NOVEL RISK FACTORS AND PREVENTION FOR STROKE

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DEDICATION

For my parents, Guijing Wang and Suzhen Zhao, who have always been my strongest supporters and encouraged me to follow my ambitions.

ABSTRACT

Stroke is a major public health problem and is a leading cause of long-term disability. Identifying novel risk factors could potentially aid in understanding the mechanisms and pathophysiology of stroke. In addition, atrial fibrillation (AF) is a strong risk factor for stroke and there is a need to understand the risks associated with stroke prevention therapies.

Using data from the Atherosclerosis Risk in Communities (ARIC) study, the first manuscript analyzed the cross-sectional association of atrial myopathy—characterized by abnormalities in left atrial (LA) function and size—and brain MRI markers of silent cerebrovascular disease. Several measures of lower LA function were associated with cerebral microbleeds and brain infarcts.

In the second manuscript, we explored the relationship of 4,877 plasma proteins to risk of incident ischemic stroke in the ARIC study. Among the proteins that were identified in ARIC, three proteins were validated in an external cohort, the Cardiovascular Health Study. Mendelian randomization analysis suggested that NT-proBNP may be causally related to ischemic stroke.

The third manuscript used data from the Medicare 20% sample to assess stroke prevention among patients with AF. The Watchman implant is a minimally invasive procedure for patients with AF who are at an elevated risk for stroke and have an oral anticoagulant contraindication. Compared to oral anticoagulant users, patients with the Watchman device did not have an increased risk of stroke or death. However, an increased risk for bleeding was noted, though this risk was more pronounced in the initial 180 days after the procedure.

Collectively, this dissertation identifies novel risk factors for stroke that could contribute to future stroke prevention efforts and also assesses risks associated with current prevention therapies.

TABLE OF CONTENTS

List of Tables

List of Figures

Chapter 1. Introduction

Stroke is a leading cause of serious long-term disability in adults and the fifth leading cause of death in the US.[1] The prevalence of stroke increases with age, and it is projected that 3.9% of the adult population in the US will have a stroke.[1] Lifetime risk of stroke in the US, based on estimates from the Framingham Heart Study (FHS), is approximately 1 in 5 for women and 1 in 6 for men.[2]

Vascular brain injury seen on brain MRI scans, including brain infarcts, cerebral microbleeds, and white matter hyperintensities (WMH), increases the risk for clinical stroke, dementia, and death.[3] Given that the presence of brain MRI markers of vascular brain injury may lead to adverse events, identifying risk factors is of importance. Atrial myopathy, defined by left atrial (LA) functional and structural abnormalities, has been shown to be associated with dementia.[4] Mechanisms for this association are currently not well-characterized, but it is plausible that vascular brain injury underlies this association.

Several risk factors for stroke (e.g., age, hypertension, and atrial fibrillation [AF]) have been identified[1] and research has shown that plasma biomarkers are linked to higher stroke risk.[5-9] Newer technology now allows for a large number of proteins to be screened using proteomic approaches. Therefore, proteomic analyses have the potential to discover novel protein associations, which could provide further insight into the pathophysiology of stroke.

Individuals with AF have a 5-fold increased risk of stroke.[1,10] Pharmacological options (warfarin, direct oral anticoagulants [DOACs]) are often initiated for stroke prevention among those with AF. More recently, percutaneous left atrial appendage occlusion (LAAO) has emerged as an alternative stroke prevention strategy in those with

rationale for a nonpharmacological option.[11] Clinical trials have shown that patients who

undergo LAAO with the Watchman may have similar rates of adverse events (e.g.,

stroke, death) as oral anticoagulants (OACs) users,[12-17] but this relationship has not yet

been reported in a real-world population.

Chapter 2. Pathophysiology of Stroke

A. Natural History of Stroke

The history of stroke spans several centuries, with the first stroke, then called

"apoplexy," being recognized over 2,400 years ago. In the mid-1600s, it was found that

apoplexy could be caused from bleeding in the brain or from blockage in one of the

brain's vessels.[18] Several advances in the pathophysiology of stroke were made

throughout the years, but in the 20th century, the link between carotid bifurcation disease

and stroke was observed and the first successful carotid endarterectomy was

performed.[18,19] Since then, several therapies for prevention and treatment of stroke have

been used, which will be discussed in a later section.

In 1958, the National Institute for Neurological Disorders and Blindness

(NINDB) originally classified strokes into four major etiologic subtypes: thrombosis with

atherosclerosis, cerebral embolism, other causes, and cerebral infarction of undetermined

origin.[20,21] As computed tomography (CT) of the brain was used more frequently, the

Harvard Cooperative Stroke Registry developed another classification system in 1978

that further categorized stroke etiology into large artery thrombosis, lacunar infarcts, and

embolism.[22] Additional advances in brain imaging and ultrasonography in the 1980s and

1990s allowed more specific stroke subtypes to be defined. In 1986, the Stroke Data

Bank classification categorized ischemic strokes into several different subtypes: large

artery thrombosis, infarct with tandem arterial pathology or arterial embolism, embolism

attributed to cardiac or transcardiac source, lacunar infarction, infarct of undetermined

cause, and infarct with a normal angiogram.[23] Another classification system was created

in 1993 by the Trial of ORG 10172 in Acute Stroke Treatment (TOAST). TOAST

categorized ischemic stroke into five mutually exclusive subtypes: large-artery

atherosclerosis, cardioembolism, small-vessel occlusion (lacune), stroke of other

determined etiology, and stroke of undetermined etiology.[24]

B. Stroke Types and Subtypes

There are two main stroke types: ischemic stroke and hemorrhagic stroke.

Ischemic strokes occur when a blood vessel becomes blocked and impairs blood flow to

the brain, while hemorrhagic strokes occur when a blood vessel ruptures and bleeds.

Ischemic and hemorrhagic strokes can be further classified into various stroke subtypes.

A breakdown of stroke types and subtypes are shown in **Figure 2.1**.
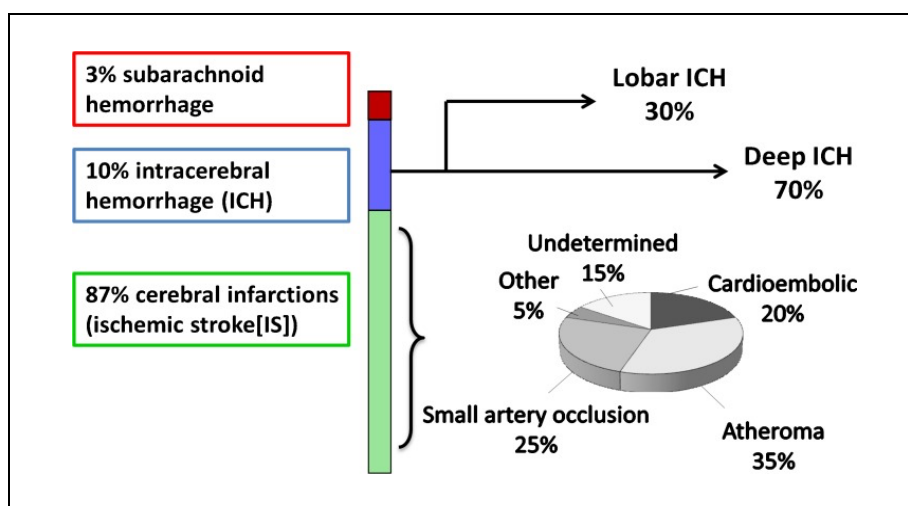
Figure 2.1. Percentage Breakdown of Stroke Types and Subtypes[25]

B.1.   Ischemic Stroke

In the US, the majority of strokes, approximately 87%, are ischemic.[1] Ischemic strokes consists of several subtypes: large artery atherosclerosis, cardioembolism, small artery occlusion, other causes, and undetermined causes.[20] Strokes due to large artery atherosclerosis are diagnosed by having a stenosis of at least 50% or occlusion of a major brain artery, which is likely due to atherosclerosis.[24] Cerebral small vessel disease consists of numerous subtypes, including lacunar infarcts, white matter lesions, and cerebral microbleeds. Lacunar infarcts account for approximately a quarter of ischemic strokes.[26] Lacunar infarcts are smaller in size than those caused by large-vessel occlusions and are often "silent" with no clinical symptoms.[27] Lacunes are often missed on CT scans given their small size, but MRIs generally have higher sensitivity in detecting lacunes.[20] Cardioembolic strokes account for 20-30% of ischemic strokes in the US and are diagnosed by the presence of a cardiac source of embolism, which can be identified by electrocardiograms (ECG), indicating presence of AF, or by transthoracic and transesophageal echocardiograms.[20] AF is a common cause of cardioembolic strokes[20] and will be discussed in a later chapter. Cryptogenic strokes, also known as strokes due to an unknown cause, account for approximately 15% of all strokes.[20,25]

B.2.   Hemorrhagic Stroke

Hemorrhagic strokes occur when a blood vessel ruptures and bleeds. They can be further classified as an intracerebral hemorrhage (ICH), in which the bleeding occurs in the brain,[23] or a subarachnoid hemorrhage (SAH), in which the bleeding occurs in the subarachnoid space. Although only 10% of all strokes are ICH and 3% are SAH,[1]

mortality rates for both ICH and SAH are high.[20] ICH largely occurs in the deep portions of the cerebral hemispheres, with the putamen being the most common site.[23] Causes of ICH include deep perforating vasculopathy and cerebral amyloid angiopathy. SAH often occur due to trauma or an aneurysmal rupture. When excluding trauma, the rupture of a saccular aneurysm at the base of the brain causes 85% of SAH. Major modifiable risk factors of SAH include cigarette smoking and hypertension. Hypertension contributes to hemodynamic stress, which has been considered a key mediator of aneurysm development.[20]

C. Diagnosis and Treatment

Common symptoms of ischemic stroke are unilateral weakness and speech disturbance,[28] while patients with hemorrhagic stroke often present with headaches, nausea, neck stiffness, and vomiting.[23,29] Headaches associated with SAH are particularly intense and sudden, and patients often refer to it as "the worst headache of my life."[20,29,30] Several clinical symptoms (e.g., headache, vomiting, severe hypertension, neck stiffness) are more common among patients with hemorrhagic stroke than ischemic stroke; however, neuroimaging is needed to reliably confirm the diagnosis.[29] A noncontrast head CT scan or brain magnetic resonance imaging (MRI) are used for initial evaluation in patients with suspected strokes and can differentiate between ischemic and hemorrhagic strokes.[20,28,31,32] If a patient with suspected SAH has a negative head CT, a lumbar puncture is performed as it is considered the gold standard to detect SAH.[20] Other diagnostic tests that are also performed in patients with suspected strokes include blood glucose, oxygen saturation, complete blood count, and an electrocardiogram (ECG).[31]

Intravenous recombinant tissue plasminogen activator (rtPA) is a systemic thrombolytic agent that is an US Food and Drug Administration (FDA) approved treatment for ischemic stroke. However, rtPA should be administered within 3 hours of symptom onset.[33,34] Given that many patients are not at the hospital, or their stroke diagnosis is not confirmed with 3 hours of symptom onset, many are unfortunately unable to receive rtPA treatment. For patients who did not respond to rtPA treatment or missed the short timeframe to receive rtPA, the Merci Retriever, Solitaire flow restoration device, and Trevo clot retrieval device are FDA-approved devices that remove the blood clot in patients with ischemic strokes. The Merci Retriever, which was approved by the FDA in 2004, was the first device to be used in patients with ischemic strokes. In 2012, the FDA approved both the Solitaire and Trevo devices after randomized trials reported these devices showed better outcomes than the Merci Retriever.[35,36] More recently, the FDA expanded the treatment window for the Trevo device from 6 hours to up to 24 hours after symptom onset, allowing for the device to be used in a broader group of patients.[37]

The American Heart Association (AHA)/American Stroke Association (ASA) have created guidelines for the diagnosis and treatment of ICH and aneurysmal SAH.[30,32] Controlling elevated blood pressure (BP) quickly is the most promising acute treatment for hemorrhagic strokes. Neurosurgical procedures, such as a craniectomy or insertion of an external ventricular drain, can reduce the effect of ICH; however, this generally only improves survival rates and does not reduce disability.[20] Among patients who have an aneurysm, a cerebral angiography continues to be the gold standard test to identify the location of the aneurysm.[20] If the aneurysm is ruptured, surgical clipping of the aneurysm should be done as soon as possible to reduce the possibility of rebleeding.[30]

Chapter 3. Epidemiology of Stroke

A. Incidence and Prevalence

In the US, the prevalence of stroke increases with advancing age in both men and women.[1] By 2030, it is projected that 3.9% of the adult population in the US will have had a stroke.[1] In addition, over 600,000 individuals in the US experience their first stroke each year.[1] The Atherosclerosis Risk in Communities (ARIC) study reported that the crude incidence rate for stroke was 4.10 (95% CI: 3.89, 4.33) per 1,000 person-years.[38] When stratified by race, Black participants had an incidence rate of 6.26 per 1,000 person-years, while White participants had an incidence rate of 3.39 per 1,000 person-years.[38] Similarly, the Greater Cincinnati/Northern Kentucky Stroke Study (GCNKSS) and the Reasons for Geographic and Racial Differences in Stroke (REGARDS) study reported adjusted incidence rates for stroke to be greater in Black participants than in Whites.[39,40] The incidence of stroke is greater in older individuals, as well. Those <65 years had an incidence rate of 2.19 per 1,000 person-years and those ≥65 years had an incidence rate of 5.58 per 1,000 person-years.[38] More recently, several population-based cohorts have reported that stroke incidence rates are decreasing in both men and women.[38,41,42]

B. Economic Burden

The direct and indirect cost of stroke in the United States was estimated to be $49.8 billion in 2016-2017. In 2035, the total direct medical cost of stroke, which includes hospital outpatient visits and inpatient stays, emergency room visits, prescribed

medications, and home health care, is projected to increase to $94.3 billion.[43] AF, a strong risk factor for stroke that will be discussed in a later chapter, further contributes to healthcare costs associated with strokes.[44,45] A prior MarketScan analysis reported that patients with AF have higher stroke-related costs than non-AF patients.[45] Although the prevalence of stroke is higher in older populations, AF-related ischemic strokes are still more costly than non-AF-related ischemic strokes in those <65 years.[44]

The costs of informal caregiving further add to the already high cost of stroke. Informal caregivers are often family members who provide care without payment. Compared to other cardiovascular diseases (hypertension, coronary heart disease [CHD], heart failure [HF]), stroke was found to be the most expensive condition in terms of informal caregiving.[46] In addition, patients with stroke require more hours of care per week from informal caregivers than other cardiovascular diseases.[46] By 2035, the total cost of informal caregiving attributable to stroke is projected to be $66 billion.[46] Post-stroke care is complex and informal caregivers must quickly learn how to assist with activities of daily living as 50% of stroke survivors have hemiparesis and 30% are unable to walk without assistance.[47] Given the demands of caregiving, caregivers' have an increased risk of depressive symptoms.[48,49] Caregivers for patients with acute neurological injury, which includes stroke, have more depressive symptoms than caregivers for patients with cancer.[50] The impact of a stroke not only affects the patient, but also their families, highlighting the importance of additional research into primary and secondary prevention of stroke.

Chapter 4. Risk Factors

A. Introduction

Several risk factors for stroke have been recognized and include demographics, clinical, behavioral, and genetic risk factors. Major risk factors include age, hypertension, diabetes, smoking, AF, and HF. This chapter will discuss these and other risk factors, as well as possible mechanisms in which they may lead to stroke.

B. Demographics

Demographic characteristics are known to be related to stroke. It has been well-established that risk of stroke increases with increasing age, regardless of sex or race.[51,52] Among both Black and White participants in the REGARDS study, women had a lower risk of stroke between the ages of 45-64 years; however, among those 75 years or older, men and women had similar risks for stroke.[51] Previous studies report that men have a higher age-specific stroke risk than women,[53,54] but women have a higher lifetime risk of stroke likely due to their longer life expectancies.[53] FHS cohorts have reported the lifetime risk of stoke to be approximately 1 in 5 for women and 1 in 6 for men.[2,55] In addition, stroke-related outcomes, such as disability and quality of life, are often worse in women than men.[53]

Racial differences in risk of stroke have been noted. Cohort studies have reported that Black participants have a greater risk of both ischemic and hemorrhagic strokes compared to White participants.[56-58] When assessing other racial groups, American Indian, Hispanic, and Asian/Pacific Islander participants have a higher risk of stroke when compared to White participants.[58-60]

C. Clinical and Behavioral

C.1.   Diabetes

Diabetes is an established risk factor for stroke. Several studies have reported that individuals with diabetes are at an increased risk of stroke compared to those without diabetes.[61-65] A meta-analysis of prospective studies conducted by the Emerging Risk Factors Collaboration reported that diabetes increases the risk of both ischemic and hemorrhagic strokes (HRs [95% CIs]: 2.27 [1.95, 2.65] and 1.56 [1.19, 2.05], respectively).[65] In addition to prevalent diabetes, duration of diabetes has also been found to be independently associated with ischemic stroke. In the Northern Manhattan Study (NOMAS), each additional year of diabetes increased the risk of ischemic stroke by 3% (HR [95% CI]: 1.03 [1.02, 1.04]).[63] Compared to those without diabetes, participants with ≥10 years of diabetes had a 3.2-fold greater risk of ischemic stroke (95% CI: 2.36, 4.51).[63] Moreover, risk of stroke is elevated even among those who are prediabetic.[66,67]

Diabetes may lead to stroke through several mechanisms. Inflammatory biomarkers (e.g., C-reactive protein [CRP], interleukin-6 [IL-6], tumor necrosis factor-α) are often elevated in those with diabetes.[68,69] Additionally, individuals with diabetes have stiffer arteries, which could lead to atherosclerosis.[68] Elevated levels of inflammatory biomarkers and arterial stiffness are risk factors for stroke and will be discussed in a later chapter. Diabetes may also lead to stroke through endothelial dysfunction[68,70] or hypoglycemia.[71,72] Hypoglycemia is a side effect of insulin treatment and may lead to a stroke as TIAs are known sequela of hypoglycemia.[71,72]

C.2.   Hypertension

Hypertension is a major risk factor for stroke,[73-75] especially hemorrhagic strokes.[52,64] In a worldwide case-control study consisting of 22 countries, hypertension was found to be the strongest risk factor for stroke (OR [95% CI]: 2.64 [2.26, 3.08]).[64] BP control has been recommended for primary and secondary stroke prevention[76] and several randomized clinical trials have shown the benefit of BP reduction and control in lowering risk of stroke.[77-81] A meta-analysis of 14 trials reported that compared to participants on a less intensive BP regimen, those on intensive BP lowering regimens were associated with a 22% (95% CI: 10%, 32%) reduction in risk of stroke.[78] Another meta-analysis found that a decrease of 10 mmHg in systolic BP or 5 mmHg in diastolic BP is associated with a 41% (95% CI: 33%, 48%) reduction in stroke.[79] Mechanisms through which hypertension may lead to stroke include arterial stiffness,[75,82] formation and rupture of atherosclerotic plaques, or disruption of the blood-brain barrier.[75] In addition to overt hypertension, studies have reported that stroke risk is elevated even among those who are prehypertensive.[74,83] Results from a meta-analysis of 19 prospective cohorts found that prehypertension increases the risk of stroke (RR [95% CI]: 1.66 [1.51, 1.81]).[83]

C.3.   Cigarette Smoking

Over the past several decades, the prevalence of cigarette smoking in the US has declined; however, it continues to be the leading cause of preventable disease and death in the US.[84,85] Several observational studies have found that current smoking, even as little as one cigarette per day,[86] increases the risk of stroke.[87-91] Furthermore, a dose-

response relationship for the amount smoked (e.g., number of cigarettes per day or pack-years smoked) has been noted.[64,88] In addition to current smokers, individuals exposed to secondhand smoke have also been found to have an increased risk of stroke.[92] Cigarette smoke, as well as secondhand smoke, increases platelet activity, which can increase the possibility of atherosclerosis, inflammation, and thrombosis.[93-95]

Smoking cessation should be promoted as it lowers one's risk for stroke compared to current smokers. Compared to never smokers, former smokers did not have a significantly increased risk of stroke, further suggesting that smoking cessation likely has potential benefits in reducing risk of stroke.[87,88,90,91] In addition, the risk of stroke starts to decline relatively quickly after smoking cessation (<5 years).[88]

C.4.   Obesity

Various measures of obesity have been shown to be associated with stroke. Several studies have found that BMI is associated with an increased risk of stroke.[96-98] Furthermore, measures of central obesity, including higher waist circumference,[99,100] waist to hip ratio,[64,99,100] waist to height ratio,[100] and waist to stature ratio,[99] are also associated with stroke. Obesity likely leads to stroke through mechanisms, such as diabetes, hypertension, atherosclerosis, and AF. Because weight loss is associated with improvements in BP, inflammatory biomarkers levels, and insulin levels,[101] it is possible that weight loss may be effective in prevention of stroke.

C.5.   Physical Activity

Higher level of physical activity is inversely associated with stroke.[64,102-105] A meta-analysis of prospective cohort studies reported a dose-response relationship in which higher level of physical activity are associated with lower risk of ischemic stroke.[103] Among older adults (mean age: 73 years) in the Cardiovascular Health Study (CHS), greater leisure-time activity, exercise intensity, and walking distance and pace were associated with a lower risk of stroke.[102] Additionally, a dose-response relationship was observed among women in the Women's Health Study, in which more time spent walking per week was associated with a lower risk of all stroke and hemorrhagic stroke.[104] Another meta-analysis reported that moderately or highly active individuals have a lower risk of incident stroke (ischemic and hemorrhagic) than those with low activity levels.[105]

There is evidence that among patients with strokes, higher level of physical activity pre-stroke may reduce the risk of adverse outcomes after their stroke.[106-108] The ExStroke Pilot Trial retrospectively assessed pre-stroke physical activity levels in 265 ischemic stroke patients. Those with pre-stroke physical activity levels in the highest quartile were more likely to have a less severe stroke and more favorable long-term outcomes compared to those in the lowest quartile.[106] Furthermore, a prior ARIC analysis assessed pre-stroke physical activity over 6 years and found that participants in the highest tertile of pre-stroke physical activity had a lower risk of all-cause mortality after incident stroke compared to those in the lowest tertile.[107] Similarly, a study in Taiwan reported that those who were active were more likely to have lower stroke severity, fewer post-stroke complications, and better long-term outcomes.[108]

D. Cardiovascular Conditions and Disease

D.1. Transient Ischemic Attack (TIA)

Both the short- and long-term risk of stroke is substantial among individuals who have a TIA.[109-111] Initiating treatments earlier after a TIA reduces the risk of a stroke.[110] More recently, data have suggested that prognosis after a TIA has improved likely due to more urgent care post-TIA; however, patients who have a TIA continue to remain at an elevated risk of stroke.[112]

D.2. Cardiac Arrhythmias

AF is an influential risk factor for stroke and increases the risk of stroke by 5-fold.[1,10] Both clinical and subclinical AF are risk factors for stroke. A meta-analysis of 7 studies reported that device-detected subclinical AF is associated with a 2.41-fold increased risk of stroke (95% CI: 1.78, 3.26).[113] In addition to AF, other cardiac arrhythmias, including paroxysmal supraventricular tachycardia,[114] excessive supraventricular ectopic activity,[115] premature atrial contractions,[116] and premature ventricular complexes,[117,118] are also associated with stroke.

D.3. Cardiovascular Comorbidities

Individuals with cardiovascular comorbidities are at an increased risk of having a stroke. HF is a risk factor for stroke,[119-122] and risk is greatest in the 30 days after HF diagnosis.[121] A systematic review reports that clinically diagnosed HF increases the risk of stroke up to 3-fold.[119] Studies have also reported that patients who had a myocardial

infarction (MI) are at an elevated risk of stroke.[123,124] CHD, which includes MI, may lead to stroke as a source of embolism from the heart, by having shared risk factors, or through atherosclerotic disease.[23]

D.4. *Subclinical Atherosclerosis*

Several measures of subclinical atherosclerosis are risk factors for stroke. Elevated carotid intima media thickness (cIMT) and cIMT progression have both been found to be associated with stroke.[125-128] Other measures of atherosclerosis, including ankle-brachial index (ABI) and coronary artery calcification, are also predictors of stroke.[127,129-131] Furthermore, peripheral artery disease (PAD), defined by an abnormal ABI, is associated with an increased risk of stroke.[132,133] Arterial and carotid stiffness both occur during the aging process and can lead to an elevated risk of stroke. A meta-analysis of prospective cohort studies reported that greater carotid stiffness was associated with a 1.18-fold (95% CI: (1.05, 1.33) increased risk of stroke.[134] In addition, measures of arterial stiffness (e.g., aortic pulse wave velocity, Young's elastic modulus, arterial distensibility) have also been found to be associated with incident stroke.[135-137]

Various mechanisms may explain the association between measures of subclinical atherosclerosis and stroke. Increased carotid and arterial stiffness may lead to elevated cIMT and the development of plaques, which could rupture and result in a stroke.[138,139] Furthermore, stiffening of arteries can lead to elevated pulse pressure, which in turn can promote arterial remodeling and plaque formation.[134]

E. Cardiac Imaging Measures

Numerous echocardiographic measures of left ventricular (LV) and LA remodeling have been associated with stroke. Community-based studies have reported that LV size,[140-142] LV hypertrophy,[143,144] LV dysfunction,[145] LA size,[146-148] and LA function,[149,150] have been associated with an increased risk of stroke. LA structural and functional remodeling may lead to blood stagnation, which could result in blood clots and ultimately lead to a stroke.[151]

F. Electrocardiogram (ECG) Measures

ECG-diagnosed variables, including LV hypertrophy[143,152] and prolonged corrected QT interval,[153,154] have been associated with an increased risk of stroke. In CHS, ECG-defined LV hypertrophy was associated with a 68% (95% CI: 1.23, 2.28) increased risk of stroke.[143] Several P-wave parameters have also been associated with incident stroke. A meta-analysis found that increased P-wave terminal force in lead V1 (PTFV$_1$), prolonged P-wave duration, and increased maximum P-wave area were all associated with an increased risk of ischemic stroke.[155] Furthermore, heart rate variability, a marker for autonomic nervous system dysfunction, has been associated with stroke.[156]

G. Circulating Biomarkers

Various circulating biomarkers are associated with incident stroke. Markers of inflammation (interleukin-6, CRP),[5,8,157,158] myocardial ischemic (troponin T and

I),[6,159,160] atrial overload (B-type natriuretic peptide),[6,161-164] and coagulation (D-dimer, fibrinogen),[9,165-168] have been found to be associated with incident stroke.

