in AAs are *non*existent.

The Apolipoprotein E Gene

Description of the Apolipoprotein Gene

The *APOE* gene is a member of the apolipoprotein gene family and is located at chromosome 19q13.2 (Eichner, 2002). The *APOE* gene has three common alleles (ε2, ε3, ε4), which produce three isoforms of the protein: ApoE2, ApoE3, and ApoE4. These isoforms differ in amino acid sequence at positions 112 and 158. There are three homozygous (ε4/4, ε3/3, and ε2/2) and three heterozygous (ε2/4, ε3/4, and ε2/3) genotypes. ApoE2 has two cysteines in the position of 112

and 158. ApoE3, which is the most common polymorphism, has cysteine in the position of 112 and arginine at 158. ApoE4 has two arginines in these positions. This amino substitution results in not only structural differences, but also physiologic differences such as their binding affinity for specific lipoprotein receptors, antioxidant properties, and inflammatory responses (Jofre-Moseny, Minihane, & Rimbach, 2008). Additionally, each of the *APOE* alleles is associated with differing risks and outcomes for disease. The heterozygous *APOE* genotypes result in co-dominant inheritance. ApoE2 is associated with higher concentrations of plasma lipoproteins and ApoE4 with lower concentrations. ApoE2 is defective in binding lipoproteins and consequently, is associated with hyperlipoproteinemia. ApoE3 is the most common form and is considered "protective" against atherosclerosis. ApoE4 is the least favorable with studies demonstrating increased risk, greater severity, and worse outcomes in various nervous system disorders such as Alzheimer disease (Farrer et al., 1997; Murrell et al., 2006) and acute brain injuries such as intracerebral hemorrhage (Alberts et al, 1995), traumatic brain injury (Zhou et al., 2008), and ischemic stroke (Baranska et al., 2003; Gromadzka, Baranska-Gieruszczak, Ciesielska, Sarzynaka-Dlugosz, & Czlongkowska 2005; Gromadzka, Baranska-Gieruszczak, Ciesielska, Sarzynaka-Dlugosz, & Czlongkowska, 2007).

Associations of Inflammatory Markers and APOE Genotype

Although the immune modulating properties of the *APOE* gene were first described almost two decades ago (Joffre-Monsey, 2008), there is limited data available on leukocyte activation and *APOE* genotypes. The majority of studies has focused on immune modulating properties of the *APOE* gene and has included in vivo as well as in vitro models. Nevertheless, this growing body of literature supports the immune modulating role of specific *APOE* genotypes. The data

further support *APOE4* carriers as associated with an enhanced inflammatory response or a pro-inflammatory phenotype. *APOE4* carriers exhibit distinct differences from the other *APOE* genotypes, which include: microglial morphology, macrophage response, cytokine expression, and systemic inflammation.

Inflammatory markers and APOE genotypes in animal models. The microglia plays an active role in the brain's innate immune response and studies demonstrate *APOE* genotype specific differences in microglial production of cytoactive factors (Vitek, Brown, & Colton, 2007). Data support that microglia derived from *APOE4* mice demonstrates a pro-inflammatory phenotype that included altered cell morphology and increased cytoactive factor production (Vitek et al., 2007). These genotype specific effects are global in nature and extend to peripheral macrophage function. In a study that examined the microglia from *APOE4* mice, findings demonstrated amorphous, amoeboid morphology characteristic of both in vivo and in vitro "activated microglia" and increased nitric oxide production and higher cytokine production (Vitek et al., 2007). Recent evidence further suggests that these immune modulating effects may have particular relevance to cerebral edema and injury. Lynch et al. (2003) examined the role of the *APOE* gene in modifying systemic and brain inflammatory responses in *APOE* deficient mice and *APOE* mice. *APOE4* mice had significantly greater systemic and brain elevations of pro-inflammatory cytokines (TNFa and IL-6) as compared to *APOE3* mice. Moreover, following administration of *APOE* mimetic peptide, both systemic and cerebral inflammation was effectively suppressed. The precise mechanisms by which *APOE* genotypes alter immune response are not known. But peptides derived from the *APOE* receptor binding region were developed that mimicked the anti-inflammatory and neuroprotective function of the *APOE*

protein (Laskowitz, Fillit, Yeung, Toku, & Vitek, 2006). In animal models these peptides improved the functional and histological outcomes in animal models of brain injury.

APOE can alter macrophage response. The impact of *APOE* genotypes on overall immune response is evident by several studies. Rosellar and Daugherty (1998) investigated whether *APOE* deficiency altered immune responses following the administration of Listeria monocytogense, which provokes a well-defined inflammatory response. *APOE* deficient mice were compared to mice with the *APOE* gene. Findings revealed that serum levels of TNFa were significantly increased in *APOE* deficient mice compared to *APOE* mice. Another notable finding was that *APOE* deficient mice failed to suppress proliferation of Listeria monocytogense in the early stages of infection, which resulted in premature death. These findings demonstrate that *APOE* genotypes can alter immune responses, particularly lymphocytes and macrophages, affecting the overall immune status and thereby, altering disease outcome.

Another study examined the role of T lymphocyte activation by comparing responses between *APOE* deficient mice and mice with the *APOE* gene (Tenger & Zhou, 2003). Macrophages of *APOE* deficient mice stimulated T cell activation more effectively than the *APOE* mice. Because T cell activation is dependent upon interactions between cell surface molecules and proteins, the expression after in vivo stimulation with interferon-ỳ was assessed. Expression of cell surface molecules and CD40 and CD80 was increased on macrophages in *APOE* deficient mice compared to *APOE* mice. This data suggests that the *APOE* gene controls T-cell activation by down-regulating the expression of cell surface molecules on the antigen presenting cell.

Inflammatory markers and APOE genotypes in cell culture models. *APOE* can increase cytokine expression in an isoform specific manner. Jofre-Monseny et al. (2007) using a murine

macrophage cell line, which had been transfected with *APOE3* or *APOE4* gene, examined the association of *APOE* genotypes and inflammatory responses. Pro-inflammatory measurements included TNFα, IL1ß, andIL-6, and macrophage inflammatory protein-1alpha. Anti-inflammatory measurements included IL-10. Additionally, heme oxygenase-1, a stress induced anti-inflammatory protein was also measured to determine mRNA levels of these inflammatory mediators. Following lipopolysaccharide stimulation, *APOE4* macrophages revealed higher concentrations of TNF-ą, IL-1ß, and macrophage inflammatory protein-1alpha with lower concentrations of IL-10 at both the mRNA and protein levels. No differences were found for IL-6, which is known to act as both a pro and anti-inflammatory mediator. Increased expression of heme oxygenase-1 was also observed in the *APOE4* cells. These findings were similar to another study performed by Tsoi, Wong, Liu, and Ho (2007), which used a similar cell culture model and showed *APOE4* and *APOE2* macrophages produced higher amounts of TNFα. These studies suggest pro-inflammatory cytokine expression is *APOE* isoform specific with *APOE4* demonstrating greater systemic increases in TNFα and IL-6. Additionally, the *APOE4* genotype evidenced an altered inflammatory response as well as an inflammatory imbalance between pro and anti-inflammatory mediators, affecting the overall immune response (Michelsen, Doherty, Shah, & Arditi, 2004).

Inflammatory markers and APOE genotypes in human studies. There is limited data available in humans, which investigates *APOE* genotype and cytokine expression, but findings include studies from cardiovascular surgery and cardiology. In a study to determine unconventional risk factors for preoperative assessment, Drabe et al. (2001) investigated the *APOE4* carriers on cytokine release after cardiopulmonary bypass. Twenty-two cardiopulmonary

bypass patients were enrolled at 48 hours after surgery and 27% (6) were *APOE4* carriers. Findings revealed that *APOE4* carriers were associated with increased levels of TNFα and IL-8, which is a potent activator of neutrophils as well as T-lymphocytes during myocardial ischemia. These findings were consistent with a similar study conducted by Grunenfelder et al. (2004). The purpose of this study was to examine whether *APOE* genotypes affect cytokine release after cardiopulmonary bypass. Thirty eight cardiopulmonary bypass patients were enrolled after 48 hours of surgery. Findings revealed 24% of *APOE4* carriers evidenced higher systemic inflammatory levels of TNFα and IL-8. These findings support that *APOE4* carriers demonstrate an inflammatory imbalance, particularly with cytokine expression, resulting in an altered inflammatory response.

There are several studies examining *APOE* and CRP. Because increased levels of acute phase reactants, such as CRP predict future cardiovascular events, while IL-10 levels have atheroprotective actions, Tziakas et al. (2006) investigated the *APOE* genotype and levels of CRP and IL-10 in 166 acute coronary syndrome and 70 chronic stable angina patients from Greece. Findings revealed that acute coronary syndrome and chronic stable angina patients that were *APOE4* carriers had lower levels of IL-10 and CRP. These levels were also reduced in comparison to other *APOE* genotypes. This was the first study to investigate the association of *APOE* genotypes and anti-inflammatory mediators. But this study did have limitations. Although there were no differences in inflammatory mediator levels between coronary artery patients with stable disease versus unstable disease, acute coronary syndrome patients demonstrated higher levels of IL-10 and lower CRP levels. This finding may have represented the clinical stability of their disease. Differences may also not have been detected due to the small sample size.

Another study investigated the relationship of CRP and *APOE* genotype in a European population (Marz et al., 2004). Seven hundred thirty nine patients with stable angiographic coronary artery disease (defined as stable clinical condition with > 20% coronary artery stenosis) and 539 controls (< 20% coronary artery stenosis) were enrolled and CRP, fibrinogen, and white blood cell count drawn. Findings revealed that patients with stable coronary artery disease were significantly older than controls. Additionally, conventional risk factors were more prevalent in the coronary artery disease participants. CRP levels were higher in subjects with the *APOE2* and *APOE3* carriers than in *APOE4* carriers. Fibrinogen and white blood cell count was not related to *APOE* genotypes. The difference in CRP due to *APOE* genotype was approximately half the difference between smokers and nonsmokers and equal in diabetics or the presence of CAD by angiography. The major noteworthy finding of this study was that *APOE* is significantly associated with CRP levels in both patients with and without coronary artery disease and was independent of conventional risk factors. Moreover, the major strength of this study was angiography-based controls, which ruled out individuals with significant, yet clinically unapparent coronary artery disease. The second strength lies with the frequency of the major conventional risk factors in the control group were similar to the general population. Limitations included the possibility of selection bias as only individuals without coronary artery disease or with clinically stable coronary artery disease without lipid lower drugs were included. Thus, a lower prevalence of subjects with the *APOE4* genotype may have been included.

The *APOE* Genotype and Stroke

The *APOE* gene has been shown to have a protective function in cerebral ischemia (Horsburgh et al., 1999; Sheng, Laskowitz, Mackensen, Kudo, Pearlstein, & Warner, 1999;

McColl et al., 2007), specifically in regulating calcium homeostasis, promoting neuron survival, protecting neurons from oxidative stress, inhibiting microglial activation, and down-regulating the brain and systemic inflammatory response (Gromadzka et al., 2007; Laskowitz, Horsburgh, & Roses, 1998). Laskowitz, Horsburgh, and Roses (1998) investigated *APOE3 a*nd *APOE4* mice following 60 minutes of middle cerebral artery occlusion and 24 hours recovery. Infarct volumes and neurological injury was greater in *APOE4* mice as compared to *APOE3* mice, suggesting an *APOE* isoform specific response. *APOE* deficient animal models had larger infarct volume following middle cerebral artery occlusion (Laskowitz et al., 1997), increased neurological impairment, and higher mortality rates (Sheng et al., 1999; McColl et al., 2007). Animal studies that have examined *APOE* genotypes and cerebral ischemia found that transgenic mice expressing *APOE4* have larger infarct volumes than transgenic mice with *APOE3* (Sheng et al., 1998; Mori, Kobayashi, Town, Fujita, & Asano, 2003). Several human studies investigating associations of *APOE* genotypes and stroke outcomes reveal similar findings. Liu et al. (2006) investigated whether *APOE* genotypes influences magnetic resonance imaging in acute stroke. Eight *APOE4* carriers and 15 controls with acute ischemic stroke in the anterior circulation underwent diffusion and perfusion-weighted and magnetic resonance angiography within 24 hours of stroke and then one week later. Findings revealed that in the ischemic core and area of infarct and cerebral blood flow values were significantly higher in *APOE4* carriers compared to controls. Collateral blood flow was also better in *APOE4* carriers. But despite these observations, *APOE4* carriers had progression of their cerebral insult to infarction with greater lesion growth in comparison to other genotypes. These findings indicate that among *APOE4* carriers, the brain is more vulnerable and develops permanent damage at milder levels of hypo-perfusion than carriers

of other *APOE* genotypes. Consequently, the threshold of *APOE4* carriers to survive stroke may be different from non-*APOE4* carriers.

Another study found that *APOE4* carriers in European men and *APOE2* carriers in European women was associated with an increased 30-day stroke mortality (Baranska, et al., 2003; Gromadzka et al., 2005) and appeared independent of cholesterol, so mechanisms other than arteriosclerosis may contribute to deleterious outcomes of stroke (Gromadzka et al.,, 2005). In one study of 657 European ischemic stroke patients, *APOE4* carriers were a significant predictor of death within one year following stroke in male European patients (Gromadzka et al., 2007). Additionally, association studies examining the *APOE* gene and stroke outcome among various ethnic populations are scarce and among AAs are *non*existent. Nevertheless, the association of the *APOE* gene and stroke mortality implies that certain *APOE* genotypes may contribute to stroke pathology and recovery through various mechanisms, which may include an antioxidant and an inflammatory modulating effect. These mechanisms have led to the description of the *APOE* gene as an "injury factor" for cerebrovascular disease (McCarron et al., 2000).

APOE genotype associations with other inflammatory biomarkers have been limited, but, to date, there have been a few studies to examine the association of *APOE* genotype and inflammatory marker levels in ischemic stroke patients. Park, Kim, Kang, Suh, and Lee (2007) investigated this association in 275 Korean stroke patients with large artery atherosclerosis, 106 with small artery occlusion, and 119 age matched controls from the neurology department. Three inflammatory markers: matrix metalloproteinase, tissue inhibitor of metalloproteinase-1, and hs-CRP were measured. Findings revealed no significant differences in inflammatory markers levels among the *APOE* genotypes, but all markers showed elevated levels in the *APOE2* and *APOE4*

carriers compared to the *APOE3* carriers. In the stroke group, matrix metalloproteinase showed significantly increased levels in *APOE2* carriers compared to other *APOE* carriers. The findings of this study differed from the previously discussed studies that found lower CRP levels in *APOE4* carriers. Study weaknesses included the limited discussion of methodology, lack of sex-matched comparison groups, and the inclusion of different stroke subtypes used for comparison. The primary conclusion of this study is that certain *APOE* carriers may be more vulnerable to the inflammatory processes associated with ischemic stroke.

APOE Genotypes in African Americans

Several studies have demonstrated that the *APOE* gene frequency differs among ethnic groups (Howard, Gidding, & Liu, 1998). Certain populations report a higher *APOE4* frequency, including AAs, Finnish, and Swedish populations, while several Asian populations have a lower *APOE4* genotype frequency. The Coronary Artery Risk Development in Young Adults (CARDIA) Study was a longitudinal study initiated in 1983, which investigated the development of cardiovascular risk factors in a biracial sample of young urban adults from four geographic areas (Birmingham, Alabama; Chicago, Illinois; Minneapolis, Minnesota; and Oakland, California). CARDIA was the first study to examine *APOE* gene frequency and compare the effect of the *APOE* genotype on lipoprotein and apolipoprotein concentrations. The sample included 1612 AAs. Findings revealed that AA men and women had significantly higher frequencies of the *APOE2* and *APOE4*. No significance difference was found between *APOE4* carriers and lipoproteins among AAs and EAs. A similar study examined the *APOE* gene and its influence on lipoprotein concentrations in 8-17 year old AAs and EAs from Bogalusa, Louisiana

(Srinivasan, Ehnholm, Wattigney, & Berenson, 1993). Findings were similar to the CARDIA study in terms of *APOE* frequency and also lipoprotein concentrations.

