CORONARY ARTERY CALCIUM AND INCIDENT STROKE IN THE
MULTI-ETHNIC STUDY OF ATHEROSCLEROSIS (MESA) COHORT

BY

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LIST OF ABBREVIATIONS

ABI - Ankle-Brachial Index
ACC - American College of Cardiology
AHA - American Heart Association
ARIC - Atherosclerosis Risk in Communities
ASCVD - atherosclerotic cardiovascular disease
BMI - Body Mass Index
CT - Computed Tomography
CAC - Coronary Artery Calcium
CHD - Coronary Heart Disease
CVD - Cardiovascular Disease
ERC - Emerging Risk Factors Collaboration
FHS - Framingham Heart Study
FSR - Framingham Stroke Risk
gm - gram
HR - hazard ratio
HNR - Heinz Nixdorf Recall
hs-CRP - ultra-sensitive C-reactive protein
IMT - Intimal Medial Thickness
MESA - Multi Ethnic Atherosclerosis
mSv - millisievert
NHANES - National Health and Nutrition Examination Survey

NRAF - National Registry of Atrial Fibrillation

NRI - Net Reclassification Index

ROC - Receiver Operating Curve

SD - standard deviation

WHS - Women’s Health Study
ABSTRACT

In the published literature, coronary artery calcium (CAC) score is a well-documented independent predictor of incident coronary heart disease (CHD) events, including myocardial infarction, death related to cardiovascular disease, and cardiac arrest. In addition to the progression of disease in the coronary arteries, CAC has also been associated with the progression of atherosclerotic disease in the cerebrovascular system and has been shown to predict the presence of significant/overt carotid stenosis in an asymptomatic cohort. Despite the relationship between carotid stenosis and risk of future stroke, the prognostic value of CAC to predict incident stroke remains unclear. The MESA cohort provides a unique opportunity to study the association between CAC and the risk of incident stroke as the cohort contains over 6800 participants with no known coronary or cerebrovascular disease. The participants have been followed for over 10 years and a wide range of data has been collected including demographics, assessment of cardiovascular risk factors, and adjudication for various clinical outcomes. Using the available MESA participant risk factor and outcome data, the purpose of this thesis is to examine the relationship between CAC score and the risk of incident stroke in a multi-ethnic population free of cerebrovascular disease.
Chapter 1: Coronary Artery Calcium, a Surrogate for Subclinical Disease

INTRODUCTION

Cerebrovascular disease and its clinical consequences remain a leading cause of morbidity and mortality around the world. Currently cerebrovascular disease is the 4th leading cause of death in the United States. According to the Heart Disease and Stroke Statistics 2011 Update, each year approximately 800,000 people in the United States experience a stroke. In addition, more than 600,000 of these people are experiencing their first cerebrovascular event. The impact of cerebrovascular disease was recently highlighted by a member of the American Heart Association 2013 Guidelines writing group who made the following statement, “Cardiovascular disease caused by atherosclerosis remains the number one cause of death, a major cause of disability and a huge source of health care costs. We must do a better job of preventing it. By including stroke in our new algorithm, we can better calculate overall cardiovascular risk, especially in women and African-Americans.”

There has been a significant decline in both coronary heart disease deaths as well as strokes in the United States since the 1970s which can be attributed to the decline in traditional CHD risk factors including improvement in cholesterol, blood pressure, smoking, and physical activity. While the time to first CHD event has decreased across age groups, approximately 10% of all strokes occur in people ages 18 to 50 years old. The rate of stroke death has declined in this age group, however unfortunately the rate of decline has been less substantial than the rate in older age groups.
An attempt to decrease the stroke rate across all age groups is easily dependent upon being able to identify people at highest risk of disease. Decades of cardiovascular disease research has identified conventional risk factors such as hypertension, diabetes and smoking. Moving beyond the established risk factors, researchers have turned their attention to identifying surrogate markers that are able to detect subclinical disease early enough to allow preventative strategies to reverse or delay the onset of disease. Over the last 10 years, CAC has become a well-established, non-invasive measure of subclinical atherosclerotic disease and has been proven to reliably predict future coronary heart disease events such as angina and myocardial infarct. CAC may also have the ability to predict events due to other forms of atherosclerotic disease such as stroke.

Atherosclerosis is a systemic process linked to substantial pathology in both the heart and the brain. During the progressive stages of atherosclerotic plaque formation, the lipid rich layer developing in the arterial wall experiences multiple internal disruptions and replacement by thrombi. One of the subsequent pathways in plaque formation involves the addition of a subintimal layer of coronary calcification which either replaces or is added to a lipid core.

For many years CAC was felt to be a benign process of little clinical consequence, however studies over the last decade have confirmed that coronary artery medial calcification is associated with arterial stiffness. Stiffness of the arterial wall has long been known to significantly increase the risk of adverse cardiovascular events.