H. Genetics

Recent research has recognized several genetic variants that are associated with risk of stroke. A large multiethnic genome wide association study (GWAS) utilizing data from MEGASTROKE identified 32 genetic loci related to stroke, 22 of which were considered novel, and many of these loci are related to vascular risk traits.[169] A GWAS meta-analysis utilizing data from European-only participants in the UK Biobank and MEGASTROKE identified 3 more novel loci associated with stroke.[170] Furthermore, another study assessed genetic loci with the different subtypes of ischemic and hemorrhagic strokes.[171] Though several loci were associated with multiple ischemic stroke subtypes, only one locus (12q24) was associated with all ischemic stroke subtypes.[171] Many genetic studies are conducted in European populations, but Black individuals have a greater risk of cardiovascular disease, including stoke. Therefore, the MESA study conducted an analysis in Black participants, in which they found that variants in the *SERGEF* and *PRMT3* gene was associated with stroke.[172]

Chapter 5. Adverse Outcomes Associated with Stroke

A. Functional Impairment

Stroke is a leading cause of both physical and cognitive long-term disability.[1] An analysis by the Global Burden of Diseases Study found that out of 15 neurological disorders, stroke was the largest contributor to disability-adjusted life-years, which is a

measure of years of life lost and years lived with disability.[173] Several studies report that both short- and long-term functional outcomes are poor in stroke survivors.

The Montreal Stroke Cohort study found that at 6 months post-stroke, 39% of participants had limitations in performing activities of daily living, such as bathing and walking, and 54% had difficulty with at least 1 household task, such as shopping or cooking.[174] Among stroke survivors in NOMAS, declines in functional status and quality of life were observed up to 5 years after ischemic stroke, especially among those who were uninsured or insured with Medicaid.[175,176] Similar results were noted in the CHS, in which a gradual increase in disability occurs years after stroke.[177] Among patients in the North East Melbourne Stroke Incidence study, health-related quality of life was poor 5 years after their stroke.[178]

B. Recurrent Stroke

Recurrent stroke is common[1] and occurs more commonly than other cardiac events.[179-181] Each year, approximately 185,000 adults in the US experience a recurrent stroke.[1] The Oxfordshire Community Stroke Project, a community-based stroke register, reported that stroke patients were most at risk of stroke recurrence in the first 6 months; however, risk continued to be elevated up to 5 years after their first stroke.[182,183] In NOMAS, risk of a recurrent stroke is approximately 20% 5 years after the first stroke.[179]

More recently, evidence suggests stroke recurrence rates may be decreasing. A study using Medicare data reported that recurrent ischemic stroke incidence rates were declining for both Black and White individuals between 1999-2013,[184] while the South

London Stroke Register reported that stroke recurrence rates decreased between 1995 and 2005 but have not changed since then.[185]

C. Cardiovascular Events

Cardiovascular complications, such as HF, coronary artery disease, or MI, are common after a stroke and can contribute to increased healthcare-related costs and mortality rate, as well as decreased quality of life. Risk of cardiovascular events are generally highest within the first 30 days post-stroke and then decreases, though risk continues to remain elevated years after the index stroke. Among older adults (mean age: 77 years), 9% of patients experienced a cardiovascular event (i.e., acute MI, HF, coronary artery disease, coronary revascularization, or cardiac death) at 1-year post ischemic stroke.[186] This elevated risk of incident cardiovascular events was similar in both men and women.[187] In patients with ICH, 4% had a cardiac event (acute MI, ventricular arrhythmias, HF, or cardiac death) in the acute hospitalization phase.[188] Younger adults (aged 18-45 years) also had elevated readmission rates for cardiac events up to 1-year post-stroke.[180]

D. Cognitive Impairment and Dementia

Stroke is a strong risk factor for adverse neurocognitive outcomes. Meta-analyses have shown that stroke increases the risk for all-cause dementia[189] and for dementia subtypes (e.g., Alzheimer's disease).[190] In general, prior research has suggested that stroke doubles the risk of dementia.[191-193] Risk of mild cognitive impairment is also increased in patients with stroke. The South London Stroke Register reported that the

prevalence of cognitive impairment 3 months after stroke is 24% and remains relatively unchanged at 22% up to 15 years after stroke.[194] Among a population-based sample of cognitively normal individuals from Olmstead County, Minnesota, stroke increases the odds of mild cognitive impairment.[195] Furthermore, there is accelerated cognitive decline years after an individual has a stroke. The REGARDS study has found that incident stroke was associated with an accelerated decline in global cognition and executive function up to 8 years post-stroke.[196,197]

E. Mortality

Several studies have reported that hemorrhagic strokes are associated with a greater risk of death than ischemic strokes.[56,198,199] A study in South Carolina reported that patients with hemorrhagic strokes are 65% more likely to have vascular death and 49% more likely to have all-cause death compared to those with ischemic stroke.[199] In patients with ischemic stroke, results from a meta-analysis indicated that the risk of cardiac and vascular death is 1.38%/year and 2.17%/year, respectively.[181] Over one-third of patients with hemorrhagic stroke die within 30 days of their stroke.[200] Among those who survive a hemorrhagic stroke, risk of death continues to remain elevated a year after the stroke when compared to matched controls.[200]

Chapter 6. Stroke Prevention in AF

A. Risk Stratification Scores

AF increases one's risk of stroke by 5-fold;[1,10] therefore, preventing thromboembolic events is a high priority among patients with AF. Several scoring

systems have been created using established stroke risk factors to estimate risk of stroke in patients with AF. **Table 6.1** lists in chronological order risk scores for stroke prediction and the variables included in each score. Although not the most recent, the CHA_2DS_2-VASc risk score is the most widely used clinically. For instance, the 2019 AHA/ACC/HRS guideline recommends OACs to men with a CHA_2DS_2-VASc score ≥ 2 and women with a score ≥ 3.[201]

Table 6.1. Risk Scores for the Prediction of Stroke in Patients with AF

Risk Score Model	Year Created	Variables
AFI[202]	1994	Age, hypertension, diabetes, prior stroke/TIA
$CHADS_2$[203]	2001	Age, heart failure, hypertension, diabetes, prior stroke/TIA
Framingham Heart Study[204]	2003	Age, sex, systolic blood pressure, diabetes, prior stroke/TIA
CHA_2DS_2-VASc[205]	2010	Age, sex, heart failure, hypertension, diabetes, prior stroke/TIA, vascular disease
ATRIA[206]	2013	Age, sex, heart failure, hypertension, diabetes, proteinuria, eGFR/end stage renal disease
ABC-stroke[207]	2016	Age, cardiac troponin, NT-proBNP, prior stroke/TIA

B. Medications

Several pharmacological options for stroke prevention in patients with AF are currently used: aspirin, vitamin K antagonists (e.g., warfarin in the US) and DOACs (e.g., dabigatran, rivaroxaban, apixaban, and edoxaban). Aspirin reduces the relative risk of stroke by approximately 20%, but is less effective than other medications in preventing stroke in patients with AF.[208,209] Warfarin, which was approved by the FDA in 1954, reduces the relative risk of stroke by 64% and 37% compared to placebo or aspirin, respectively.[209] Until relatively recently, warfarin was the only FDA-approved drug for

stroke prevention in patients with AF. Since 2010, four DOACs have been approved by the FDA and several randomized trials have shown that DOACs are as effective as warfarin.[210-213]

Although OACs reduce the risk of stroke, there are several limitations to this therapy. First, there is an increased risk of bleeding.[208,214] Second, among those taking warfarin, continuous monitoring for international normalized ratio (INR) is needed and the target INR range is often difficult to maintain.[215,216] An INR below or above the target range increases the risk for stroke and bleeding events.[215,217] Additionally, adherence to warfarin is often poor, and it takes time for patients to obtain a stable INR in the proper range.[218,219] Third, patient non-compliance results in poorer anticoagulation control and increases the risk for adverse events. Suboptimal adherence is not only an issue with warfarin; it is also common with DOACs and associated with an increased risk of stroke (HR [95% CI]: 1.39 [1.06, 1.81]).[220] Fourth, regardless of the anticoagulant therapy selected, patients will likely need to discontinue the therapy prior to surgeries, certain procedures, or tests.[208]

C. Left Atrial Appendage Occlusion

Among patients with AF who have contraindications to DOACs or warfarin, percutaneous LAAO has emerged as a potential alternative treatment for stroke prevention.[11] The left atrial appendage is a finger-like extension of the LA and up to 90% of AF-related thrombi may originate from the left atrial appendage.[221] Given that the left atrial appendage is a common source of AF-related thrombi, percutaneous LAAO may be a feasible stroke prevention strategy for patients with AF.

Currently, there are several devices that are used for percutaneous LAAO procedures. Many of these devices are available in Europe under CE Mark, but only two are approved in the US: the Watchman device (Boston Scientific, Marlborough, Massachusetts) and the Amplatzer Amulet device (Abbott, Plymouth, Minnesota). The Watchman and Amplatzer Amulet devices were approved by the FDA in March 2015 and August 2021, respectively.

The Watchman device was approved by the FDA following the results of two randomized clinical trials.[12-15,222] This device is approved for patients with AF who 1) have an indication for long-term oral anticoagulation (e.g., increased CHA_2DS_2-VASc score), 2) are deemed safe for short-term oral anticoagulation by their physician (given that patients are required to take an OACs for at least 45 days after implant), and 3) have rationale for a nonpharmacologic alternative, such as contraindication to long-term anticoagulation (e.g., presence of falls, increased risk of bleeding, or poor adherence to medications).[223] The Watchman device prevents embolization of thrombi by sealing the ostium of the left atrial appendage.[12] Currently, patients are required to take an OAC for at least 45 days after Watchman implantation; however, a randomized clinical trial is ongoing to assess the safety and effectiveness of the Watchman device in those who are considered unsuitable for short-term OACs.[224] In this trial, potential participants were deemed unsuitable for OACs by two study physicians and took aspirin and/or clopidogrel after their Watchman implant.[224]

Stroke prevention can be costly in patients with AF, but research suggests that LAAO with the Watchman device may be more cost-effective than warfarin and DOACs.[225,226] LAAO is more costly upfront; however, a cost-effectiveness analysis using

US Medicare data suggests that the Watchman device reaches cost-effectiveness relative to DOACs and warfarin by year 6. Additionally, by year 10, it becomes more cost-effective than DOACs and warfarin.[225]

Chapter 7. Proteomics

A. Proteomic Profiling

A wide range of proteins can be detected in plasma and plasma proteins constitutes can reflect diseased cells and tissues.[227,228] Therefore, the assessment of the plasma proteome holds promise for the discovery of novel biomarkers and may lead to the development of new diagnostic tools for cardiovascular disease.[228,229] The majority of proteomic studies for cardiovascular disease have been based on mass spectrometric analyses, though this is limited by lower sample throughput due to the need for multiple preparation steps.[228] New technology, such as aptamer microarrays, microbead-based multiplexed immunoassays, or proximity extension assays, will likely produce an extensive list of novel proteins that may be risk factors for cardiovascular disease.

B. Proteomic Profiling for Stroke

Recent studies have used proteomic approaches to identify proteins associated with incident ischemic stroke. A study of two community-based cohorts of elderly participants in Sweden used a proximity extension assay (Olink Proseek Multiplex Cardiovascular 96x96 kit) and evaluated 84 proteins, in which 8 of these proteins were found to be associated with ischemic stroke.[230] Another study of 826 male participants in

Sweden assessed 742 proteins. Over a median follow-up of 12.5 years, 135 ischemic stroke events occurred and 13 proteins were associated with ischemic stroke.[231]

C. SomaScan Assay

SomaScan is an aptamer microarray that measures proteins using a Slow Off-rate Modified Aptamer (SOMAmer)-based capture array.[227] The SomaScan assay transforms individual protein concentrations into a corresponding modified aptamer (SOMAmer reagent) concentration that can be quantified by DNA microarrays in relative fluorescence units.[227] The SomaScan assay has high sensitivity and specificity[232] and has been used in prior cohort studies to identify proteins associated with cardiovascular disease.[233-235]

Chapter 8. Study Designs

Manuscripts 1 and 2 of this dissertation used data from the ARIC study to assess the relationship between atrial myopathy and brain MRI measures and to analyze proteins associated with incident ischemic stroke. Manuscript 3 used the Medicare 20% sample databases to examine the association between percutaneous LAAO with the Watchman device and stroke among patients with AF.

A. The Atherosclerosis Risk in Communities (ARIC) Study

The ARIC study is a prospective, community-based study that was developed to study the etiology and natural history of atherosclerosis, as well as conduct community surveillance of cardiovascular disease.[236,237] Since inception in 1987, the ARIC study has

expanded its research beyond cardiovascular disease to include several other chronic conditions, such as chronic kidney disease, diabetes, cancer, cognitive decline/dementia, and others.

A.1.   Study Design and Population

The ARIC study is a multi-center prospective cohort that enrolled 15,792 adults aged 45-64 years in 1987-1989 from four US communities (Forsyth County, North Carolina; Jackson, Mississippi; northwest suburbs of Minneapolis, Minnesota; and Washington County, Maryland). Participants were selected by probability sampling in each community. In Forsyth County, households were identified by area sampling, while other communities sampled age-eligible lists to identify their households. Lists used included: driver's license or state identification cards in Jackson, jury duty eligibility with driver's license, identification cards, or voter registration cards in Minneapolis, and driver's license or listed in a 1975 private county health census in Washington County. The Jackson center only recruited Black participants, while the other 3 centers recruited participants representative of the local population. In the Minneapolis and Washington County centers, the vast majority of participants were White, while in the Forsyth County center approximately 15% of participants were Black and 85% White.

Eight clinic visits have been completed (visit 1 [1987-98], visit 2 [1990-92], visit 3 [1993-95], visit 4 [1996-98], visit 5 [2011-13], visit 6 [2016-17], visit 7 [2018-19]), with visit 8 by phone (2020) and visit 9 currently on going (**Figure 8.1**). In addition to clinic visits, regular telephone calls (annual prior to 2012, twice-yearly thereafter) were made to continue contact with participants and obtain medical events that may have

occurred. The baseline visits for manuscripts 1 and 2 of this dissertation are visit 5 (2011-13) and visit 3 (1993-95), respectively.
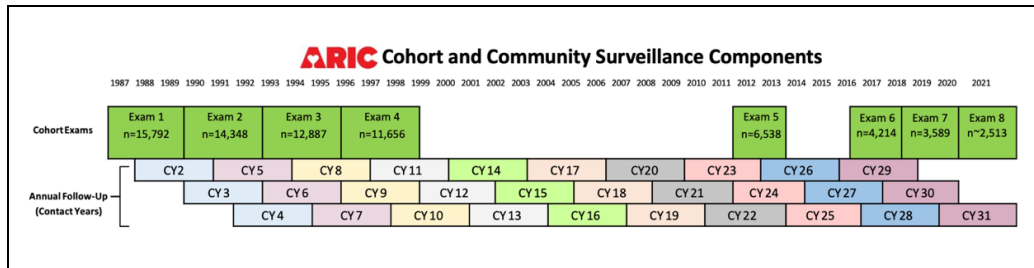
Figure 8.1. ARIC Study Visits and Annual Follow-Up, 1987-present

A.2.   Echocardiograms

Trained technicians performed 2D-echocardiograms at visit 5 as previously described.[238] Briefly, echocardiograms were performed by trained sonographers using Philips iE33 Ultrasound systems with Vision 2011. Studies were transferred from each field center to a secure server at the Echocardiography Reading Center (Brigham and Women's Hospital, Boston, MA).

The Simpson method was used to generate LA time-volume curves by calculating LA volume at each phase of the cardiac cycle (maximal and minimal LA volumes). Volumes were indexed to body surface area to derive maximal and minimal LA volume index. LA function measures, which included LA reservoir strain, conduit strain, and contractile strain, was measured using a speckle tracking vendor-dependent software using R-R gating with an auto-strain algorithm (QLAB Advanced Quantification Software 13.0, Philips Ultrasound, Inc.). Speckles were tracked during a cardiac cycle frame by frame.

A.3.   Brain MRI

At visit 5, a subset of participants who had no brain MRI contraindications and met specific criteria were selected to undergo a brain MRI. Inclusion criteria for a brain MRI included those who: 1) participated in a prior ARIC brain MRI scan in 2004-06, 2) had evidence of cognitive impairment and/or declines on longitudinally administered tests, and 3) selected from an age-stratified random sample of cognitively normal participants to approximate the age distribution of cognitively impaired participants.[239] Using standardized protocols, 3-T Siemens scanners were used at each study site and scans were read centrally at the ARIC MRI Reading Center at the Mayo Clinic (Rochester, MN).

A.4.   Proteomics

Plasma samples were obtained from blood that was collected from participants using standardized protocols. The plasma samples were stored at -80°C and transferred to the ARIC central laboratory. Plasma proteins were measured using a DNA aptamer-based capture array (SomaScan, Somalogic, Inc., Boulder, CO).[240] Protein concentrations were quantified into relative fluorescence units.[227] The SomaScan assay was performed as previously described[227] and standard Somalogic quality control and data normalization were applied.[232] Median intra- and inter-assay coefficients of variation were ~5%.[232]

In quality control, 422 samples from visit 3 were run in duplicate. The median inter-assay coefficient of variation was 6.3%. Of the 5,824 available aptamer measures, 94 were excluded due to a Bland-Altman coefficient of variation >50% or a variance of <0.01 on the log scale. An additional 313 measures were excluded because of nonspecific

binding to nonproteins. After excluding quality control outliers, the median split sample reliability coefficient was 0.85. In total, 4,877 aptamer measures that corresponded to 4,697 unique proteins were included in this analysis. Plasma protein measures were log base 2 transformed to correct for skewness.

A.5.   Stroke Ascertainment

In ARIC, stroke events were identified by hospitalization records or death certificates.[56] All potential cases of stroke were independently reviewed by a physician. Using criteria from the National Survey of Stroke,[241] stroke events were further classified by a computer algorithm into four categories: SAH, ICH, thrombotic brain infarction, or embolic brain infarction. Final diagnosis was determined by an agreement of the physician reviewer and computer algorithm. If there was a disagreement in diagnosis, a second physician reviewer adjudicated the event.[56]

A.6.   Covariate Ascertainment

Similar methods were used for covariate measures at all study visits. Participants self-reported their sociodemographic and lifestyle characteristics (e.g., age, sex, race, smoking status). Medication use was recorded based on review of medication bottles that participants brought or by self-report for some medications. Trained technicians obtained participant's anthropometric measures, blood pressure, and a blood draw. Height and weight were used to derive BMI. Blood pressure was measured three times after a 5-minute rest using a random zero sphygmomanometer. Systolic and diastolic blood pressure were calculated as the mean of the final two measurements. Diabetes was

defined as a fasting glucose ≥126 mg/dL or a non-fasting glucose ≥200 mg/dL, antidiabetic medication use in the past two weeks, or a self-reported physician diabetes diagnosis. Plasma total cholesterol and HDL cholesterol were measured by the enzymatic method.[242]

CHD was defined by self-reported physician diagnoses at visit 1, MI diagnosis by ECG, or adjudicated cases after visit 1.[243] HF was identified by the Gothenburg criteria (visit 1 only), HF medication use within the past two weeks, or ICD codes for HF from hospitalization records during follow-up.[244] AF was ascertained from ECGs conducted during study visits and ICD codes from hospitalization discharge and death records.[245] Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.[246] Genotyping the apolipoprotein ε4 (APOE ε4) was done using the TaqMan assay (Applied Biosystems).[247] LV ejection fraction and LV mass index were obtained from 2D-echocardiograms. LV ejection fraction was calculated as 100 × (LV end-diastolic volume – LV end-systolic volume)/LV end-diastolic volume, while LV mass index was calculated from LV linear measures and indexed to body surface area, per recommendations by the American Society of Echocardiography.[248]

B. Medicare Databases

Medicare is a health insurance program in the US and people qualify for Medicare coverage if any of the following criteria apply: 1) aged ≥65 years, 2) aged <65 years with certain disabilities, and 3) have end-stage renal disease. There are several plans that are available in Medicare: Part A (hospital insurance), Part B (medical services insurance),

and Part D (prescription drug coverage). The US Centers for Medicare and Medicaid Services (CMS) compiles Medicare data and standardized datasets representing a 5% random sample, a 20% random sample, and the 100% sample are created.

B.1. *Database Population*

Claims data (inpatient, outpatient, and carrier files) from a nationally representative 20% sample of Medicare beneficiaries from 2015-2018 was used. The inpatient files contained institutional claims (submitted by healthcare facilities) for inpatient services covered by Medicare Part A. Outpatient files contained institutional claims for outpatient services covered by Medicare Part B and carrier files contained noninstitutional providers (e.g., physicians, social workers, nurse practitioners) for services covered by Medicare Part B.

B.2. *AF Ascertainment*

To identify patients with AF, we required at least one inpatient code for AF or two outpatient codes for AF that were 7-365 days apart using International Classification of Disease, Ninth or Tenth Revision, Clinical Modification (ICD-9-CM: 427.31, 427.32 or ICD-10-CM: I48 in any position).[249] The positive predictive value (PPV) and sensitivity of the ICD-9-CM codes for AF were approximately 90% and 80%, respectively.[249]

Chapter 9. Manuscript 1: Association of Atrial Myopathy with Brain MRI Measures: The Atherosclerosis Risk in Communities Neurocognitive Study

A. Overview

Background: Atrial myopathy, defined by left atrial (LA) abnormalities, is associated with increased dementia risk and it may be possible that vascular brain injury underlies this association. Using data from the Atherosclerosis Risk in Communities (ARIC) study, we assessed the cross-sectional association between atrial myopathy, measured by 2D echocardiogram, and brain MRI measures (brain infarcts, cerebral microbleeds, white matter hyperintensity volume, deep gray matter, and temporal lobe volume region of interest).

Methods: ARIC participants who attended visit 5 (2011-13) and had both an echocardiogram and a brain MRI were included. Linear regression was used for brain volume outcomes. Logistic regression was used for dichotomous outcomes, while negative binomial regression was used for count outcomes.

Results: We included 1327 participants (mean [SD] age 76 [5], 61% female, 29% Black participants) in this analysis. Lower LA reservoir strain and conduit strain was associated with higher odds of the presence of cerebral microbleeds (lowest vs. highest quartile ORs [95% CIs]: 1.74 [1.37, 2.21] and 1.42 [1.12, 1.79], respectively). In addition, participants in the lowest LA conduit strain quartile had higher odds of brain infarcts compared to those in the highest quartile (OR [95% CI]: 1.33 [1.05, 1.69]). When assessing brain

volumes, lower LA contractile strain was associated with greater white matter hyperintensity volume (per 1-SD decrease β [95% CI]: 0.07 [0.01, 0.12]).

Conclusion: In this community-based cohort, measures of lower LA function were cross-sectionally associated with vascular brain injury. Prospective studies are needed to confirm these findings.

B. Introduction

Atrial fibrillation (AF) and clinical ischemic stroke are associated with an increased risk of dementia.[250-252] In addition, recent evidence indicates that atrial myopathy, which is characterized by left atrial (LA) dysfunction and enlargement, is also associated with elevated dementia risk, independent of AF and stroke.[4] Currently, reasons for this association are unclear and may include vascular brain injury.

Prior studies have characterized the neuroimaging correlates of atrial myopathy; however, they have often defined atrial myopathy with ECG markers.[253-256] Few studies have used echocardiogram-defined atrial myopathy measures and they consisted of smaller sample sizes.[257,258] Results from these studies suggest atrial myopathy and vascular brain injury may be related, but additional research with echocardiogram-defined atrial myopathy is warranted. Therefore, using data from the Atherosclerosis Risk in Communities (ARIC) study, a large, community-based cohort, we propose to assess the cross-sectional association of atrial myopathy (measured by 2D echocardiograms) with vascular brain injury.