APOE Genotypes and Interactions

There may be additive effects with *APOE4*. In other words, the study of single polymorphisms may have a weak effect on particular phenotypes when studied individually, but their influence may be more pronounced when studied with synergistic co-effects. This is a particular consideration in genotype association studies and multifactorial, complex disease such as ischemic stroke (Talmud, 2004). Interactions with *APOE* and age, gender, risk factors, and their effect on inflammatory markers have remained largely unexplored, particularly among AAs with ischemic stroke.

Non-modifiable risk factors and APOE interactions. Age, gender, and *APOE* genotype interactions have been investigated primarily with Alzheimer's disease, cholesterol levels, and cardiovascular disease risk (Jarvik, 1997). Although most studies present the *APOE* genotype independent of age, *APOE* genotype and allele frequencies have been found to vary with age (Zerba, Ferrell, & Sing, 1996; Jarvik, 1997). Zerba and colleagues (1996) investigated age dependence, plasma *APOE* levels, and *APOE* genotypes. Their findings revealed that the most significant role of the *APOE* genotype is in younger and middle aged adults. This finding was consistent with other studies that found *APOE4* allele was associated with earlier age in myocardial infarction and with the presence of coronary angiographic evidence of coronary artery disease (Cummings & Robertson, 1984; Lenzen, Assman, Buchwalky, & Schulte, 1986; Kuusi et al., 1989). In several aging populations, the frequency of *APOE4* alleles decreases with age (Divig*non*, Bouthillier, Nestruck, & Sing, 1987; Jarvick, et al., 1995; Cauley, Eichner,

Kamboh, Ferrell, & Kulier, 1993). The decline in *APOE4* with age may be due to differential survival based on differences among individuals as *APOE4* carriers (Jarvik, 1997). Additionally, interactions between age and *APOE* genotype have been demonstrated. A study that examined the effects of the *APOE* genotype on plasma apoE concentration was found to change with age in both men and women with decreasing variance due to the *APOE* genotype in older ages (Zerba, Ferrel, & Sing, 1996). Age is certainly an important consideration with the *APOE* genotype and has not been studied in relation to inflammatory response.

Gender has also been reported in the association with *APOE* genotype in various pathological conditions, such as cognitive decline (Fleisher et al., 2005), Alzheimer's disease (Dal Forno et al., 2002), Parkinson's disease (Buchanan, Silburn, Prince, & Mellick, 2007), colorectal cancer (Watson et al., 2003), and coronary artery disease (Frikke-Schmidt, Tybjaerb-Hansen, Steensen, Jensen, & Nordestgaard, 2000). In most of these diseases, a significant effect was found in *APOE4* female carriers. However, the association between *APOE4* and coronary events was men-specific. This observation was supported by a study that examined gender and *APOE* genotype in European subjects with ischemic stroke. Findings revealed *APOE4* male carriers had worse neurological outcomes than females and were a significant independent positive predictor of death at 1 month, 3 months, and 1 year following stroke (Gromadzka et al. (2007). Plausible explanations for this observation include direct or indirect modulation of *APOE* and gender hormone effects. But most of the subjects included in this study were post- menopausal and it is known that estrogen is produced by other sites other than gonads. Another plausible explanation includes gender specificity noted in *APOE4* carriers and immune activation. In animal studies, increased immune responsiveness was noticed in adult *APOE4* mice, but not in females, even

after ovariectomization. Researchers hypothesized that increased immune system reactivity in *APOE4* ovariectomized female mice was prevented by lifelong exposure to estrogen (Colton, Brown, & Vitek, 2005). This explanation conflicts with stroke data that demonstrated the same mortality rates between AA women and men at one year following stroke between the ages of 40-69 (AHA, 2009). Over all age groups, AA women have worse mortality and morbidity outcomes following stroke than their male counterparts. Consequently, gender related specificity with *APOE4* female carriers following stroke may be much more complicated than the explanations offered.

Modifiable risk factors and *APOE* interactions. Studies examining modifiable risk factors and *APOE* genotype interactions have also been few and primarily focused on disease risk. One study investigated risk factor-gene interaction and carotid atherosclerosis (measured by carotid intima-media thickness via ultrasound) in 205 healthy Japanese. Several gene polymorphisms were determined for each subject and included angiotensin-converting enzymes (*ACE*), angiotensinogen (*AGT*), angiotensin II type 1 receptor (*ATIR*), and *APOE*. Findings revealed that the association with risk factors and carotid intima-media thickness were genotype specific. Interactions revealed that the age**ACE* genotype interactions, the systolic blood pressure**AGT* genotype interactions, and BMI**APOE* genotype interactions were significantly associated with carotid intima-media thickness (Tabara, Kohara, Nakura, & Miki, 2001). Another study examined the modifying effects of various genetic polymorphisms and modifiable risk factors for ischemic stroke risk. Several unfavorable effects were found when interaction with *APOE4* carriers and the combination of hypertension, diabetes mellitus type 2, smoking, and alcohol consumption was examined on the susceptibility of ischemic stroke in a European population

(Szolnoki et al., 2003). These findings suggest that genetic factors when present alone may be insignificant, but when co-variance exists, an additive effect may result. Their co-variance can therefore result in a highly significant risk of ischemic stroke with particular genotypes and clinical risk factors.

The modifiable risk factors that have been selected for this study are based on literature that supports these conditions as a chronic inflammatory state, thus supporting leukocyte activation or primed neutrophil states. Additionally, these modifiable risk factors represent clinical states that are known to accelerate the atherosclerosis process and contribute to increased mortality or morbidity associated with cardiovascular and cerebrovascular disease (Mazor et al. 2008). Kristal et al. (2001) investigated the neutrophil state of hypertension, diabetes type 2, and cigarette smoking and found that polymorphonuclear leukocytes were primed, exposing individuals to increased oxidative stress and chronic inflammation. Kim and associates (2007) investigated the pro-inflammatory state in obese rats and found evidence of spontaneous leukocyte activation and increased integrin expression before the development of diabetes or hypertension. This finding supports a link between oxidative stress, inflammation, and obesity. Findings from these studies suggest that leukocyte activation and primed neutrophils may be the common denominator in these risk factors, which ultimately contribute to endothelial damage, vascular injury, and inflammatory response. Interactions with *APOE4* carriers, non-modifiable and modifiable risk factors, and inflammatory markers have not been investigated with ischemic stroke. Moreover, studies investigating interactions with *APOE4* carriers, inflammatory markers, and *non*-modifiable and modifiable risk factors in AAs with ischemic stroke are *non*existent.

Summary

In summary, the literature review provides evidence of inflammatory processes associated with stroke. Unique inflammatory marker levels among AAs with stroke may exist and have relevance for primary and secondary prevention of stroke. Neutrophil priming and activation has been hypothesized to have a significant role in the neutrophil response to injury. Evidence also supports *APOE4* carriers may be associated with a pro-inflammatory phenotype with specific differences in microglial morphology, macrophage response, cytokine expression, and systemic inflammation. Interaction of the *APOE4* genotype with *non*-modifiable risk factors (age and gender) and risk factors of chronic inflammatory states that reflect leukocyte activation and primed neutrophil states, such as hypertension, diabetes type 2, hyperlipidemia, obesity, and smoking may modulate the *APOE4'*s effect on the inflammatory response associated with ischemic stroke. Research to date has not investigated the relationships among *APOE* genotypes, inflammatory markers, and risk factors in AAs with ischemic stroke.

CHAPTER III: METHODOLOGY

The overall purpose of this research was to determine the relationships among *APOE* genotypes, inflammatory and anti-inflammatory markers, and risk factors in AAs with ischemic stroke. Inflammatory markers studied were CD11β, PLA, TNFα, IL-1β, IL-6, IL-8, hs-CRP, and fibrinogen. The anti-inflammatory marker, IL-10 was also studied. Risk factors included hypertension, type 2 diabetes, hyperlipidemia, obesity, and smoking. The specific aims and related hypotheses were:

Specific Aim 1: Determine the *APOE* carriers among AAs after ischemic stroke.

Hypothesis 1. Consistent with the frequency of *APOE4* carriers among the AA population, the frequency of non-*APOE4* carriers will be greater than *APOE4* carriers among AAs with ischemic stroke.

Specific Aim 2: Determine the levels of inflammatory markers and the anti-inflammatory marker between *APOE4* carriers and non-*APOE4* carriers among AAs at 3 days after ischemic stroke.

Hypothesis 2. Inflammatory markers will be increased and the anti-inflammatory marker will be decreased in *APOE4* carriers than with non-*APOE4* carriers among AAs at 3 days after ischemic stroke.

Specific Aim 3*:* Examine *APOE4* carriers as a predictor for a higher inflammatory state (composite scores of inflammatory markers and the anti-inflammatory marker) among AAs at 3 days after ischemic stroke.

Hypothesis 3. *APOE4* carriers will predict a higher inflammatory state (composite scores of inflammatory markers plus the anti-inflammatory marker) as compared to non-*APOE4* carriers among AAs at 3 days after ischemic stroke.

Design

This study utilized a descriptive, comparative, design. Participants were recruited at 3 days after ischemic stroke while in the acute care setting. At the time of recruitment, the investigator obtained a complete medical and personal health history and baseline physiologic variables (height, weight, waist circumference, body mass index (BMI), and blood pressure). In addition, the participants were assessed for baseline inflammatory markers (CDllβ, TNFα, IL-6, IL-8, and IL10, fibrinogen, hsCRP) and *APOE* polymorphisms.

Setting

The setting for this study was an academic research health science center in the southeastern United States. A 20-bed inpatient neurology medical unit in a 900 bed regional stroke center was used for this study.

Sample

The participants consisted of a convenience sample of AAs. The inclusion criteria for this study included the following:

1. Self- identified AAs
2. Adults 21 or older who were English speaking.
3. First time ischemic stroke confirmed and documented by computed tomography or magnetic resonance imaging of the brain admitted to the inpatient neurology medical unit and 3 days post stroke (Coull et al., 1991).

The exclusion criteria for this study included the following (Coull et al., 1991):

1. Cerebral hemorrhage, multiple infarcts, sickle cell stroke, or other *non*-ischemic neurological condition (e.g. seizure).
2. Cognitive limitations prior to the ischemic stroke (reported by subject or family, or other).
3. Co-morbidity such as acute myocardial infarction within the last 30 days, respiratory failure requiring mechanical ventilation, psychiatric condition, or liver disease.
4. A diagnosis of acute bacterial infection.
5. Autoimmune disorders or malignancy.
6. Use of nonsteroidal anti-inflammatory medications or steroid use within the last 30 days.
7. Pregnancy (positive pregnancy test)

Sample Size

Power calculations for comparative analysis were performed using power analysis and sample size (NCSS, LLC, 2008) software. The power calculation assumed a type I error rate of 0.05 and a two-sided statistical test. The projected sample size of *APOE4* carriers and non-*APOE4* carriers, the standard deviation (SD), and the difference (D) between group means to test the hypotheses was calculated. The standard deviation of 0.9 was selected from previous statistical analysis of CRP values in the CARDIA study (personal communication statistician, June 19, 2009). For the purpose of preserving statistical power and to test the hypotheses, *APOE* genotypes were dichotomized into two groups: *APOE4* carriers (e2/4, e3/4, and e4/4) and non-*APOE4* carriers (e2/2, e2/3, and e3/3) (Jarvik, 1997). While a sample size of 200 or greater represented the optimal size, serious consideration was given to the time and financial constraints for this proposal as well as having preliminary data for future research. For these reasons, a

sample size of 30 *APOE4* carriers and 70 non-*APOE4* carriers was determined to achieve 80% power to detect a difference of 0.6 between group means with a SD of 0.9. A sample size of 100 participants was anticipated based on this analysis.

Methods

Recruitment Strategy

The investigator was notified via pager of all stroke admissions to the neurology medical unit and determined if inclusion and exclusion criteria were met by screening the patient in person. The recruitment strategy for participants was to utilize an inpatient neurology medical unit in the southeastern U.S. to recruit AAs with ischemic stroke. The center admits approximately 1200 strokes annually. Six hundred to 700 admissions are ischemic strokes. Forty five percent (n=270) of ischemic stroke admitted to this unit are AAs. Projected enrollment of 20 subjects per month was anticipated (personal communication Stroke Center Director, March 31, 2009) with 30% (n=6) expected to be *APOE4* carriers. Estimates of *APOE* carriers were based on the findings from the CARDIA Study (Howard, Gidding, & Lieu, 1998), which was the first study to investigate *APOE* polymorphisms among AAs and included participants from this metropolitan area.

Data Collection Overview

Once the inclusion criterion was established, informed consent was obtained at three days of ischemic stroke event. While the patient was in the neurology unit, the following measures were obtained by the investigator: a complete medical and personal health history, height, weight, waist circumference, blood pressure, inflammatory markers (CD11b, TNFa, IL-6, IL-8, fibrinogen, and hsCRP), anti-inflammatory marker, IL-10, and *APOE* genotype.

Detailed Methods

Medical and personal health history questionnaire. A data tool designed specifically for this study to collect demographic, personal history, familial and individual health history was developed. These questions were derived from questionnaires from the Cardiovascular Health Study (NHLBI, 2009), REGARD Study (University of Alabama at Birmingham, 2003), and World Health Organization Risk Surveillance Stroke Questionnaire (WHO, 2005). Questionnaire variables included: age, gender, marital status, education level, and income (defined by the U.S. Federal government). Personal history included information regarding life style behaviors such as smoking and alcohol information. In addition, familial history in relation to stroke and risk factor assessment was obtained. Individual health history included medical diagnosis, co-morbidities, and medications. Modifiable risk factors such as hypertension, diabetes mellitus type 2, obesity, hyperlipidemia, and smoking were categorized as present if the condition existed prior to stroke. Diabetes type 2 was determined by self- report or the use of antidiabetic medications. Hypertension was determined by self- report or the use of antihypertensive medications. Obesity was determined by BMI measurements according to the National Heart Lung and Blood Institute (NHLBI) recommendations (NHLBI, 1998) and defined as a BMI > 30 kg/m^2. Smokers were measured by pack years (py) defined as the number of packs of cigarettes per day multiplied by the number of years smoked during their lifetime. Subjects who smoked < 100 cigarettes in their lifetime (equivalent of 0.001 py) were considered nonsmokers and designated 0 py (McCulloch et al., 2008). The health history also included type of stroke, National Institute of Health stroke severity scale (Brott et al., 1989) results and the following laboratory results: complete blood count, lipid profile, hematocrit, and glycated

hemoglobin. These variables were selected based on literature review and the possibility of future sub-analysis.

Physiological Measures

Weight. Weight was obtained on each subject without shoes and measured in kilograms using a balanced beam scale to the nearest 0.1 kilograms (Kg) for participants who were able to stand and an electronic bed scale for participants who were unable to stand.

Height. Height was measured to the nearest centimeter using a stadiometer with the participant in their socks for participants who were able to stand and a measuring tape in the supine position for participants who were unable to stand.