The extent of calcium in the coronary artery bed is directly proportional to the degree of atherosclerosis and has been shown to correlate strongly with future cardiac
events. Calcification due to atherosclerotic disease occurs mostly in the intimal layer of the arterial wall and is linked to advanced age, diabetes, dyslipidemia, hypertension, male gender, smoking and renal disease.\textsuperscript{40} Conversely, calcification of the media is a result of reduced glomerular filtration rate, electrolyte disturbances such as hypercalcemia and hyperphosphatemia and the duration of dialysis.\textsuperscript{41}

The calcified plaque can be detected by noninvasive imaging such as a computed tomography (CT) scan. Radiologists utilize a cardiac-gated, non-contrast CT scan to identify discrete calcific foci located throughout the coronary artery bed and calculate a calcium score in terms of volume of calcium (mm\textsuperscript{3}), area-density of calcium (Agatston units) or calibrated mass of calcium (gm).\textsuperscript{23} The imaging report provides a total CAC score as well as a CAC score percentile which is based on a nomogram comparing an individual’s CAC score to others of the same age and gender. The CAC score has been shown to have good inter-observer reproducibility, especially at higher CAC values.\textsuperscript{24}

Proponents of cardiac CT imaging believe the exam offers valuable information with relatively low risk to the patient compared to other more invasive cardiac testing techniques such as coronary angiography. In addition, cardiac CT scans do not require intravenous contrast and are performed with near negligible radiation exposure to the patient (1 mSv).

The ideal surrogate marker would establish the presence of subclinical disease prior to a clinical event. In the published literature, CAC score is a well-documented independent predictor of incident CHD events, including myocardial infarction, death related to cardiovascular disease, and cardiac arrest.\textsuperscript{22} In addition to the progression of
disease in the coronary arteries, CAC has also been associated with the progression of atherosclerotic disease in the cerebrovascular system, and is able to predict the presence of significant/overt carotid stenosis in previous cohort studies. Finally, the presence of CAC has been linked to an increased risk of ischemic stroke. Despite the relationship between severe carotid stenosis and the risk of future stroke, models utilizing CAC to predict incident stroke have been limited in significance and remain inconclusive.

PREDICTION MODELS TO ESTIMATE RISK OF DISEASE

Disease prediction models are a useful tool that aid both physicians and patients in making informed clinical decisions based upon the predicted risk for development of a disease. A disease prediction model is an analytic framework, typically a statistical regression model, which uses a person’s individual characteristics and relates these characteristics to the development of a specific disease. Typically a number of risk factors known to be associated with the disease are included in a model and a risk score is calculated based on these risk factors. A higher risk score is interpreted as being at higher risk for developing disease compared to other people in the population being studied who have a lower risk score. In clinical use, risk scores are often used to classify individuals into groups according to level of risk, i.e. low, intermediate or high risk for development of disease.

A successful prediction model accurately differentiates between people at risk and those who are not at risk for disease, and can provide guidance regarding potential screening and preventative therapies.
A risk prediction model must be validated prior to being used in clinical decision making. According to Altman and colleagues, a validated test must be both calibrated and able to discriminate between those who will development disease in the future and those who will not develop disease. Calibration is defined as a model’s ability to accurately predict risk among a group of individuals. If a prediction model is well calibrated, the mean predicted risk among individuals of a group should be equal to the observed cumulative incidence of disease in the group. Discrimination on the other hand, is a prediction model’s ability to accurately differentiate individuals who will develop disease from those who will not. Sensitivity and specificity are two terms used to quantify discrimination. A prediction model is sensitive if it estimates high risk in individuals who develop disease, and conversely a model is specific if it assigns low risk to individuals who will not develop disease.

The relationship between sensitivity and specificity can be visualized using a graph coined the Receiver Operating Characteristic (ROC) curve. The ROC curve is a plot of sensitivity versus 1 - specificity for a model constructed to predict disease above a specified threshold of risk. The area under the ROC curve, termed the c-statistic, represents a model’s ability to accurately distinguish between individuals who will and will not develop disease. A model with perfect discrimination will have a c-statistic approximating 1. Furthermore, the ROC curve transforms the accuracy of a prediction model into a relevant scale based on its performance which can then be utilized to make meaningful comparisons with other prediction models.
Until recently, change in the c-statistic was the most common method used to quantify improvement in the accuracy of a prediction model after the addition of a new variable. Over the last ten years, researchers have begun to question whether clinically relevant changes in risk prediction models were being rejected due to an insignificant change in the c-statistic. For example, if the addition of a new risk factor to an existing disease prediction model took an individual from intermediate to high risk for disease, the clinical implication of this change in risk would result in more aggressive primary disease prevention measures. Unfortunately, if the c-statistic did not identify a significant change in the prediction model, high risk individuals inaccurately identified as intermediate risk would be less likely to receive targeted preventative therapies.