C. Methods

C.1.   *Study Population*

The ARIC study is a community-based cohort of predominately Black and White adults that began in 1987-1989. At inception, 15,792 men and women aged 45-64 years were recruited from four US communities: Forsyth County, NC; Jackson, MS; Washington County, MD; northwest suburbs of Minneapolis, MN.[237] Since the baseline exam, participants have attended several additional follow-up visits and are also followed continuously for hospitalizations. For this analysis, data from visit 5 (2011-13) was used since echocardiograms and brain MRIs were performed at this visit.

Participants who attended visit 5 and had both an echocardiogram and brain MRI were included in this analysis. Exclusion criteria included those with prevalent dementia, AF, or stroke at visit 5 or missing covariates, as well as those whose race was other than Black or White and non-Whites in the Minneapolis and Washington County centers due to small numbers (**Figure 9.1**). After all exclusions, 1327 participants were included in our analytic sample.

Institutional Review Boards at each center approved the study and participants provided written informed consent at each visit.

C.2.   *Echocardiogram Measures*

The exposures of interest included measures of LA function (LA reservoir strain, conduit strain, and contractile strain) and LA size (maximal and minimal volume index). These measures were evaluated by 2D-echocardiograms at visit 5 as previously described.[238] Briefly, echocardiograms were performed by trained sonographers using

Philips iE33 Ultrasound systems with Vision 2011. Studies were transferred from each field center to a secure server at the Echocardiography Reading Center (Brigham and Women's Hospital, Boston, MA).

Using the Simpson method, LA time-volume curves were generated by calculating LA volume at each phase of the cardiac cycle (maximal and minimal LA volumes). Maximal and minimal LA volume index were derived by indexing the volumes to body surface area.

LA function was measured using a speckle tracking vendor-dependent software using R-R gating with an auto-strain algorithm (QLAB Advanced Quantification Software 13.0, Philips Ultrasound, Inc.). Speckles were tracked during a cardiac cycle frame by frame. For this analysis, the absolute values of LA conduit and contractile strain were used. Intra-reader and inter-reader variability for LA reservoir strain was assessed in a sample of 40 randomly selected subjects. The intraclass correlation coefficient for inter-reader and intra-reader variability were 0.91 and 0.98, respectively.

C.3.   Brain MRI Measures

The ARIC brain MRI imaging protocol has been previously described.[239] Briefly, a subset of visit 5 participants who had no brain MRI contraindications and met any of the following criteria were invited to undergo a brain MRI: 1) participated in a prior ARIC brain MRI scan in 2004-2006, 2) had evidence of cognitive impairment and/or declines on longitudinally-administered tests, and 3) selected from an age-stratified random sample of cognitively normal participants to approximate the age distribution of

cognitively impaired participants. Sampling weights were assigned based on inverse sampling fractions and the probability of completing the exam.[239]

3-T Siemens scanners were used at each study site using standardized protocols. Scans were read centrally at the ARIC MRI Reading Center at the Mayo Clinic (Rochester, MN). Total intracranial volume and distinct regional volumes were measured using FreeSurfer version 5.1. Deep gray matter was calculated as the combined volume of the thalamus, caudate, putamen, and pallidum. The cortical volume in a temporal-parietal meta region of interest (ROI) was calculated as the combined volume of the parahippocampal, entorhinal, inferior parietal lobules, hippocampus, and precuneus,[239] based on prior studies demonstrating the relevance of these regions in individuals with Alzheimer's disease.[259,260] White matter hyperintensity (WMH) burden was measured using an algorithm developed at Mayo Clinic, Rochester.[261]

Brain infarcts were identified and measured by a trained imaging technician and then confirmed by radiologists. Lacunar infarcts were defined as subcortical T2 fluid-attenuated inversion recovery lesions (FLAIR) with central hypointensity >3 mm and hyperintensity ≤20 mm in maximum diameter located in the caudate, lenticular nucleus, internal capsule, thalamus, brainstem, deep cerebral white matter, centum semiovale, or corona radiata.[262] Cortical infarcts were defined as lesions with a minimum extent >10 mm on T2 FLAIR.[263] Cerebral microbleeds were identified as lesions of ≤10 mm in maximum diameter on gradient-echo T2-weighted (T2*GRE) imaging sequences and were divided into lobar and subcortical microbleeds depending on the location.[264,265]

C.4.   *Covariates*

For this manuscript, covariates were obtained from visit 5, unless otherwise noted, and included: age, sex, race/center (visit 1), education (visit 1), APOE ε4 (visit 2, or occasionally visit 3), systolic blood pressure, antihypertensive medications, body mass index (BMI), diabetes, smoking status, coronary heart disease (CHD), heart failure (HF), anticoagulant use, left ventricular (LV) ejection fraction, and LV mass index.

Participants self-reported their age, sex, race, educational attainment, and smoking status. Participants brought their medication to each study visit and technicians recorded medication use. BMI was derived from height and weight. Blood pressure was measured three times and the mean of the final two measurements was used. Diabetes was defined as a fasting glucose $\geq$126 mg/dL or a non-fasting glucose $\geq$200 mg/dL, antidiabetic medication use in the past two weeks, or a self-reported physician diabetes diagnosis. [246] APOE ε4 genotyping was done using the TaqMan assay (Applied Biosystems).[247] CHD was defined by self-reported physician diagnoses at visit 1, MI diagnosis by ECG, or adjudicated cases after visit 1.[243] HF was identified by the Gothenburg criteria (visit 1 only), HF medication use within the past two weeks, or ICD codes for HF from hospitalization records during follow-up.[244] LV ejection fraction and LV mass index were obtained from 2D-echocardiograms. LV ejection fraction was calculated as 100 × (LV end-diastolic volume – LV end-systolic volume)/LV end-diastolic volume. LV mass index was calculated from LV linear measures and indexed to body surface area, per recommendations by the American Society of Echocardiography.[248]

C.5.   Statistical Analysis

Participant characteristics were described using frequencies and percentages for categorical variables and means and standard deviations for continuous variables. Infarcts and microbleeds were assessed two ways: 1) as a dichotomous outcome (presence or absence) and 2) as a count outcome. Logistic regression was used for dichotomous outcomes and negative binomial regression was used for count outcomes. For brain volume outcomes, linear regression was used.

Brain volumes were scaled based on their standard deviations in order to compare the magnitude of association across brain regions. Because WMH volume was highly skewed, log base 2 transformation was applied for normality. Weights for selection into the brain MRI study in this analysis were incorporated in all analyses. The following models were used: model 1 adjusted for age, sex, race/center, education (less than high school education, high school graduate or high school equivalent or vocational school, college or above), APOE ε4 (0 or ≥1 allele), total intracranial volume (for volume outcomes only); model 2 further adjusted for systolic blood pressure, antihypertensive medications, BMI, diabetes, smoking status (current or former/never smoker), CHD, HF, anticoagulant use; model 3 additionally adjusted for LA maximal volume index (for LA function measures only), LV ejection fraction, LV mass index. SAS software (version 9.4; Cary, NC) was used for all analyses.

D. Results

A total of 1327 participants who had an echocardiogram and brain MRI scan at visit 5 were included in this analysis (mean [SD] age 76 [5], 61% female, 29% Black

participants). Clinical characteristics, stratified by LA reservoir strain quartiles, are presented in **Table 9.1**. Those in the lowest LA reservoir strain quartile were more likely to be older, female, identify as Black, have lower educational attainment, higher prevalence of cardiovascular disease and risk factors, more imaging findings of vascular brain injury, and lower brain volumes compared to those in the higher quartiles.

D.1.   LA Measures and Brain Infarcts

Among study participants, 306 (23%) had at least one brain infarct seen on their brain MRI scan. The distribution of the number of brain infarcts are presented in **Figure 9.2**. In those with at least one brain infarct, the mean number of infarcts was 1.41.

When assessing presence of infarcts, participants in the lowest LA conduit strain quartile had higher odds of any infarcts and lacunar infarcts (**Table 9.2**; Ors [95% CIs]: 1.33 [1.05, 1.69]; 1.51 [1.15, 1.99], respectively) compared to those in the highest quartile after full model adjustment. When evaluated continuously, each 1-SD decrease in LA conduit strain was associated with 1.12 (95% CI: 1.03, 1.22) and 1.14 (95% CI: 1.03, 1.26) times higher odds of having any infarcts and lacunar infarcts, respectively. No significant association was noted between LA conduit strain and presence of cortical infarcts. Furthermore, those in the lowest LA conduit strain quartile on average had 1.10 more brain infarcts (95% CI: 0.78, 1.55) than participants in the highest quartile; however, this association was not statistically significant (**Supplemental Table 9.1**).

For LA contractile strain, compared to participants in the highest quartile, those in the lowest quartile had lower odds of the presence of any infarcts and cortical infarcts (Ors [95% CIs]: 0.73 [0.59, 0.91]; 0.67 [0.49, 0.93], respectively). Similar associations

were observed when LA contractile strain was assessed continuously: for each 1-SD decrease in LA contractile strain, there was 0.90 (95% CI: 0.83, 0.97) and 0.88 (95% CI: 0.78, 0.98) the odds of any infarcts and cortical infarcts. No associations between LA reservoir strain and brain infarcts were observed. When assessing LA size, greater LA maximal volume index was associated with brain infarcts (any infarcts and lacunar infarcts). In addition, each 1-SD increase in LA minimal volume index was associated with higher odds of any infarcts and lacunar infarcts (Ors [95% CIs]: 1.15 [1.05, 1.25], 1.13 [1.03, 1.25], respectively).

D.2.   *LA Measures and Cerebral Microbleeds*

Overall, 316 participants (24%) had at least one cerebral microbleed. Among those with at least one cerebral microbleed, mean number of microbleeds was 2.66. **Figure 9.3** shows the distribution of the number of microbleeds.

After full model adjustment, participants in the lowest LA reservoir strain quartile had higher odds of the presence of cerebral microbleeds (**Table 9.3**; OR [95% CI]: 1.74 [1.37, 2.21]) compared to those in the highest quartile. Additionally, each 1-SD decrease in LA reservoir strain was associated with 1.11-fold higher odds of having cerebral microbleeds (95% CI: 1.02, 1.22). When analyzing number of microbleeds, participants in the lowest LA reservoir strain quartile on average had 2.26 (95% CI: 1.45, 3.52) more cerebral microbleeds than those in the highest quartile (**Supplemental Table 9.2**) For types of microbleed, significant associations were observed for both subcortical and lobar microbleeds (lowest vs. highest LA reservoir strain quartile Ors [95% CIs]: 1.76 [1.37, 2.28]; 1.55 [1.05, 2.28], respectively).

Participants in the lowest LA conduit strain quartile had higher odds of the presence of cerebral microbleeds and subcortical microbleeds compared to those the highest quartile (Ors [95% CIs]: 1.42 [1.12, 1.79]; 1.57 [1.23, 2.01]). In addition, those in the lowest LA conduit strain quartile had on average 2.09 (95% CI: 1.36, 3.22) more cerebral microbleeds compared to participants in the highest quartile. No significant association between LA contractile strain and cerebral microbleeds was noted. For LA size, each 1-SD increase in LA minimal volume index was associated with a 1.12-fold (95% CI: 1.03, 1.23) higher odds of cerebral microbleeds.

D.3.   *LA Measures and Brain Volumes*

Each 1-SD decrease in LA contractile strain was associated with greater WMH volume (β [95% CI]: 0.07 [0.01, 0.12]). No other significant association was noted for any other LA measure and brain volume measures (**Table 9.4**).

E. Discussion

In this cross-sectional analysis of a US community-based cohort, measures of atrial myopathy were associated with vascular brain injury after adjustment for vascular risk factors and echocardiogram measures. Several measures of lower LA function, as well as greater LA minimal volume index, were associated with higher odds of cerebral microbleeds. Additionally, lower LA conduit strain and greater LA maximal and minimal volume index were associated with a higher odds of brain infarcts. When evaluating brain volume measures, lower LA contractile strain was associated with greater WMH volume; however, no other associations between LA function or size measures and brain volumes

were noted. Overall, our findings suggest there may be a link between atrial myopathy and vascular brain injury, in particular cerebral microbleeds.

Prior research suggests that atrial myopathy may be associated with measures of vascular brain injury, but the data are conflicting. For example, an analysis of 455 participants from the CABL (Cardiovascular Abnormalities and Brain Lesions) study, a community-based cohort, found that reduced LA function (LA emptying fraction), as measured by 3D echocardiograms, was associated with more silent brain infracts and WMH volume.[257] However, when LA function was measured using strain variables, LA strain was associated with silent brain infarcts, but not with WMH volume.[258] Furthermore, in a prior ARIC analysis, reduced LA function (measured by LA global longitudinal strain) and stiffness was associated with higher odds of subclinical brain infarcts.[150] Similarly inconsistent results have been reported between ECG-defined atrial myopathy (elevated PTFV1) and brain infarcts and WMH volume.[253,254,256] Results from our study suggest that lower LA conduit strain is associated with a higher odds of brain infarcts. In addition, contrary to our hypothesis, LA contractile strain showed an inverse association with brain infarcts. These unexpected results may reflect the influence age has on the phases of LA function. A study of 120 healthy individuals aged 20-80 years found that those in older age groups had lower LA reservoir and conduit functions, but higher booster pump (contractile) function.[266] Furthermore, it may be plausible that the decrease in conduit function (which reflects diastolic dysfunction) results in an increase in contractile function to compensate for the decrease in early diastolic filing.[266]

Little research has been reported on the relationship between atrial myopathy and cerebral microbleeds. A study in Singapore (n=408 participants) reported that elevated

PTFV1 was associated with 2.26 higher odds (95% CI: 1.33, 3.91) of the presence of cerebral microbleeds.[256] Our study adds to the literature by reporting an association between lower LA function (LA reservoir and conduit strain), measured by 2D echocardiogram, and cerebral microbleeds after adjusting for vascular risk factors, LA size, and LV function. It is plausible that biomarkers, such as beta-amyloid or tau, may underlie the association between atrial myopathy and vascular brain injury, given that atrial myopathy has been associated with elevated beta-amyloid deposition.[267] In addition, we found that lower LA reservoir and conduit strain were associated with higher cerebral microbleed count. Higher microbleed count is associated with cognitive decline[268] and baseline cerebral microbleeds are strong predictors for future cerebral microbleeds,[269,270] indicating that future research on individuals with repeat brain MRI scans is needed, particularly among those with a high baseline microbleed count.

Of note, prior evidence has shown that LV echocardiogram measures are associated with cerebrovascular disease, specifically measures such as LV mass index,[271] LV ejection fraction,[271] and LV global longitudinal strain.[272] It has been suggested that changes in LA strain often occur prior to that LV structural and functional changes,[273] highlighting the importance of measuring LA function. However, of the few studies that analyzed LA function and cerebrovascular disease, sample sizes were small.[257,258] Therefore, our results add to the literature by indicating an association between LA function measures and cerebral microbleeds in a larger cohort of individuals.

Strengths of this study include the relatively large cohort of participants that consists of both Black and White men and women, the comprehensive range of LA size and function measures assessed by echocardiogram, and the presence of an array of brain

MRI measures. Limitations also exist. First, this was a cross-sectional analysis, which does not allow us to assess the temporality of the association between atrial myopathy and brain MRI measures. Second, multiple exposures were assessed in this analysis and adjustment for multiple comparisons was not performed; therefore, these results should be interpreted as exploratory. Third, participants who attended study visits and had a brain MRI were likely a healthier subset of the cohort, which may lead to selection bias. To address this, we utilized inverse probability weighting to account for selection for the brain MRI study. Fourth, our results may not be generalizable to younger individuals as our sample consists of older adults (mean age: 76 years).

F. Conclusion

In this analysis of a US community-based cohort, measures of lower LA function were associated with cerebral microbleeds and brain infarcts. These findings suggest that reduced LA function may be a risk factor for vascular brain injury. Additional prospective research is needed to confirm these findings.

Table 9.1. Baseline Participant Characteristics Stratified by LA Reservoir Strain, The ARIC Study, 2011-2013

	LA Reservoir Strain Quartiles			
	≤27.56% (n=331)	27.57% - 32.04% (n=332)	32.05% - 37.75% (n=332)	≥37.76% (n=332)
Demographics				
Age, years	77.6 (5.5)	76.0 (5.2)	75.4 (4.7)	74.9 (5.0)
Female sex	216 (65.3%)	204 (61.4%)	191 (57.5%)	192 (57.8%)
Black race	110 (33.2%)	105 (31.6%)	81 (24.4%)	83 (25.0%)
Less than high school education	55 (16.6%)	42 (12.7%)	29 (8.7%)	34 (10.2%)
Physiologic Indications				
Body mass index, kg/m^2	28.7 (5.7)	28.3 (5.3)	28.1 (5.1)	27.4 (5.0)
Systolic blood pressure, mmHg	133.4 (19.2)	131.1 (18.5)	128.8 (16.4)	128.9 (16.5)
Diabetes	116 (35.0%)	113 (34.0%)	95 (28.6%)	83 (25.0%)
Coronary heart disease	36 (10.9%)	21 (6.3%)	26 (7.8%)	23 (6.9%)
Heart failure	59 (17.8%)	18 (5.4%)	16 (4.8%)	10 (3.0%)
≥1 APOE ε4 allele	90 (27.2%)	99 (29.8%)	77 (23.2%)	106 (31.9%)
Antihypertensive medication use	273 (82.5%)	251 (75.6%)	237 (71.4%)	217 (65.4%)
Anticoagulant use	5 (1.5%)	7 (2.1%)	6 (1.8%)	3 (0.9%)
Current smokers	13 (3.9%)	21 (6.3%)	13 (3.9%)	17 (5.1%)
Echocardiogram Measures				
LA conduit strain, %	10.3 (4.2)	13.5 (3.5)	15.5 (4.1)	19.8 (5.2)
LA contractile strain, %	13.2 (4.5)	16.4 (3.5)	19.2 (4.0)	22.4 (4.9)
Maximal LA volume index, ml/m^2	39.2 (12.8)	35.8 (9.9)	32.0 (9.1)	29.2 (7.4)
Minimal LA volume index, ml/m^2	20.7 (9.0)	15.8 (5.1)	12.6 (4.3)	9.7 (3.1)
Left ventricular ejection fraction, %	64.2 (8.4)	66.7 (5.3)	66.1 (5.6)	67.2 (4.8)
Left ventricular mass index, g/m^2	85.8 (23.9)	76.9 (16.4)	75.6 (15.9)	72.0 (15.2)
Brain MRI Measures				
Any infarcts	86 (26.0%)	76 (22.9%)	80 (24.1%)	64 (19.3%)

Cortical infarcts	35 (10.6%)	30 (9.0%)	29 (8.7%)	27 (8.1%)
Lacunar infarcts	62 (18.7%)	50 (15.1%)	57 (17.2%)	46 (13.9%)
Any microbleeds	105 (31.7%)	78 (23.5%)	70 (21.1%)	63 (19.0%)
Subcortical microbleeds	86 (26.0%)	62 (18.7%)	53 (16.0%)	52 (15.7%)
Lobar microbleeds	34 (10.3%)	26 (7.8%)	23 (6.9%)	26 (7.8%)
White matter hyperintensity volume, cm^3	20.3 (19.8)	15.9 (14.4)	15.0 (15.7)	14.2 (13.8)
Deep gray matter volume, cm^3	42.3 (4.5)	42.6 (4.1)	43.1 (4.2)	43.3 (4.1)
Temporal lobe volume meta ROI, cm^3	66.4 (8.3)	67.6 (8.3)	69.2 (7.9)	70.0 (8.5)

[*]Data are expressed as mean ± SD or n (%).

Abbreviations: APOE = apolipoprotein E; LA = left atrial; LV = left ventricular; ROI = region of interest

Table 9.2. Association of Left Atrial Measures with Brain Infarcts, ARIC-NCS, 2011-2013 (n=1,327)

	LA Reservoir Strain Quartiles				LA Reservoir Strain per 1-SD decrease (7.4%)
	≤27.56% (n=331)	27.57% - 32.04% (n=332)	32.05% - 37.75% (n=332)	≥37.76% (n=332)	
Any Infarcts					
N, ≥1 infarct*	86	76	80	64	306
OR (95% CI)					
Model 1	1.38 (1.11, 1.72)	1.19 (0.96, 1.48)	1.35 (1.10, 1.66)	Reference	1.17 (1.08, 1.27)
Model 2	1.16 (0.92, 1.45)	1.12 (0.90, 1.40)	1.32 (1.08, 1.62)	Reference	1.09 (1.01, 1.18)
Model 3	0.89 (0.70, 1.14)	0.99 (0.79, 1.24)	1.21 (0.98, 1.49)	Reference	0.99 (0.90, 1.08)
Lacunar Infarcts					
N, ≥1 infarct*	62	50	57	46	215
OR (95% CI)					
Model 1	1.51 (1.17, 1.94)	1.05 (0.81, 1.36)	1.50 (1.18, 1.90)	Reference	1.21 (1.10, 1.32)
Model 2	1.24 (0.95, 1.61)	0.99 (0.77, 1.29)	1.46 (1.15, 1.85)	Reference	1.11 (1.01, 1.22)
Model 3	1.01 (0.76, 1.35)	0.90 (0.69, 1.18)	1.37 (1.07, 1.74)	Reference	1.04 (0.93, 1.15)
Cortical Infarcts					
N, ≥1 infarct*	35	30	29	27	121
OR (95% CI)					
Model 1	1.07 (0.78, 1.47)	1.04 (0.76, 1.41)	0.93 (0.69, 1.25)	Reference	1.06 (0.95, 1.19)
Model 2	0.95 (0.69, 1.31)	0.98 (0.71, 1.33)	0.89 (0.66, 1.21)	Reference	1.01 (0.90, 1.13)
Model 3	0.76 (0.54, 1.09)	0.91 (0.66, 1.25)	0.84 (0.62, 1.14)	Reference	0.92 (0.81, 1.05)
	LA Conduit Strain Quartiles				LA Conduit Strain per 1-SD decrease (5.6%)
	≤10.84% (n=331)	10.85% - 14.42% (n=332)	14.43% - 18.15% (n=332)	≥18.16% (n=332)	
Any Infarcts					
N, ≥1 infarct*	96	66	83	61	306
OR (95% CI)					

Model 1	1.90 (1.53, 2.36)	1.14 (0.91, 1.43)	1.70 (1.38, 2.09)	Reference	1.28 (1.18, 1.38)
Model 2	1.60 (1.28, 2.00)	1.01 (0.81, 1.27)	1.59 (1.29, 1.96)	Reference	1.20 (1.10, 1.30)
Model 3	1.33 (1.05, 1.69)	0.92 (0.73, 1.16)	1.52 (1.23, 1.88)	Reference	1.12 (1.03, 1.22)
Lacunar Infarcts					
N, ≥1 infarct[*]	67	39	66	43	215
OR (95% CI)					
Model 1	2.14 (1.67, 2.76)	0.91 (0.69, 1.20)	2.20 (1.73, 2.79)	Reference	1.29 (1.18, 1.42)
Model 2	1.75 (1.35, 2.27)	0.79 (0.60, 1.06)	2.06 (1.62, 2.63)	Reference	1.19 (1.09, 1.31)
Model 3	1.51 (1.15, 1.99)	0.74 (0.55, 0.99)	1.99 (1.56, 2.54)	Reference	1.14 (1.03, 1.26)
Cortical Infarcts					
N, ≥1 infarct[*]	42	31	22	26	121
OR (95% CI)					
Model 1	1.29 (0.95, 1.74)	1.09 (0.81, 1.47)	0.66 (0.47, 0.92)	Reference	1.17 (1.04, 1.32)
Model 2	1.15 (0.84, 1.57)	0.99 (0.74, 1.35)	0.61 (0.44, 0.85)	Reference	1.13 (0.99, 1.27)
Model 3	0.97 (0.70, 1.35)	0.93 (0.68, 1.26)	0.59 (0.42, 0.83)	Reference	1.06 (0.93, 1.20)

	LA Contractile Strain Quartiles				LA Contractile Strain
	≤14.43% (n=331)	14.44% - 17.62% (n=332)	17.63% - 21.04% (n=333)	≥21.05% (n=331)	per 1-SD decrease (5.5%)
Any Infarcts					
N, ≥1 infarct[*]	89	63	63	91	306
OR (95% CI)					
Model 1	0.93 (0.76, 1.13)	0.61 (0.50, 0.75)	0.65 (0.53, 0.80)	Reference	0.98 (0.91, 1.06)
Model 2	0.85 (0.69, 1.04)	0.64 (0.52, 0.79)	0.64 (0.52, 0.79)	Reference	0.96 (0.88, 1.03)
Model 3	0.73 (0.59, 0.91)	0.59 (0.48, 0.73)	0.62 (0.50, 0.76)	Reference	0.90 (0.83, 0.97)
Lacunar Infarcts					
N, ≥1 infarct[*]	66	45	46	58	215
OR (95% CI)					