Waist circumference. Waist circumference was measured to detect the presence of central obesity. A Gulick II tape measure was used to measure waist circumference. A waist measurement was obtained using the NHLBI recommendations (1998).

Body mass index (BMI). BMI was calculated according to NHLBI (1998) as weight in kilograms divided by height in meters squared.

Blood pressure (BP). BP was measured two times 10 minutes apart during the beginning phase of the visit prior to having blood drawn. A manual sphygmomanometer set at eye level using a cuff bladder that covers at least two-thirds of the upper right arm and at least half the circumference was used. The participants were asked to sit quietly for at least two minutes prior to each measurement.

Blood collection. The blood sampling procedure included the collection of blood from a vein in the upper extremity with the arm resting on a pillow or supportive prop. A proper vein was selected after placing a tourniquet around the arm, inspecting and palpating the vein, and

sterilizing the site. The needle was inserted into the skin, bevel upward, smoothly and quickly into the vein to avoid hemolysis. Immediately after insertion, the tourniquet was released. The order in which the tubes were collected was based on risk of contamination and coagulation. Clinical Laboratory and Standards Institute (CLSI) (2009) recommended the following order: 1) tubes for serum, 2) citrated serum tubes, 3) gel tubes, 4) heparin filled tubes, and 5) ethylene diamine tetra-acetic acid (EDTA) filled tubes. Consideration to the priority of the assay may also affect the order of tube filling.

Vacuum tubes were used for blood collection and the tube was placed in the adapter. When taking several tubes, the next tube was placed immediately after the previous one was filled. Once complete, the needle was withdrawn and pressure held to the site with a band aide applied over the area. Before leaving the examination site, the tubes were properly labeled with the subject's identification code.

Inflammatory Marker Analysis

CD11B and PLAs levels. The investigator obtained blood by venipuncture at 3 days after stroke. CD11β and PLA levels can be collected any time of day without fasting. A sample of 4.0 ml venous blood was collected into an EDTA lavender top tube, inverted gently and transported immediately at room temperature to the University of Alabama at Birmingham Immunology lab for immediate processing. Samples were immediately shipped via overnight mail to the University of Arizona, Dr. Ritter's lab for flow cytometry analysis of neutrophil activation (CD11β) and PLAs. All data were acquired via FACSCaliber flow cytometer (Becton Dickenson); CellQuest Pro software was used to analyze FACSCaliber data.

Validity of flow cytometry is dependent upon the manufacturer's recommendations for specific instrumentation, the testing laboratory, the reagents, and the staining procedure (Owens, Vall, Hurley, & Wormsley, 2000; BD Bioscience, 2005; Shapiro, 2003). Both internal and external quality controls were utilized. Optimal fluorescent intensity was achieved by demonstrating adequate saturation with antibody titration (antibody binding capacity) and examining the curve prior to testing. Reproducibility relied on monoclonal antibody staining with adequate proportion of antibodies to cells. Absolute cell counts should be performed to ensure correct proportions of cells to monoclonal antibodies and thus, accurate staining patterns (Owens et al., 2000). Inter-laboratory reproducibility has been reported as a variation of 11% when an internal standard is used (Zenger et al, 1998). In this study, data acquisition utilized mean fluorescence after forward scatter/side scatter dot-plot gates. Five thousand gated events were acquired for single fluorescence analysis, which is comparable to methods used in similar studies (Fusman et al., 2001).

Plasma cytokines (TNFα, IL-6, IL-8, IL-10) levels. The investigator obtained venous blood by venipuncture at 3 days after stroke. Cytokine levels can be collected any time of day without fasting. A sample of 2.0 ml whole blood was collected into a red top tube. The sample was transported to the University of Alabama at Birmingham, Immunology lab where it was processed. The sample was kept at room temperature for 10 minutes, and then placed on ice for up to 50 minutes. Within 1 hour of collection, the sample was centrifuged at 3000 rpm for 10 minutes. Removal of 1.0 ml aliquots of serum from top of tube was collected and placed in plastic containers. The samples were then frozen in -80 C freezer until processing. Samples were shipped via overnight mail to the University of Arizona, Dr. Ritter's lab for flow cytometry

analysis of cytokines in batches of 25. An AMSD multi-array cytokine flow cytometry assay was used to quantify cytokine levels. Detection limits ranged from 0.3 pg/ml for IL-6 and TNFα to 1.0 pg/ml for IL-8 to 1.9 pg/ml for IL-10.

Hs-CRP levels. Hs-CRP can be collected any time of day without fasting. A total of 1.0 ml of venous blood was collected by the investigator in a red top tube with cytokine levels. Samples were sent to the University of Alabama at Birmingham Hospital Research lab for processing and analysis. The Stanbio WR™ CRP assay was run using the Stanbio CRP Multi-Calibrator Set. A multi-point calibration curve using a Stanbio WR™ CRP multi-calibrator set was used when performing this assay. CRP levels were determined using the precalibration curve.

b) For quality controls, routine use of controls containing assayed levels of CRP by immunotrubidimetric assay was used. These controls were assayed in every run and treated in the same manner as patient samples. Sensitivity (defined as the concentration at three standard deviations above the CRP calibrator of 0.0 mg/dL) range is 0.005mg/dL – 0.02mg/dL depending on the calibrator set.

Fibrinogen levels. Fibrinogen can be collected any time of day without fasting. A sample of 4.0 ml of venous blood was collected in a blue top tube containing 3.2% sodium citrate.

a) The specimen was gently inverted 6 times and transported immediately to the University of Alabama Hospital at Birmingham Coagulation laboratory. Laboratory personnel centrifuged and separated plasma and test within one hour of collection during daytime hours or quick freeze at -80 if sent after 4 pm. Samples were measured using the Sta-R Evolution Series Analyzer (Diagnostica Stago, Inc., Gennevilliers, France).The standard reference range for fibrinogen is 220-498 mg/dl (University of Alabama Hospital at Birmingham, Coagulation Laboratory, 2009).

APOE genotype analysis. Blood sample for genetic analysis were obtained and performed at the Genetic Core Laboratory by Dr. Michael Crowley at the University of Alabama at Birmingham under the direction of Dr. Jose Fernandez. Three 5-ml blood samples were obtained from each subject and collected in an EDTA purple tube by the investigator. The sample was transported immediately to the Genetic Core Laboratory by the investigator. Blood was prepared, and DNA extracted by the Genetic Core Lab personnel. The samples were then frozen in -80 C freezer until processing. *APOE* genotypes were determined by two single nucleotide polymorphisms (SNP ID rs7412 and rs429358). Pyrosequencing was the molecular technique used for *APOE* genotyping.

Summary

This study utilized a descriptive, comparative design. The overall purpose was to examine relationships among *APOE* genotypes, inflammatory markers, and risk factors in AA with ischemic stroke. Participants were recruited from the inpatient neurology medical intensive care unit. A sample size of 100 participants was anticipated. The participants were screened in person. Once a participant had been approved for the study and agreed to participate, a complete medical and personal health history, physiological variables, and inflammatory markers, and *APOE* genotypes was collected at 3 days post stroke. All risks, costs, and benefits were discussed with participants. The participant was assured of their right to withdraw at any time without prejudice to their care.

Data Analysis and Management

Data Analysis Plan

Descriptive statistics for all study variables were generated for the overall group and

then for each genotype group. Categorical data was expressed as percentages. Data between groups was expressed as means and standard deviation (SD) where appropriate. Means and SD were determined for each inflammatory marker. Values for each inflammatory marker were compared between groups along with demographic characteristics, stroke characteristics, and risk factors, using the Statistical Package for Social Sciences software (SPSS 22.0, 2013), considering a p-value < 0.05 as significant. Chi square was applied to categorical variables. Paired t-tests was applied to within groups for continuous variables where appropriate. T-tests or Mann-Whitney test was applied to between groups for continuous variables where appropriate.

For Aim 1, to test if the frequency of non-*APOE* carriers were greater than *APOE4* carriers, we analyzed data from three national studies for comparison. These studies included: NHANES Study by Audrey et al., 2009, CARDIA study by Howard et al.1998), and the MESA study by Liang et al. (2013). An average of the three studies was computed. Chi square was used to compare frequencies between the genotype groups.

For Aim 2, levels of the inflammatory markers and the anti-inflammatory marker between *APOE4* carriers and non-*APOE4* carriers was analyzed. For values that did not have normal distribution; Mann-Whitney test was used. For values that had normal distribution, paired t-test was used for comparison within groups and t-test was used for comparison of continuous variables between groups.

For Aim 3**:** to examine *APOE4* carriers as a predictor for a higher inflammatory state, all inflammatory markers were converted to z scores. A composite score of inflammatory markers and the anti-inflammatory marker was then determined and t test conducted to compare groups.

Protection of Human Subjects

Human Involvement and Characteristics.

The investigator was notified of potential participants by stroke pager. Participants were recruited from the inpatient neurology medical intensive care unit. Preapproval from the neurologists of the stroke research team was obtained before the study was discussed with subject or family who met the inclusion criteria. Informed consent procedures were initiated if the potential participant or family member agreed. Informed consent forms were available for participants and for families in the case of a patient who was unable to communicate. Self-identified AAs adults, age 21 or older, and English speaking were eligible for this study. Participants consisted of first time ischemic stroke confirmed and documented by computed tomography or magnetic resonance imaging of the brain admitted to the inpatient neurology medical unit and 3 days post stroke (Coull et al., 1991). The exclusion criteria for this study included the following (Coull et al., 1991): 1) cerebral hemorrhage, multiple infarcts, sickle cell stroke, or other non-ischemic neurological condition (e.g. seizure), 2) cognitive limitations prior to the ischemic stroke (reported by subject or family, or other), 3) co-morbidity such as acute myocardial infarction, respiratory failure requiring mechanical ventilation, psychiatric condition, or liver disease, 4) a diagnosis of acute bacterial infection, 5) autoimmune disorders or malignancy, 6) use of *non*steroidal anti-inflammatory medications or steroid use, or 7) pregnancy (positive pregnancy test).

Security of materials. To protect the privacy of participants, each participant was assigned an accession number. Confidentiality was maintained by coding all specimens and questionnaire results with the participant's identification number. All data from this

study was kept in a 1) notebook and 2) a computer spreadsheet in the investigator's locked office. The notebook was kept in a locked cabinet. The computer was password protected and encrypted by the University of Alabama Data Security Office AB Data Security Office. In the notebook and the computer spreadsheet, only numbers were used to identify patients. Only the PI had access to the list of patient initials and numbers, which was kept in a separate locked file in the investigator's office (located at the University of Alabama at Birmingham, School of Nursing, office #544, Birmingham, AL). All identifying information was shredded and destroyed at the completion of the study.

Only qualified research personnel had access to the database containing the participant's information. All of the participant's data that is entered into statistical analyses and publication reports will refer to individuals only by number rather than name. This procedure maintained confidentiality. Only key investigative and data entry personnel were aware of participant's identity. All physiological data was drawn by the investigator or qualified research personnel. Participants were advised that they were free to stop the study procedures and exit the study at any time.

Potential risks. The risk of venipuncture was minimal. There is some minor discomfort and risk of mild bruising during venipuncture. Bruises from venipuncture would heal in several days. Discomfort associated with the venipuncture procedure included needle insertion, blood withdrawal, and needle withdrawal. Bleeding may also occur at the puncture site, but is alleviated with direct pressure and a band aide.

Adequacy of protection against risks. All laboratory tests were conducted under the supervision of trained personnel. Since the tests are not inherently hazardous, hazard was likely

to occur only as the result of impaired participant confidence or sudden unwillingness to complete the test. To avoid such hazards, study personnel thoroughly explained all tests to potential participant before the participant was offered the consent form to sign. Potential participants had the opportunity to see all equipment and facilities before giving consent or undergoing testing.

Handling of blood samples. All blood component and materials were handled as potentially hazardous with universal precautions followed per established guidelines by the Center for Disease Control and Prevention, (2005) and Occupational Safety and Health Standards (Occupational Safety and Health Administration, 2008). In addition, pursuant to the standards for discarding blood products, a standard protocol for discarding such waste was followed according to the institution policies.

Recruitment and informed consent. Participants were recruited from the inpatient neurology medial unit. The investigator was notified of potential participants by stroke pager. Medical charts were screened by the investigator for potential participants. Preapproval from the neurologists of the stroke research team was obtained before the study was discussed with subject or family who met the inclusion criteria. Informed consent procedures were initiated if the potential participant agreed. Informed consent forms were available for participants and for families in the case of a patient who was unable to communicate. The information provided to each participant included the purpose of the study and routine and potential risks associated with the study procedures. All risks, costs, and benefits were discussed. The participant was assured of their right to withdraw at any time without prejudice to their care. The participant was also assured of confidentiality in maintaining records and reporting of results.

Protection against risk. Prior to beginning this study the investigator obtained approval from both the University of Alabama at Birmingham and the University of Arizona Institutional Review Board. All materials including questionnaires and consent forms were reviewed and approved by the Institutional Review Board. Additional approval was obtained from the stroke research members and physicians. Data collection was for the sole purpose of the proposed study. Any adverse event was investigated and determined if further action should be taken (*e.g.*, reporting the event to the Institutional Review Board). The investigator conferred with stroke research team if revisions to the research protocol were necessary.

Potential benefits for the participants. The participant may gain personal satisfaction in contributing to knowledge leading to improved, ethnic specific means for preventing or treating stroke. In addition, participants received information regarding their specific inflammatory state and ApoE genotype, if requested. This information was particularly desirable to participants who would have difficulty gaining this information or undergoing the expense without participating in a research study. *Non*etheless, direct personal benefit to the participant was minimal.

Inclusion of women, children, and minorities. The entire sample of participants was self-identified AAs of both genders. The investigator selected an under representative research population due to the high mortality and morbidity associated with stroke in this population. Children were not included in this study due to the focus of this study was with AA adults. The investigator ensured that participants received culturally sensitive care during their experiences in the study. Enrollment of study participants was evaluated on a monthly basis. If the sample diverged from the planned enrollment, alternative strategies were explored with consultation

services from the Stroke Research Team and or Health Disparity Research Center at the University of Alabama as deemed appropriate.

Summary

This study utilized a descriptive, comparative design. The overall purpose was to examine the relationships among *APOE* genotypes, inflammatory marker levels, and risk factors in AAs with ischemic stroke. Participants were recruited from the inpatient neurology medical intensive care unit. A sample size of 100 participants was anticipated for this study. Medical and personal health history, physiologic variables (height, weight, waist circumference, BMI, and blood pressure), inflammatory marker levels (CD11β, PLAs, TNFa, IL-6, IL-8, fibrinogen, hs-CRP), and anti-inflammatory marker (IL-10), and *APOE* genotypes were obtained at 3 days post stroke. Data analysis included descriptive analysis in addition to Chi square, Mann-Whitney, t-tests where appropriate to test the hypotheses. The specific research procedure and data management was described. The protection of human subjects was discussed in detail.

CHAPTER IV: DATA ANALYSIS

Screening

One hundred and seventy five patients with acute ischemic stroke were screened during April 2011 through April 2012. A total of 14% (n=25) stroke patients met inclusion criteria and were enrolled. Forty percent (n=70) of those screened were excluded due to intracerebral hemorrhage and 25% (n=43) were excluded due to other causes, such as history of autoimmune disorders, chronic renal failure on dialysis, or taking anti-inflammatory medications. Twenty one percent (n=37) of those screened who met inclusion criteria declined to participate.

APOE Genotypes

For the purpose of preserving statistical power and to test the hypotheses, *APOE* genotypes were dichotomized into two groups: *APOE4* carriers (e2/4, e3/4, and e4/4) and non-*APOE4* carriers (e2/2, e2/3, and e3/3) (Jarvik, 1997). There were 12 patients in the *APOE4* group and 13 patients in the non-*APOE* group. Table 1 summarizes the *APOE* groups.