Cook and colleagues were one of the first groups to recognize the c-statistic was an insensitive measurement of improvement in model accuracy and introduced the reclassification method as a more relevant way to quantify improvement in risk prediction. In the results reported by Cook, et al., a predicted risk was termed “reclassified” if the risk categories were different between the new and old models. The group calculated the observed event rates for the people reclassified and called the reclassification more accurate if the observed rate was in the new model’s risk category. In 2008, Pencina, et al. took this concept a step further by measuring the degree of discrimination afforded by the addition of a new risk factor to an old model. These writers suggested that when comparing diseased versus non diseased people, a “correct reclassification” would include people who moved up a risk category using the new
model and developed disease as well as people who moved down a risk category that did not develop disease.

At this time there is no general consensus on the most reliable way to compare prediction models. Questions regarding potential flaws in reclassification also exist and there are concerns that methods used to quantify improvement after reclassification may actually lead to uninformative variables being deemed predictive. Some researchers have argued that reclassification of risk for each person does not necessarily translate into improved risk prediction for a population. In addition, statistical methods for validating significance have not been clearly defined.48

CORONARY ARTERY CALCIUM IN PREDICTION MODELS

Coronary Artery Calcium has been widely studied and several investigators have confirmed its utility as a noninvasive tool for assessing a person’s risk for future CHD events. Raggi, et al. published one of the largest assessments estimating the risk of all-cause mortality associated with CAC using data collected from 35,388 asymptomatic patients.16 Study participants included 3,570 older than 70 years at initial screening and 50% of the participants were women. Participants were referred from their primary care physicians for a CAC screening exam and any patients with known or suspected coronary disease were excluded from the study. CAC score was assessed using the Agatston method and expressed in Cox models as a categorical variable (CAC score 0-10, 11-100, 101-399, 400-999, or ≥1,000). After a mean follow-up time of 5.8 ± 3 years, the investigators found that the cumulative survival for CAC subsets varied by age however
increased CAC score was associated with decreased survival across all age groups (p < 0.0001). Cumulative unadjusted survival for <40-year-old and ≥80-year-old men with a CAC score ≥400 was 88% and 19% respectively. Women with a CAC score ≥400 also had a significant, but less dramatic decrease in survival (95% for women <40-years, 44% for women ≥80-years, p < 0.0001). Among the 20,562 patients with CAC score 0-10, annual mortality rates ranged from 0.3% to 2.2% for the youngest patients ages 40 to 49 years or the oldest patients ≥70 years, respectively (p < 0.0001).

In addition to studying the association between CAC and cardiovascular disease mortality, investigators of several large cohorts have also studied whether CAC adds diagnostic benefit beyond traditional CHD risk factors. The ideal method to best quantify the improvement in risk prediction models after the addition of a new variable is a focus of ongoing research efforts. Net Reclassification Index (NRI) is one proposed method to quantify the incremental improvement in prognostic value when CAC is added to traditional CHD risk factors. Table 1 reports net reclassification findings from several large cohorts after CAC, carotid IMT, and CRP were added independently to a model predicting stroke using traditional risk factors.

<table>
<thead>
<tr>
<th></th>
<th><strong>NRI</strong></th>
<th><strong>NRI</strong> (Intermediate Risk)</th>
<th>Δ C statistic</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAC</td>
<td>14-25%</td>
<td>31-55%</td>
<td>+</td>
<td>HNR, Rotterdam, MESA</td>
</tr>
<tr>
<td>Carotid IMT</td>
<td>7.6-9.9%</td>
<td>21%</td>
<td>+</td>
<td>FHS, ARIC</td>
</tr>
<tr>
<td>CRP</td>
<td>1.5-5.7%</td>
<td>15%</td>
<td>-/+</td>
<td>ERC, WHS</td>
</tr>
</tbody>
</table>

NRI = Net Reclassification Index, HNR = Heinz Nixdorf Recall, FHS = Framingham Heart Study, ARIC = Atherosclerosis Risk in Communities, ERC = Emerging Risk Factors Collaboration, WHS = Women’s Health Study
Table 2 presents the improved Net Reclassification Index (NRI), specifically for participants in the intermediate risk group, after CAC was added to the traditional CHD risk model in the MESA, Heinz Nixdorf Recall (HNR) and Rotterdam cohorts.  