	≤26.52 ml/m² (n=331)	26.53 – 32.55 ml/m² (n=332)	32.56 – 39.64 ml/m² (n=332)	≥39.65 ml/m² (n=332)	LA Maximal Volume Index per 1-SD increase (10.7 ml/m²)
Model 1	1.00 (0.80, 1.26)	0.64 (0.51, 0.82)	0.68 (0.53, 0.86)	Reference	1.01 (0.93, 1.10)
Model 2	0.89 (0.71, 1.13)	0.68 (0.53, 0.87)	0.68 (0.53, 0.86)	Reference	0.98 (0.90, 1.07)
Model 3	0.80 (0.63, 1.03)	0.65 (0.51, 0.83)	0.66 (0.52, 0.84)	Reference	0.94 (0.85, 1.03)
Cortical Infarcts					
N, ≥1 infarct*	30	24	25	42	121
OR (95% CI)					
Model 1	0.81 (0.60, 1.09)	0.67 (0.50, 0.91)	0.77 (0.57, 1.03)	Reference	0.94 (0.84, 1.05)
Model 2	0.75 (0.55, 1.02)	0.67 (0.49, 0.91)	0.73 (0.54, 0.99)	Reference	0.92 (0.82, 1.03)
Model 3	0.67 (0.49, 0.93)	0.65 (0.48, 0.89)	0.72 (0.53, 0.97)	Reference	0.88 (0.78, 0.98)

	LA Maximal Volume Index Quartiles				**LA Maximal Volume Index per 1-SD increase (10.7 ml/m²)**
	≤26.52 ml/m² (n=331)	26.53 – 32.55 ml/m² (n=332)	32.56 – 39.64 ml/m² (n=332)	≥39.65 ml/m² (n=332)	
Any Infarcts					
N, ≥1 infarct*	69	64	75	98	306
OR (95% CI)					
Model 1	Reference	0.65 (0.52, 0.81)	0.83 (0.67, 1.02)	1.59 (1.29, 1.94)	1.23 (1.14, 1.32)
Model 2	Reference	0.64 (0.51, 0.80)	0.78 (0.63, 0.97)	1.40 (1.14, 1.73)	1.17 (1.08, 1.26)
Model 3	Reference	0.64 (0.51, 0.80)	0.77 (0.62, 0.96)	1.30 (1.04, 1.62)	1.12 (1.03, 1.22)
Lacunar Infarcts					
N, ≥1 infarct*	45	45	57	68	215
OR (95% CI)					
Model 1	Reference	0.71 (0.55, 0.91)	1.03 (0.81, 1.31)	1.60 (1.27, 2.03)	1.21 (1.11, 1.31)
Model 2	Reference	0.70 (0.54, 0.91)	0.97 (0.76, 1.25)	1.39 (1.09, 1.77)	1.13 (1.03, 1.24)
Model 3	Reference	0.72 (0.55, 0.93)	0.99 (0.77, 1.27)	1.38 (1.07, 1.78)	1.12 (1.02, 1.23)
Cortical Infarcts					
N, ≥1 infarct*	29	26	26	40	121
OR (95% CI)					

	LA Minimal Volume Index Quartiles				LA Minimal Volume Index per 1-SD increase (7.1 ml/m^2)
	≤9.83 ml/m^2 (n=331)	9.84 – 13.19 ml/m^2 (n=332)	13.20 – 17.65 ml/m^2 (n=332)	≥17.66 ml/m^2 (n=332)	
Model 1	Reference	0.75 (0.55, 1.03)	0.62 (0.45, 0.86)	1.45 (1.09, 1.93)	1.13 (1.01, 1.26)
Model 2	Reference	0.75 (0.55, 1.03)	0.62 (0.44, 0.86)	1.34 (0.99, 1.81)	1.09 (0.97, 1.22)
Model 3	Reference	0.74 (0.54, 1.02)	0.59 (0.42, 0.83)	1.18 (0.87, 1.61)	1.00 (0.89, 1.13)
Any Infarcts					
N, ≥1 infarct[*]	73	59	75	99	306
OR (95% CI)					
Model 1	Reference	0.59 (0.47, 0.73)	0.92 (0.75, 1.14)	1.51 (1.23, 1.86)	1.28 (1.19, 1.38)
Model 2	Reference	0.61 (0.49, 0.76)	0.87 (0.71, 1.08)	1.34 (1.08, 1.65)	1.21 (1.11, 1.31)
Model 3	Reference	0.59 (0.47, 0.73)	0.84 (0.68, 1.04)	1.20 (0.97, 1.50)	1.15 (1.05, 1.25)
Lacunar Infarcts					
N, ≥1 infarct[*]	45	41	62	67	215
OR (95% CI)					
Model 1	Reference	0.66 (0.51, 0.86)	1.34 (1.06, 1.69)	1.51 (1.19, 1.92)	1.25 (1.14, 1.36)
Model 2	Reference	0.70 (0.54, 0.91)	1.26 (0.99, 1.60)	1.30 (1.02, 1.67)	1.15 (1.06, 1.26)
Model 3	Reference	0.69 (0.52, 0.90)	1.25 (0.98, 1.59)	1.25 (0.96, 1.61)	1.13 (1.03, 1.25)
Cortical Infarcts					
N, ≥1 infarct[*]	32	29	20	40	121
OR (95% CI)					
Model 1	Reference	0.67 (0.49, 0.92)	0.54 (0.39, 0.75)	1.39 (1.04, 1.84)	1.20 (1.07, 1.33)
Model 2	Reference	0.70 (0.51, 0.96)	0.52 (0.37, 0.73)	1.29 (0.96, 1.73)	1.15 (1.03, 1.28)
Model 3	Reference	0.67 (0.49, 0.92)	0.49 (0.35, 0.69)	1.12 (0.82, 1.52)	1.05 (0.93, 1.19)

Model 1: adjusted for age, sex, race/center, APOE ε4, education

Model 2: adjusted for model 1 plus body mass index, smoking status, diabetes, systolic blood pressure, antihypertensive medications, coronary heart disease, heart failure, anticoagulant use

Model 3: adjusted for model 2 plus LA max volume index (for LA function measures only), left ventricular ejection fraction, left ventricular mass index

Abbreviations: LA = left atrial; OR = odds ratio; APOE = apolipoprotein E

*Number of participants with ≥1 infarct present

Table 9.3. Association of Left Atrial Measures with Cerebral Microbleeds, ARIC-NCS, 2011-2013 (n=1,327)

	LA Reservoir Strain Quartiles				LA Reservoir Strain per 1-SD decrease (7.4%)
	≤27.56% (n=331)	27.57% - 32.04% (n=332)	32.05% - 37.75% (n=332)	≥37.76% (n=332)	
Cerebral Microbleeds					
N, ≥1 microbleed[*]	105	78	70	63	316
OR (95% CI)					
Model 1	1.96 (1.57, 2.43)	1.20 (0.96, 1.51)	1.29 (1.04, 1.60)	Reference	1.19 (1.10, 1.29)
Model 2	1.89 (1.51, 2.36)	1.21 (0.96, 1.51)	1.29 (1.04, 1.60)	Reference	1.16 (1.07, 1.26)
Model 3	1.74 (1.37, 2.21)	1.17 (0.93, 1.48)	1.25 (1.01, 1.56)	Reference	1.11 (1.02, 1.22)
Subcortical Microbleeds					
N, ≥1 microbleed[*]	86	62	53	52	253
OR (95% CI)					
Model 1	1.85 (1.47, 2.34)	1.09 (0.85, 1.38)	1.17 (0.93, 1.48)	Reference	1.16 (1.06, 1.26)
Model 2	1.87 (1.47, 2.38)	1.09 (0.85, 1.39)	1.17 (0.93, 1.48)	Reference	1.15 (1.06, 1.26)
Model 3	1.76 (1.37, 2.28)	1.08 (0.84, 1.39)	1.15 (0.91, 1.45)	Reference	1.12 (1.01, 1.23)
Lobar Microbleeds					
N, ≥1 microbleed[*]	34	26	23	26	109
OR (95% CI)					
Model 1	1.71 (1.21, 2.41)	1.29 (0.91, 1.84)	1.29 (0.92, 1.81)	Reference	1.16 (1.02, 1.31)
Model 2	1.55 (1.08, 2.23)	1.35 (0.95, 1.93)	1.31 (0.93, 1.85)	Reference	1.11 (0.98, 1.26)
Model 3	1.55 (1.05, 2.28)	1.38 (0.96, 1.99)	1.31 (0.93, 1.85)	Reference	1.11 (0.96, 1.28)
	LA Conduit Strain Quartiles				LA Conduit Strain per 1-SD decrease (5.6%)
	≤10.84% (n=331)	10.85% - 14.42% (n=332)	14.43% - 18.15% (n=332)	≥18.16% (n=332)	
Cerebral Microbleeds					
N, ≥1 microbleed[*]	103	76	75	62	316

OR (95% CI)					
Model 1	1.66 (1.33, 2.06)	1.15 (0.92, 1.42)	1.23 (0.99, 1.52)	Reference	1.17 (1.08, 1.26)
Model 2	1.58 (1.26, 1.98)	1.14 (0.92, 1.43)	1.21 (0.97, 1.50)	Reference	1.15 (1.06, 1.24)
Model 3	1.42 (1.12, 1.79)	1.09 (0.87, 1.36)	1.18 (0.95, 1.47)	Reference	1.10 (1.01, 1.20)
Subcortical Microbleeds					
N, ≥1 microbleed[*]	89	61	51	52	253
OR (95% CI)					
Model 1	1.73 (1.38. 2.18)	1.04 (0.83, 1.32)	1.01 (0.80, 1.27)	Reference	1.19 (1.10, 1.30)
Model 2	1.72 (1.36, 2.17)	1.06 (0.83, 1.34)	0.99 (0.79, 1.26)	Reference	1.19 (1.09, 1.30)
Model 3	1.57 (1.23, 2.01)	1.02 (0.80, 1.30)	0.99 (0.78, 1.25)	Reference	1.15 (1.05, 1.26)
Lobar Microbleeds					
N, ≥1 microbleed[*]	29	27	31	22	109
OR (95% CI)					
Model 1	1.29 (0.90, 1.84)	1.31 (0.93, 1.84)	1.45 (1.05, 2.02)	Reference	1.08 (0.95, 1.22)
Model 2	1.15 (0.78, 1.67)	1.27 (0.90, 1.81)	1.45 (1.04, 2.03)	Reference	1.03 (0.90, 1.17)
Model 3	1.07 (0.72, 1.60)	1.24 (0.87, 1.77)	1.44 (1.03, 2.02)	Reference	1.00 (0.87, 1.15)
	LA Contractile Strain Quartiles				**LA Contractile Strain per 1-SD decrease**
	≤14.43% (n=331)	14.44% - 17.62% (n=332)	17.63% - 21.04% (n=333)	≥21.05% (n=331)	(5.5%)
Cerebral Microbleeds					
N, ≥1 microbleed[*]	83	81	79	73	316
OR (95% CI)					
Model 1	1.03 (0.83, 1.28)	1.18 (0.96, 1.45)	1.01 (0.82, 1.25)	Reference	1.09 (1.01, 1.17)
Model 2	0.97 (0.78, 1.21)	1.19 (0.96, 1.46)	1.02 (0.82, 1.26)	Reference	1.07 (0.99, 1.16)
Model 3	0.91 (0.73, 1.15)	1.15 (0.93, 1.43)	1.00 (0.81, 1.24)	Reference	1.04 (0.96, 1.13)
Subcortical Microbleeds					
N, ≥1 microbleed[*]	62	66	63	62	253

53

OR (95% CI)					
Model 1	0.84 (0.67, 1.07)	1.04 (0.83, 1.29)	0.93 (0.74, 1.17)	Reference	1.02 (0.94, 1.11)
Model 2	0.82 (0.65, 1.04)	1.05 (0.84, 1.32)	0.94 (0.75, 1.18)	Reference	1.02 (0.94, 1.11)
Model 3	0.78 (0.61, 0.99)	1.04 (0.83, 1.30)	0.94 (0.75, 1.18)	Reference	1.00 (0.92, 1.09)
Lobar Microbleeds					
N, ≥1 microbleed[*]	29	28	25	27	109
OR (95% CI)					
Model 1	1.34 (0.97, 1.86)	1.13 (0.81, 1.58)	0.97 (0.69, 1.36)	Reference	1.14 (1.01, 1.29)
Model 2	1.27 (0.91, 1.78)	1.17 (0.83, 1.64)	1.01 (0.71, 1.42)	Reference	1.12 (0.99, 1.27)
Model 3	1.29 (0.91, 1.83)	1.19 (0.84, 1.68)	1.01 (0.71, 1.43)	Reference	1.13 (0.99, 1.29)

	LA Maximal Volume Index Quartiles				LA Maximal Volume
	≤26.52 ml/m^2 (n=331)	26.53 - 32.55 ml/m^2 (n=332)	32.56 - 39.64 ml/m^2 (n=332)	≥39.65 ml/m^2 (n=332)	Index per 1-SD increase (10.7 ml/m^2)
Cerebral Microbleeds					
N, ≥1 microbleed[*]	69	71	85	91	316
OR (95% CI)					
Model 1	Reference	0.84 (0.68, 1.05)	1.25 (1.01, 1.53)	1.16 (0.94, 1.44)	1.10 (1.02, 1.19)
Model 2	Reference	0.84 (0.67, 1.04)	1.24 (1.00, 1.53)	1.11 (0.89, 1.38)	1.08 (0.99, 1.17)
Model 3	Reference	0.85 (0.69, 1.06)	1.25 (1.01, 1.55)	1.08 (0.86, 1.37)	1.07 (0.99, 1.17)
Subcortical Microbleeds					
N, ≥1 microbleed[*]	57	55	70	71	253
OR (95% CI)					
Model 1	Reference	0.68 (0.54, 0.86)	1.03 (0.82, 1.28)	0.96 (0.77, 1.21)	1.04 (0.96, 1.14)
Model 2	Reference	0.69 (0.54, 0.87)	1.02 (0.81, 1.27)	0.94 (0.74, 1.19)	1.03 (0.95, 1.13)
Model 3	Reference	0.69 (0.55, 0.87)	1.01 (0.80, 1.26)	0.88 (0.69, 1.12)	0.99 (0.91, 1.10)
Lobar Microbleeds					
N, ≥1 microbleed[*]	26	26	27	30	109
OR (95% CI)					

	≤9.83 ml/m² (n=331)	9.84 - 13.19 ml/m² (n=332)	13.20 - 17.65 ml/m² (n=332)	≥17.66 ml/m² (n=332)	LA Minimal Volume Index per 1-SD increase (7.1 ml/m²)
Model 1	Reference	1.13 (0.80, 1.59)	1.48 (1.06, 2.06)	1.20 (0.84, 1.71)	1.04 (0.92, 1.18)
Model 2	Reference	1.06 (0.75, 1.50)	1.38 (0.98, 1.94)	1.06 (0.74, 1.54)	0.99 (0.87, 1.12)
Model 3	Reference	1.07 (0.75, 1.52)	1.40 (0.99, 1.98)	1.05 (0.72, 1.53)	0.97 (0.85, 1.12)
LA Minimal Volume Index Quartiles					
Cerebral Microbleeds					
N, ≥1 microbleed*	69	66	77	104	316
OR (95% CI)					
Model 1	Reference	0.81 (0.65, 1.00)	1.07 (0.87, 1.32)	1.33 (1.07, 1.65)	1.17 (1.08, 1.26)
Model 2	Reference	0.82 (0.66, 1.02)	1.06 (0.86, 1.31)	1.28 (1.03, 1.59)	1.14 (1.05, 1.24)
Model 3	Reference	0.81 (0.65, 1.01)	1.05 (0.85, 1.31)	1.24 (0.99, 1.55)	1.12 (1.03, 1.23)
Subcortical Microbleeds					
N, ≥1 microbleed*	60	49	62	82	253
OR (95% CI)					
Model 1	Reference	0.67 (0.53, 0.84)	0.91 (0.73, 1.14)	1.03 (0.82, 1.30)	1.10 (1.01, 1.19)
Model 2	Reference	0.67 (0.53, 0.85)	0.92 (0.73, 1.15)	1.02 (0.81, 1.29)	1.09 (1.00, 1.19)
Model 3	Reference	0.65 (0.51, 0.82)	0.89 (0.71, 1.12)	0.94 (0.74, 1.20)	1.05 (0.95, 1.15)
Lobar Microbleeds					
N, ≥1 microbleed*	26	23	26	34	109
OR (95% CI)					
Model 1	Reference	0.96 (0.68, 1.36)	1.23 (0.88, 1.72)	1.36 (0.97, 1.92)	1.11 (0.98, 1.25)
Model 2	Reference	0.95 (0.67, 1.34)	1.13 (0.81, 1.59)	1.21 (0.84, 1.73)	1.04 (0.92, 1.18)
Model 3	Reference	0.94 (0.66, 1.33)	1.13 (0.80, 1.59)	1.18 (0.82, 1.70)	1.02 (0.89, 1.17)

Model 1: adjusted for age, sex, race/center, APOE ε4, education

Model 2: adjusted for model 1 plus body mass index, smoking status, diabetes, systolic blood pressure, antihypertensive medications, coronary heart disease, heart failure, anticoagulant use

Model 3: adjusted for model 2 plus LA max volume index (for LA function measures only), left ventricular ejection fraction, left ventricular mass index

Abbreviations: LA = left atrial; OR = odds ratio; APOE = apolipoprotein E

[*]Number of participants with $\geq$1 microbleed present

Table 9.4. Association of Left Atrial Measures with Brain Volume Measures, ARIC-NCS, 2011-2013 (n=1,327)

	LA Reservoir Strain Quartiles				LA Reservoir Strain per 1-SD decrease (7.4%)
	≤27.56% (n=331)	27.57% - 32.04% (n=332)	32.05% - 37.75% (n=332)	≥37.76% (n=332)	
Log$_2$(WMH Volume), cm^3					
β (95% CI)					
Model 1	0.15 (0.01, 0.29)	0.09 (-0.05, 0.23)	0.09 (-0.04, 0.22)	Reference	0.06 (0.01, 0.12)
Model 2	0.08 (-0.07, 0.22)	0.06 (-0.08, 0.20)	0.09 (-0.04, 0.22)	Reference	0.03 (-0.02, 0.09)
Model 3	0.07 (-0.08, 0.23)	0.06 (-0.08, 0.20)	0.09 (-0.04, 0.22)	Reference	0.04 (-0.02, 0.09)
Deep Gray Matter Volume, cm^3					
β (95% CI)					
Model 1	-0.06 (-0.16, 0.05)	0.02 (-0.08, 0.12)	0.04 (-0.06, 0.13)	Reference	-0.02 (-0.05, 0.02)
Model 2	-0.05 (-0.16, 0.05)	0.02 (-0.08, 0.12)	0.04 (-0.05, 0.14)	Reference	-0.01 (-0.05, 0.02)
Model 3	-0.03 (-0.14, 0.08)	0.03 (-0.07, 0.14)	0.05 (-0.05, 0.15)	Reference	-0.004 (-0.05, 0.04)
Temporal Lobe Volume ROI, cm^3					
β (95% CI)					
Model 1	-0.13 (-0.22, -0.03)	-0.10 (-0.19, -0.01)	-0.04 (-0.13, 0.04)	Reference	-0.05 (-0.08, -0.01)
Model 2	-0.10 (-0.19, -0.0004)	-0.08 (-0.17, 0.01)	-0.04 (-0.13, 0.05)	Reference	-0.04 (-0.07, -0.001)
Model 3	-0.09 (-0.19, 0.02)	-0.08 (-0.18, 0.01)	-0.03 (-0.12, 0.05)	Reference	-0.03 (-0.07, 0.004)
LA Conduit Strain Quartiles					LA Conduit Strain per 1-SD decrease (5.6%)
	≤10.84% (n=331)	10.85% - 14.42% (n=332)	14.43% - 18.15% (n=332)	≥18.16% (n=332)	

Log$_2$(WMH Volume), cm^3					
β (95% CI)					
Model 1	0.09 (-0.05, 0.23)	0.02 (-0.11, 0.16)	0.13 (-0.002, 0.27)	Reference	0.02 (-0.03, 0.07)
Model 2	0.02 (-0.13, 0.16)	-0.05 (-0.18, 0.09)	0.07 (-0.06, 0.21)	Reference	-0.01 (-0.07, 0.04)
Model 3	-0.001 (-0.15, 0.15)	-0.05 (-0.19, 0.08)	0.07 (-0.06, 0.21)	Reference	-0.02 (-0.08, 0.03)
Deep Gray Matter Volume, cm^3					
β (95% CI)					
Model 1	-0.07 (-0.17, 0.03)	-0.07 (-0.17, 0.03)	0.04 (-0.05, 0.14)	Reference	-0.02 (-0.06, 0.02)
Model 2	-0.06 (-0.17, 0.04)	-0.08 (-0.18, 0.02)	0.02 (-0.08, 0.12)	Reference	-0.02 (-0.06, 0.02)
Model 3	-0.05 (-0.16, 0.06)	-0.07 (-0.17, 0.03)	0.02 (-0.07, 0.13)	Reference	-0.01 (-0.05, 0.03)
Temporal Lobe Volume ROI, cm^3					
β (95% CI)					
Model 1	-0.13 (-0.23, -0.04)	-0.07 (-0.16, 0.02)	-0.12 (-0.21, -0.03)	Reference	-0.04 (-0.07, -0.01)
Model 2	-0.10 (-0.20, -0.01)	-0.04 (-0.13, 0.05)	-0.11 (-0.20, -0.02)	Reference	-0.03 (-0.06, 0.01)
Model 3	-0.08 (-0.18, 0.02)	-0.04 (-0.13, 0.06)	-0.11 (-0.20, -0.02)	Reference	-0.02 (-0.06, 0.01)
	LA Contractile Strain Quartiles				LA Contractile Strain per 1-SD decrease (5.5%)
	≤14.43% (n=331)	14.44% - 17.62% (n=332)	17.63% - 21.04% (n=333)	≥21.05% (n=331)	
Log$_2$(WMH Volume), cm^3					
β (95% CI)					
Model 1	0.17 (0.03, 0.31)	0.09 (-0.05, 0.22)	0.06 (-0.08, 0.20)	Reference	0.07 (0.02, 0.12)
Model 2	0.13 (-0.004, 0.27)	0.11 (-0.02, 0.25)	0.06 (-0.08, 0.19)	Reference	0.06 (0.01, 0.11)
Model 3	0.14 (-0.003, 0.28)	0.12 (-0.01, 0.25)	0.06 (-0.07, 0.19)	Reference	0.07 (0.01, 0.12)

Deep Gray Matter Volume, cm³					
β (95% CI)					
Model 1	0.003 (-0.10, 0.10)	0.01 (-0.09, 0.11)	0.11 (0.01, 0.21)	Reference	0.001 (-0.04, 0.04)
Model 2	0.12 (-0.09, 0.11)	0.02 (-0.08, 0.11)	0.11 (0.01, 0.21)	Reference	0.004 (-0.03, 0.04)
Model 3	0.03 (-0.08, 0.13)	0.02 (-0.08, 0.12)	0.11 (0.01, 0.21)	Reference	0.01 (-0.03, 0.05)
Temporal Lobe Volume ROI, cm³					
β (95% CI)					
Model 1	-0.05 (-0.15, 0.04)	-0.04 (-0.13, 0.05)	-0.02 (-0.11, 0.07)	Reference	-0.02 (-0.06, 0.01)
Model 2	-0.03 (-0.13, 0.06)	-0.05 (-0.14, 0.04)	-0.02 (-0.11, 0.07)	Reference	-0.02 (-0.05, 0.02)
Model 3	-0.03 (-0.13, 0.06)	-0.05 (-0.14, 0.04)	-0.02 (-0.11, 0.07)	Reference	-0.02 (-0.05, 0.02)
	LA Maximal Volume Index Quartiles				**LA Maximal Volume Index per 1-SD increase (10.7 ml/m²)**
	≤26.52 ml/m² (n=331)	26.53 - 32.55 ml/m² (n=332)	32.56 - 39.64 ml/m² (n=332)	≥39.65 ml/m² (n=332)	
Log₂(WMH Volume), cm³					
β (95% CI)					
Model 1	Reference	-0.06 (-0.19, 0.08)	0.01 (-0.04, 0.24)	0.02 (-0.13, 0.16)	0.03 (-0.02, 0.08)
Model 2	Reference	-0.06 (-0.08, 0.07)	0.06 (-0.08, 0.19)	-0.06 (-0.20, 0.08)	-0.005 (-0.06, 0.05)
Model 3	Reference	-0.08 (-0.21, 0.06)	0.04 (-0.10, 0.18)	-0.11 (-0.26, 0.04)	-0.03 (-0.08, 0.03)
Deep Gray Matter Volume, cm³					
β (95% CI)					
Model 1	Reference	-0.04 (-0.14, 0.06)	0.01 (-0.09, 0.11)	-0.06 (-0.16, 0.04)	-0.01 (-0.05, 0.02)
Model 2	Reference	-0.05 (-0.15, 0.05)	-0.01 (-0.11, 0.09)	-0.08 (-0.18, 0.03)	-0.02 (-0.06, 0.02)