Table 1: *APOE* Genotypes

***APOE* Genotypes**	**ALL**	***APOE4***	**Non-*APOE4***
ε2, n (%)	4 (16)		
ε3, n (%)	9 (36)		
ε4, n (%)	12 (48)	12 (48)	
ε2 + ε3, n (%)			13 (52)

Characteristics of Participants

Gender

Sixty-four percent (n=16) were male and 36% (n=9) were female in the overall sample. In the *APOE4* group there were 75% (n=9) males and 25% (n=3) females. In the non-*APOE4* group there were 54% (n=7) males and 46% (n=6) females. A Chi-square test for independence indicated no significant association between gender and ApoE genotype, χ^2=1.2, p =.27.

Age

The average age of patients was 61 ± 14 years. The overall male (n=16) age was 57 ± 10. The overall female (n=9) age was 70 ± 16.The average age in the *APOE4* group was 56.5 ± 9.0. The male (n=9) age in the *APOE4* group was 56 ± 11. The female (n=3) age in the *APOE4* group was 57 ± 5.The average age in the *non-APOE4* group was 66 ± 16. The male (n=7) age in the non-*APOE4* group was 57 ± 10. The female (n=6) age in the non-*APOE4* group was 76 ± 17. An independent t-test was conducted to compare means for age between males and females. There was no significant difference between the ages for males and *APOE* genotypes (t = -.26, p =.79). Although females were older in the non-*APOE4* group, there was no significant difference for female age and genotypes (t= -1.3, p =.23).

Marital Status

In the overall group: 60% (n=15) of patients were married, 24% (n=6) of patients were widowed, and two patients were separated. One patient was divorced, and one patient was cohabitating. In the *APOE4* group, 58% (n=7) of patients were married and two patients were widowed. One patient was separated, one patient was divorced, and one patient was cohabitating. In the non-*APOE4* group, 62% (n=8) of patients were married and 31% (n=4) of patients were

widowed. One patient was separated. A Chi-square test for independence indicated no significant association between the status of married and *APOE* genotype, $\chi^2 = 2.6$, $p = .61$.

Education Level

In the overall sample: two patients had some level of vocational training and 20% (n=5) of patients had completed a GED. Of those who completed grade school, 16% (n=4) of patients completed the highest level, 12th grade, which was followed by 56 % (n=14) of patients for under the 12th grade. In the *APOE4* group, one patient had completed some level of vocational training and two patients had completed a GED. One patient had completed the 12th grade, and 67% (n=8) of patients completed grades less than 12th grade. In the non-*APOE4* group: one patient had completed some type of vocational training and 23% (n=3) of patients completed the 12th grade. Of those who completed grade school, 23% (n=3) of patients completed the 12th grade, and 46% (n=6) of patients had completed less than 12th grade. A Chi-square test for independence indicated no significant association between education level and *APOE* genotype, $\chi^2 = .07$, $p = .79$.

Income Level

In the overall sample, one patient earned 5,000-7,999.00 annually. Forty-four percent (n=11) of patients earned 12,000-15,999.00 annually. Twenty eight percent (n=7) of patients earned 16,000-24,999.00 annually. Twenty percent (n=5) of patients earned 25,000-34,999.00 annually with one patient earning 35,000-49,999.00. In the *APOE4* group, one patient earned 8,000-11,999.00. Forty-two percent (n=5) of patients earned 12,000-15,999.00 annually followed by 33% (n=4) of patients earning 16,000-24,999.00 annually. One patient earned 25,000-34,999.00 annually, and one patient earned 35,000-49,999.00 annually. In the non-*APOE4* group, no

patient earned less than 12,000 annually. Forty six percent (n=6) of patients earned 12,000-15,999.00 annually. Twenty three percent (n=3) of patients earned 16,000-24,999 followed by 30% (n=4) of patients who earned 25,000-34,999. No patient earned more than 50,000 annually.

Risk Factors

None of the patients had a reported history of myocardial infarction or valvular heart disease. One patient had had a history of congestive heart failure and was in the *APOE4* group. Two patients had a history of left ventricular hypertrophy and were in the *APOE4* group. Two patients had a history of atrial fibrillation and each person was in one of the *APOE* genotype groups. One person had a history renal insufficiency and was in the non-*APOE4* group. Thirty two percent (n=8) of patients had a history of alcohol use. Fifty four percent (n=6) of patients with alcohol use were in the *APOE4* group and 15% (n=2) of patients were in the non-*APOE4* group. Sixty five percent (n=16) of patients had a family history of stroke with 75% (n=9) in the *APOE4* group and 54% (n=7) of patients in the non-*APOE4* group. Sixty-eight percent (n=17) of patients had a family history of heart disease with 58% (n=7) of patients in the *APOE4* group and 77% (n=10) of patients in the non-*APOE4* group. A Chi-square test for independence indicated no significant association between family history of stroke and *APOE* genotype, χ^2= .1.2, p =.27 and family history of heart disease and *APOE* genotype, χ^2= .99, p =.31.

Medications Prior to Stroke

In the overall sample, 32% (n=8) of patients were on aspirin. Twenty-four percent (n=6) of patients were on a beta blocker and 24% (n=6) of patients were on a calcium channel blocker. One person was on an angiotensin receptive blocker. Thirty-two percent (n=8) of patients were on an ace inhibitor. Forty eight percent (n=12) of patients were on a statin before their stroke.

Twelve percent (n=3) of patients were on an antiplatelet agent, excluding aspirin. No patients were on an anticoagulant. Of the female patients, none were on an estrogen supplement.

In the *APOE4* group: 25% (n=3) of patients were on aspirin. Thirty three percent (n=4) of patients were on a beta blocker and 33% (n=4) of patients were on a calcium channel blocker. No patient was on an angiotensin receptive blocker. Thirty-three percent (n=4) of patients were on an ace inhibitor. Forty-two percent (n=5) of patients were on a statin before their stroke. No patients were on an antiplatelet agent, excluding aspirin, or anticoagulant. Of the female patients, none were on an estrogen supplement.

In the non-*APOE4* group: 38% (n=5) of patients were on aspirin. Two people were on a beta blocker and two people were on a calcium channel blocker. One person was on an angiotensin receptive blocker. Thirty percent (n=4) of patients were on an ace inhibitor. Fifty-four percent (n=7) of patients were on a statin before their stroke. Twenty three percent (n=3) of patients were on an antiplatelet agent, excluding aspirin. No patient was on an anticoagulant. Of the female patients, none were on an estrogen supplement.

A Chi-square test for independence indicated no significant association between use of statin before stroke and *APOE* genotype, $\chi^2 = .37$, $p = .54$.

Stroke and In-hospital Treatment

Stroke type and severity. All patients had an ischemic stroke of a major large cerebral or carotid artery. Utilizing the NIH stroke scale, in the overall group 80% (n=20) of patients were classified with a mild stroke with NIH stroke scale scores in the range of 0-5 and 20% (n=5) of patients had a moderate stroke with NIH stroke scores in the range of 6-13. In the *APOE4* group 83% (n=10) of patients were classified with a mild stroke and 16% (n=2) of patients had a

moderate stroke. In the non-*APOE4*, 77% (n=10) of patients had a mild stroke and 23% (n=3) of patients had a moderate stroke. A Chi-square test for independence indicated no significant association between stroke severity and *APOE* genotype, χ^2= .07, p =.79

Diagnostic tests. Eighty percent (n=20) of patients had an initial head CT scan. Twenty eight percent (n=7) of patients had both a head CT scan and MRI. Eighty percent (n=20) of patients had a transthoracic echocardiogram. Seventy-six percent (n=19) of patients had a carotid doppler performed. Among the *APOE4* group, 92% (n=11) of patients had an initial head CT scan performed. Twenty-five percent (n=3) of patients had both a CT scan and MRI. All *APOE4* patients (100%, n=12) had a transthoracic echocardiogram performed and 83% (n=10) of patients had a carotid doppler performed. In the non-*APOE4* group, 70% (n=9) of patients had an initial head CT scan performed. Thirty percent (n=4) of patients had both a head CT scan and MRI performed. Sixty-one percent (n=8) of patients had a transthoracic echocardiogram performed. Seventy percent (n=9) of patients had a carotid doppler performed. A Chi-square test for independence indicated no significant association between initial head CT scan and *APOE* genotype, χ^2= .42, p =.51.

In-hospital treatment and adverse events. Three patients received thrombolytics prior to arrival to the stroke unit; one person was in the *APOE4* group versus two people (15%) in the non-*APOE4* group. Eighty eight percent (n=22) of patients did not require any intervention such as surgery, or stent placement, and were treated with medications only. In the *APOE4* group, 92% (n=11) of patients were treated with medications only. In the non-*APOE4* group, 85% (n=11) of patients were treated with medications only. A Chi-square test for independence

indicated no significant association between stroke patients treated with medications only and *APOE* genotype, χ^2= .08, p =.93.

One person developed heart failure during their hospital stay in the overall group and was in the *APOE4* group. No adverse events occurred in the non-*APOE4* group.

Length of stay. The average length of stay was 3.68 ± 0.63 for the overall sample. The *APOE4* group's length of stay was 3.66 ± 0.50 and with the non-*APOE4* group, the average length of stay was 3.6 ± 0.75. There was no statistical difference between length of stay among the groups (t = 1.84, p = 0.92).

Discharge disposition. Seventy two percent (n=18) of patients were discharged home with the remaining 20% (n=5) of patients discharged to a rehab facility. Two people were discharged to a skilled nursing facility. In the *APOE4* group 58% (n=7) of patients were discharged home and 25% (n=3) of patients were discharged to a rehab facility. Two people were discharged to a skilled nursing facility. In the non-*APOE4* group 85% (n=11) of patients were discharged home and two people were discharged to a rehab facility. No patients in the non-*APOE4* were discharged to a skilled nursing facility. A Chi-square test for independence indicated no significant association between discharge disposition and *APOE* genotype, χ^2= 1.6, p =.44.

Medications after stroke. In the overall sample, 96% (n=24) of patients were discharged on aspirin. Sixty-four percent (n=16) of patients were discharged on a beta blocker and 40% (n=10) of patients were on a calcium channel blocker. No subjects were discharged on an angiotensin receptive blocker. Forty-eight percent (n=12) of patients were on an ace inhibitor. All stroke patients were discharged on a statin. Sixty-four percent (n=16) of patients were discharged on an

antiplatelet agent, excluding aspirin. Forty-eight percent (n=12) of patients were discharged on an anticoagulant.

In the *APOE4* group all stroke patients were discharged on aspirin. Sixty percent (n=8) of patients were discharged on a beta blocker. Thirty-three percent (n=4) of patients were discharged on a calcium channel blocker. No patient was discharged on an angiotensin receptive blocker. Fifty percent (n=6) of patients were on an ace inhibitor. All stroke patients were discharged on a statin. Sixty percent (n=8) of patients were discharged on an antiplatelet agent, excluding aspirin. Fifty-eight percent (n=7) of patients were discharged on an anticoagulant.

In the non-*APOE4* group 92% (n=12) of patients were discharged on aspirin. Sixty-two percent (n=8) of patients were discharged on a beta blocker and 46% (n=6) of patients were discharged on a calcium channel blocker. No patient was discharged on an angiotensin receptive blocker. Forty-six percent (n=6) of patients were discharged on an ace inhibitor. All stroke patients were discharged on a statin. Sixty-two percent (n=8) of patients were discharged on an antiplatelet agent, excluding aspirin. Thirty-eight percent (n=5) of patients were discharged on an anticoagulant. A Chi-square test for independence indicated no significant association between aspirin as a discharged medication and APOE genotype, $\chi^2 = .96$, $p = .32$. Table 2 summarizes the characteristics of the sample.

TABLE 2: CHARACTERISTICS

Characteristics	**ALL**	***APOE4***	**Non-*APOE4***	**Statistic, p**
Gender, n (%)				
Male	16 (64)	9 (75)	7 (54)	χ^2=1.2, p = 0.27
Female	9 (36)	3 (25)	6 (46)	
Age, mean (SD)				
Male	57 (10)	56 (11)	57 (10)	t= -.26, p =.79
Female	70 (16)	57 (5)	76 (17)	t= -1.3, p =.23
Marital Status, n (%)				
Married	15 (60)	7 (58)	8 (62)	χ^2=2.6, p=.61
Widow(ed)	6 (24)	2 (16)	4 (31)	
Separated	2 (8)	1 (8)	1 (8)	
Divorced	1 (4)	1 (8)		
Cohabitating	1 (4)	1 (8)		
Education, n (%)				
Vocational Ed	2 (8)	1 (8)	1 (8)	
GED	5 (20)	2 (16)	3 (23)	
High School	4 (16)	1 (8)	3 (23)	
< High School	14(56)	8 (67)	6 (46)	χ^2=.07, p =.79
Income, n (%)				
35-49,000	1 (4)	1 (8)		
25-34,999	5 (20)	1 (8)	4 (30)	
16-24,999	7 (28)	4 (33)	3 (23)	χ^2= .99, p =.31
12-15,999	11 (44)	5 (42)	6 (46)	
5- 7,999	1 (4)	1 (8)		
Risk Factors, n (%)				
MI	0	0	0	
Valvular Heart Disease	0	0	0	
CHF	1 (4)	1 (8)	0	
Left Ventricular Hypertrophy	2 (8)	2 (16)	0	
Atrial Fibrillation	2 (8)	1 (8)	1 (8)	
Renal Insufficiency	1 (4)	1 (8)	0	
Alcohol Use	8 (32)	6 (54)	2 (15)	
Family History Stroke	16 (65)	9 (75)	7 (54)	χ^2= 1.2, p = .27
Family History Heart Disease	17 (68)	7 (58)	10 (77)	
Medications				
Prior to Stroke, n (%)				
Aspirin	8 (32)	3 (25)	5 (38)	χ^2=.07, p = .79
Beta Blocker	6 (24)	4 (33)	2 (15)	
Calcium Blocker	6 (24)	4 (33)	2 (15)	
ARB	1 (4)	0	1 (8)	
Ace Inhibitor	8 (32)	4 (33)	4 (30)	
Statin	12 (48)	5 (42)	7 (54)	χ^2= .37, p =.54

Antiplatelet Anticoagulation Estrogen	3(12) 0 0	0 0 0	3 (23) 0 0	
Stroke Type, n (%) Ischemic	25 (100)	12 (100)	13 (100)	
Stroke Severity NIH Score, n (%) 0-5 Mild 6-13Moderate >14 Severe	 20 (80) 5 (20) 0	 10 (83) 2 (16) 0	 10 (77) 3 (23) 0	χ^2= .07, p =.79
Diagnostic Tests, n (%) Initial CT Scan CT scan & MRI TTE Echo Carotid Doppler	 20 (80) 7 (28) 20 (80) 19 (76)	 11 (92) 3 (25) 12 (100) 10 (83)	 9 (70) 4 (30) 8 (61) 9 (70)	χ^2= .42, p = .51
In-hospital Treatment, n (%) TPA No Intervention/ Medically Treated	 3 (12) 22 (88)	 1 (8) 11(92)	 2 (15) 11(85)	χ^2=.08, p = .93
Adverse Event, n (%) Heart Failure	 1 (4)	 1 (8)	 0	
Length of Stay, n (SD)	3.68 (.63)	3.66 (.50)	3.69 (.75)	
Discharge Disposition, n (%) Home Rehab Facility Skilled Unit	 18 (72) 5 (20) 2 (8)	 7 (58) 3 (25) 2 (16)	 11 (85) 2 (15)	χ^2=1.6, p = .44
Medications After Stroke, n (%) Aspirin Beta Blocker Calcium Blocker ARB Ace Inhibitor Statin Antiplatelet Anticoagulation Estrogen	 24 (94) 16 (64) 10 (40) 0 12 (48) 25 (100) 16 (64) 12 (48) 0	 12 (100) 8 (60) 4 (33) 0 6 (50) 12 (100) 8 (60) 7 (58) 0	 12 (92) 8 (60) 6 (46) 0 6 (46) 13 (100) 8 (62) 5 (38) 0	χ^2= .96, p = .32

Risk Factors

In the overall group all patients had hypertension. Fifty two percent (n=13) of patients were smokers with a mean of 21 pack years. Forty eight percent (n=12) of patients had hyperlipidemia and 48% (n=12) of patients had diabetes mellitus Type II. Twenty percent (n=5) of patients had documented obesity.