<table>
<thead>
<tr>
<th></th>
<th>Adjusted HR CAC ≥ 400*</th>
<th>NRI</th>
<th>NRI (Intermediate Risk)</th>
<th>Δ c statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>MESA (n=6814)</td>
<td>9.7</td>
<td>25%</td>
<td>55%</td>
<td>.77 → .81</td>
</tr>
<tr>
<td>HNR (n=4129)</td>
<td>6.0</td>
<td>24%</td>
<td>31%</td>
<td>+</td>
</tr>
<tr>
<td>Rotterdam (n=2028)</td>
<td>4.6</td>
<td>14%</td>
<td>52%</td>
<td>.72 → .76</td>
</tr>
</tbody>
</table>

NRI = Net Reclassification Index, HNR = Heinz Nixdorf Recall  
*Hazard ratio for incident CHD event adjusted for all CHD risk factors for CAC ≥ 400 vs. 0

The current literature contains limited analysis describing the association between CAC score and risk of future cerebrovascular event in the general population. In 2002, investigators of the retrospective population-based Rotterdam Coronary Calcification Study reported that when participants with the lowest calcium score (0-100) were compared to participants with a calcium score of 101-500, after adjusting for age, individuals in the higher score category were twice as likely to have experienced a stroke (OR 2.1, 95% CI 0.9-4.7). After adjusting for age and gender, participants with a calcium score above 500 were 3.3 times more likely to have a stroke than those individuals in the lowest score category (95% CI 1.5-7.2, p=0.001).

Folsom, et al. published the results of a multivariable model that included CAC score, carotid IMT, age, race and sex on incident cardiovascular disease (CVD) and incident stroke in the MESA cohort. The study reported that a 1 SD increase in [ln(CAC
was associated with a Hazard Ratio (HR) of 2.1 for CVD incidence \( (p < 0.05) \). In contrast, the HR for incident stroke in this same population was not statistically significant; however, the data did suggest a trend towards an association between CAC and risk of stroke \( (HR 1.1, \ p= 0.41) \) \( \text{(Table 3)} \). Notably, the investigators acknowledged the significant limitation that only 59 adjudicated strokes occurred over a follow-up of 3.9 years.

### Table 3. Incident Stroke in Relation to 1-SD Increment (+/-2.5) of CAC

<table>
<thead>
<tr>
<th></th>
<th>Model 1§</th>
<th>Model 2§§</th>
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<tr>
<td></td>
<td>N</td>
<td>Events</td>
</tr>
<tr>
<td>CAC score [ln(CAC+1)]</td>
<td>6698</td>
<td>59</td>
</tr>
<tr>
<td>( p ) value ( (a=0.05) )</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

§ Model 1 – Hazard Ratio (HR) risk is adjusted for age, race and sex
§§ Model 2 – Hazard Ratio (HR) risk is adjusted for age, sex, race/ethnicity, smoking, total cholesterol, HDL, hypertension, diabetes, and statin medication

One of the few studies designed specifically to evaluate the ability of CAC to predict stroke was published by Hermann, \textit{et al.}, who followed a German population cohort of individuals ages 45 to 75 years old. After 7.9±1.6 years of follow-up, they concluded that patients who experienced a stroke after enrollment had significantly higher CAC scores compared to participants who did not experience a stroke \( (\text{median 104 vs. 11.2, p<0.001}) \). In a multivariable Cox regression model, CAC was found to be independently associated with stroke after adjusting for traditional risk factors including age, blood pressure, and smoking. The German cohort was composed of a very
homogeneous population, therefore it is unclear if the predictive ability of CAC translates to diverse, multi-ethnic populations, such as those found in the United States.

**CORONARY ARTERY CALCIUM AND STROKE**

The difficulty in identifying patients at risk for stroke may be in part due to the heterogeneity of stroke. In general, the term stroke includes two broad categories of cerebrovascular injury; ischemia resulting from occlusion or reduced flow in the arteries supplying the brain, or hemorrhage which is a manifestation of arterial leakage or rupture. Furthermore, strokes can be grouped according to pathophysiology including embolism, thrombosis, decreased perfusion, leakage or rupture. While many stroke risk factors such as gender and hypertension are true regardless of stroke type, other risk factors which may be inherently related to the pathology leading to the stroke have not been generalizable across all stroke subtypes. For example, smoking and heavy alcohol use are more strongly correlated with the risk of hemorrhagic stroke. In comparison, validated risk factors for atherosclerotic cardiovascular disease, specifically diabetes and atrial fibrillation, appear to be more strongly associated with the risk of ischemic stroke. Due to the inconsistency of stroke risk factors, the potential exists for a statistically significant association to be overlooked if all stroke events are combined into a single disease outcome when formulating disease prediction models.

One of the few stroke prediction models available is the widely used and validated Framingham Stroke Risk Score (FSR) score. The FSR score is composed of data initially collected in the 1960s from participants enrolled in the Framingham cohort.
who were ages 55-84 years and free of cardiovascular disease during the baseline exam.