	LA Minimal Volume Index Quartiles				LA Minimal Volume Index per 1-SD increase (7.1 ml/m²)
Model 3	Reference	-0.06 (-0.16, 0.04)	-0.01 (-0.11, 0.09)	-0.09 (-0.20, 0.01)	-0.03 (-0.07, 0.01)
Temporal Lobe Volume ROI, cm³ β (95% CI)					
Model 1	Reference	-0.01 (-0.10, 0.08)	-0.05 (-0.14, 0.04)	-0.02 (-0.11, 0.08)	-0.01 (-0.04, 0.03)
Model 2	Reference	-0.01 (-0.10, 0.08)	-0.04 (-0.13, 0.05)	0.01 (-0.09, 0.10)	-0.002 (-0.03, 0.04)
Model 3	Reference	-0.01 (-0.10, 0.08)	-0.03 (-0.12, 0.06)	0.03 (-0.07, 0.13)	0.01 (-0.02, 0.05)
	LA Minimal Volume Index Quartiles				
	$\leq$9.83 ml/m² (n=331)	9.84 - 13.19 ml/m² (n=332)	13.20 - 17.65 ml/m² (n=332)	$\geq$17.66 ml/m² (n=332)	
Log$_2$(WMH Volume), cm³ β (95% CI)					
Model 1	Reference	-0.04 (-0.17, 0.10)	0.03 (-0.10, 0.17)	0.06 (-0.08, 0.21)	0.05 (0.0001, 0.11)
Model 2	Reference	-0.03 (-0.16, 0.11)	0.004 (-0.13, 0.14)	-0.01 (-0.15, 0.13)	0.02 (-0.03, 0.07)
Model 3	Reference	-0.04 (-0.17, 0.09)	-0.01 (-0.15, 0.12)	-0.05 (-0.20, 0.09)	-0.003 (-0.06, 0.05)
Deep Gray Matter Volume, cm³ β (95% CI)					
Model 1	Reference	-0.09 (-0.18, 0.01)	0.01 (-0.09, 0.10)	-0.03 (-0.13, 0.07)	-0.01 (-0.05, 0.03)
Model 2	Reference	-0.10 (-0.20, -0.001)	0.003 (-0.10, 0.10)	-0.04 (-0.14, 0.07)	-0.02 (-0.03, 0.02)
Model 3	Reference	-0.10 (-0.20, -0.001)	-0.003 (-0.10, 0.10)	-0.04 (-0.15, 0.07)	-0.02 (-0.06, 0.02)
Temporal Lobe Volume ROI, cm³ β (95% CI)					
Model 1	Reference	-0.05 (-0.14, 0.03)	-0.10 (-0.19, -0.01)	-0.06 (-0.15, 0.04)	-0.02 (-0.06, 0.02)

Model 2	Reference	-0.06 (-0.15, 0.03)	-0.09 (-0.18, 0.01)	-0.03 (-0.13, 0.07)	-0.01 (-0.04, 0.03)
Model 3	Reference	-0.05 (-0.14, 0.03)	-0.08 (-0.17, 0.02)	-0.01 (-0.10, 0.09)	0.01 (-0.03, 0.05)

Model 1: adjusted for age, sex, race/center, APOE ε4, education, total intracranial volume

Model 2: adjusted for model 1 plus body mass index, smoking status, diabetes, systolic blood pressure, antihypertensive medications, coronary heart disease, heart failure, anticoagulant use

Model 3: adjusted for model 2 plus LA max volume index (for LA function measures only), left ventricular ejection fraction, left ventricular mass index

Abbreviations: LA = left atrial; ROI = region of interest; WMH = white matter hyperintensity

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┌─────────────────────────────────┐
│ Atherosclerosis Risk in Communities │
│ study population at Visit 5 (2011-13) │
│           n = 6,538              │
└─────────────────────────────────┘
                 │
                 ▼
┌─────────────────────────────────┐
│ Visit 5 participants who had brain MRIs │
│            (2011-13)             │
│           n = 1,978              │
└─────────────────────────────────┘
                 │
                 │───────────────────────►  ┌──────────────────────────────────────────┐
                 │                           │ Missing LA function measures: n=384        │
                 ▼                           │ Prevalent atrial fibrillation or stroke: n=107 │
┌─────────────────────────────────┐          │ Prevalent dementia: n=84                   │
│   Final Analytic Sample Size    │          │ Missing covariates: n=45                   │
│          n = 1,327              │          │ Missing brain MRI measures: n=16           │
└─────────────────────────────────┘          │ Race exclusions: n=15                      │
                                             └──────────────────────────────────────────┘
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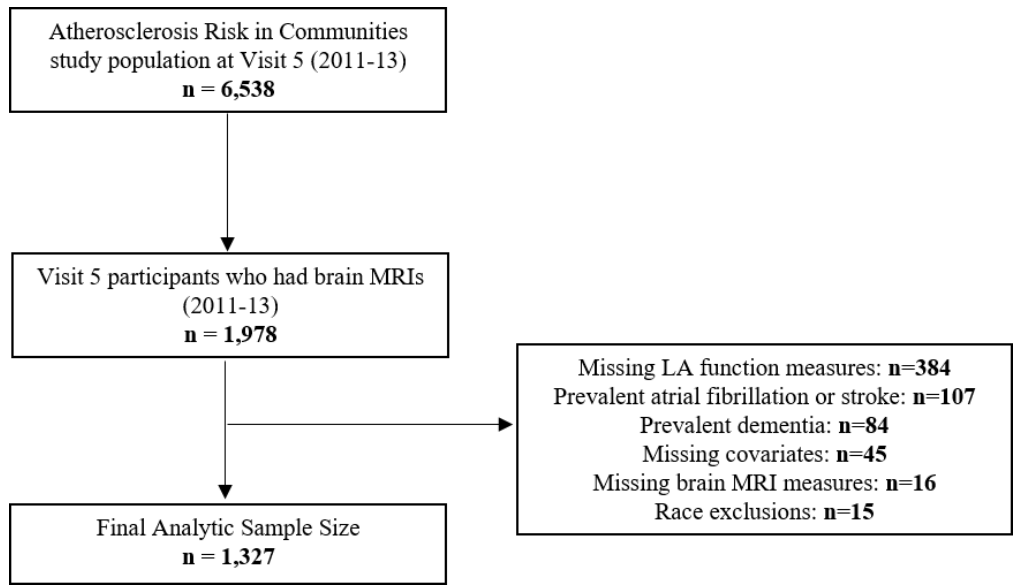
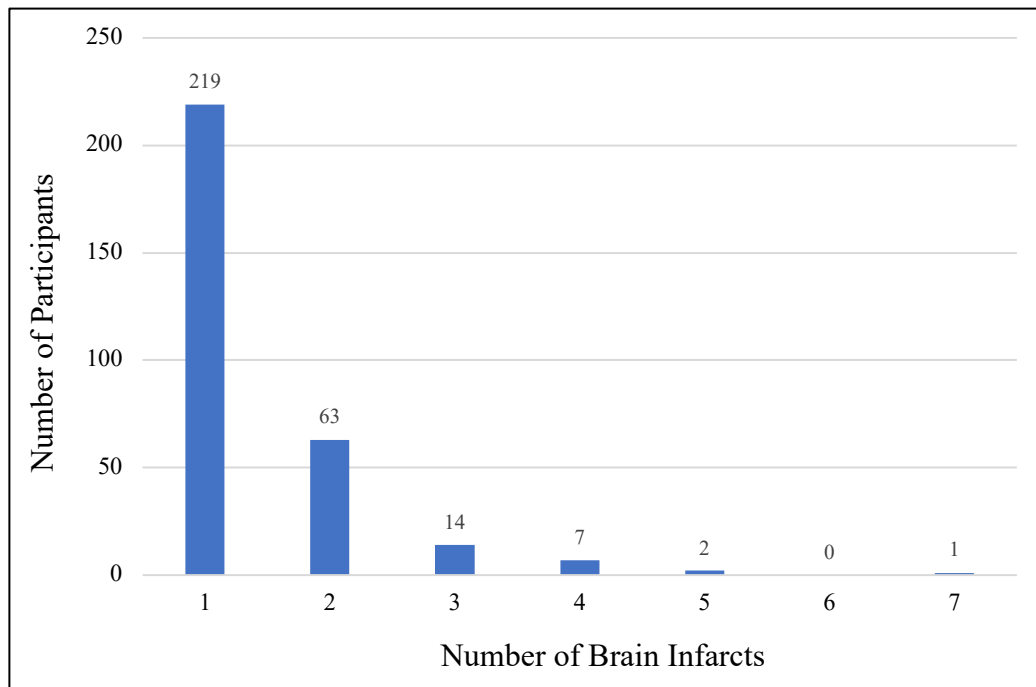**Figure 9.1**. Participant Exclusion Flowchart, ARIC-NCS

Figure 9.2. Frequency of Brain Infarcts[*], ARIC-NCS, 2011-2013
[*]Among participants with at least one brain infarct
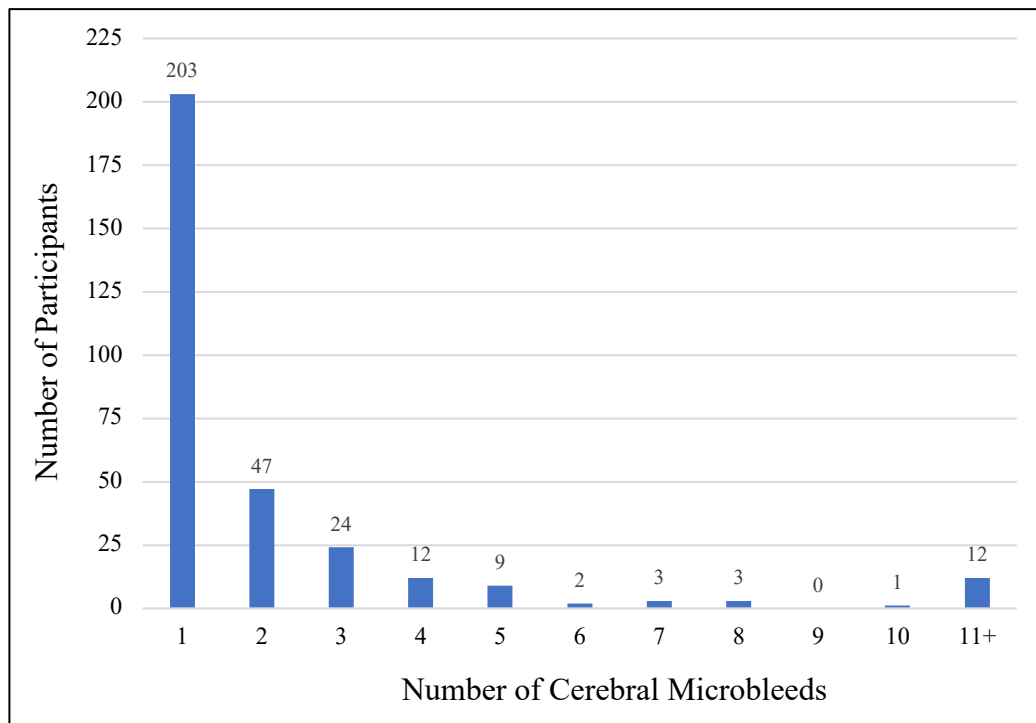
Figure 9.3. Frequency of Cerebral Microbleeds[*], ARIC-NCS, 2011-2013
[*]Among participants with at least one cerebral microbleed

Supplemental Table 9.1. Association of Left Atrial Measures with Number of Brain Infarcts, ARIC-NCS, 2011-2013 (n=1,327)

	LA Reservoir Strain Quartiles				LA Reservoir Strain per 1-SD decrease (7.4%)
	≤27.56% (n=331)	27.57% - 32.04% (n=332)	32.05% - 37.75% (n=332)	≥37.76% (n=332)	
N, ≥1 infarcts	86	76	80	64	306
Mean no. infarcts[*]	1.44	1.39	1.45	1.34	1.41
RR (95% CI)					
Model 1	1.40 (0.99, 1.96)	1.24 (0.89, 1.74)	1.36 (0.98, 1.88)	Reference	1.12 (1.00, 1.26)
Model 2	1.13 (0.80, 1.59)	1.15 (0.82, 1.60)	1.29 (0.93, 1.78)	Reference	1.04 (0.92, 1.17)
Model 3	1.00 (0.69, 1.44)	1.07 (0.76, 1.51)	1.24 (0.89, 1.72)	Reference	0.99 (0.87, 1.12)
	LA Conduit Strain Quartiles				LA Conduit Strain per 1-SD decrease (5.6%)
	≤10.84% (n=331)	10.85% - 14.42% (n=332)	14.43% - 18.15% (n=332)	≥18.16% (n=332)	
N, ≥1 infarcts	96	66	83	61	306
Mean no. infarcts[*]	1.45	1.26	1.42	1.51	1.41
RR (95% CI)					
Model 1	1.48 (1.07, 2.05)	0.90 (0.63, 1.27)	1.33 (0.96, 1.84)	Reference	1.17 (1.04, 1.32)
Model 2	1.21 (0.86, 1.68)	0.77 (0.54, 1.09)	1.19 (0.86, 1.64)	Reference	1.08 (0.96, 1.23)
Model 3	1.10 (0.78, 1.55)	0.73 (0.52, 1.04)	1.16 (0.84, 1.61)	Reference	1.05 (0.92, 1.19)
	LA Contractile Strain Quartiles				LA Contractile Strain per 1-SD decrease (5.5%)
	≤14.43% (n=331)	14.44% - 17.62% (n=332)	17.63% - 21.04% (n=333)	≥21.05% (n=331)	
N, ≥1 infarcts	89	63	63	91	306
Mean no. infarcts[*]	1.38	1.41	1.59	1.32	1.41
RR (95% CI)					

		LA Maximal Volume Index Quartiles			LA Maximal Volume Index per 1-SD increase (10.7 ml/m^2)
Model 1	1.02 (0.75, 1.39)	0.76 (0.55, 1.06)	0.85 (0.62, 1.17)	Reference	1.02 (0.91, 1.14)
Model 2	0.91 (0.67, 1.25)	0.77 (0.55, 1.06)	0.83 (0.60, 1.13)	Reference	0.99 (0.88, 1.10)
Model 3	0.84 (0.61, 1.16)	0.73 (0.53, 1.02)	0.80 (0.58, 1.10)	Reference	0.95 (0.85, 1.07)
	≤26.52 ml/m^2 (n=331)	26.53 - 32.55 ml/m^2 (n=332)	32.56 - 39.64 ml/m^2 (n=332)	≥39.65 ml/m^2 (n=332)	
N, ≥1 infarcts	69	64	75	98	306
Mean no. infarcts[*]	1.41	1.31	1.47	1.44	1.41
RR (95% CI)					
Model 1	Reference	0.84 (0.59, 1.18)	1.16 (0.83, 1.60)	1.52 (1.11, 2.09)	1.17 (1.04, 1.31)
Model 2	Reference	0.81 (0.57, 1.13)	1.06 (0.76, 1.47)	1.30 (0.94, 1.79)	1.09 (0.97, 1.22)
Model 3	Reference	0.81 (0.57, 1.14)	1.05 (0.76, 1.47)	1.27 (0.91, 1.78)	1.07 (0.95, 1.21)
		LA Minimal Volume Index Quartiles			LA Minimal Volume Index per 1-SD increase (7.1 ml/m^2)
	≤9.83 ml/m^2 (n=331)	9.84 - 13.19 ml/m^2 (n=332)	13.20 - 17.65 ml/m^2 (n=332)	≥17.66 ml/m^2 (n=332)	
N, ≥1 infarcts	73	59	75	99	306
Mean no. infarcts[*]	1.37	1.44	1.40	1.43	1.41
RR (95% CI)					
Model 1	Reference	0.82 (0.58, 1.15)	1.04 (0.75, 1.45)	1.46 (1.06, 2.01)	1.21 (1.08, 1.35)
Model 2	Reference	0.82 (0.59, 1.15)	0.94 (0.68, 1.30)	1.23 (0.89, 1.70)	1.12 (0.99, 1.25)
Model 3	Reference	0.82 (0.58, 1.14)	0.93 (0.67, 1.29)	1.19 (0.85, 1.66)	1.10 (0.97, 1.24)

Model 1: adjusted for age, sex, race/center, APOE ε4, education
Model 2: adjusted for model 1 plus body mass index, smoking status, diabetes, systolic blood pressure, antihypertensive medications, coronary heart disease, heart failure, anticoagulant use
Model 3: adjusted for model 2 plus LA max volume index (for LA function measures only), left ventricular ejection fraction, left ventricular mass index
Abbreviations: LA = left atrial; APOE = apolipoprotein E
[*]Among participants with ≥1 infarct

Supplemental Table 9.2. Association of Left Atrial Measures with Number of Cerebral Microbleeds, ARIC-NCS, 2011-2013 (n=1,327)

	LA Reservoir Strain Quartiles				LA Reservoir Strain per 1-SD decrease (7.4%)
	≤27.56% (n=331)	27.57% - 32.04% (n=332)	32.05% - 37.75% (n=332)	≥37.76% (n=332)	
N, ≥1 microbleed	105	78	70	63	316
Mean no. microbleeds*	3.35	2.04	2.11	2.90	2.66
RR (95% CI)					
Model 1	2.15 (1.43, 3.23)	0.95 (0.63, 1.42)	1.10 (0.73, 1.66)	Reference	1.25 (1.08, 1.44)
Model 2	2.29 (1.53, 3.45)	1.04 (0.70, 1.57)	1.16 (0.76, 1.77)	Reference	1.26 (1.09, 1.46)
Model 3	2.26 (1.45, 3.52)	1.05 (0.69, 1.61)	1.18 (0.77, 1.81)	Reference	1.25 (1.06, 1.47)
	LA Conduit Strain Quartiles				LA Conduit Strain per 1-SD decrease (5.6%)
	≤10.84% (n=331)	10.85% - 14.42% (n=332)	14.43% - 18.15% (n=332)	≥18.16% (n=332)	
N, ≥1 microbleed	103	76	75	62	316
Mean no. microbleeds*	3.37	2.14	2.40	2.45	2.66
RR (95% CI)					
Model 1	1.93 (1.29, 2.88)	0.96 (0.64, 1.45)	1.06 (0.71, 1.59)	Reference	1.25 (1.08, 1.45)
Model 2	2.14 (1.42, 3.21)	1.11 (0.73, 1.68)	1.12 (0.75, 1.70)	Reference	1.29 (1.11, 1.49)
Model 3	2.09 (1.36, 3.22)	1.15 (0.75, 1.76)	1.13 (0.74, 1.70)	Reference	1.28 (1.10, 1.50)
	LA Contractile Strain Quartiles				LA Contractile Strain per 1-SD decrease (5.5%)
	≤14.43% (n=331)	14.44% - 17.62% (n=332)	17.63% - 21.04% (n=333)	≥21.05% (n=331)	
N, ≥1 microbleed	83	81	79	73	316
Mean no. microbleeds*	2.19	2.99	3.01	2.47	2.66
RR (95% CI)					
Model 1	1.27 (0.84, 1.93)	1.22 (0.82, 1.80)	1.55 (1.04, 2.31)	Reference	1.11 (0.95, 1.30)
Model 2	1.16 (0.76, 1.76)	1.19 (0.79, 1.79)	1.44 (0.97, 2.15)	Reference	1.08 (0.92, 1.27)

	≤26.52 ml/m² (n=331)	26.53 - 32.55 ml/m² (n=332)	32.56 - 39.64 ml/m² (n=332)	≥39.65 ml/m² (n=332)	LA Maximal Volume Index per 1-SD increase (10.7 ml/m²)
Model 3	0.99 (0.64, 1.55)	1.12 (0.74, 1.71)	1.32 (0.88, 1.98)	Reference	1.03 (0.87, 1.21)
LA Maximal Volume Index Quartiles					
N, ≥1 microbleed	69	71	85	91	316
Mean no. microbleeds*	3.00	2.21	2.58	2.85	2.66
RR (95% CI)					
Model 1	Reference	0.83 (0.55, 1.25)	1.40 (0.93, 2.08)	1.18 (0.78, 1.80)	1.08 (0.94, 1.25)
Model 2	Reference	0.89 (0.59, 1.34)	1.47 (0.98, 2.21)	1.27 (0.83, 1.95)	1.13 (0.96, 1.31)
Model 3	Reference	0.84 (0.56, 1.28)	1.30 (0.86, 1.98)	1.09 (0.70, 1.69)	1.05 (0.89, 1.24)
LA Minimal Volume Index Quartiles	≤9.83 ml/m² (n=331)	9.84 - 13.19 ml/m² (n=332)	13.20 - 17.65 ml/m² (n=332)	≥17.66 ml/m² (n=332)	LA Minimal Volume Index per 1-SD increase (7.1 ml/m²)
N, ≥1 microbleed	69	66	77	104	
Mean no. microbleeds*	2.86	2.02	2.39	3.15	316
RR (95% CI)					2.66
Model 1	Reference	0.63 (0.42, 0.95)	1.20 (0.80, 1.79)	1.44 (0.96, 2.14)	1.15 (0.99, 1.35)
Model 2	Reference	0.71 (0.46, 1.07)	1.23 (0.82, 1.83)	1.57 (1.04, 2.36)	1.19 (1.01, 1.40)
Model 3	Reference	0.70 (0.46, 1.06)	1.17 (0.78, 1.76)	1.40 (0.92, 2.14)	1.12 (0.94, 1.33)

Model 1: adjusted for age, sex, race/center, APOE ε4, education

Model 2: adjusted for model 1 plus body mass index, smoking status, diabetes, systolic blood pressure, antihypertensive medications, coronary heart disease, heart failure, anticoagulant use

Model 3: adjusted for model 2 plus LA max volume index (for LA function measures only), left ventricular ejection fraction, left ventricular mass index

Abbreviations: LA = left atrial; APOE = apolipoprotein E

*Among participants with ≥1 microbleed

Chapter 10. Manuscript 2: Proteomics of Incident Ischemic Stroke: The Atherosclerosis Risk in Communities Study

A. Overview

Background: Proteomics profiling can discover novel biomarkers related to ischemic stroke, which may provide further insight into the pathophysiology of stroke. Using data from the Atherosclerosis Risk in Communities (ARIC) study, we examined the relationship between proteomics and incident ischemic stroke.

Methods: We included 10,822 ARIC participants (mean [SD] age 60 [6] years, 55% female; 21% Black participants) who had proteomic data at visit 3 (1993-95). Cox proportional hazards models were used to analyze the association between protein levels and incident ischemic stroke. A replication analysis in the Cardiovascular Health Study was conducted. In addition, we performed a two-sample Mendelian randomization (MR) analysis to further investigate the relationship between associated proteins and ischemic stroke.

Results: Over a median follow-up time of 22 years, 882 ischemic stroke events were ascertained. After adjustment for demographics and clinical risk factors for stroke, 7 proteins were significantly associated with an increased risk for ischemic stroke, with growth/differentiation factor 15 (GDF15) having the lowest p-value (HR [95% CI] per doubling of the protein level: 1.57 [1.36, 1.82]). When follow-up was restricted to 10 years, 5 proteins were significantly associated with ischemic stroke after full model

adjustments. Furthermore, 3 of the 5 proteins (NT-proBNP, GDF15, WAP four-disulfide core domain protein 2 [WFDC2]) replicated in CHS. MR analysis suggested that NT-proBNP (OR [95% CI] per 1-SD: 0.03 [0.002, 0.50]), but not GDF15 OR [95% CI] per 1-SD: 0.93 [0.18, 4.73]), may be causally related to ischemic stroke.

Discussion: In this community-based cohort, 3 proteins (NT-proBNP, GDF15, WFDC2) were associated with incident ischemic stroke in 2 independent cohorts. MR analysis suggests there may be a causal relationship between NT-proBNP and ischemic stroke. Additional research should assess whether these proteins could be therapeutic targets for stroke prevention or whether these proteins could be included in risk scores for stroke prediction.

B. Introduction

Stroke is the fifth leading cause of death in the US and the leading cause of long-term disability.[1] Many risk factors for stroke such as elevated blood pressure, obesity, diabetes, atrial fibrillation (AF), and smoking have been identified;[1] however, it is possible there are new stroke risk factors that have yet to be identified. Recent studies have reported that novel biomarkers can improve risk prediction for stroke.[274,275] Although these studies suggest that plasma protein biomarkers may provide novel insights into the pathophysiology of stroke, they have used a targeted approach that assess a limited set of proteins.[274,275] More recently, proteomic approaches have been used to screen large numbers of proteins to discover novel protein associations with incident ischemic stroke.[230,231,276]

In a collaboration with SomaLogic, the Atherosclerosis Risk in Communities (ARIC) study screened 4,877 plasma proteins in a population of Black and White adults and is well suited to conduct a comprehensive interrogation of the plasma proteome in relation to incident ischemic stroke. Using data from the ARIC study, we aimed to evaluate the prospective association of plasma proteins with incident ischemic stroke.