In the *APOE4* group all patients had hypertension. Fifty-eight percent (n=7) of patients were smokers with a mean of 43 pack years. Fifty percent (n=6) of patients had history of hyperlipidemia. Twenty five percent (n=3) of patients had diabetes mellitus Type II. Two patients had obesity.

In the non-*APOE4* group all patients had hypertension. Forty-six percent (n=6) of patients were smokers with a mean of 43 pack years. Forty-six percent (n=6) of patients had hyperlipidemia. Sixty-nine percent (n=9) of patients had diabetes mellitus Type II. Three patients had obesity.

A Chi-square test for independence was conducted for risk factors with 5 or more patients. The Chi-square test indicated no significant association between tobacco use and *APOE* genotype, $\chi^2 = .37$, $p = .54$ and hyperlipidemia and *APOE4* genotype, $\chi^2 = .37$, $p = .54$.

Risk Factor Clusters (Hypertension, Diabetes, Obesity, Hyperlipidemia, and Smoking)

Risk factor clusters included hypertension and one or more variable risk factors. The following risk factor clusters were evident in the overall sample: one risk factor 12% (n=3), two risk factors 32% (n=8), three risk factors 32% (n=8), and four risk factors 24% (n=6). No patient had all five risk factors.

In the *APOE4* group, the following risk factor clusters were evident: one risk factor 16% (n=2), two risk factors 42% (n=5), three risk factors 16% (n=2), and four risk factors 25% (n=3). Among the men in the group, 25% (n=3) had two risk factors (hypertension and tobacco dependence). Among the women in the group, one person had four risk factors: hypertension, hyperlipidemia, diabetes, and obesity.

In the non-*APOE4* group, the following risk factor clusters were evident: one risk factor 8% (n=1), two risk factors 23% (n=3), three risk factors 46% (n=6), and four risk factors 23% (n=3). Three risk factors (hypertension, diabetes mellitus Type 2, and tobacco dependence was found in 36% (n=5) among both men and women in this group.

Table 3 and 4 summarizes the risk factors and risk factor clusters in the overall group and between groups.

Table 3: Risk Factors

Risk Factors (%)	**ALL**	***APOE4***	**Non-*APOE4***
Hypertension	25 (100)	12 (100)	13 (100)
Tobacco Use	13 (52)	7 (58)	6 (46)
Hyperlipidemia	12 (48)	6 (50)	6 (46)
Diabetes Type 2	12 (48)	3 (25)	9 (69)
Obesity	5 (20)	2 (16)	3 (23)

Table 4: Risk Factor Clusters

Number of Risk Factors (%)	**ALL**	***APOE4***	**Non-*APOE4***
One Risk Factor	3 (12)	2 (16)	1 (7)
Two Risk Factors	8 (32)	5 (42)	3 (23)
Three Risk Factors	8 (32)	2 (16)	6 (42)
Four Risk Factors	6 (24)	3 (25)	3 (23)
Five Risk Factors	0	0	0

Physiological Characteristics of Participants

BMI

The mean BMI was 27.7 kg/m^2 ± 7.2.in the overall group. In the *APOE4* group, the mean BMI was 27 kg/m^2 ± 7.0. In the non-*APOE4* group, the mean BMI was 28 kg/m^2 ± 7.7. Males in the overall group had a mean BMI of 26.3± 6.4. The mean BMI for males in the *APOE4* group was 28 ± 7.7 and in the non-*APOE4* group was 24 ± 3.2. The mean BMI for females in the *APOE4* group was 30 ±8.4 and in the non-*APOE4* group was 33 ± 8.5. A t-test was conducted to compare BMI between *APOE* groups and also BMI and gender within *APOE* genotypes. For males, there was no significant differences in BMI and *APOE* genotypes ($t = 0.39$, $p = 0.69$) in the overall group and between BMI and *APOE* genotypes ($t = -1.21$, $p = 0.25$).

There *was* a statistical significant difference between BMI for females and *APOE* genotypes ($t = 2.56$, $p = 0.04$).

Twelve percent (N=3) of the subjects had a BMI ≥ 35 kg/m^2. In the *APOE4* group, one patient had a BMI ≥ 35 kg/m^2. In the non-*APOE4* group, two patients had a BMI ≥ 35 kg/m^2.

Blood Pressure

The mean systolic blood pressure was 155 mmHg ± 22.6 in the overall group. The mean diastolic blood pressure was 85 mmHg ±12.2. In the *APOE4* group, the mean systolic blood

pressure was 149 mmHg ± 23. In the *APOE4* group, the mean diastolic blood pressure was 83 mmHg ± 11. In the non-*APOE4* group, the mean systolic blood pressure was 160mmHg ± 22. In the non-*APOE4* group, the mean diastolic blood pressure was 84 mmHg ± 11.5.

White Blood Cell Count

The mean white blood cell count was 7.2 cells/milliliter (cells/ml) ± 2.9. In the *APOE4* group, the mean white blood cell count was 6.7 cells/ml ± 3.2. In the non-*APOE4* group, the mean white blood cell count was 7.7 cells/ml ± 2.8. There was no statistical difference between groups ($t = -0.83$, $p = 0.41$).

Hematocrit Level

The mean hematocrit level was 37.% ± 5.6. In the *APOE4* group, the mean hematocrit level was 37.8% ± 5.5. In the non-*APOE4* group, the mean hematocrit level was 36.6 ± 5.8. There was no statistical difference between groups ($t = 0.49$, $p = 0.62$).

Cholesterol/Triglycerides/HDL/LDL

The mean cholesterol level was 166.40 milligrams/deciliter (mg/dL) ± 41.32. In the *APOE4* group, the mean cholesterol level was 171mg/dL ± 31. In the non-*APOE4* group, the mean cholesterol level was 161 mg/dL ± 49. There was no statistical difference between groups ($t = 0.57$, $p = 0.57$).

The mean triglyceride level was 147.4 mg/dL ± 66.7. In the *APOE4* group, the mean triglyceride level was 144.5 mg/dL ± 59. In the non-*APOE4* group, the mean triglyceride level was 150 mg/dL ± 75. There were no statistical difference between groups ($t = -0.2$, $p = 0.84$).

The mean HDL level was 39.4 mg/dL ± 13.3. In the *APOE4* group, the mean HDL level was 37 mg/dL ± 11. In the non-*APOE4* group, the mean HDL level was 41 mg/dL ± SD 15. There

were no statistical difference between groups (t = -0.3, p = 0.74). The mean LDL level was 93.8 mg/dL ± 42.8. In the *APOE4* group, the mean LDL level was 101.5 mg/dL ± 34. In the non-*APOE4* group, the mean LDL level was 86 mg/dL ± 50. There were no statistical difference between groups (t = -0.7, p = 0.46).

HgA1C

The mean Hb/A1C was 6.6 % ± 1.3. In the *APOE4* group, the mean Hb/A1C was 6.3 % ± 1.3. In the non-*APOE4* group, the mean Hb/A1C was 6.9% ±1.3. There was no statistical difference between groups (t = -0.9, p = 0.33).

Table 5 summarizes the physiologic variables for the overall group and *APOE* groups.

Table 5: Physiologic Characteristics

Physiologic Characteristic	**ALL**	***APOE4***	***Non-APOE4***	**Statistic, p**
BMI, Kg/m^2, mean (SD) ≥ 35 Kg/m^2, n (%)	27.7 (7.23) 3 (12)	27 (7) 1 (8)	28 (7.72) 2 (16)	t = 0.39, p = 0.69
Blood Pressure, mean (SD) Systolic BP mmHg Diastolic BP mmHg	 155 (22.68) 85 (12.28)	 149 (23) 83 (11)	 160 (22) 84 (11.5)	
White Blood Cell Count, mean(SD)	7.2 (2.98)	6.7 (3.2)	7.7 (2.8)	t =-0.83, p =0.41
Hematocrit % (SD)	37.2 (5.64)	37.8 (5.5)	36.6 (5.8)	t = 0.49, p =0.62
Cholesterol mg/dL, mean(SD)	166.40 (41)	171 (31)	161 (49)	t = -0.02, p = 0.57
Triglycerides mg/dL, mean(SD)	147.40 (66.7)	144.5 (59)	150 (75)	t = -0.20, p = 0.84
HDL mg/dL, mean (SD)	39.48 (13.3)	37 (11)	41 (15)	t = -0.30, p = 0.74
LDL mg/dL, mean (SD)	93.88 (42.8)	101.5 (34)	86 (50)	t = -0.70, p = 0.46
HgbA1C, %, mean (SD)	6.65 (1.37)	6.36 (1.3)	6.9 (1.37)	t = -0.90, p = 0.33

Results for Specific Aims

AIM 1: Determine the *APOE* carriers among African Americans after ischemic stroke.

Hypothesis 1. Consistent with the frequency of non-*APOE4* carriers among the African American population, the frequency of non-*APOE4* carriers will be greater than *APOE4* carriers among African American with ischemic stroke.

A Chi-square goodness of fit test indicated there was no significant differences in the frequency of non-*APOE4* carriers identified in the current sample (52%) as compared to 64%, which is the average from three national studies: n= 1599, 62% were in the non-*APOE4* group (Audrey et al., 2009); n=696, 64% were in the non-*APOE4* group (Howard et al., 1998), and n=554, 66% were in the non-*APOE4* group (Liang et al., 2013), $\bar{x}$ = 64%, χ^2 == 1.5, p = .21). In addition, there was no significant differences in the frequency of the *APOE4* carriers (48%) as compared to 36%, which is the average from three national studies: n = 1599, 38% were in the *APOE4* group (Audrey et al., 2009), n=696, 36% were in the *APOE4* group (Howard et al., 1998), n = 544, 34% were in the *APOE4* group (Liang, et al., 2013), $\bar{x}$ =36%.

The hypothesis for AIM 1 was supported with the frequency of non-*APOE4* carriers as 52% compared to *APOE4* carriers as 48% among AAs with ischemic stroke.

AIM 2: Determine the levels of inflammatory markers and the anti-inflammatory marker between *APOE4* carriers and non-*APOE4* carriers among African Americans at 3 days after ischemic stroke.

Hypothesis 2: Inflammatory markers will be increased and the anti-inflammatory marker will be decreased in *APOE4* carriers than with non-*APOE4* carriers among African Americans at 3 days after ischemic stroke.

Table 6 summarizes the normal values for the variables listed below.

Table 6: Normal Values of Markers

Inflammatory Marker/Anti-Inflammatory Marker	**Normal Value**
Fibrinogen	145-348 mg/dl
hs-CRP	< 3 mg/l
TNFα	5-27 pg/ml
IL-1β	< 3 pg/ml
IL-6	0-4.72 pg/ml
IL-8	0-63 pg/ml
IL-10	< 2.0 pg/ml

Fibrinogen

The overall mean fibrinogen level among all patients was 422 mg/dL ± 24.3. Among the *APOE4* carriers, the mean fibrinogen level was 406 mg/dL ± 37.5. Among the non-*APOE4* carriers, the mean fibrinogen level was 434 ± 37.5. A Mann Whitney U Test revealed no significant difference in fibrinogen level and *APOE* genotypes (U= 67, z -.60, p = 0.54).

hsCRP

The overall mean hsCRP level among all patients was 25.6 mg/dL ± 6.8. Among the *APOE4* carriers, the mean hsCRP level was 19.5 mg/dL ± 9.1. Among the non-*APOE4* carriers, the mean

hsCRP level was 31.2 ± 10. A Mann Whitney U Test revealed no significant difference in hsCRP level and *APOE* genotypes (U= 48, z -1.60, p = 0.10).

TNFα and IL1β

For TNFα and IL1β, the values lied below detectable limits of the assay.

IL6

The overall mean IL6 level among all patients was 34.8 picogram per milliliter (pg/ml) ± 7.4. Among the *APOE4* carriers, the mean IL6 level was 29.0 pg/ml ± 9.1. Among the non-*APOE4* carriers, the mean IL6 level was 40.1 ± 11.7. A Mann Whitney U Test revealed no significant difference in IL6 levels and *APOE* genotypes (U= 61, z -.92, p = 0.35).

IL8

The overall mean IL8 level among all patients was 15.7 pg/ml ± 2.3. Among the *APOE4* carriers, the mean IL8 level was 18.5 pg/ml ± 3.87. Among the non-*APOE4* carriers, the mean IL8 level was 13.1 ± 2.6. A Mann Whitney U Test revealed no significant difference in IL8 levels and *APOE* genotypes (U= 54, z -.1.3, p = 0.19).

IL10

The overall mean IL10 level among all patients was 0.11 pg/ml ± 0.04. Among the *APOE4* carriers, the mean IL10 level was 0.10 pg/ml ± 0.06. Among the non-*APOE4* carriers, the mean IL10 level was 0.13 ± 0.07. A Mann Whitney U Test revealed no significant difference in IL10 levels and *APOE* genotypes (U= 77, z -.60, p = 0.95).

CD11β

The overall mean CD11β total fluorescent intensity (TFI) among all patients was 19.1 ± 2.6. The overall mean CD11β and Formyl-Methionyl-Leucyl-Phenlalanin (fMLP) TFI among was

23.0 ± 2.4. Among the *APOE4* carriers, mean CD11β TFI was 23.4 ± 3.9. The mean CD11β and fMLP TFI among *APOE4* carriers was 25.7 ± 4.2. Among the non-*APOE4* carriers, mean CD11β TFI was 17.1 ± .32. The mean CD11β and fMLP TFI among non-*APOE4* carriers was 21.9 ± 2.8.

Paired sample t-tests and independent t-tests were conducted where appropriate to compare values of CD11β and CD11β + fMLP for the overall group, *APOE4* carriers, and non-*APOE4* carriers among African Americans with ischemic stroke at 3 days with the following statistical significant findings:

- There *was* a statistically significant difference in overall CD11β (mean = 19, ± 12.5) and overall CD11β + fMLP (mean = 23, ± 11.6 with SEM 2.4); t = -3.2, *p = 0.003.*
- There *was* a significant difference among *APOE4* carriers in CD11β (mean= 18.8, ± 14, with SEM 4.2) and CD11β + fMLP (mean=23, ± 12.5, with SEM 3.7); t = -2.25, *p =0.04.*
- There *was* a statistically significant difference among non-*APOE4* carriers in CD11β *(mean* = 19.5, ± 11.5, with SEM 3.3) and CD11β + fMLP (mean =23, ± 11.25, with SEM 3.25); t = -2.31, *p =0.04.*

There were no statistical differences found in the following:

- CD11β among *APOE4* (mean = 18.8, ± 14, with SEM 4.2) and non-*APOE4* carriers (mean= 19, ± 11.5, with SEM 3.32); t = -.128, *p* = 0.90.
- CD11β + fMLP among *APOE4* (mean = 23, ± 12.5, with SEM 3.7) and non-*APOE4* carriers (mean= 23, ± 11.25, with SEM 3.2); t = -0.007, *p* = 0.99.