The cohort participants were followed closely for 10 years and several cardiovascular
disease risk factors and incident events were recorded over time.\textsuperscript{22} The investigators
utilized several risk factors to compute stroke probabilities using Cox proportional
hazards models stratified by gender. Figure 1 illustrates the individual risk factors along
with their associated relative risk. These risk factors are combined to estimate a person’s
10 year risk of having a stroke compared to the average risk of stroke for a person of the
same age and sex. The FSR score has been validated in several large population studies
across the United States as well as Europe.\textsuperscript{26}

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|}
\hline
Risk Factor & Men Relative Risk\textsuperscript{*} & Women Relative Risk\textsuperscript{*} \\
\hline
Age & 1.66 & 1.93 \\
Systolic Blood Pressure & 1.91 & 1.68 \\
Antihypertensive therapy & 1.39 & § \\
Diabetes mellitus & 1.40 & 1.72 \\
Cigarette Smoking & 1.67 & 1.70 \\
Cardiovascular disease\textsuperscript{§§} & 1.68 & 1.54 \\
Atrial fibrillation & 1.83 & 3.16 \\
Left ventricular hypertrophy\textsuperscript{§§§} & 2.32 & 2.34 \\
\hline
\end{tabular}
\caption{Components of the Framingham Stroke Risk Score}
\end{table}

Source: Wolf 1991; Each relative risk is adjusted for effects of other risk factors.
\textsuperscript{*}Relative risk for age and systolic blood pressure are given for 10-unit changes; all other variables are dichotomous (1 if
yes and 0 if no). All variables are significant at p<0.05.
\textsuperscript{§} Because blood pressure x therapy interaction is significant for women, relative risks here depend on levels of blood
pressure and are not summarized as a single value.
\textsuperscript{§§} Cardiovascular disease includes a diagnosis or prior history of coronary heart disease, cardiac failure, or intermittent
claudication.
\textsuperscript{§§§} Left ventricular hypertrophy determined by electrocardiogram

Although the FSR score provides valuable information, its ability to discriminate
between stroke and non stroke events remains low, especially in ethnic minority
populations.\textsuperscript{47} Some authors have proposed that a change in the prevalence of important
stroke risk factors over the last few decades has led to the FSR score being less accurate. In addition, others have proposed that successful implementation of targeted strategies aimed at reducing the impact of FSR score identified risk factors have resulted in a reduction in the incidence of stroke. Current areas of research include focus to improve current prediction models and specifically to identify novel risk factors in order better predict individuals at greatest risk for incident stroke.
Chapter 2: Coronary Artery Calcium and Incident Cerebrovascular Events in an Asymptomatic Cohort. The MESA Study

Coronary Artery Calcium and Incident Cerebrovascular Events in an Asymptomatic Cohort

The MESA Study

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ABSTRACT

OBJECTIVES This study assessed the predictive value of coronary artery calcium (CAC) score for cerebrovascular events (CVE) in an asymptomatic multiethnic cohort.

BACKGROUND The CAC score, a measure of atherosclerotic burden, has been shown to improve prediction of coronary heart disease events. However, the predictive value of CAC for CVE is unclear.

METHODS CAC was measured at baseline examination of participants (N = 6,779) of MESA (Multi-Ethnic Study of Atherosclerosis) and then followed for an average of 9.5 ± 2.4 years for the diagnosis of incident CVE, defined as all strokes or transient ischemic attacks.

RESULTS During the follow-up, 234 (3.5%) adjudicated CVE occurred. In Kaplan-Meier analysis, the presence of CAC was associated with lower CVE event-free survival versus the absence of CAC (log-rank chi-square: 39.8, p < 0.0001). Log-transformed CAC was associated with increased risk for CVE after adjusting for age, sex, race/ethnicity, body mass index, systolic and diastolic blood pressure, total cholesterol, high-density lipoprotein cholesterol, cigarette smoking status, blood pressure medication use, statin use, and interatrial fibrillation (hazard ratio [HR]: 1.33 [95% confidence interval (CI): 1.07 to 1.62], p = 0.0061). The American College of Cardiology/American Heart Association-recommended CAC cutoff was also an independent predictor of CVE and strokes (HR: 1.70 [95% CI: 1.24 to 2.32], p = 0.001, and HR: 1.59 [95% CI: 1.11 to 2.27], p = 0.01, respectively). CAC was an independent predictor of CVE and was stratified by sex or race/ethnicity and improved discrimination for CVE when added to the full model (C-statistic: 0.744 vs. 0.735; CAC improved the discriminative ability of the Framingham stroke risk score for CVE.

CONCLUSIONS CAC is an independent predictor of CVE and improves the discrimination afforded by current stroke risk factors or the Framingham stroke risk score for incident CVE in an initially asymptomatic multiethnic adult cohort. (J Am Coll Cardiol 2014;7:108-115) © 2014 by the American College of Cardiology Foundation.