C. Methods

C.1. *Study Population*

The ARIC study is a prospective, community-based cohort that began in 1987-89. At baseline, 15,792 Black and White adults aged 45-64 years were recruited from four US communities: Forsyth County, NC; Jackson, MS; Washington County, MD; northwest suburbs of Minneapolis, MN.[237] Since then, participants have attended several additional follow-up clinic visits. For this manuscript, visit 3 (1993-95) served as the baseline.

Of the 12,887 participants who attended visit 3, we excluded those with missing or low-quality proteomic data, races other than Black or White and non-White participants in the Minneapolis and Washington County center (due to low numbers), or missing covariates. We also excluded participants with prevalent ischemic stroke, which was defined as an event prior to visit 3. The final analytic sample included 10,822 participants (**Figure 10.1**). Institutional Review Boards at each participating center approved the study and participants provided written informed consent at each visit.

For replication, we evaluated associations in an external cohort: the Cardiovascular Health Study (CHS). CHS is a population-based study of adults aged 65

years and older that began in 1989-90.[277] A total of 5,201 participants were recruited at baseline from four US communities: Forsyth County, NC; Sacramento County, CA; Washington County, MD; Pittsburgh, PA. An additional 687 predominantly African American participants were enrolled in 1992-93. For the replication analysis, the 1992-93 visit served as the baseline. Among the 5,265 participants who attended the 1992-93 visit, 3,188 participants were included in the proteomics sample. Prior ancillary studies in CHS had partially depleted the number of unthawed plasma samples, which accounts for the difference in the number of participants who attended the 1992-93 visit and those who were included in the proteomics sample.[278] Among the 3,188 participants included in the proteomics sample, we excluded those with low-quality proteomic data, missing covariates, races other than Black or White, or prevalent ischemic stroke. Ischemic stroke events were adjudicated by an events committee that was comprised of neurologists from each study site, a neuroradiologist from the MRI Reading Center, and an internist or neurologist representing the coordinating center.[279] Prevalent ischemic stroke was defined as an event prior to the baseline visit for this analysis (1992-93 visit). After all exclusions, 3,004 CHS participants were included in the replication analysis (**Figure 10.2**). All CHS participants provided written informed consent and each study site approved the study.

C.2. *Proteomics Profiling*

In ARIC, blood was collected from participants at visit 3 using a standardized protocol at each study center. Plasma was obtained from the blood samples and the relative concentration of plasma proteins was measured using a DNA aptamer-based

capture array (SomaScan, Somalogic, Inc., Boulder, CO).[240] The SomaScan assay was performed as previously described.[227] Plasma samples were stored at -80°C, transferred to the ARIC central laboratory, and then incubated with the SomaScan array. The SomaScan array then quantified individual protein concentrations into relative fluorescence units.[227] Standard Somalogic quality control and data normalization was applied.[232] A total of 422 samples from visit 3 were run in duplicate. Median inter-assay coefficient of variation across all proteins was 6.3%.

Of the 5,824 available aptamer measures, 94 were excluded due to a Bland-Altman coefficient of variation >50% or a variance of <0.01 on the log scale. Furthermore, an additional 313 measures were excluded because of nonspecific binding to nonproteins. After quality control measures, a total of 4,877 aptamer measures that corresponded to 4,697 unique proteins were included in this analysis. In addition, plasma protein measures were log base 2 transformed to correct for skewness.

For CHS, plasma proteins were measured from blood samples that were collected in 1992-1993. Plasma samples were shipped to the University of Vermont Core Laboratory on dry ice for processing. Samples were initially stored at -70°C freezers and then stored at -80°C for the last 20 years.[278] The SomaScan assay platform was also used in CHS.

C.3.   *Ischemic Stroke Ascertainment*

In ARIC, ischemic stroke events were identified by hospital records and death certificates. Potential events were independently reviewed by a physician. In addition, a computer algorithm classified stroke events using criteria adapted from the National

Survey of Stroke.[241] The computer algorithm classified stroke events into four categories: subarachnoid hemorrhage, intracerebral hemorrhage, thrombotic brain infarction, or embolic brain infarction. Final diagnosis was determined by an agreement of the physician reviewer and computer algorithm. If there was a disagreement in diagnosis, a second physician reviewer adjudicated the event.[56] Ischemic stroke included all thrombotic and cardioembolic strokes (definite and probable).

In CHS, potential events were identified through review of medical records, physician outpatient records, death certificates, obituaries, and the Health Care Financing Administration (HCFA) health care utilization database for hospitalizations.[279] The Cerebrovascular Adjudication Committee, which was comprised of neurologists from each study site, an internist or neurologist representing the coordinating center, and a neuroradiologist from the MRI reading center, reviewed all events and deaths that were through to be related to TIA or stroke. The committee would then decide if a TIA, nonfatal stroke, or fatal stroke had occurred. Then, when appropriate, the committee would assign all stroke events as either an ischemic stroke, hemorrhagic stroke, or uncertain.

C.4.   Covariate Ascertainment

Covariates for this analysis were obtained from visit 3 and included the following ischemic stroke risk factors: age, sex, race/center, body mass index (BMI), diabetes, smoking status, systolic blood pressure, antihypertensive medication use, anticoagulant use, total cholesterol, high-density lipoprotein (HDL) cholesterol, coronary heart disease (CHD), heart failure (HF), and atrial fibrillation (AF). Estimated glomerular filtration rate

(eGFR) was also included because renal function impacts plasma protein turnover and protein levels.

Participants self-reported their age, sex, race, and smoking status. Medication use was either self-reported (antidiabetic or antihypertensive medications) or recorded by technicians based on medication bottles that participants brought to study visits. Height and weight were measured to derive BMI. Blood pressure was measured three times after a 5-minute rest and the mean of the final two measurements was calculated. Diabetes was defined as a fasting glucose $\geq$126 mg/dL or a non-fasting glucose $\geq$200 mg/dL, self-reported antidiabetic medication use in the past two weeks, or a self-reported physician diabetes diagnosis. Plasma total cholesterol and HDL cholesterol were measured by the enzymatic method.[242] CHD was defined by self-reported physician diagnoses at visit 1, myocardial infarction diagnosis by ECG, or adjudicated cases after visit 1.[243] HF was identified by the Gothenburg criteria (visit 1 only), HF medication use within the past two weeks, or ICD codes for HF from hospitalization records during follow-up.[244] AF was ascertained from ECGs conducted during study visits and ICD codes from hospitalization discharge and death records.[245] eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.[246]

C.5.   *Statistical Analysis*

Baseline characteristics were described using mean $\pm$ SD for continuous variables and count (%) for categorical variables. Cox proportional hazards models were used to relate each protein level to incident ischemic stroke. Follow-up time was defined as time from visit 3 to the occurrence of incident ischemic stroke, loss to follow-up, or December

31, 2019, whichever occurred first. Multivariable models were adjusted as follows: model 1 adjusted for age, sex, race/center; model 2 further adjusted for eGFR; model 3 additionally adjusted for BMI, diabetes, smoking status (current, former, or never smoker), systolic blood pressure, antihypertensive medication use, anticoagulant use, total cholesterol, HDL cholesterol, CHD, HF, AF. The p-value threshold for significance was defined using the Bonferroni correction to account for the number of proteins analyzed ($p<0.05/4877$).

We also conducted an analysis in which follow-up was restricted to 10 years. Because follow-up time in CHS was closer to 10 years, we used the proteins discovered in this analysis to perform a replication analysis in CHS. The proteins measured from plasma samples that were collected at the 1992-93 CHS visit were used. The SomaScan assay platform was also used in CHS. Analyses were conducted using SAS software (version 9.4; SAS Institute Inc., Cary, NC).

To further investigate the relation between associated proteins and ischemic stroke, a two-sample Mendelian randomization (MR) analysis was performed. Protein quantitative trait loci (pQTL) were obtained from the deCODE study[280] to identify single nucleotide polymorphisms (SNPs) associated with Somascan-quantified levels of ischemic stroke-associated proteins. For the MR analysis, regression coefficients and standard errors for SNP-protein associations were obtained from summary results made publicly available by the deCODE study (sample 1; N=35,559). Regression coefficients and standard errors for SNP-incident stroke associations were estimated in White ARIC participants (sample 2; N=235 stroke cases over a median follow-up of 10 years). Black

ARIC participants were excluded from the MR analysis since the deCODE study consisted of participants from European ancestry (Icelandic).

Linkage disequilibrium-based clumping was performed to retain a set of independent variants ($r^2<0.1$). SNPs were pruned using the PLINK clumping algorithm within the "TwoSampleMR" package in R. SNPs with a genome-wide significance of p<5e-06 for association with the candidate protein and within the cis-region of the corresponding gene, defined as 1Mb downstream or upstream of the structural gene of the candidate protein, were selected. The 1000 Genomes Project (European population) was used as the linkage disequilibrium reference panel.[281] The clumping algorithm selects significant index SNPs and forms clumps of other SNPS within 10,000kb from the index SNP that were in linkage disequilibrium below a threshold of $r^2=0.1$. The SNP with the lowest p-value was retained.

The primary MR analysis was the inverse variance weighted (IVW) method, whereas the MR-Egger and weighted median methods were performed as sensitivity analyses to assess possible pleiotropic effects of the SNPs. MR analysis was performed for proteins that had ≥3 SNPs. Additionally, Cook's distance was calculated to identify potential SNP outliers.[282] An outlier was defined as having a Cook's distance >4/n, where n is the total number of data points. If outliers were detected, a corrected IVW analysis was then conducted after removing SNP outliers. MR analyses were performed using R software (version 4.0.4) and the R package "TwoSampleMR."

D. Results

In this analysis, 10,822 participants with protein levels measured at visit 3 were included (mean [SD] age 60 [6] years, 55% female, 21% Black participants). Median follow-up time of 22 years and 882 ischemic stroke events occurred. Baseline characteristics, stratified by incident ischemic stroke status, are shown in **Table 10.1**. Compared to those who did not have an ischemic stroke event, participants who did were older, more likely to identify as Black participants, and have more cardiovascular risk factors.

D.1. *Association of Protein Levels with Incident Ischemic Stroke*

Over a median follow-up of 22 years, proteins that were significantly associated with incident ischemic stroke after adjusting for demographics (model 1) and eGFR (model 2) are presented in **Supplemental Table 10.1**. After additionally adjusting for cardiovascular disease risk factors (model 3), 7 proteins were significantly associated ($p < 1.03 \times 10^{-5}$) with incident ischemic stroke (**Table 10.2**). The proportional hazards assumption held for all significant proteins ($p > 0.05$), except for NT-proBNP ($p = 0.001$). Growth/differentiation factor 15 (GDF15) was the protein with the lowest p-value: for each doubling of the protein level, the risk of ischemic stroke was 1.57 times higher (95% CI: 1.36, 1.82). Of the 7 significant proteins, 2 showed an inverse relationship with ischemic stroke: epidermal growth factor receptor (EGFR; HR [95% CI]: 0.43 [0.30, 0.61]) and alpha-2-HS-glycoprotein (AHSG; HR [95% CI]: 0.49 [0.36, 0.67]). When follow-up was restricted to 10 years, 5 proteins were significantly associated with ischemic stroke after full model adjustment. The proportional hazards assumptions held

for all significant proteins (p>0.05). The protein with the lowest p-value was N-terminal pro-brain natriuretic peptide (NT-proBNP). For each doubling of NT-proBNP level, there was a 1.31-fold (95% CI: 1.18, 1.44) increased risk for ischemic stroke.

D.2. Replication in the Cardiovascular Health Study

The CHS replication cohort included 3004 participants (mean [SD] age 74 [5] years, 61% female, 15% identify as Black). Median follow-up time was 12.5 years, and 459 ischemic stroke events were ascertained. In this replication analysis, 3 of the 5 proteins replicated and were significantly associated with ischemic stroke (**Table 10.3**). The proteins associated with ischemic stroke were NT-proBNP, GDF15, and WAP four-disulfide core domain protein 2 (WFDC2) and the magnitudes of effect were in the same direction as the discovery analysis in ARIC. NT-proBNP was the protein with the lowest p-value and associated with a 1.36-fold increased risk for ischemic stroke (95% CI: 1.22, 1.52) for each doubling of the protein level.

D.3. Mendelian Randomization

Results from the MR analysis for the significant proteins that replicated in CHS and had at least 3 instrumental variables are presented in **Table 10.4**. Using the IVW method, there was evidence that suggested a causal relationship between NT-proBNP and ischemic stroke (OR [95% CI] per 1-SD: 0.03 [0.002, 0.50]). No clear evidence suggestive of a causal effect for GDF15 and ischemic stroke was observed (OR [95% CI] per 1-SD: 0.93 [0.18, 4.73]). To identify SNP outliers, Cook's distance for NT-proBNP

and GDF15 was calculated (**Figure 10.3**). After identifying and removing SNP outliers, results remained similar to the primary IVW method (**Table 10.5**).

E. Discussion

In this community-based cohort, 7 of 4,877 proteins were associated with ischemic stroke at a Bonferroni corrected significance level over a median follow-up time of 22 years. After restricting follow-up time to 10 years, 5 proteins were associated with ischemic stroke, and 3 of these replicated in CHS (NT-proBNP, GDF15, WFDC2). MR analysis provided evidence that suggested NT-proBNP and ischemic stroke may be causally associated; however, this was not observed for GDF15.

NT-proBNP is a marker for atrial overload and is used clinically as a biomarker for cardiovascular disease.[283] Several community-based cohorts and proteomic analyses have reported that elevated NT-proBNP levels are associated with an increased risk for incident ischemic stroke.[6,161,163,164,230,231] Prior MR analyses did not provide evidence to suggest a causal relationship between NT-proBNP and ischemic stroke.[284,285] However, results from our study suggested that NT-proBNP may be causally related to ischemic stroke. NT-proBNP may potentially add value to risk prediction scores. The ABC-stroke risk score is used to predict stroke in patients with AF and NT-proBNP is included in the score. Additional research assessing whether adding NT-proBNP to other scores, such as the CHA_2DS_2-VASc score, improves risk prediction in patients regardless of AF status.

GDF15 is a marker of oxidative stress and inflammation[286] and is strongly induced in response to inflammation and myocardial injury.[287] Prior observational studies suggest that elevated GDF15 levels may be associated with stroke, though results are

somewhat mixed. Elevated GDF15 levels have been associated with incident stroke in a Japanese study,[288] as well as among patients with acute coronary syndrome[289] and coronary heart disease.[290] However, in the Framingham Offspring Study, GDF15 was not associated with ischemic stroke.[291] In proteomics analyses, GDF15 was significantly associated with ischemic stroke.[230,231] Our results and other MR studies did not suggest that GDF15 and ischemic stroke were causally related.[292,293]

WFDC2, also known as human epididymis protein 4 (HE4), is a diagnostic biomarker for ovarian cancer[294,295] and is expressed in several tissues, including the respiratory tract, lung, prostate, thyroid, and kidney.[296] In a recent discovery proteomics analysis that measured 742 proteins in 826 participants of the Uppsala Longitudinal Study of Adult Men, WFDC2 was associated with incident ischemic stroke, as well as HF and MI.[231] To our knowledge, no observational studies have reported the relationship between WFDC2 and ischemic stroke yet, but there is evidence that suggests WFDC2 and cardiovascular disease are linked. WFDC2 levels are higher in patients with HF than in controls,[297] and among patients with chronic obstructive pulmonary disease, elevated WFDC2 was associated with higher risk of cardiovascular events (composite outcome of MI, stroke, HF, and cardiovascular death).[298] Additionally, WFDC2 may be a potential biomarker of renal fibrosis and play a role in the progression of chronic kidney disease.[299,300] Therefore, it may be plausible that elevated WFDC2 levels leads to ischemic stroke through impaired renal function given that kidney function is a risk factor for stroke.[301]

Strengths of this analysis include the use of a high sample throughput array in a large, community-based cohort of adults, the comprehensive assessment of proteins

identifiable by SomaScan, the prospective design, and the replication of our results in an external cohort (CHS). However, this analysis also has limitations. pQTL data were obtained from the deCODE study, which consisted of participants from Iceland, limiting the generalizability of our results. Additionally, the SomaScan platform does not capture the entire human proteome and we were only able to assess proteins that are included in this platform. Furthermore, SomaScan has not fully validated their assay against traditional assays yet. A prior ARIC analysis of a subset of participants compared clinical assay measures and SomaScan measures for nine proteins, one of which was NT-proBNP and it was found to be highly correlated across platforms.[240] There is also the possibility of protein degradation, given the long-term storage of the samples; however, an ARIC validation study did not support widespread protein degradation.[240]

F. Conclusion

In conclusion, we conducted proteomic profiling to analyze the relationship between proteomics and incident ischemic stroke in a community-based cohort. Of the proteins associated with ischemic stroke, three proteins (NT-proBNP, GDF15, and WFDC2) were replicated in an external cohort (CHS). MR analysis suggested that NT-prBNP, but not GDF15, may be causally related to ischemic stroke. Additional studies should further evaluate these proteins as they may be potential therapeutic targets for stroke prevention.

Table 10.1. Baseline Participant Characteristics by Incident Ischemic Stroke Status, The ARIC Study, 1993-1995[*]

	Incident ischemic stroke through 2019 (n=882)	No incident ischemic stroke through 2019 (n=9940)
Demographics		
Age, years	61.6 (5.5)	60.0 (5.7)
Female sex	448 (50.8%)	5475 (55.1%)
Black race	244 (27.7%)	2018 (20.3%)
Clinical Characteristics		
Body mass index, kg/m^2	29.2 (5.4)	28.4 (5.6)
Systolic blood pressure, mmHg	131.1 (21.3)	123.9 (18.8)
Diabetes	224 (25.4%)	1402 (14.1%)
Coronary heart disease	88 (10.0%)	685 (6.9%)
Heart failure	10 (1.1%)	114 (1.1%)
Atrial fibrillation	34 (3.9%)	144 (1.4%)
Total cholesterol, mg/dL	210.8 (38.6)	206.6 (36.8)
HDL cholesterol, mg/dL	49.4 (16.2)	52.5 (18.2)
eGFR, mL/min per m^2	86.7 (17.1)	88.6 (15.0)
Antihypertensive medications	439 (49.8%)	3627 (36.5%)
Anticoagulant use	16 (1.8%)	104 (1.0%)
Current smokers	177 (20.1%)	1761 (17.7%)

[*]Data are expressed as mean ± SD or n (%).
Abbreviations: HDL = high-density lipoprotein; eGFR = estimated glomerular filtration rate

Table 10.2. Protein Biomarkers Associated with Incident Ischemic Stroke, The Atherosclerosis Risk in Communities Study, 1993-2019 (n=10,822)

Protein	Gene Name	Follow-up through 2019		Follow-up to 10 years	
		HR (95% CI)[*]	p-value	HR (95% CI)[*]	p-value
Growth/differentiation factor 15	GDF15	1.57 (1.36, 1.82)	**9.32E-10[†]**	1.65 (1.34, 2.03)	**2.64E-06[†]**
WAP four-disulfide core domain protein 2	WFDC2	1.67 (1.41, 1.99)	**6.28E-09[†]**	1.97 (1.53, 2.53)	**1.53E-07[†]**
Triggering receptor expressed on myeloid cells 1	TREM1	1.54 (1.30, 1.83)	**7.92E-07[†]**	1.70 (1.32, 2.18)	2.96E-05
Marginal zone B- and B1-cell-specific protein	PACAP	1.37 (1.20, 1.56)	**1.97E-06[†]**	1.37 (1.13, 1.67)	0.002
Epidermal growth factor receptor	EGFR	0.43 (0.30, 0.61)	**2.57E-06[†]**	0.36 (0.21, 0.61)	0.0001
N-terminal pro-BNP	NPPB	1.18 (1.10, 1.26)	**4.49E-06[†]**	1.31 (1.18, 1.44)	**9.84E-08[†]**
Alpha-2-HS-glycoprotein	AHSG	0.49 (0.36, 0.67)	**8.58E-06[†]**	0.47 (0.29, 0.76)	0.002
cGMP-dependent protein kinase 1, beta isozyme	PRKG1	0.39 (0.25, 0.61)	3.18E-05	0.16 (0.08, 0.31)	**1.36E-07[†]**
Adrenomedullin	ADM	0.47 (0.31, 0.72)	0.0004	0.17 (0.08, 0.36)	**4.95E-06[†]**

[*]Hazard ratio expressed as per doubling of protein level. Adjusted for age, sex, race/center, eGFR, body mass index, smoking status, diabetes, systolic blood pressure, antihypertensive medications, total cholesterol, HDL cholesterol, coronary heart disease, heart failure, atrial fibrillation, anticoagulant use
[†]Significance level of $p<0.05/4877 = 1.03 \times 10^{-5}$

Table 10.3. Replication of Significant Protein Biomarkers in the Cardiovascular Health Study, 1992-2015 (n=3,004)

Protein	Gene Name	HR (95% CI)*	p-value†
N-terminal pro-BNP	NPPB	1.36 (1.22, 1.52)	1.93E-08
Growth/differentiation factor 15	GDF15	1.48 (1.17, 1.89)	0.001
WAP four-disulfide core domain protein 2	WFDC2	1.48 (1.10, 1.98)	0.009
cGMP-dependent protein kinase 1, beta isozyme	PRKG1	0.72 (0.46, 1.15)	0.168
Adrenomedullin	ADM	1.18 (0.78, 1.80)	0.430

*Hazard ratio expressed as per doubling of protein level. Adjusted for age, sex, race/center, eGFR, body mass index, smoking status, diabetes, systolic blood pressure, antihypertensive medications, total cholesterol, HDL cholesterol, coronary heart disease, heart failure, atrial fibrillation, anticoagulant use
†Significance level of $p<0.05/5 = 0.01$

Table 10.4. Mendelian Randomization Analysis of Significant and Replicated Protein Biomarkers, The ARIC Study

Protein	Method	N, SNPs	MR beta	MR p-value	OR (95% CI)
N-terminal pro-BNP	Inverse variance weighted	29	-3.35	0.01	0.03 (0.002, 0.50)
	MR-Egger	29	-3.86	0.12	0.02 (0.0002, 2.29)
	Weighted median	29	-3.72	1.25E-19	0.02 (0.01, 0.05)
Growth/ differentiation factor 15	Inverse variance weighted	88	-0.07	0.93	0.93 (0.18, 4.73)
	MR-Egger	88	-2.33	0.07	0.10 (0.01, 1.13)
	Weighted median	88	-2.40	6.45E-17	0.09 (0.05, 0.16)
WAP four-disulfide core domain protein 2	Inverse variance weighted	2	--	--	--
	MR-Egger	2	--	--	--
	Weighted median	2	--	--	--

Abbreviations: SNPs = single nucleotide polymorphisms; MR = Mendelian randomization

Table 10.5. Mendelian Randomization Analysis of Significant and Replicated Protein Biomarkers After Removing Outliers,[*] The ARIC Study

Protein	Method	N, SNPs	MR beta	MR p-value	OR (95% CI)
N-terminal pro-BNP	Inverse variance weighted	28	-3.95	0.002	0.02 (0.002, 0.23)
Growth/ differentiation factor 15	Inverse variance weighted	85	0.76	0.49	2.13 (0.25, 18.29)

[*]Outliers were detected based on Cook's distance.