Platelet-Leukocyte Aggregates

The overall mean CD42β total fluorescent intensity (TFI) among all patients was 1.4 ± 0.39. The overall mean CD42β and platelet activating factor (PAF) among all patients was 1.3 ±0.38. Among the *APOE4* carriers, mean CD42β TFI was 1.2 ± 0.18. The mean CD42β and PAF TFI among *APOE4* carriers was 1.04 ± 0.13. Among the non-*APOE4* carriers, mean CD42β TFI was 1.7 ± 0.73. The mean CD42β and PAF TFI among non-*APOE4* carriers was 1.7 ± 0.70. There was no statistical difference between groups.

Paired sample t-tests and independent t-tests were conducted where appropriate to compare values of CD42β and CD42β + PAF for the overall group, *APOE4* carriers, and non-*APOE4* carriers among African Americans with ischemic stroke at 3 days with the following results:

- There was no significant difference in the overall group for CD42 (mean= 1.4, ± 1.8, with SEM 0.39) and CD42β + PAF (mean=1.3, ± 1.8, with SEM 0.37); t = 1.48, p = 1.51.
- There was no significant difference among *APOE4* carriers in CD42β (mean = 1.9, ± 2.6, with SEM 0.80) and CD42β + PAF (mean =1.8, ± 2.5, with SEM 0.76); t = 0.90, p =0.40.
- There was no significant difference among non-*APOE4* carriers in CD42β (mean = 0.9, ± 0.5, with SEM 0.15) and CD42β + PAF (mean = 0.8, ± 0.5, with SEM 0.14); t = 1.28, p = 0.22.
- There was no significant difference in CD42β among *APOE4* (mean = 1.9, ± 2.6, with SEM 0.79) and non-*APOE4* carriers (x^2 = 0.9, SD 0.5, with SEM 0.15); t = 1.24, p = 0.22.

- There was no significant difference in CD42β + PAF among *APOE4* (mean = 1.8, ± 2.5, with SEM 0.76) and non-*APOE4* carriers (mean = 0.9, ± 0.5, with SEM 0.14); t = 1.2, *p* =0.24.

Although there were higher inflammatory marker levels for IL-8, and CD11β with and without fMLP, and lower levels of the anti-inflammatory marker, IL-10 in the *APOE4* carriers, these were not statistically significant between *APOE* groups. The hypothesis for AIM 2 was not supported.

Table 7 summarizes the inflammatory markers for the overall group and *APOE* groups.

Table 7: Inflammatory and Anti-Inflammatory Markers

Inflammatory and Anti-inflammatory Markers	**ALL**	***APOE4***	***Non-APOE4***	**Statistic, p**
Fibrinogen mg/dL, mean(SD)	422 (24.3)	406 (37.5)	434 (37.5)	U= 67, z = -.60, p = .54
hsCRP mg/dL, mean(SD)	25.66 (6.8)	19.5 (9.1)	31.2 (9.1)	U = 48, z = -1.6, p = .10
IL 6 pg/ml, mean (SD)	34.8 (7.4)	29.0 (9.1)	40.1 (11.7)	U = 61, z = -.92, p = .35
IL 8 pg/ml, mean (SD)	15.72 (2.3)	18.5 (3.8)	13.1 (3.8)	U = 54, z = -1.3, p = .19
IL 10 pg/ml, mean (SD)	.11 (0.04)	0.10 (0.06)	0.13 (0.06)	U = 77, z = -.06, p = .95
CD11β (TFI), mean (SD)	19.19 (2.6)	23.4 (3.9)	17.1 (3.2)	t = 0.007, p =0.99
PLA (TFI), mean (SD)	1.44 (0.39)	1.20 (0.18)	1.76 (0.73)	t = 1.21, p =0.24

AIM 3: Examine APOE4 carriers as a predictor for a higher inflammatory load (composite scores of inflammatory markers and the anti-inflammatory marker) as compared to non-APOE4 carriers among African Americans at 3 days after ischemic stroke.

Hypothesis 3: *APOE4* carriers will predict a higher inflammatory load (composite scores of inflammatory markers plus the anti-inflammatory marker) as compared to non-*APOE4* carriers among African Americans at 3 days after ischemic stroke.

Values for the each inflammatory marker were translated to z scores divided by the number of variables to formulate a total composite score of inflammatory markers: fibrinogen, CRP, IL6, IL8, CD11β, CD42β. plus anti-inflammatory marker IL-10, which was converted to a negative number.

The inflammatory composite score for the *APOE4* group was 0.10 and for the non-*APOE4* group was .07. Although a higher composite score was noted for the *APOE4* carriers, it was not statistically significant (t = -.26, p = .79).

The hypothesis for AIM 3 was not supported.

Correlations of Risk Factors and Inflammatory Markers among ApoE Genotypes

Due to the lack of statistical significance for inflammatory load among *APOE4* genotypes, we examined correlations among risk factors and inflammatory markers among the *APOE* genotypes. Effect size was determined by Cohen guidelines (Pallant, 2013). A coefficient of determination was then calculated to determine how much variance was shared between the two variables. Findings included the following:

Among the *APOE4* carriers:

- There was a small positive relationship between CRP and fibrinogen (r = .53, p =.03) with a 28% shared variance.
- There was a large positive relationship between age and fibrinogen (r = .77, p = .001) with a 60% shared variance.
- There was a large positive relationship between PLA and BMI (r = .95, p = .012) with a 90% shared variance.

Among the non-*APOE4* carriers:

- There was a large negative relationship between PLA and IL-6 ($r = -.88$, $p = .021$) with a 77% shared variance.
- There was a large negative relationship between CD11β and HDL ($r = -.94$, $p = .005$) with an 88% shared variance.

CHAPTER V: DISCUSSION

This study was the first to examine risk factors and inflammatory markers with *APOE* genotypes among African Americans with ischemic stroke. The major findings of this study were: 1) there were no statistical differences between inflammatory markers and *APOE* genotypes among AAs with ischemic stroke and 2) the *APOE4* carrier is not a predictor for overall inflammatory load among AAs with ischemic stroke. Although the major hypotheses were not supported, the study provides a unique opportunity to examine inflammatory markers, risk factors, and *APOE4* genotypes among AAs with ischemic stroke. Further, the study was underpowered and small effect sizes were not sufficient to create statistical significant findings.

Age, Gender, and Socioeconomic Status

Age

Our sample was consistent with national data that showed stroke occurs at a much earlier age in males and at an older age for women (Howard et al., 2011; Koton et al., 2014). In-hospital data from 2010 demonstrated that from ages 45 to 65, 57% were men. After 65 year of age, women were the majority (Go et al., 2013). In general, women tend to be approximately 4 years older than men at the age of first ischemic stroke (Gibson, 2013). A meta-analysis on data from 2,566 patients revealed the mean age of onset of first ischemic stroke was 66.6 years for men compared to 70 years in women (Gargano, Wehner, & Reeves, 2009). In this study, we did not find an age difference among males and females in the *APOE4* group. In the *APOE4* group, women were younger with an average age comparable to men (mean age of 57). Due to insufficient sample size, we were not able to examine if this was an incidental or a significant

finding. Similar to other studies, men in both genotypes groups were younger (mean age of 57) and women in the non-*APOE4* group had an average age of 76. The proportion of patients aged > 75 was significantly higher in women in the non-*APOE* group in this study than in men, which was consistent with previous studies (Gargano, Wehner, & Reeves, 2008; Reid et al., 2008; Testai, Cursio, & Gorelick, 2010).

Gender

The number of studies that has examined gender differences with acute stroke care is relatively small. Older women in our study were in the non-*APOE4* group and women in both *APOE* groups were post- menopausal (> 49 years of age), (McKnight et al., 2011). More females were widowed in the non-*APOE4* group and had a grade school education. Although some studies have found women receive less brain imaging, carotid ultrasound, and echocardiogram than men (DiCarlo et al, 2003; Smith, Lisabeth, Brown, Morgenstern, 2005), this was not a finding in our study. In addition, data from the African American Antiplatelet Stroke Prevention Study (AAASPS) (Worrall et al., 2002) demonstrated that there are major differences in stroke risk factors between AA women and men. AA men had a higher incidence of hypertension, tobacco dependence, and alcohol use in this study, which is consistent with findings from AAASPS. In contrast to men, AA women had a higher incidence of hypertension, diabetes mellitus Type 2, and obesity in this study, which is also consistent with findings from AAASPS.

Socioeconomic Status

Our patient profile indicated a group near or at poverty level with education predominantly at the grade school level and multiple risk factors. According to U.S. Census guidelines, poverty level is defined as an income < 23,850 for a family of four (U.S. Department of Health and

Human Services, 2014). The majority of patients in the *APOE4* group earned < 16,000 per year. In the non-*APOE4* group, the majority of patients earned < 24,000 per year. Our patient profile is consistent with data from the Greater Cincinnati/Northern Kentucky Stroke Study (GCNKSS), one of the largest population-based surveillance studies of racial differences, which found lower community socioeconomic status (SES) to be significantly associated with higher stroke incidence and that this effect was similar for AAs and European Americans, alike (Kleindorfer et al., 2006). Amarenco et al. (2014) found among 516 patients from 17 countries unemployment status, living in a rural area, not living in a fully serviced accommodations (e.g. house or apartment with electricity and water supply), no health insurance coverage, and low education level were predictors of a major vascular event. Lower SES was also associated with a higher prevalence of stroke risk factors, which was consistent with our findings. Our patient profile supports SES as a risk factor for stroke and is consistent with the idea that low SES may contribute to excess stroke mortality in the southern states (Amarenco et al., 2014; Bravata et al., 2005; Howard et al., 2007; Signorello et al., 2014).

Risk Factors

Hypertension

All patients in this study had a history of hypertension. The non-*APOE4* group had a higher systolic blood pressure than the *APOE4* group, but in both groups, blood pressure was not controlled. Racial and gender differences in severity of hypertension, as reflected by systolic blood pressure and the use of anti-hypertensive medications, are well recognized. When examining medications prior to stroke; only a small percentage of patients were prescribed anti-hypertension drugs. This study did not explore the causal pathway for the lack of prescribed

medications for hypertension, for example, if this finding was due to insurance status, health care access, or potential transportation difficulties to visit primary care physician. Inconsistencies with hypertensive treatment guidelines were found in a study by Henderson, Bretsky, DeQuattro, and Henderson (2003). The investigators found more than 40% of Latinos and AAs were not being treated for hypertension. In a more recent study, investigators who examined prescribing patterns among AA elderly (Yazdanshenas et al., 2014) found that nearly one third of aged AA were not prescribed anti-hypertensive medications based on established clinical guidelines.

Diabetes Mellitus Type II

In the non-*APOE4* group, there were more female patients with a history of diabetes mellitus type II. The normal range for hemoglobin A1Cas a measure of glycemic control for the preceding 2-3 months is < 7.0% (Bazerbachi, Nazarian, Alraiyes, & Alraies, 2014). AAs tend to have higher HgA1C levels by 0.65% in comparison to *non*-Hispanic whites (Kirk et al., 2006). The non-*APOE4* group had a higher HgA1c level (6.9 versus 6.3), indicating poor glycemic control. Fiorentino et al. (2014) examined the inflammatory profile of individuals with pre-diabetes by HbA1C levels and found that among individuals with HgA1C levels of 5.7-6.4%, a significant increase of hsCRP, fibrinogen, and WBC was evident. Studies also suggest AAs tend to have poorer glycemic self-management and glycemic control than *non*-Hispanic whites (Sayda, Cowie, Eberhardt, De Rekeneire, & Narayan, 2007; Campbell, Walker, Smalls, Egede, 2012). The significance of poor glycemic control is that these patients develop micro- and macro-vascular complications earlier. These patients also demonstrate poor control with other co-morbidities, such as blood pressure and lipid control (Campbell, Walker, Smalls, Egede, 2012), further predisposing them to a major vascular event.

Obesity

The women in the non-*APOE4* group who predominantly had diabetes also had greater BMI, which was statistically significant. A BMI > 30 kg/m^2 is classified as obese and its incidence is higher in minority populations, especially AA women (Smith et al., 2005). Previous studies have shown that almost 50% of the excess risk of diabetes in AA women, but not men can be explained by BMI (Brancata, Kao, Folsom, Watson, & Szklo, 2000). Henry-Okafor et al. (2012) investigated the association between obesity and risk factors among AA women and found obese AA women had elevated systolic and diastolic BP, HDL, triglycerides, hsCRP, and fibrinogen in comparison to normal weight women. In addition, the ARIC study investigated whether the association between obesity and ischemic stroke differed for AAs versus European Americans. They found that obesity was a significant risk factor for ischemic stroke regardless of race or gender (Yatsuya et al., 2010).

Hyperlipidemia

Triglycerides and HDL levels are usually evaluated together and typically are inversely correlated. For example, if triglyceride levels are high, HDL levels are low. Generally, AAs have lower triglyceride levels and higher HDL levels than European Americans and this lipid profile is more often seen in AA men than women (Sophia, Castillo, Courville, & Sumner, 2012). This was not a finding in the *APOE4* group, which exhibited normal triglyceride levels and low HDL levels. Normal triglyceride levels and low HDL levels is the characteristic lipid profile of insulin resistance that has been found in not only AAs, but also West Africans, and black South Africans (Sumner et al., 2010; Goedecke et al., 2010). In contrast, patients in non-*APOE4* group had higher triglyceride levels and low HDL levels. This lipid profile is highly atherogenic and is

often referred to as the “dyslipidemia of insulin resistance” (Sophia, Castillo, Courville, & Sumner, 2012).). Elevated triglyceride levels commonly leads to HDL reduction and increase in atherogenic LDL levels. Triglycerides may also stimulate atherogenesis by production of pro-inflammatory cytokines, fibrinogen, coagulation factors, and impairment of fibrinolysis (Tenebaum, Klempfner, & Fisman, 2014). Further, this lipid profile was not surprising given that there were more women in this group. The lipid profile for women is different compared to men. HDL decreases and LDL increases post menopause. LDL particles become more dense and subsequently, more atherogenic (Mottillo et al., 2010). Further, evidence suggests that elevated triglycerides are more highly associated with vascular disease in women than men. In a meta-analysis, it was shown that an increase in triglycerides of 18 mg/dL was associated with a 76% increased cardiovascular risk in women compared with 32% increased risk in men (Hokanson & Austin, 1996).

Smoking

Consistent with national data, we found that men reported higher rates of cigarette use in this study, though pack years between groups was the same. There were more smokers in the *APOE4* group than the non-*APOE4* group. Smokers in this study all had hypertension. Studies demonstrated that hypertension and cigarette use were two of the most significant risk factors for young AAs with vascular disease (Rohr et al., 1996; Worrall et al., 2002). While we did not investigate inflammatory differences among smokers and non-smokers, Kawada (2015) found that after adjusting for age, fibrinogen, WBC, and CRP were elevated among smokers compared to non-smokers and ex-smokers among 5102 working men aged 30-60 years old.