Coronary artery calcium (CAC) is an independent predictor of cardiovascular disease (CVD) events (1-3), a composite that often include strokes and has also been shown to improve discrimination for CVD events in the general population beyond current risk prediction tools such as the Framingham risk score and Reynolds score (4-6). However, in almost all of these studies (1-3), the association between CAC and stroke failed to achieve statistical significance due to relatively small sample sizes. Some authors have questioned the use of CAC to improve stroke risk.
prediction in the general population based on these data (7).

The recent American College of Cardiology (ACC)/American Heart Association (AHA) guidelines for risk prediction adopted a new composite: atherosclerotic cardiovascular disease (ASCVD), which includes coronary death, nonfatal myocardial infarction, and fatal and nonfatal stroke (8). The new AHA/ACC ASCVD risk score does not consider current subclinical atherosclerosis measures. Given persuasive data on the improvement of discrimination for CVD by subclinical atherosclerotic measures (4,5) and the similarity of the constituents of the pooled ASCVD risk prediction tool with the Framingham risk score (8,9), there are ongoing efforts to improve the risk prediction afforded by the new pooled ASCVD risk tool with these subclinical atherosclerotic measures in the general population. However, adding subclinical atherosclerotic measures to the new pooled ASCVD risk tool would only make sense if these measures were associated with strokes. A recent publication from the HNR (Heinz Nixdorf Recall) study with a larger number of strokes than that of prior published data (1-3) showed an independent association between CAC and strokes in low- to intermediate-risk Caucasian subjects (10). However, the racial homogeneity of the HNR cohort limits its external validity. Thus, the association between CAC and strokes in the general population remains unclear.

In this report, we examined the relationship of CAC measured during the baseline examination to adjudicated cerebrovascular events (CVE) in participants of the MESA (Multi-Ethnic Study of Atherosclerosis) over a 10-year follow-up.

METHODS

STUDY POPULATION AND DATA COLLECTION. A detailed description of the study design for MESA has been published (11). In brief, MESA is a cohort study that began in July 2000 to investigate the prevalence, correlates, and progression of subclinical CVD. At baseline, the cohort included 6,814 women and men age 45 to 84 years recruited from 6 U.S. communities (Baltimore, Maryland; Chicago, Illinois; Forsyth County, North Carolina; Los Angeles County, California; northern Manhattan, New York; and St. Paul, Minnesota). MESA participants were 38% white, 28% black, 22% Hispanic, and 12% Chinese. Individuals with a history of physician-diagnosed myocardial infarction, angina, heart failure, stroke, or transient ischemic attack (TIA) or who had undergone an invasive procedure for CVD (coronary artery bypass graft, angioplasty, valve replacement, pacemaker placement, or other vascular surgeries) were excluded.

Demographics, medical history, and anthropometric and laboratory data for these analyses were obtained at the first MESA examination (July 2000 to August 2002). Current smoking was defined as having smoked a cigarette in the past 30 days. Diabetes mellitus was defined as fasting glucose ≥126 mg/100 dl or use of hypoglycemic medications. Use of antihypertensive and other medications was based on the review of prescribed medication containers. Resting blood pressure was measured 3 times in a seated position, and the average of the second and third readings was used. Hypertension was defined as a systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, or use of medication prescribed for hypertension. Body mass index was calculated as weight (kg)/height² (m²). Total and high-density lipoprotein cholesterol were measured from blood samples obtained after a 12-h fast. Low-density lipoprotein cholesterol was estimated by the Friedewald equation (12). The MESA study was approved by the institutional review boards of each study site, and written informed consent was obtained from all participants.

MEASUREMENT OF CAC SCORE. Details of the MESA computed tomography (CT) scanning and interpretation methods have been reported by Carr et al. (13). Scanning centers assessed CAC by noncontrast cardiac CT with either an electron-beam CT scanner (Chicago, Illinois; Los Angeles, California; and New York, New York field centers) or a multidetector CT system (Baltimore, Maryland; Forsyth County, North Carolina; and St. Paul, Minnesota field centers). Certified technologists scanned all participants twice over phantoms of known physical calcium concentration. A radiologist or radiologist read all CT scans at a central reading center (Los Angeles Biomedical Research Institute at Harbor-University of California, Los Angeles, Torrance, California). We used the mean Agatston score for the 2 scans in all analyses (14). Intraobserver and interobserver agreements were excellent (κ = 0.93 and 0.90, respectively).