Abbreviations: SNPs = single nucleotide polymorphisms; MR = Mendelian randomization

Supplemental Table 10.1. Protein Biomarkers Associated with Incident Ischemic Stroke, The Atherosclerosis Risk in Communities Study, 1993-2019 (n=10,822)

Protein	Gene Name	Model 1		Model 2	
		HR (95% CI)	p-value	HR (95% CI)	p-value
Growth/differentiation factor 15	GDF15	1.80 (1.57, 2.06)	**1.33E-17**[†]	1.77 (1.55, 2.03)	**2.30E-16**[†]
Neurocan core protein	NCAN	0.55 (0.47, 0.64)	**8.71E-15**[†]	0.55 (0.47, 0.63)	**4.99E-15**[†]
Cartilage intermediate layer protein 2	CILP2	0.52 (0.44, 0.61)	**2.24E-14**[†]	0.51 (0.43, 0.61)	**7.61E-15**[†]
WAP four-disulfide core domain protein 2	WFDC2	1.81 (1.56, 2.10)	**3.47E-15**[†]	1.79 (1.54, 2.09)	**1.11E-13**[†]
Amyloid-like protein 1	APLP1	0.60 (0.52, 0.69)	**6.52E-13**[†]	0.59 (0.51, 0.68)	**2.55E-13**[†]
Glycosaminoglycan xylosylkinase	FAM20B	0.29 (0.20, 0.41)	**2.41E-12**[†]	0.28 (0.20, 0.40)	**1.03E-12**[†]
Matrix-remodeling-associated protein 8	MXRA8	0.45 (0.36, 0.57)	**2.89E-12**[†]	0.45 (0.36, 0.56)	**1.10E-12**[†]
Epidermal growth factor receptor	EGFR	0.30 (0.21, 0.41)	**6.33E-13**[†]	0.31 (0.22, 0.43)	**4.34E-12**[†]
Angiopoietin-2	ANGPT2	1.74 (1.49, 2.03)	**3.79E-12**[†]	1.72 (1.47, 2.01)	**9.40E-12**[†]
Triggering receptor expressed on myeloid cells 1	TREM1	1.72 (1.47, 2.01)	**1.14E-11**[†]	1.68 (1.43, 1.98)	**1.72E-10**[†]
N-terminal pro-BNP	NPPB	1.25 (1.17, 1.33)	**6.83E-11**[†]	1.24 (1.16, 1.32)	**2.18E-10**[†]
Neural cell adhesion molecule 1, 120 kDa isoform	NCAM1	0.55 (0.45, 0.66)	**5.85E-10**[†]	0.55 (0.45, 0.66)	**4.75E-10**[†]
Ectonucleotide pyrophosphatase/ phosphodiesterase family member 5	ENPP5	0.66 (0.57, 0.75)	**5.19E-10**[†]	0.66 (0.57, 0.75)	**6.19E-10**[†]
Thrombospondin-2	THBS2	1.40 (1.26, 1.56)	**6.07E-10**[†]	1.40 (1.25, 1.55)	**9.13E-10**[†]
Vesicular, overexpressed in cancer, prosurvival protein 1	VOPP1	0.53 (0.43, 0.65)	**8.75E-10**[†]	0.53 (0.44, 0.65)	**1.09E-09**[†]

Sushi, von Willebrand factor type A, EGF and pentraxin domain-containing protein 1	SVEP1	1.63 (1.38, 1.92)	**5.99E-09**[†]	1.66 (1.41, 1.95)	**1.71E-09**[†]
Brevican core protein	BCAN	0.57 (0.48, 0.69)	**4.13E-09**[†]	0.57 (0.47, 0.68)	**2.11E-09**[†]
Collagen alpha-3(VI) chain	COL6A3	2.13 (1.71, 2.66)	**2.36E-11**[†]	2.09 (1.63, 2.67)	**3.78E-09**[†]
Collagen alpha-1(XXVIII) chain	COL28A1	2.06 (1.66, 2.56)	**7.57E-11**[†]	2.02 (1.60, 2.56)	**4.88E-09**[†]
Ribonuclease pancreatic	RNASE1	1.43 (1.28, 1.60)	**1.12E-10**[†]	1.42 (1.26, 1.60)	**5.56E-09**[†]
Beta-1,4-galactosyltransferase 2	B4GALT2	0.33 (0.23, 0.48)	**5.06E-09**[†]	0.33 (0.23, 0.48)	**6.49E-09**[†]
Semaphorin-6B	SEMA6B	1.84 (1.48, 2.27)	**2.33E-08**[†]	1.87 (1.51, 2.31)	**1.02E-08**[†]
Tumor necrosis factor receptor superfamily member 1A	TNFRSF1A	1.80 (1.50, 2.16)	**2.82E-10**[†]	1.78 (1.46, 2.16)	**1.18E-08**[†]
ADAMTS-like protein 2	ADAMTSL2	1.65 (1.38, 1.96)	**2.39E-08**[†]	1.66 (1.40, 1.99)	**1.38E-08**[†]
Marginal zone B- and B1-cell-specific protein	PACAP	1.46 (1.28, 1.65)	**6.15E-09**[†]	1.44 (1.27, 1.64)	**1.44E-08**[†]
Serine protease HTRA1	HTRA1	1.76 (1.43, 2.16)	**7.14E-08**[†]	1.79 (1.46, 2.20)	**2.32E-08**[†]
Sushi, von Willebrand factor type A, EGF and pentraxin domain-containing protein 1	SVEP1	1.60 (1.34, 1.90)	**9.49E-08**[†]	1.63 (1.37, 1.93)	**2.86E-08**[†]
Secretogranin-3	SCG3	0.60 (0.50, 0.73)	**3.65E-07**[†]	0.57 (0.47, 0.70)	**3.01E-08**[†]
Adenosine deaminase CECR1	CECR1	1.40 (1.24, 1.58)	**5.22E-08**[†]	1.41 (1.25, 1.59)	**3.85E-08**[†]
Neuronal pentraxin receptor	NPTXR	0.67 (0.57, 0.78)	**4.92E-07**[†]	0.64 (0.55, 0.75)	**4.27E-08**[†]
Matrilysin	MMP7	1.46 (1.28, 1.65)	**4.56E-09**[†]	1.43 (1.26, 1.62)	**4.31E-08**[†]
Macrophage metalloelastase	MMP12	1.38 (1.23, 1.54)	**1.68E-08**[†]	1.37 (1.22, 1.53)	**4.38E-08**[†]
Cystatin-C	CST3	2.14 (1.68, 2.72)	**7.87E-10**[†]	2.17 (1.64, 2.87)	**4.52E-08**[†]
Transmembrane protein 132B	TMEM132B	0.48 (0.36, 0.62)	**4.55E-08**[†]	0.48 (0.37, 0.62)	**5.14E-08**[†]
Kit ligand	KITLG	0.61 (0.51, 0.74)	**2.51E-07**[†]	0.59 (0.49, 0.72)	**5.90E-08**[†]
Hepatitis A virus cellular receptor 1	HAVCR1	1.25 (1.15, 1.35)	**3.25E-08**[†]	1.24 (1.15, 1.35)	**6.73E-08**[†]

Anthrax toxin receptor 2	ANTXR2	0.63 (0.54, 0.75)	**6.21E-08**[†]	0.64 (0.54, 0.75)	**7.66E-08**[†]
Oligodendrocyte-myelin glycoprotein	OMG	0.73 (0.65, 0.82)	**9.04E-08**[†]	0.73 (0.65, 0.82)	**1.01E-07**[†]
Insulin-like growth factor-binding protein 4	IGFBP4	1.91 (1.53, 2.39)	**1.73E-08**[†]	1.85 (1.48, 2.33)	**1.13E-07**[†]
Protein S100-A12	S100A12	1.35 (1.21, 1.51)	**9.64E-08**[†]	1.35 (1.21, 1.51)	**1.23E-07**[†]
Cartilage acidic protein 1	CRTAC1	0.62 (0.52, 0.74)	**1.09E-07**[†]	0.62 (0.52, 0.74)	**1.24E-07**[†]
Voltage-dependent calcium channel subunit alpha-2/delta-3	CACNA2D3	0.59 (0.48, 0.72)	**2.05E-07**[†]	0.58 (0.48, 0.71)	**1.53E-07**[†]
Heparan-sulfate 6-O-sulfotransferase 3	HS6ST3	0.41 (0.29, 0.57)	**1.05E-07**[†]	0.41 (0.29, 0.57)	**1.63E-07**[†]
Netrin-4	NTN4	1.57 (1.32, 1.86)	**1.95E-07**[†]	1.56 (1.32, 1.85)	**2.35E-07**[†]
cGMP-dependent protein kinase 1, beta isozyme	PRKG1	0.30 (0.19, 0.46)	**6.86E-08**[†]	0.31 (0.20, 0.49)	**2.77E-07**[†]
Arfaptin-2	ARFIP2	0.70 (0.60, 0.80)	**6.11E-07**[†]	0.69 (0.60, 0.79)	**2.96E-07**[†]
Receptor-type tyrosine-protein phosphatase delta	PTPRD	0.63 (0.52, 0.76)	**1.97E-06**[†]	0.60 (0.50, 0.73)	**3.18E-07**[†]
Seizure 6-like protein	SEZ6L	0.52 (0.40, 0.68)	**2.29E-06**[†]	0.50 (0.38, 0.65)	**4.53E-07**[†]
Endosialin	CD248	0.62 (0.51, 0.75)	**9.01E-07**[†]	0.61 (0.50, 0.74)	**4.65E-07**[†]
Tetranectin	CLEC3B	0.45 (0.33, 0.62)	**6.20E-07**[†]	0.45 (0.33, 0.62)	**5.00E-07**[†]
Coiled-coil domain-containing protein 126	CCDC126	0.64 (0.54, 0.77)	**1.01E-06**[†]	0.63 (0.53, 0.76)	**5.33E-07**[†]
Inactive tyrosine-protein kinase 7	PTK7	1.85 (1.45, 2.35)	**6.52E-07**[†]	1.86 (1.46, 2.38)	**5.59E-07**[†]
SLIT and NTRK-like protein 5	SLITRK5	0.64 (0.52, 0.77)	**5.81E-06**[†]	0.60 (0.49, 0.73)	**5.63E-07**[†]
Beta-defensin 4A	DEFB4A	1.15 (1.09, 1.22)	**4.93E-07**[†]	1.15 (1.09, 1.22)	**5.80E-07**[†]
Asialoglycoprotein receptor 1	ASGR1	1.78 (1.45, 2.19)	**5.34E-08**[†]	1.72 (1.39, 2.13)	**6.93E-07**[†]
Ubiquitin-conjugating enzyme E2 G2	UBE2G2	1.75 (1.42, 2.17)	**1.89E-07**[†]	1.71 (1.38, 2.11)	**7.68E-07**[†]

Interleukin-1 receptor-like 2	IL1RL2	0.43 (0.31, 0.60)	**8.13E-07**[†]	0.43 (0.31, 0.60)	**7.75E-07**[†]
Mannosyl-oligosaccharide 1,2-alpha-mannosidase IB	MAN1A2	0.41 (0.29, 0.58)	**4.03E-07**[†]	0.42 (0.30, 0.60)	**9.62E-07**[†]
Transmembrane emp24 domain-containing protein 10	TMED10	1.69 (1.41, 2.03)	**2.42E-08**[†]	1.64 (1.35, 2.01)	**1.03E-06**[†]
P-selectin	SELP	1.54 (1.28, 1.84)	**2.95E-06**[†]	1.57 (1.31, 1.88)	**1.06E-06**[†]
Serine/arginine-rich splicing factor 7	SRSF7	1.62 (1.35, 1.95)	**2.41E-07**[†]	1.59 (1.32, 1.92)	**1.23E-06**[†]
Alpha-2-HS-glycoprotein	AHSG	0.46 (0.34, 0.63)	**1.24E-06**[†]	0.46 (0.34, 0.63)	**1.23E-06**[†]
Inositol monophosphatase 3	IMPAD1	0.60 (0.49, 0.75)	**4.26E-06**[†]	0.59 (0.47, 0.73)	**1.52E-06**[†]
Superoxide dismutase [Mn], mitochondrial	SOD2	0.62 (0.51, 0.75)	**9.80E-07**[†]	0.62 (0.52, 0.76)	**1.65E-06**[†]
C1GALT1-specific chaperone 1	C1GALT1C1	0.56 (0.45, 0.70)	**6.04E-07**[†]	0.57 (0.45, 0.72)	**1.73E-06**[†]
Potassium voltage-gated channel subfamily E regulatory beta subunit 5	KCNE5	0.57 (0.45, 0.73)	**6.07E-06**[†]	0.55 (0.43, 0.71)	**1.99E-06**[†]
Tumor necrosis factor receptor superfamily member 1B	TNFRSF1B	1.70 (1.40, 2.08)	**1.43E-07**[†]	1.65 (1.34, 2.03)	**2.40E-06**[†]
Protein S100-A9	S100A9	1.36 (1.19, 1.54)	**2.33E-06**[†]	1.35 (1.19, 1.53)	**2.72E-06**[†]
Glypican-3	GPC3	0.63 (0.52, 0.76)	**7.10E-07**[†]	0.65 (0.54, 0.78)	**3.21E-06**[†]
Angiostatin	PLG	0.49 (0.37, 0.65)	**8.86E-07**[†]	0.51 (0.38, 0.68)	**3.62E-06**[†]
Insulin	INS	1.38 (1.20, 1.57)	**3.05E-06**[†]	1.37 (1.20, 1.57)	**3.92E-06**[†]
Contactin-1	CNTN1	0.52 (0.40, 0.68)	**1.91E-06**[†]	0.54 (0.41, 0.70)	**4.20E-06**[†]
Neurogenic locus notch homolog protein 1	NOTCH1	0.35 (0.22, 0.54)	**4.42E-06**[†]	0.35 (0.22, 0.54)	**4.62E-06**[†]
S-phase kinase-associated protein 1	SKP1	0.33 (0.22, 0.52)	**9.80E-07**[†]	0.36 (0.23, 0.56)	**5.17E-06**[†]

Inter-alpha-trypsin inhibitor heavy chain H2	ITIH2	0.48 (0.36, 0.66)	**2.64E-06**[†]	0.49 (0.37, 0.67)	**5.31E-06**[†]
IgLON family member 5	IGLON5	0.63 (0.51, 0.78)	2.11E-05	0.61 (0.49, 0.76)	**6.25E-06**[†]
Ganglioside GM2 activator	GM2A	1.70 (1.39, 2.07)	**1.87E-07**[†]	1.64 (1.32, 2.04)	**6.55E-06**[†]
SLIT and NTRK-like protein 1	SLITRK1	0.70 (0.59, 0.83)	6.90E-05	0.66 (0.55, 0.79)	**6.68E-06**[†]
Lysosomal Pro-X carboxypeptidase	PRCP	1.53 (1.25, 1.87)	4.11E-05	1.60 (1.30, 1.97)	**6.98E-06**[†]
Insulin-like growth factor-binding protein complex acid labile subunit	IGFALS	0.65 (0.54, 0.79)	**6.34E-06**[†]	0.66 (0.54, 0.79)	**7.35E-06**[†]
Fructose-bisphosphate aldolase C	ALDOC	0.61 (0.49, 0.75)	**2.07E-06**[†]	0.62 (0.51, 0.77)	**7.53E-06**[†]
Carbohydrate sulfotransferase 12	CHST12	1.58 (1.29, 1.95)	1.54E-05	1.61 (1.31, 1.98)	**7.93E-06**[†]
Sialic acid-binding Ig-like lectin 7	SIGLEC7	1.56 (1.26, 1.93)	4.55E-05	1.62 (1.31, 2.00)	**8.01E-06**[†]
Receptor tyrosine-protein kinase erbB-3	ERBB3	0.55 (0.42, 0.71)	**3.77E-06**[†]	0.56 (0.43, 0.72)	**8.08E-06**[†]
Peroxidasin homolog	PXDN	1.26 (1.15, 1.38)	**1.08E-06**[†]	1.24 (1.13, 1.36)	**8.78E-06**[†]
NT-3 growth factor receptor	NTRK3	0.62 (0.50, 0.77)	1.16E-05	0.61 (0.50, 0.76)	**9.79E-06**[†]
Desmoglein-2	DSG2	0.62 (0.49, 0.77)	2.25E-05	0.60 (0.48, 0.75)	**1.02E-05**[†]
Receptor-type tyrosine-protein phosphatase S	PTPRS	0.54 (0.41, 0.72)	1.83E-05	0.53 (0.40, 0.70)	**1.02E-05**[†]
Vitamin K-dependent protein C	PROC	0.54 (0.42, 0.70)	**3.47E-06**[†]	0.56 (0.43, 0.72)	**1.02E-05**[†]
Gamma-aminobutyric acid receptor-associated protein	GABARAP	1.90 (1.48, 2.45)	**6.72E-07**[†]	1.82 (1.39, 2.37)	1.03E-05
Ephrin-A4	EFNA4	1.64 (1.35, 2.00)	**9.13E-07**[†]	1.59 (1.29, 1.95)	1.29E-05
Neuroblastoma suppressor of tumorigenicity 1	NBL1	1.48 (1.25, 1.74)	**3.82E-06**[†]	1.43 (1.19, 1.70)	9.26E-05
IGF-like family receptor 1	IGFLR1	1.31 (1.17, 1.47)	**4.24E-06**[†]	1.29 (1.14, 1.45)	3.91E-05
Beta-2-microglobulin	B2M	1.71 (1.36, 2.15)	**4.74E-06**[†]	1.62 (1.27, 2.08)	0.00012

Trafficking protein particle complex subunit 3	TRAPPC3	1.44 (1.23, 1.69)	**4.84E-06**[†]	1.41 (1.20, 1.66)	2.31E-05
Gamma-aminobutyric acid receptor-associated protein-like 1	GABARAPL1	1.63 (1.32, 2.01)	**5.19E-06**[†]	1.57 (1.27, 1.96)	4.51E-05
Hypoxanthine-guanine phosphoribosyltransferase	HPRT1	0.49 (0.36, 0.67)	**6.81E-06**[†]	0.51 (0.37, 0.69)	2.42E-05
Signaling lymphocytic activation molecule	SLAMF1	1.35 (1.18, 1.54)	**7.80E-06**[†]	1.32 (1.16, 1.51)	4.12E-05
Semenogelin-1	SEMG1	0.66 (0.55, 0.79)	**7.89E-06**[†]	0.67 (0.56, 0.81)	2.32E-05
Apolipoprotein M	APOM	0.59 (0.47, 0.74)	**7.98E-06**[†]	0.59 (0.47, 0.75)	1.15E-05
EGF-containing fibulin-like extracellular matrix protein 1	EFEMP1	1.82 (1.40, 2.37)	**8.85E-06**[†]	1.74 (1.33, 2.28)	4.92E-05
Thioredoxin domain-containing protein 5	TXNDC5	1.48 (1.24, 1.76)	**9.90E-06**[†]	1.45 (1.22, 1.74)	3.33E-05

[*]Hazard ratio expressed as per doubling of protein level.
[†]Significance level of $p<0.05/4877 = 1.03 \times 10^{-5}$
Model 1: adjusted for age, sex, race/center
Model 2: adjusted for model 1 plus eGFR
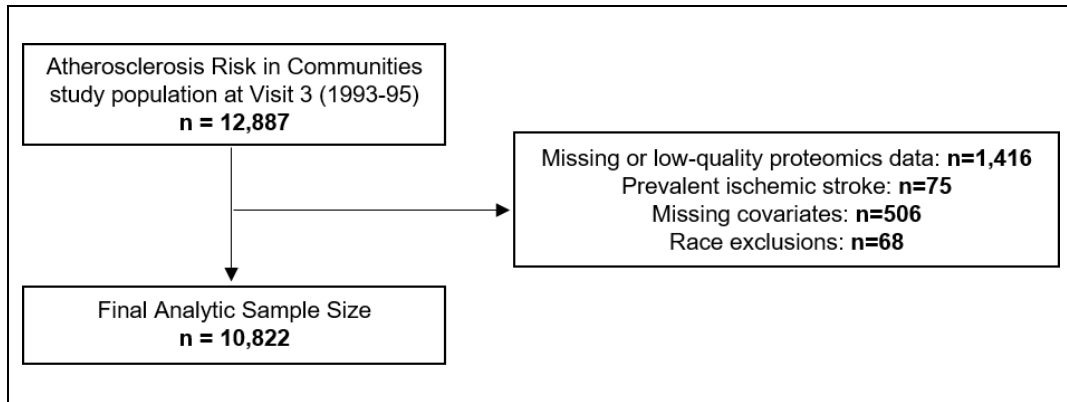
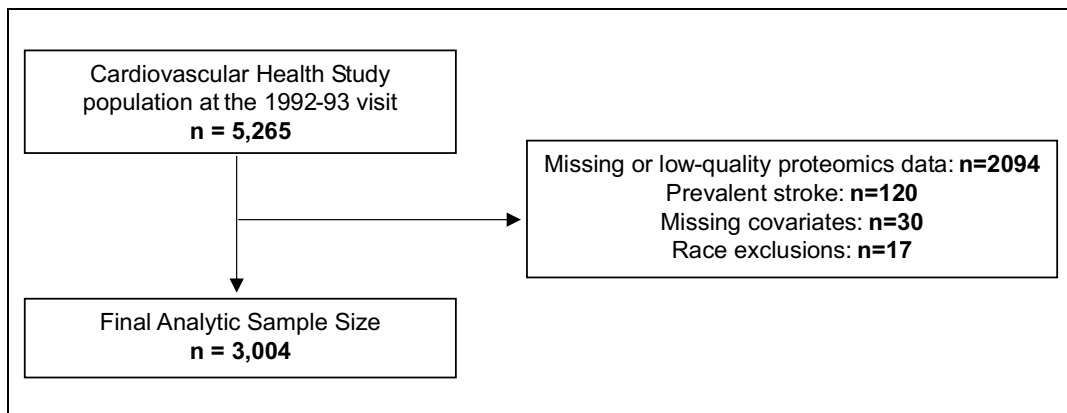**Figure 10.1**. Participant Exclusion Flowchart, The ARIC Study

Figure 10.2. Participant Exclusion Flowchart, The Cardiovascular Health Study
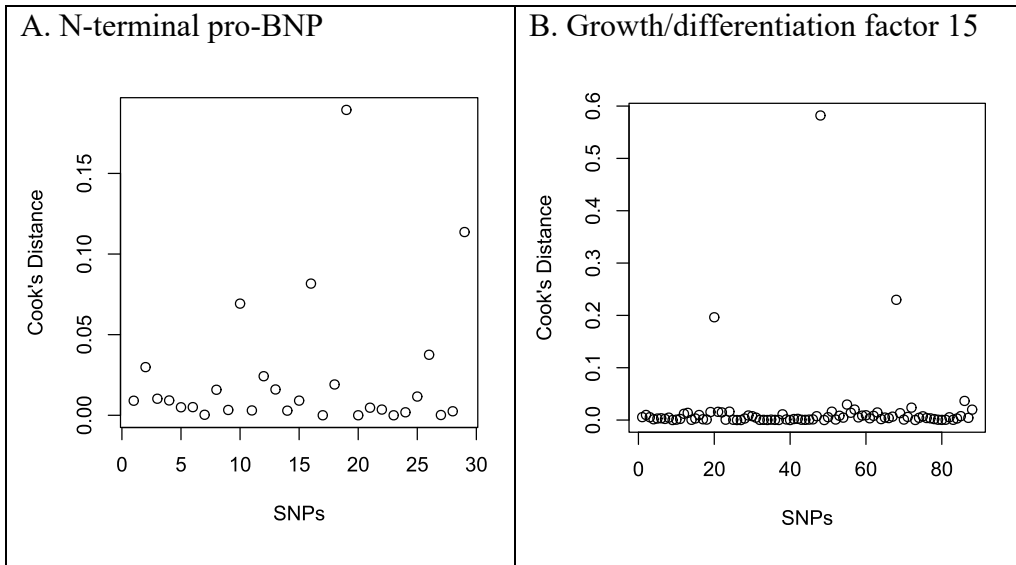
Figure 10.3. Cook's Distance Plots of Significant Protein Biomarkers

Chapter 11. Manuscript 3: Left Atrial Appendage Occlusion and Risk of Stroke in Medicare Beneficiaries

A. Overview

Background: Atrial fibrillation (AF) is a significant risk factor for stroke and medications, such as oral anticoagulants, are often used for stroke prevention. However, a nonpharmacologic option is needed for patients with contraindications for oral anticoagulants (OACs). Recently, left atrial appendage occlusion (LAAO) with the Watchman device has emerged as an alternative treatment option. Although clinical trials have shown that LAAO is non-inferior to warfarin and direct oral anticoagulants, few studies using real-world data have been done.

Methods: Using data from the Medicare 20% sample databases (2015-18), we assessed the association between percutaneous LAAO with the Watchman device vs. OAC use and risk of stroke among patients with AF who had an elevated CHA_2DS_2-VASc score. Secondary outcomes included death and hospitalized bleeding. Patients with the Watchman device were matched with up to 5 other patients who were taking OACs. A total of 17,514 patients with AF (2,927 with the Watchman device) were matched (average [SD] 78 [6] years, 44% female). Cox proportional hazards model was used to estimate hazard ratios.

Results: Over a median follow-up of 10.3 months, 293 stroke events, 1,925 deaths and 618 major bleeding events occurred. Of these, 61 strokes, 317 deaths, and 240 bleeding

events were among patients with the Watchman device, respectively. After multivariable

adjustments, no significant difference for risk of stroke or death was noted when patients

with the Watchman device was compared to those taking OACs (HRs [95% CIs]: 1.25

[0.95, 1.67] and 0.94 [0.83, 1.06], respectively). However, there was a 3.24-fold (95% CI:

2.75, 3.81) increased risk for hospitalized bleeding among patients with the Watchman

device.

Conclusion: Using data from the Medicare 20% sample databases, no significant

difference in risk of stroke or death were noted when LAAO with the Watchman device

was compared to OAC users. However, there was an increased risk for bleeding, but

elevated bleeding risk may have been the initial contraindication to OACs. Our results

confirm the results of randomized trials that among older patients with AF and a high-risk

for stroke, the Watchman device may be an alternative to OAC use; however, patients

should be aware of potential risks associated with the implantation.