Risk Factor Clusters

In order to understand the risk factor burden and inflammatory load, we examined risk factor clusters. Hypertension affected all patients in this study. The proportions of diabetes were high overall with more women affected than men. Histories of hyperlipidemia were similar. More men were affected by cigarette use, though pack years did not differ between *APOE* genotype groups. Over 83% of the women in the non-*APOE* group were obese (BMI > 30). Additionally, more than 92% of participants in this study had two or more risk factors. The *APOE4* group had the greatest percentage of two risk factors; while the non-*APOE4* group had the greatest percentage of three risk factors. While this is the first report of risk factor clusters among *APOE* genotypes and AAs with ischemic stroke, we did not examine gender differences among AAs with ischemic stroke due to small sample size. AAAASPS found at least four risk factors for AA women with ischemic stroke as compared to three risk factors for AA men with ischemic stroke (Worrall et al., 2002).

Studies have also shown major differences in risk factor profiles between AAs and European Americans. While AAs had a greater incidence of hypertension and diabetes, European Americans had a greater incidence of cardiac factors such as history of myocardial infarction and congestive heart failure (Lynch, Geurgan, Raman, Barboi, & Gorelick, 2001). Hypertension and diabetes mellitus is recognized as the most widely contributing factors to the incidence of stroke among AA, accounting for one-half and one-third of the joint mediating effect of all risk factors combined respectively (Howard, 2013; Howard et al., 2011). It should also be noted that the prevalence of metabolic syndrome (the clustering of obesity, abdominal obesity, dyslipidemia, hypertension, and diabetes) is similar among men and women with stroke, but its effect on stroke

is greater in women (Reeves et al., 2014). Metabolic syndrome as a cluster of risk factors was not examined in this study, but among the non-*APOE* carriers, there were more women with greater BMI, diabetes, and hypertension. The North Manhattan study which examined metabolic syndrome in a multiethnic population found that metabolic syndrome on stroke risk was greater in women than men, suggesting that metabolic syndrome may be more potent among women (Boden-Albala et al., 2008). These sample characteristics may be a consideration for the lack of statistical significance between inflammatory load and *APOE* genotypes among AA with ischemic stroke.

APOE Genotypes

The frequency of *APOE* genotypes found in this study was consistent with comparable studies that examined *APOE* genotypes among African Americans. The hypothesis for AIM 1 was supported by averaging the *APOE* genotype frequencies from three national studies and using it as a reference for comparing the frequency of non-*APOE* carriers versus *APOE4* carriers in this study (Audrey et al., 2009; Liang et a., 2013). Our findings were similar to these studies.

Inflammatory Markers and APOE Genotypes

Although our study found no statistical significant differences inflammatory and anti-inflammatory markers between *APOE* genotypes and did not support Aim 2, it is noteworthy that to the best of our knowledge, this was the first study to report inflammatory markers and *APOE* genotypes among AA with ischemic stroke.

Fibrinogen

This was the first study to examine fibrinogen levels and *APOE* genotypes among AAs with ischemic stroke. The standard normal range for fibrinogen is 145-348 mg/dl. Findings from the

ARIC study showed that AAs had higher fibrinogen levels than European Americans (Folsom et al., 1992). Additionally, data from the Multi-Ethnic Study of Atherosclerosis (MESA) showed that fibrinogen levels were 2.7% higher in AAs with greater African ancestry as compared to other ethnic groups. Elevated fibrinogen levels increases the risk for cardiovascular disease and stroke through its role in platelet aggregation, plasma viscosity, and fibrin formation, thus, contributing to the health disparity among AAs (Lutsey et al., 2012; Smith et al., 2005). Fibrinogen is also known to mediate the thrombogenic effect of other risk factors. For example, fibrinogen levels increase with the number of cigarettes smoked and then quickly falls after smoking cessation (Kannel, 2005). Fibrinogen levels in this study were elevated in both *APOE* genotypes in comparison to the reference range and were higher in the non-*APOE4* carriers, but the finding was not statistically significant and again, could be the result of inadequate power and small effect size.

Fibrinogen is an acute phase protein and is elevated in inflammatory states. Studies measuring fibrinogen during stroke are conflicting. Some investigators found higher levels of fibrinogen in acute stroke patients compared to stroke-free control group (Marquaradt et al., 2005; Taman, Iltumur, & Apak, 2005). Beamer et al. (1998) found fibrinogen levels were elevated up to one year after acute stroke compared to healthy control subjects. In some studies fibrinogen level increased during stroke course (Marquardt et al., 2005) whereas in other studies there was no evidence of a time trend seen (Tamam, Iltumur, & Apak, 2005; Elkind et al., 2006).

Stroke severity is also a major determinant of acute phase reactions after stroke. Fibrinogen levels have been shown to positively correlate with stroke severity on days 1, 14, and 90 after stroke (Marquardt et al., 2005). In contrast, patients in this study had elevated fibrinogen levels,

but the majority of patients had mild strokes evidenced by NIH stroke scale of 0-5. Perhaps fibrinogen levels were mediated by risk factor clusters as previously discussed. Gender differences are also a consideration. Thorand et al. (2006) investigated systemic inflammation and measures of obesity (BMI and fat mass) and abdominal adiposity (waist circumference) among men and women aged 55-74. The investigators found obesity measures highly correlated with CRP, fibrinogen, and IL-6 in participants. In women, fat mass in percentage explained the highest percentage of variability with acute phase proteins (CRP and fibrinogen). Adiposity, was found to strongly associate with low grade inflammation, but was particularly stronger in women. Further, age is also a consideration. Spada et al. (2004) investigated fibrinogen levels among the elderly and found that fibrinogen changes with normal aging. There was a 19% increase in fibrinogen between patients in the 50-59 age group compared to patients in the 70-79 age group. The non-*APOE* group had older women and more risk factor clusters in comparison to the *APOE* group. These characteristics may explain the higher fibrinogen level found in the non-*APOE4* group.

CRP

CRP is a marker of systemic inflammation and also a non-specific acute phase protein. The standard normal range for CRP is 0-10 mg/ml and for hsCRP is < 3 mg/l. Although there is limited data available on ethnic differences in CRP levels, CRP levels have been found to be higher in AAs and females (Kelley-Hedgepeth et al., 2008; Lakoski et al., 2006; Khera et al., 2005). CRP levels have also been shown to be 30% higher in AAs than European Americans (Khera et al., 2005). There is conflicting data on associations of CRP with risk factors. Some studies show AA women tend to have the highest levels of CRP, even after controlling for risk

factors that influence cardiovascular risk, such as BMI, smoking status, hypertension, diabetes mellitus, and high cholesterol (Doumatey et al., 2010; Laksoski et al., 2006; Morris & Ferdinand, 2009); while other studies show an association between AA women with higher CRP levels and modifiable risk factors, particularly BMI (Kelley-Hedgepeth et al., 2008; Khera et al., 2005). The findings of this study found CRP levels were 1½ times higher with the non-*APOE4* carriers in comparison to the *APOE* carriers, but both groups had elevated values in comparison to reference range. The non-*APOE* carriers had older women with more risk factors, particularly higher BMI.

There are many studies that have examined CRP and stroke, but studies examining levels among AAs with stroke are scarce. Increases in CRP may reflect a systemic inflammatory response following ischemic stroke, the extent of tissue injury, or concurrent infection (Hertog et al., 2009). Studies that have examined the temporal course of CRP after stroke in European Americans demonstrate different findings. Acalovschi et al. (2003) found CRP levels increased on days 3 and 7. Emsley et al. (2003) studied changes in IL-6 and CRP in 36 patients with ischemic stroke and 36 control subjects matched for not only age and gender, but also for degree of atherosclerosis. The investigators found CRP level was higher on admission and remained elevated until three months compared to control group. The greatest elevation occurred at days 5 and 7. Taman, Iltumur, and Apak. (2005) found CRP level was elevated in stroke patients on day 1, reached a peak at day 3, and started to decrease on day 10 after stroke. Di Napoli, Pappa, and Bocola (2001) found CRP levels in ischemic stroke patients changed between admission and discharge from the hospital. Different patterns were observed: persistently normal values were found in 19.5% of all patients (n=128), increasing values in 6.3%, decreasing values

in 28.1%, and persistent elevation in 46% of patients. Marquart et al. (2005) measured CRP level in 50 patients with acute ischemic stroke, in 30 healthy control subjects, and 20 controls matched for stroke risk factors. The majority of patients had mild stroke. CRP level measured on days 1, 14, and 90 was higher in stroke patients compared to healthy controls, but not to risk factors control subjects. Normal CRP levels were found in 38% of patients on days 1 and 90. Similarly, Elkind et al. (2006) found no evidence of time trend of CRP in 21 patients with mild ischemic stroke, however, CRP level in these patients was higher than in 1776 stroke-free subjects from the same community. Audebert, Rott, Eck, and Haberl (2004) found successful thrombolysis reduced CRP concentrations on days 3 and 5. Beamer et al (1998) investigated the changes of acute phase proteins during one year after stroke. CRP levels declined gradually after stroke. Longitudinal concentrations of CRP levels were markedly elevated compared with healthy elderly, but not compared with risk factor group.

The majority of patients in this study were classified as having mild stroke evidenced by NIH stroke scale scores of 0-5 with elevated CRP values found in both groups. The non-*APOE4* group had more patients with moderate stroke based on NIH stroke scale scores of 6-13 in comparison to the *APOE4* group (23% versus 16%). This finding differed from previous studies. Christensen and Boysen (2004) observed significant CRP increase within 24 hours after admission only in severe stroke, but not in those with mild or moderate stroke. Similarly, Idicula, Brogger, Naess, Waje-Andreassen, & Thomassen (2009) found elevated CRP levels were associated with more severe stroke. Additionally, only a few studies have analyzed the relationship between elevated admission CRP levels and stroke severity or stroke etiology. All of the patients in this study had ischemic strokes caused by large artery atherosclerosis. Zing et al.

(2013) found CRP was closely associated with stroke subtype and that plasma CRP was higher in those with large artery atherosclerosis. Additionally, the investigators found that IL-6 and fibrinogen correlated with the plasma CRP. More studies are needed to clarify CRP with ischemic stroke, particularly among AAs and other ethnic groups.

Cytokines

TNFα. This was the first study to examine TNFα and *APOE* genotypes among AAs with ischemic stroke. The standard normal range for TNFα is 5-27 pg/ml. With healthy AAs, TNFα was not found to be elevated (Doumatey et al., 2010). Studies of TNFα serum levels after stroke have not been conclusive. A few studies found that serum TNFα was not increased or any time between 12 hours and 10 days after stroke (Doll, Barr, & Simpkins, 2014; Montaner et al, 2003; Fassbender, Schmidt, Mossner, Daffertshofer, & Hennerici, 1994; Ormstad, Aass, Lund-Sorensen, Amthor, & Sandvik, 2011). Other studies, however, have shown that TNFα is increased in the serum of stroke patients compared to controls within 6 hours and stay elevated for 10 days post-stroke (Doll, Barr, & Simpkins, 2014; Intiso et al., 2003; Zaremba & Losey, 2001). Furthermore, studies measuring TNFα in plasma observed a significant increase of TNFα in stroke patients at admission and within 24 hours post stroke (Doll, Barr, & Simpkins, 2014; Castellanos et al., 2002; Vila, Castil, Davalos, & Chamorro, 2003). Our findings showed TNFα was not detectable in AAs with ischemic stroke. Consideration for this finding must be given to sampling, processing, and/or assay error.

IL-1β. This was the first study to examine IL-1β and *APOE* genotypes among AAs with ischemic stroke. The standard normal range for plasma IL-1β is < 0-3 pg/ml. Studies examining IL-1B among AAs are scarce. IL-1β was also not quantifiable in our study. Studies examining

peripheral blood found elevated levels of IL-1β in plasma and serum of stroke patients (Ormstad, Aass, Lund-Sorensen, Amthor, & Sandvick, 2011; Fassbender, Schmidt, Mossner, Daffertshofer, & Hennerici, 1994; Tarkowski et al., 1995) However, other studies show no increase in IL-1β in serum or plasma (Licata et al., 2009; Mazzotta et al., 2004). IL-1β plays a highly localized role at the site of inflammation and is considered a neurotoxic mediator (Kim, Kawabori, & Yenari, 2014). This localized role may be why IL-1β is not seen in plasma or serum of stroke patients. Additionally, as with TNFa, consideration for this finding must be given to sampling, processing, and/or assay error.

IL-6._ This was the first study to examine IL-6 and *APOE* genotypes among AAs with ischemic stroke. IL-6 is a key mediator of acute phase reaction. The standard normal range for IL-6 is 0-4.72 pg/ml. IL-6 is known to be elevated in AAs as compared to European Americans (Paalini, Lee, Haddad, & Tonstad, 2011; Stowe, Peek, Cutchin, & Goodwin, 2010). IL-6 is also known to increase with age (Stowe, Peek, Cutchin, & Goodwin, 2010). Although IL-6 was 4 times more elevated in *APOE4* carriers, it was not statistically significant. Studies consistently show IL-6 to be elevated after stroke. In one of the earliest study investigating acute phase proteins after stroke, Fassbender et al. (1994) measured serum IL-6 level in 19 ischemic stroke patients without pre-existing infection admitted to the hospital within 4 hours after symptoms onset. They observed a significant increase in IL-6 levels on hours 6, 8, 10, 14, and days 1, 3, and 5 compared to IL-6 concentrations measured 4 hours after symptoms onset. On day 7, levels did not differ significantly from those obtained initially after hour 4. In the study by Tarkowsi et al. (1995) serum IL-6 level was significantly higher in stroke patients compared with healthy controls during the whole observation period (day 0, 1, 2, 3, 7-9, 21-26, > 90). However, it did

not display any distinct time-related variation in contrast to changes in cerebrospinal fluid where IL-6 peak level was observed on days 2 and 3. Similarly, Szczudlik, Dziedzic, Bartus, Slowik, and Kieltyka (2004) measured IL-6 level in 22 patients with ischemic stroke and 17 controls. Serum samples were collected on the second day of stroke at 6:00, 10:00, 18:00, and 22:00 hours and at the same time points in control group. Serum IL-6 levels were significantly higher in stroke patients than in controls at each time point. Three months after stroke IL-6 concentrations did not differ significantly between groups. While there is evidence to demonstrate elevated IL-6 levels in stroke patients during the week after stroke onset, the timing of which IL-6 levels peak appear to be dependent on stroke severity and stroke type (Doll, Barr, & Simpkins, 2014). Beamer, Coull, Clark, Hazel, and Silberger (1995) found higher IL-6 levels in patients with infarcts of greater than 3 cm and lowest in patients with lacunar strokes. The majority of our patients had mild stroke, which may explain the lack of statistical significance in levels of IL-6. Additionally, elevated levels of IL-6 and IL-1β support previous findings that IL-6 suppresses the effects of TNFα and IL-1β by inhibiting their production (Doll, Barr, & Simpkins, 2014; Ormstad, Aass, Lund-Sorensen, Amthor, & Sandvik, 2011).

IL-8. This was the first study to examine IL-8 and *APOE* genotypes among AAs with ischemic stroke. IL-8 is a potent chemoattractant and pro-inflammatory cytokine that has been demonstrated to recruit in vivo polymorphonuclear leukocyte (PMNL) (Colditz, Zwahlen, Dewald, &Baggiolini, 1989). IL-8 is also known to delay PMNL apoptosis, indicating that IL-8 can further prolong and amplify the effects of PMN (Kettritz et al., 1998). The standard normal range for IL-8 is 0-63 pg/ml. Studies of IL-8 are to the best of our knowledge, nonexistent in AAs with ischemic stroke. Although there was no statistical significance, IL-8 levels were 2.5

times higher with *APOE4* carriers. Few studies found a significant elevation of plasma IL-8 in ischemic stroke patients (AL-Bahrani, Taha, Shaath, & Bakhiet, 2007; M.; Kostulas, Pelidou, Kivisakk, Kostulas, & Link, 1999; Ormstad, Aass, Lund-Sorensen, Amthor, & Sandvik, 2011). Grau et al. (2001) found IL-8 level was increased on day 1 after stroke and remained elevated on days 3 and 7 in patients with ischemic stroke as compared to age and gender matched healthy control subjects. Although not examined in this study, Ormstad, Aass, Lund-Sorensen, Amthor, and Sandvik (2011) found IL-8 was elevated in ischemic stroke and was significant after adjusting for age. This finding indicated that IL-8 may be a more prominent cytokine in older patients. Higher IL-8 levels in the *APOE* group who were much younger than the non-*APOE4* group did not support this finding. It is not known if IL-8 may contribute to older age and predicts poor functional outcomes and mortality after stroke Ormstad, Aass, Lund-Sorensen, Amthor, & Sandvik (2011). More studies are needed to verify the role of IL-8 in older AAs with ischemic stroke.