ASSESSMENT OF CVE. Strokes, TIAS, and other cerebrovascular events were adjudicated by a MESA committee that included cardiologists, physician epidemiologists, and neurologists. A detailed description of the adjudication process has been published (11). For the purposes of this study, we defined CVE as fatal or nonfatal strokes due to hemorrhage or infarcts or TIA. TIAS and strokes
were also used individually as secondary outcomes for this analysis. Interim incident atrial fibrillation, which occurred during the follow-up period, was adjusted for in the full model as a time-varying covariate. Interim atrial fibrillation in MESA is a combination of adjudicated; International Classification of Diseases, Ninth Revision code; and self-reported cases.

**STATISTICAL ANALYSIS**. Demographic and other characteristics were compared according to cerebrovascular event. CAC was introduced into models as a binary variable (CAC present/absent), as a continuous variable (ln [CAC + 1]), or as 4 categories (CAC: 0, 0 to 100, >100 to 400, and >400 Agatston units). Kaplan-Meier and Cox proportional hazards analyses were used to evaluate the association between CAC and incident CVE. Among participants with more than 1 type of event adjudicated during the follow-up period, the first event was used in this analysis. Covariates entered in models were chosen on the basis of their association with incident CVE in the present analyses and in published data. The covariates include age, sex, race/ethnicity, body mass index, systolic and diastolic blood pressure, total cholesterol, high-density lipoprotein, cigarette smoking status, blood pressure medication use, statin use, and interim atrial fibrillation that occurred during the follow-up period. The full multivariable model was then stratified by sex and race/ethnicity. The preceding analysis was repeated with all strokes and TIA's as the outcome.

The improvement of discrimination for incident CVE afforded by the addition of CAC to our full model was evaluated using the receiver-operating curve analysis. The Framingham stroke risk score (FSRS) was calculated for each MESA participant (using baseline data only) using the following variables: age, systolic blood pressure, diabetes mellitus, cigarette smoking, prior CVD, atrial fibrillation, left ventricular hypertrophy (EKG criteria), and blood pressure medications. No MESA participant had prior CVD or atrial fibrillation during the baseline examination. The improvement in discrimination afforded by the addition of CAC to the FSRS (and also using the constituents in the model) was also assessed using receiver-operating curve analysis. A 2-tailed value of \( p < 0.05 \) was considered significant. All statistical analyses were performed using SAS version 9.2 (SAS Institute, Cary, North Carolina).

## RESULTS

After a mean of 9.5 ± 2.4 years, 234 (3.5%) adjudicated CVE (180 strokes and 67 TIA's) were identified. Ischemic CVE (cerebral infarcts and TIA's) were observed in 206 (3.4%) participants, of whom 152 (2.2%) had cerebral infarcts. Participants who developed a cerebrovascular event were older, had a worse cardiovascular risk profile, and developed atrial fibrillation more often (17.1% vs. 5.6%) during the follow-up period than those who did not have a cerebrovascular event (Table 1). As shown in Table 2, similar proportions of the participants within each CAC category had hemorrhagic strokes during the follow-up period. However, an increased proportion of participants had cerebral infarcts, TIA's, and atrial fibrillation with higher CAC category.

**CAC AND CEREBROVASCULAR EVENT PREDICTION.** In Kaplan-Meier analyses, participants with CAC present during the baseline examination had a lower cerebrovascular event-free survival rate compared with participants with CAC absent at baseline (log-rank chi-square: 59.84, \( p < 0.0001 \) (Figure 1). When participants were divided into 4 groups according to baseline CAC (CAC: 0, 0 to 100, >100 to 400, and >400 Agatston units), a significant graded cerebrovascular event-free survival rate was observed.
Table 2. Occurrence of Cerebrovascular Events, Hemorrhagic Strokes, Cerebral Infarcts, and TIAs

<table>
<thead>
<tr>
<th>CAC Category (Agatston Units)</th>
<th>Cerebrovascular Events</th>
<th>Hemorrhagic Strokes</th>
<th>Cerebral Infarcts</th>
<th>TIAs</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3,399</td>
<td>69 (2.0)</td>
<td>13 (0.4)</td>
<td>40 (1.3)</td>
</tr>
<tr>
<td>&gt;0-100</td>
<td>726</td>
<td>67 (3.5)</td>
<td>9 (0.5)</td>
<td>41 (2.3)</td>
</tr>
<tr>
<td>&gt;100-400</td>
<td>923</td>
<td>52 (5.6)</td>
<td>4 (0.4)</td>
<td>36 (3.6)</td>
</tr>
<tr>
<td>&gt;400</td>
<td>671</td>
<td>46 (6.9)</td>
<td>2 (0.3)</td>
<td>35 (5.2)</td>
</tr>
<tr>
<td>Total</td>
<td>234</td>
<td>28</td>
<td>152</td>
<td>67</td>
</tr>
</tbody>
</table>

Values are n (%). Events occurred within each CAC category after a mean of 9.5 ± 2.4 years of follow-up in the MESA cohort.

*TIAs — transient ischemic attack; other abbreviations as in Table 1.