B. Introduction

Stroke prevention among individuals with atrial fibrillation (AF) is of importance

since AF is associated with a 5-fold increased risk of stroke.[1,10] Several pharmacological

options, such as warfarin or direct oral anticoagulants (i.e., rivaroxaban, apixaban,

dabigatran, edoxaban), are often used for stroke prevention in patients with AF. However,

a nonpharmacologic alternative is needed for patients with contraindications to oral

anticoagulants (OACs; e.g., unable to adhere to medications, increased risk of bleeding,

presence of frequent falls). Left atrial appendage occlusion (LAAO) has recently emerged

as an alternative stroke prevention strategy.[11] The left atrial appendage is the most common source of thrombus formation in AF.[221,302] In sinus rhythm, the left atrial appendage has pulsatile flow, which prevents stasis and clot formation.[208] However, during AF, the function of the left atrial appendage is reduced, leading to stagnation and thrombosis.[302]

The Watchman is FDA-approved for patients with AF who meet the following criteria: 1) have an indication for long-term oral anticoagulation (e.g., increased CHA_2DS_2-VASc score), 2) are deemed safe for short-term oral anticoagulation by their physician (patients are required to take an OAC for at least 45 days after implant), and 3) have rationale for a nonpharmacologic alternative, such as contraindication to long-term anticoagulation (e.g., presence of falls, increased risk of bleeding, or poor adherence to medications).[223] Randomized clinical trials have assessed how percutaneous LAAO with the Watchman compares with warfarin or a DOAC in preventing adverse events. These clinical trials have reported that LAAO with the Watchman is noninferior to warfarin and DOACs in preventing stroke, cardiovascular death, and significant bleeding.[12,13,15-17] However, clinical trials often tend to have stringent inclusion criteria and may not be representative of the full clinical spectrum of individuals who receive the Watchman device.

The availability of Medicare 20% sample data allows us to assess the effectiveness and safety associated with the Watchman in a large real-world population that is generalizable to individuals with AF aged $\geq$65 years. Therefore, using Medicare 20% sample databases, we evaluated the risk of stroke, death, and major bleeding

associated with percutaneous LAAO with the Watchman device vs. oral anticoagulation use among patients with AF who are at an elevated risk for stroke.

C. Methods

C.1. Study Population

For this analysis, we used claims data (inpatient, outpatient, and carrier files) from a nationally representative 20% sample of Medicare data from 2015-2018. Medicare is a health insurance program and people in the US qualify for Medicare coverage if they meet any of the following criteria: 1) aged $\geq$65 years, 2) aged <65 years with certain disabilities, and 3) have end-stage renal disease. For this analysis, we included individuals with a diagnosis of AF who were $\geq$65 years old, had an elevated CHA_2DS_2-VASc score ($\geq$2 in males and $\geq$3 in females), per the AHA/ACC/HRS guidelines,[201] and had $\geq$90 days of continuous enrollment prior to their LAAO Watchman implantation or OAC prescription (among those who did not get the Watchman) (**Figure 11.1**).

AF was defined based on at least one inpatient or two outpatient encounters (between 7-365 days) for AF using International Classification of Disease, Ninth or Tenth Revision, Clinical Modification (ICD-9-CM: 427.31, 427.32 or ICD-10-CM: I48 in any position).[249] The positive predictive value (PPV) and sensitivity of the ICD-9-CM codes were approximately 90% and 80%.[249] Percutaneous LAAO with the Watchman device is an inpatient procedure and was defined using ICD-10-procedure code 02L73DK. Use of OACs was defined, using National Drug Codes, by the date of first outpatient OAC prescription fill after their AF diagnosis. To take into account when a patient is considered stable on OACs, we added a constant of 2 months to the date of first

outpatient OAC prescription. For those who received the Watchman, index date was defined as date of procedure, while OACs user index date was defined as date of first OAC prescription plus the 2-month constant. Patients with AF undergoing percutaneous LAAO were matched, using a greedy algorithm,[303] to up to 5 patients with AF who had filled prescriptions for OACs (warfarin, rivaroxaban, apixaban, dabigatran, or edoxaban) by sex, age (±3 years), date of enrollment (±90 days), index date (±90 days) and CHA_2DS_2-VASc score. The final analytic sample included 2,927 AF patients with the Watchman device who were matched to 14,587 OAC users.

C.2.   *Outcome Ascertainment*

All outcomes were defined using ICD-9-CM or ICD-10-CM codes. For this analysis, the primary outcome of interest was incident stroke. Stroke was defined based on inpatient claims as the primary diagnoses using validated algorithms that had PPVs >80%.[304] Secondary outcomes included death and hospitalized bleeding events. Death information in the Medicare 20% sample comes from linkage to Social Security Administration data and is believed to be virtually complete. Bleeding events included intracranial hemorrhage, gastrointestinal bleeding, and other major bleeding events from inpatient claims based on the Cunningham algorithm.[305] Gastrointestinal bleeding and other major bleeding events were based on inpatient claims as primary or secondary diagnoses, and presence of transfusion codes. PPV for this algorithm was >89%.

C.3. *Covariates*

Covariates were defined using inpatient, outpatient, and carrier files prior to the time of index date. We included covariates that are in the CHA_2DS_2-VASc score, which is used clinically to predict stroke risk among AF patients.[205] The CHA_2DS_2-VASc score includes the following variables: congestive heart failure, hypertension, age, diabetes, prior stroke or TIA, vascular disease (MI or PAD), and female sex.[205] The HAS-BLED score, which is used to estimate major bleeding risk among AF patients on OACs, was also calculated and consisted of the following variables: hypertension, abnormal renal/liver function, stroke, bleeding, age, and drug/alcohol use.[306] International Normalized Ratio is typically included in the HAS-BLED score; however, this variable is not available in the Medicare 20% sample dataset and was therefore not included.

C.4. *Statistical Analysis*

Baseline characteristics were described as mean (SD) or percent. Incidence rates per 100 person-years were calculated for each outcome. Cox proportional hazards model was used to assess the association of LAAO with incident stroke, and (separately) secondary outcomes. Analyses adjusted for variables included in the CHA_2DS_2-VASc score: age, sex, hypertension, diabetes, prior stroke or TIA, heart failure, and vascular disease (MI or PAD). Interactions by sex and age (median split) were evaluated and stratified models were reported when appropriate. For the outcome of bleeding events, analyses were stratified to assess risk by different timepoints (**Figure 11.2**). SAS software (version 9.4; SAS Institute Inc., Cary, NC) was used for all analyses.

D. Results

Overall, 2,929 patients with AF underwent a percutaneous LAAO procedure with the Watchman device. Descriptive characteristics of the full sample are provided in **Supplemental Table 11.1**. The primary analysis was matched and included 17,514 patients with AF. Baseline characteristics of matched patients are presented in **Table 11.1**. The matched patients were on average (SD) 78 (6) years old, 44% female, and the majority identified as White. Among the matched OAC patients, apixaban was the most common OAC (52%), followed by rivaroxaban (24%) and warfarin (21%).

Median follow-up time was 10.3 months, and 293 stroke events occurred, with 61 occurring in the Watchman group. Among those who received the Watchman device, the incidence rate for stroke was 1.99 per 100 person-years. Matched OAC users had an incidence rate of 1.53 per 100 person-years for stroke. Compared to those taking OACs, the risk for stroke was not significantly higher in patients who underwent the Watchman implant (**Table 11.2**; HR [95% CI]: 1.25 [0.95, 1.67]) in the matched analysis, after adjusting for CHA_2DS_2-VASc score variables (age, sex, hypertension, diabetes, prior stroke or TIA, heart failure, and vascular disease [MI or PAD]). An interaction with sex and age (median split) were observed ($p<0.10$). Stratified results are presented in **Table 11.3**. Among females, those with the Watchman had a higher risk of stroke compared to those taking OACs (HR [95% CI]: 1.62 [1.09, 2.39]). No significant association was noted for males. For age, among those in the younger age category (<78 years), patients with the Watchman had 1.89 times (95% CI: 1.33, 2.95) greater risk of stroke than those taking OACs. In patients ≥78 years, there was no significant association.

A total of 1,925 deaths occurred in the matched sample, of which 317 were in the Watchman group. No association with death was noted (HR [95% CI]: 0.94 [0.83, 1.06]). When assessing hospitalized bleeding events, those who underwent the Watchman implant had a 3.24-fold (95% CI: 2.75, 3.81) increased risk compared to matched OAC users. We further stratified results for hospitalized bleeding events according to time since Watchman implantation procedure date (**Table 11.4**). When looking at the periprocedural time frame (0-7 days) and warfarin time frame (8-45 days), those who received the Watchman implant had a significantly higher risk of a hospitalized bleed compared to OAC users. However, after 180 days from the index date, there was no longer a significant association. No interactions by sex or age were observed for the outcomes of death or hospitalized bleeding events.

E. Discussion

Using a large administrative claims database representative of older Americans with AF, there was no significant difference for risk of stroke between those who received the Watchman device and those taking OACs. Similarly, there was no increased risk for death. However, patients with the Watchman device had a higher risk of a hospitalized bleeding events than those taking OACs. Importantly, it may be possible that patients who received the Watchman implant already had an elevated bleeding risk, a common contraindication to OAC therapy.

Randomized clinical trials have shown that the Watchman device is non-inferior to OACs (warfarin or DOACs) when assessing risk of stroke, death, and bleeding events;[15-17] The clinical trials, however, randomized patients with AF to either Watchman

implantation or OACs. Therefore, OACs had to be considered safe for all trial enrollees, yet in clinical practice the Watchman device is considered a therapeutic option for AF patients who have contraindications to OAC therapy. For this reason, real-world data is particularly important. However, little research has been done in real-world populations. A study reported that patients in the National Inpatient Sample, a real-world population, had more complications associated with percutaneous LAAO with the Watchman or Amplatzer Cardiac Plug (e.g., vascular, cardiac, neurological, renal complications) than observed in clinical trials.[307] As a result, our study further advances the field by assessing the effectiveness and safety of the Watchman device among elderly patients in a real-world population.

In our analysis, we found that, overall, patients with the Watchman did not have a significantly higher risk for stroke or death compared to those taking OACs. The Watchman is approved by the FDA for patients with a contraindication to OACs and prior estimates for the number of patients with OAC contraindications have ranged between 2% to 58%.[308-314] These estimates are variable given that the definition of contraindication may be subjective and there are variations in clinical practice. As a result, a nonpharmacological stroke prevention alternative is needed, especially since those with OAC contraindications have a higher risk of bleeding, hospitalization, and death compared to those without contraindications.[313] Our results are similar to those of clinical trials in that a significantly higher risk for stroke or death was not observed when patients with the Watchman where compared to OAC users.

In a priori subgroup analyses, we also observed that among women, patients with the Watchman had a 62% higher risk of stroke than OAC users. This association was not

present among men. Similar results were observed in prior studies, in which women have a higher risk of in-hospital adverse events after LAAO with the Watchman compared to men.[315-317] This may be due to anatomical differences in the left atrial appendage between men and women.[318] Furthermore, women are often underrepresented in clinical trials.[319] In the PROTECT-AF and PREVAIL trials, approximately 30% of participants were women[12,13] compared to 44% in our study. In addition, younger patients (<78 years) in our study population with the Watchman had a higher risk of stroke compared to OAC users, while older patients did not. Other studies did not find a difference in risk of stroke based on age.[320,321] Additional research is needed to identify potential mechanisms and approaches that may reduce the risk of adverse events among women and younger patients (65-78 years) who receive the Watchman device.

Although no overall increased risk of stroke or death were observed in our analysis, there was an increased risk for bleeding among those with the Watchman device. This was not noted in clinical trials. It may be plausible that our results differ from that of clinical trials due to differences in the age distribution of our population versus the trials, given that age is a strong predictor for bleeding events among patients with AF.[322] Our study population was older (average age: 78 years) than those enrolled in prior trials (average age ranged between 72-74 years).[15,17,323] However, the incidence rate of bleeding events observed in our study was similar to that of a study of patients with the Watchman from the National Cardiovascular Data Registry,[324] as well among patients ≥75 years who received the Amplatzer Cardiac Plug, Amplatzer Amulet, or Watchman in the Iberian Registry II study.[325] Furthermore, data from an AF registry reported that those with contraindications to OACs are often sicker and more frail,[309] and high bleeding risk

is one of the most commonly listed contraindications to anticoagulant use.[309,312] Overall, this suggests that perhaps patients who received the Watchman device in the Medicare population may already be more sick and at a higher risk for bleeding than those taking OACs. Although we tried to account for this by matching on age, sex, and CHA_2DS_2-VASc score, it is likely residual confounding by factors such as frailty remained.

In addition, we observed that the risk of hospitalized bleeding varied according to time since Watchman implantation, with risk highest in the first 45 days post-implantation; a period when warfarin is recommended as the heart tissue grows over the Watchman implant and the left atrial appendage is sealed. Longer-term, 180 days after Watchman implantation, there was no difference in bleeding risk between those who received the Watchman and those who received OACs. Our results suggest that perhaps determining the safety of other treatment regimens after Watchman implant is needed. The PREVAIL and PROTECT-AF trials excluded patients who were not eligible for long-term warfarin use, likely to allow for randomization to the warfarin group.[12,13] As the FDA approved the Watchman for patients who have a contraindication to OACs, these clinical trials excluded the patient population arguably most likely to benefit from this device. Currently, the ASAP-TOO trial is ongoing to assess the safety and effectiveness of the Watchman device in those who are considered unsuitable for short-term OAC use.[224] Rather than taking warfarin after Watchman implant, participants in the ASAP-TOO trial took aspirin and/or clopidogrel.[224]

This study has several strengths, including a large sample size of Medicare beneficiaries who have undergone percutaneous LAAO with the Watchman device, the considerable number of stroke and other events, and more generalizable results than that

of clinical trials. However, there are also limitations that need to be noted. First, misclassification is possible given that ICD codes were used to identify AF, comorbidities, and outcomes. Second, uncontrolled confounding is a limitation of observational studies. Third, our results may not be generalizable to younger populations since the Medicare population consists of individuals ≥65 years; however, these results are clinically relevant as the median age of patients with AF is 75 years.[326] Fourth, we are only able to capture prescription fills by patients, and not whether the medications were actually taken.

F. Conclusion

In this population of Medicare patients with AF who were at high-risk for stroke, no difference in risk of stroke or death was observed when comparing patients who received the Watchman device compared to those taking OACs. This is in accord with RCTs, which showed the Watchman device to be non-inferior to OAC therapy. However, a higher risk for hospitalized bleeding was observed among those with the Watchman device, but this may be due to elevated bleeding risk being the indication for LAAO with the Watchman device over OAC therapy and inadequate control of confounding in our sample. LAAO with the Watchman device may be a suitable alternative for stroke prevention in patients with a contraindication to anticoagulants; however, patients should be aware of potential bleeding risks within the first 6 months after implantation. Additional research to assess the safety of the Watchman device in real-world populations is warranted.

Table 11.1. Baseline Characteristics of Matched Patients with Atrial Fibrillation, stratified by Watchmen or OAC Use, Medicare 20% Sample Databases, 2015-2018[*]

	Percutaneous (Watchman) LAAO (n=2,927)	Matched OAC Users/No LAAO[†] (n=14,587)
Age, years	78.4 (6.3)	78.3 (6.3)
Female sex	44	44
Black race	3	5
White race	90	88
Other race	7	7
Comorbidities		
Hypertension	72	77
Diabetes mellitus	31	30
Prior stroke/TIA	22	19
Heart failure	27	24
Myocardial infarction	7	8
Peripheral artery disease	21	24
Prior hospitalized bleed	34	40
Alcohol/drug use	2	3
Kidney disease	18	19
Liver disease	4	7
CHA_2DS_2-VASc score	4.1 (1.6)	4.1 (1.6)
HAS-BLED score	2.5 (1.2)	2.6 (1.2)
Oral Anticoagulants[‡]		
Apixaban	--	52
Dabigatran	--	3
Edoxaban	--	0.1
Rivaroxaban	--	24
Warfarin	--	21

[*]Data are expressed as mean ± SD or %.
[†]Patients were matched by sex, age, date of enrollment, index date, and CHA_2DS_2-VASc score.
[‡]First oral anticoagulant prescribed in the matched oral anticoagulant group.

Table 11.2. Adjusted Hazards Ratios for Incident Stroke and Secondary Outcomes Comparing LAAO vs. No LAAO Among Patients with Atrial Fibrillation, Medicare 20% Sample Databases, 2015-2018

	Percutaneous (Watchman) LAAO (n=2,927)	Matched OAC Users/No LAAO (n=14,587)
Stroke		
N, events	61	232
Incidence rate (per 100PY)	1.99	1.53
Hazard Ratio (95% CI)[*]	1.25 (0.95, 1.67)	Reference
Death		
N, events	317	1,608
Incidence rate (per 100PY)	10.22	10.47
Hazard Ratio (95% CI)[*]	0.94 (0.83, 1.06)	Reference
Hospitalized bleeding		
N, events	240	378
Incidence rate (per 100PY)	8.31	2.50
Hazard Ratio (95% CI)[*]	3.24 (2.75, 3.81)	Reference

[*]Adjusted for age, sex, race, hypertension, diabetes, prior stroke or TIA, heart failure, and vascular disease (MI or PAD)

Table 11.3. Adjusted Hazards Ratios for Incident Stroke, Stratified by Sex and Age, Comparing LAAO vs. No LAAO Among Patients with Atrial Fibrillation, Medicare 20% Sample Databases, 2015-2018

	Percutaneous (Watchman) LAAO	Matched OAC Users/No LAAO
Females		
N, events / N, total	34 / 1,288	101 / 6,423
Hazard Ratio (95% CI)[*]	1.62 (1.09, 2.39)	Reference
Males		
N, events / N, total	27 / 1,639	131 / 8,164
Hazard Ratio (95% CI)[*]	0.97 (0.64, 1.47)	Reference
<78 years		
N, events / N, total	35 / 1,336	83 / 6,682
Hazard Ratio (95% CI)[*]	1.98 (1.33, 2.95)	Reference
≥78 years		
N, events / N, total	26 / 1,591	149 / 7,905
Hazard Ratio (95% CI)[*]	0.83 (0.54, 1.26)	Reference

[*]Adjusted for age, sex, race, hypertension, diabetes, prior stroke or TIA, heart failure, and vascular disease (MI or PAD)

Table 11.4. Adjusted Hazards Ratios for Hospitalized Bleeding Events, Stratified by Different Timepoints, Comparing LAAO vs. No LAAO Among Patients with Atrial Fibrillation, Medicare 20% Sample Databases, 2015-2018

	Percutaneous (Watchman) LAAO	Matched OAC Users/No LAAO
0 - 7 days[*]		
N, events / N, total	9 / 2,927	8 / 14,587
Hazard Ratio (95% CI)[†]	5.93 (2.28, 15.46)	Reference
8 - 45 days[*]		
N, events / N, total	82 / 2,906	38 / 14,464
Hazard Ratio (95% CI)[†]	10.76 (7.31, 15.84)	Reference
46 - 180 days[*]		
N, events / N, total	95 / 2,602	110 / 13,264
Hazard Ratio (95% CI)[†]	4.36 (3.31, 5.75)	Reference
180 days - end of follow-up[*]		
N, events / N, total	54 / 1,875	222 / 9,883
Hazard Ratio (95% CI)[†]	1.26 (0.93, 1.70)	Reference

[*]Days from index date
[†]Adjusted for age, sex, race, hypertension, diabetes, prior stroke or TIA, heart failure, and vascular disease (MI or PAD)

Supplemental Table 11.1. Baseline Characteristics of Patients with Atrial Fibrillation, by LAAO or OAC User Status, Medicare 20% Sample Databases, 2015-2018[*]

	Percutaneous (Watchman) LAAO (n=2,929)	OAC Users/No LAAO (n=486,931)
Age, years	78.4 (6.3)	78.9 (7.8)
Female sex	44	52
Black race	3	5
White race	90	88
Other race	7	7
Comorbidities		
Hypertension	72	69
Diabetes mellitus	31	31
Prior stroke/TIA	22	18
Heart failure	27	27
Myocardial infarction	7	6
Peripheral artery disease	21	19
Hospitalized bleed	34	30
Alcohol/drug use	2	2
Kidney disease	18	17
Liver disease	4	4
CHA_2DS_2-VASc score	4.1 (1.6)	4.1 (1.6)
HAS-BLED score	2.5 (1.2)	2.4 (1.2)
Oral Anticoagulants[†]		
Apixaban	--	27
Dabigatran	--	6
Edoxaban	--	0.1
Rivaroxaban	--	20
Warfarin	--	47

[*]Data are expressed as mean (SD) or %.
[†]First oral anticoagulant prescribed in the matched oral anticoagulant group.
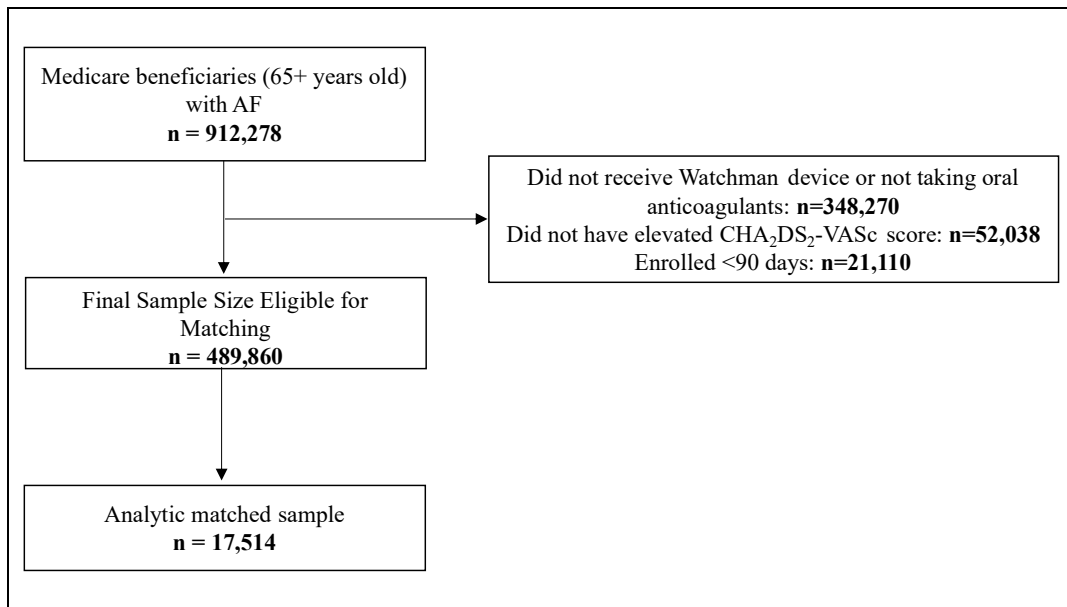
Figure 11.1. Patient Exclusion Flowchart, Medicare 20% Sample Databases

Figure 11.2. Timeline of Pharmacotherapy After Watchman Implant[327]

Chapter 12. Summary

The aims of this dissertation were to 1) assess the association between atrial myopathy and brain MRI markers of cerebrovascular disease, 2) conduct a discovery analysis to identify new proteomic markers associated with incident ischemic stroke, and 3) evaluate the efficacy and safety of stroke prevention with percutaneous left atrial appendage occlusion with the Watchman device vs. OAC use in patients with AF.

In the first manuscript, we found that lower LA function was cross-sectionally associated with brain MRI markers of vascular brain injury. Atrial myopathy (e.g., lower

LA function) is an emerging clinical risk factor and can be measured from echocardiograms post hoc. Atrial myopathy has also been associated with adverse events (e.g., clinical stroke, dementia), suggesting there is potential for public health implications, particularly if a clinical cutoff for abnormal LA function can be defined. Therefore, if future prospective studies can confirm our findings and potentially identify a cutoff to define abnormal LA function, it may be possible that patients with atrial myopathy could be enrolled in clinical trials assessing treatments that may reduce the risk for not only subclinical cerebrovascular disease, but also clinical stroke and dementia.

For the second manuscript, we conducted a proteomics discovery analysis to identify novel proteins associated with risk of incident ischemic stroke. Among the proteins associated with ischemic stroke in our discovery analysis in ARIC, three proteins (NT-proBNP, GDF15, and WFDC2) replicated in an external cohort (Cardiovascular Health Study). Risk scores that incorporate biomarkers have been shown to predict stroke and death more accurately than the CHA_2DS_2-VASc score in patients with AF.[207,328] Therefore, there may be potential in assessing whether the addition of proteins discovered in our analysis improve risk prediction for stroke above that of standard risk scores. Furthermore, it may be possible that these proteins could be potential therapeutic targets for stroke prevention.

Using data from the Medicare 20% sample databases, the third manuscript assessed stroke prevention strategies among older patients with AF who are at an elevated risk for stroke. Because the prevalence of AF continues to increase,[329] it is important to identify safe and effective stroke prevention therapies. OACs are often used for stroke prevention, and more recently, the Watchman device has emerged as a

113

nonpharmacologic option for patients with OAC contraindications. Results from our study indicate that among patients at an elevated risk for stroke, those who received the Watchman device did not have a higher risk of stroke or death compared to OAC users. A higher risk for bleeding events was observed; however, it may be possible that patients who received the Watchman device were already at a high risk for bleeding initially. Furthermore, there was no significant increased risk for bleeding events 180 days after the procedure date, suggesting there may not be a long-term risk for bleeding. Risk for bleeding was highest in the first 180 days after the procedure, and this may be due to the short course of warfarin that is required after the Watchman implant. As those who are eligible for the Watchman device have a contraindication to OACs, it may be beneficial to evaluate whether other medications, such as low-dose aspirin, can be taken after Watchman implant rather than warfarin in real-world populations.

Overall, this dissertation extends our knowledge on risk factors for stroke and helps address research gaps in stroke prevention therapies. Results from this dissertation could potentially contribute to identifying future prevention strategies for patients who are at an elevated risk for stroke.

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