IL-10. IL-10 is an anti-inflammatory cytokine. This was the first study to examine IL-10 and *APOE* gentoypes in AAs with ischemic stroke. The standard normal range for IL-10 is < 2.0 pg/ml. A study by Paalani, Lee, Haddad, and Tonstad (2011) found no ethnic differences in IL-10 in AAs compared to European Americans. IL-10 has been shown to be lower in plasma within 12 hours after stroke compared to controls and 24 hours after tPA treatment (Doll, Barr, & Simpkins, 2014; Mazzotta et al., 2004; Perini, Morra, Alecci, Galloni, Marchi, & Toso, 2001). Studies have also shown IL-10 levels to be decreased within 24 hours of stroke and increased over 72 hours post-stroke (Doll, Barr, & Simpkins, 2014). Studies also report a huge variability in the levels of IL-10, which is most likely due to the time the sample was taken and stroke

severity (Doll, Barr, & Simpkins, 2014). Chang et al. (2010) examined IL-10 levels in 135 patients with ischemic stroke, in 20 healthy control subjects, and 30 at risk control subjects. IL-10 was significantly higher in stroke patients than in both control groups. Investigators also found IL-10 levels to be higher in patients with severe neurological impairment (NIH stroke scale > 12) compared to patients with less severe neurological impairment. Also, IL-10 was strongly and independently correlated with several neurological impairment 48 hours after acute ischemic stroke and predictive of combined major clinical adverse outcomes (e.g. recurrent ischemic stroke or any cause of death, or NIH stroke scale > 12) on day 90 following stroke. Similarly, patients in this study had higher IL-10 levels in comparison to the reference range with a higher level noted in the non-*APOE* group. Unlike the Chang et al. study (2010), stroke severity was mild in the majority of patients (NIH stroke scale 0-3). Heterogeneity of co-morbidities among stroke patients could play a role in the variability levels of IL-10 and other cytokines after stroke (Doll, Barr, & Simpkins, 2014), but more studies are needed to characterize these levels in AAs with ischemic stroke.

Leukocyte Activation

Little is known about leukocyte activation among AAs with ischemic stroke. Leukocytes, which include neutrophils as a subpopulation of WBC has not been extensively studied in human stroke studies. The standard normal range for WBC is 5-10 cells/ml with neutrophils accounting for 40-60%. In this study, patients had WBC within the normal range overall with slight increase noted in the non-*APOE4* carriers. However, this finding was not statistically significant. Furthermore, to the best of our knowledge, this was the first study to examine neutrophil surface expression of CD11β among AAs and *APOE* genotypes with ischemic stroke. Neutrophil surface

expression of CD11β, a member of the B2 integrin family, is an indicator of neutrophil activation, mediates the leukocyte binding with the endothelium, causes leukocyte aggregation, and is involved with moving the cell into inflamed tissues, thus contributing to reperfusion injury. The findings from this study showed increased neutrophil expression in the overall group and within both genotypes groups. These findings were statistically significant and consistent with studies utilizing murine models that have shown neutrophil activation following stroke (Zhou et al., 2013; Morrison, McKee, & Ritter, 2011). Further, the addition of fMLP, a known neutrophil activation, increased neutrophil expression in the overall group and within both genotype groups and was statistically significant. However, neutrophil expression and with the addition of fMLP between groups was not statistically significant. Several studies have examined the integrin patterns (e.g. CD11a, CDllβ, CD11c, and CD18) of polymorphonuclear leukocytes before and after stroke. In an earlier study, Kim et al. (1995) examined leukocyte activation in patients with stroke as compared to controls. Kim et al. (1995) examined the expression of integrins, CD11a and CD18 in ten patients with ischemic stroke, 6 patients with transient ischemic attack (TIA), and 11 age and risk factor matched controls. CD11a was measured within 72 hours after onset of ischemia. Follow-up measurements were also performed at 5-7 days in 6 patients with stroke, and at 3-5 days in 3 patients with TIA. CD11a was significantly increased within 72 hours after onset of symptoms in patients with stroke as well as TIA compared with the control group. CD18 was also increased in both groups, but significance was reached only in the TIA group. Follow up measurement of CD11a and CD18 showed a trend of decrease, but CD11a remained significantly elevated compared to the control group. Similarly, Caimi et al. (2001) examined the integrin pattern using flow cytometry at baseline and during activation,

using fMLP in 19 patients with ischemic stroke. At baseline, an increase in the expression of CD11c and CD18 and a decrease in CD11β were shown in ischemic stroke patients compared to healthy control subjects. After activation, a constant and significant increase of all integrins was found in healthy control subjects, while ischemic stroke patients demonstrated an increase in CD11β and CD18, a decrease in CD11a, and no variation in CD11c. Measures of neutrophil activation may be useful indicators of clinical outcomes and reperfusion injury associated with ischemic stroke, but to date have not been thoroughly investigated in ischemic stroke. Moreover, neutrophil expression may reflect the severity of ischemic stroke. Tsai et al., 2009 evaluated whether leukocyte adhesion molecules can predict clinical outcome in patients after ischemic stroke. Investigators found in 65 acute stroke patients and 60 health control subjects. CD11b/CD18 was increased on days 1 and 7 after stroke than in controls. Leukocyte expression positively correlated with NIH stroke scale scores on admission. Additionally, diabetes mellitus and NIH stroke scale score on admission were independently associated with 3 month outcome. Further studies are needed to characterize neutrophil activation in ischemic stroke and particularly among AAs and other ethnic groups with ischemic stroke.

Platelet Leukocyte Aggregates

Platelets play a crucial role triggering arterial thrombosis and in promoting atherogenesis. The formation of PLA and leukocyte activation contributes to vascular repair and microcirculatory disturbances in ischemic tissue (Htun et al., 2006). Activated platelets degranulate and adhere to leukocytes, thus forming PLA (surface expression of CD42β). PLA, are therefore, a sensitive marker of platelet activation as suggested in studies in coronary heart disease (Marquardt et al., 2009). To the best of our knowledge, this is the first study to examine

PLA in AAs with ischemic stroke. The findings of this study showed decreased CD42β expression overall and lower values when activated with PAF in both *APOE* groups as compared to PLA expression from previous stroke studies. Although the CD42β expression was higher in the non-*APOE4* group compared to the *APOE* group, this finding was not statistical significant. The findings from this study do not support earlier findings of PLA and ischemic stroke. Zeller, Lenz, Eschenfelder, Zunker, and Deuschl (2005) investigated PLA and platelet activation in 58 stroke patients (21 with and 37 without infection) one week before acute cerebral ischemia and compared them to 58 healthy controls subjects on admission and day 7. Patients with previous infection had higher CD42β expression compared to patients without infection. On day 7, CD42β expression in patients with previous infection was near the values of other patients. Similarly, Htun et al. (2006) investigated platelet activation and PLA in 135 patients with TIA or stroke within 24 hours of clinical onset and after 3 months with 40 healthy control subjects. CD42β expression was significantly increased in the acute phase of stroke and in the acute TIA group compared to the control group. There were no significant differences between patients with TIA or stroke either in the acute phase or 3 months later. After 3 months, CD42β expression returned to a normal level, comparable to that in the control group. The findings also did not support stroke studies with murine models of acute stroke (Ritter, Stempel, Coull, & McDonough, 2005). One possible reason for our finding was the use of aspirin. Serebruany et al. (2004) investigated PLA expression (CD42β) in 120 patients divided into three groups: aspirin-free patients after ischemic stroke, post stroke patients receiving aspirin, and aspirin free subjects with multiple risk factors for vascular disease. Finding revealed that PLA was significantly lower in the aspirin treated group compared with healthy subjects with risk factors for vascular disease. Similarly,

Cao, Wang, Zhang, Zeng, and Liu (2009) investigated PLA in 40 patients with acute cerebral infarction and 20 healthy controls. The patients with stroke were assigned to two treatment groups: the aspirin group and the clopidogrel group. PLA was decreased in both groups, but the clopidogrel group was lower than the aspirin group. Platelet activation is important in ischemic stroke as aspirin serves as the primary and secondary prevention. Larger scale trials are warranted to clarify the role of PLA with ischemic stroke, particularly in AAs and other ethnic groups.

APOE4 as a Predictor of Inflammatory Load

This was the first study to examine risk factors, inflammatory markers, and *APOE* genotypes among AA with ischemic stroke The finding did show a higher composite score of inflammatory load for *APOE4* carriers, but this finding was not statistically significant. Aim 3, was therefore, not supported and *APOE4* carriers is not a predictor of inflammatory load. The higher composite score of inflammatory load found in *APOE4* carriers is in agreement with previous studies, which did show *APOE4* carriers in animal models to have a pro-inflammatory state (Lynch et al., 2003' Lo-Ming, Wong, Liu, & Ho, 2007). Further, the lack of statistical significance of inflammatory load among *APOE* genotypes is in contrast to previous studies, which did show *APOE4* carriers in animal models to have a significant difference among pro-inflammatory markers (Lynch et al., 2003' Lo-Ming, Wong, Liu, & Ho, 2007). The most plausible explanation of these findings was that the study was underpowered and small effect sizes were not sufficient to create statistical significant findings.

Correlation of Risk Factors and Inflammatory Markers Among APOE Groups

Due to the lack of statistical significance, we examined correlations among risk factors and inflammatory markers among *APOE* groups. Statistical significant findings were found for both *APOE* groups.

Among the ApoE4 carriers: There was a small positive relationship between CRP and fibrinogen and also a large positive relationship between age and fibrinogen, which were statistatically significant. Studies have supported a relationship between CRP and fibrinogen, particularly among individuals with vascular risk factors and ischemic vascular diseases Grau, Buggle, Becher, Werle, and Hacke (1996) examined inflammatory markers in individuals with vascular risk factors and also a history of ischemic vascular diseases in 197 individuals and matched for age and gender patients with ischemic stroke or TIA. Findings revealed hypertriglyceridemia, peripheral arterial disease, and diabetes mellitus were associated with CRP. Age > 65 and diabetes mellitus independently increased fibrinogen. Individuals with history of stroke or cardiovascular disease had higher fibrinogen and CRP levels compared to individuals without vascular risk factors and higher fibrinogen levels than individuals with one or more risk factors. Individuals under the age of 65 with vascular risk factors but without ischemic disease had higher fibrinogen and CRP levels. Individuals older than 65 with vascular risk factors had higher CRP levels than individuals without risk factors or ischemic diseases. The findings support the hypothesis that low grade inflammation is associated with vascular risk factors and those inflammatory mechanisms may contribute to ischemic vascular diseases.

There was also a large positive relationship between PLA and BMI, which was statistically significant. Studies in animal models have provided insight to the mechanisms involved with

obesity and ischemic diseases (Biokhin & Lentz, 2013; Schafer & Konstandtinide, 2011). Changes in platelet biology and function may underlie the thrombotic risk in obesity, which include elevated platelet counts, an increase in mean platelet volume, and an in increase PLA response. Specific adipokines mediate the prothrombotic state of obesity. In particular leptin enhances both arterial and venous thrombosis by promoting platelet adhesion, activation, and aggregation. CRP enhances the formation of PLA and platelet adhesion to endothelial cells.

Among the non-APOE4 group: There was a large negative relationship between PLA and IL-6, which was statistically significant. Increasing evidence has shown that circulating platelets interact with leukocytes and endothelial cells in inflammation, vascular remodeling, and thrombosis (McGregor, Martin, & McGregor, 2006). Leukocyte binding to activated platelets has shown increased levels of proinflammatory cytokines such as IL-6 and IL-8 (Gros, Ollivier, & Ho-Tin-Noe, 2014). Thus, the negative correlation of PLA and IL-6 is not surprising, given that neither marker was statistically significant in this study.

There was also a large negative relationship between CD11β and HDL, which was statistically significant. HDLs represent a family of particles that are able to transport cholesterol from peripheral tissues back to the liver. Additionally, HDLs also display antioxidant, anti-apoptotic, anti-inflammatory, and anti-thrombotic properties that account for their protective action on endothelial cells (Dinh et al., 2013; Meilhac, 2015). HDLs modulate the effects of neutrophil activation. Murphy et al. (2008) investigated the anti-inflammatory effects of HDL on endothelial cells and found CD11β expression was inhibited by HDLs (Murphy et al., 2008).

Limitations

There were several limitations to this study. Biomarkers were measured only at one time point (3 days post-stroke). In addition to other chronic inflammatory states, other factors need to be considered, such as confounding variables such as medications, and sampling, processing, or assay errors. Another limitation was the lack of a comparison group. However, the major limitation was the lack of power resulting from low enrollment in this study, which ultimately, may lead to Type II errors.

Enrollment of subjects in this study was lower than desired and somewhat surprising based on enrollment of AAs in other research studies conducted at this institution (personal communication with director of stroke program). However, the enrollment (14%) was similar to that reported in the Federal Drug Administration clinical trials (17%) (George, Duran, & Norris, 2014). Within two months of enrollment, we reevaluated our recruitment strategy, which included a change in script to embed altruistic reasons for participation. Enrollment improved slightly following this action, but clearly, evaluation of recruitment strategies needed to remain ongoing. During the sixth month of the study, we elicited the assistance of a Hispanic research coordinator to assist with recruitment. The ideal would have been a dedicated research coordinator of AA heritage, but we did not have access to such a member on the research team. Similar to previous findings of perceived barriers for research participation among minorities, we also found that discussion of ongoing research participation differed among subjects and their family members (George, Duran, & Norris, 2014). Twenty one percent of AA stroke patient met eligibility criteria in this study, yet declined to participate. Most family members of moderate to severe stroke patients declined participation in the study, resulting in mild stroke as the

predominant type of stroke in this study. We agree with minority health research investigators that with minority research, consent to participate in a study should be an ongoing process, rather than a singular moment in time and that it is important to include families in the consent process. Additionally, partnering with a community organization that visited research patients, or identifying a cultural broker may have offered the additional benefit of a culturally appropriate recruitment and retention strategy throughout the study.

Implications for Future Research

This was the first study to examine relationships among risk factors, inflammatory markers, and genotypes among AA with ischemic stroke. Human studies that have examined genetic influences and the various inflammatory roles and response of immune cells (damage versus repair) as a mechanism for ischemic stroke and associated outcomes among AAs are scarce. Moreover, fewer studies have examined novel risk factors for stroke among AAs. More studies are needed to not only investigate *non*traditional and novel risk factors, but also characterize inflammatory and genetic mechanisms with ischemic stroke and their associated outcomes among AAs. Such studies may lead to primary and secondary prevention of ischemic stroke and lessen the health disparities associated with ischemic stroke among AAs. Ultimately, studies of this kind may reduce mortality, morbidity, and disability from ischemic stroke among AAs.

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