Cox models but not in the multivariable Cox models (data not shown).

Figure 3 shows the effect of CAC predicting CVE across baseline Framingham stroke risk (stratified by the median: 5.7%) in this MESA cohort.

CAC and Cerebrovascular Event Discrimination.

For CVE (n = 234), c-statistic for CAC (continuous) alone was 0.642, and it was 0.744 in the full multivariable model without CAC. The addition of CAC improved discrimination of our full multivariable model (Table 3) by 0.01 (c-statistic: 0.744 vs. 0.755).

The c-statistic for the FSRS was 0.664. The addition of CAC improved its discrimination, as reflected by a c-statistic of 0.766 (p < 0.01) (Figure 4). The

![Figure 1: Incident CVE in Subjects With and Without CAC](image1)

Kaplan-Meier analysis showing the event-free survival of participants with and without coronary artery calcium (CAC) and incident cerebrovascular events (CVE) in the MESA (Multi-Ethnic Study of Atherosclerosis) cohort.
DISCUSSION

The goal of this study was to determine the predictive value of CAC for incident CVE and to assess the improvement in discrimination afforded by the addition of CAC to known risk factors for CVE in a multiethnic cohort. Our study, which is the largest and has the longest follow-up so far, shows that CAC is an independent predictor and improves the discrimination for CVE.

Compared with other subclinical and novel markers, CAC has been shown to be superior for predicting, in the general population, CHD and cardiovascular event composites, which include CVE (4,5,14,15). The predictive value of CAC with regard to CVE—which had been questionable before the present study—was clearly shown in our study.

Unlike the HNR study, we did not observe that CAC predicts stroke events in younger but not in older adults. Furthermore, our results strongly suggest that CAC improves the discrimination of incident CVE above and beyond that related to known risk factors and by a similar magnitude as the CHD risk prediction in the MESA cohort (6).

CAC is a measure of atherosclerosis burden in the coronary circulation (17). Atherosclerosis is a known systemic disease that is almost always present in other vascular beds once detected in the coronary bed (18,19). Thus, observation of atherothrombosis in the coronary bed suggests presence of atherosclerosis in the cerebral circulation and elsewhere. However, unlike CHD, the underlying pathophysiology, which is mainly atherosclerosis, cerebrovascular disease/ events have a more heterogeneous pathophysiology (20), including hemorrhage, small-vessel lacunar

c-statistic when constituents of the FSRS (risk factors) were in the model was 0.721 and when CAC was added to the model was 0.785 (data not shown).

For ischemic CVE (cerebral infarcts + TIA, n = 206), the c-statistic values related to CAC alone, our full model (minus CAC), and the addition of CAC to the full model were 0.657, 0.751, and 0.763 (p < 0.01), respectively. For cerebral infarcts (non-TIA, nonhemorrhagic strokes) (n = 132 events), these values were 0.664, 0.767, and 0.777 (p < 0.01).

<table>
<thead>
<tr>
<th>TABLE 3 Predictive Value of Coronary Artery Calcium Score [in (CAC +1)] for Incident Cerebrovascular Events in the MESA Cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Outcome</strong></td>
</tr>
<tr>
<td>----------------------</td>
</tr>
<tr>
<td>Cerebrovascular</td>
</tr>
<tr>
<td>All stroke</td>
</tr>
<tr>
<td>TIA</td>
</tr>
<tr>
<td>Sex stratified</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Race/ethnicity stratified</td>
</tr>
<tr>
<td>Caucasian</td>
</tr>
<tr>
<td>Chinese</td>
</tr>
<tr>
<td>African American</td>
</tr>
<tr>
<td>Hispanic</td>
</tr>
</tbody>
</table>

Mean follow-up was 5.5 ± 2.4 years. Multivariable model was adjusted for age, sex, race/ethnicity, body mass index, diabetes mellitus, systolic and diastolic blood pressure, total cholesterol, high-density lipoprotein, cigarette smoking status, blood pressure medication use, statin use, and interatrial block (as that occurred during the follow-up period).

CI = confidence interval, other abbreviations as in Table 2.
TABLE 4 Predictive Value of Coronary Artery Calcium (Present vs. Absent) for Incident Cerebrovascular Events in the MESA Cohort

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Events, n</th>
<th>Univariate Model</th>
<th>p Value</th>
<th>Multi variable Model*</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coronovascular</td>
<td>234</td>
<td>2.68 (1.83-3.82)</td>
<td>&lt;0.0001</td>
<td>1.13 (0.97-1.29)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Stroke</td>
<td>180</td>
<td>2.57 (1.86-3.55)</td>
<td>&lt;0.0001</td>
<td>1.45 (1.01-2.00)</td>
<td>0.043</td>
</tr>
<tr>
<td>TIA</td>
<td>67</td>
<td>2.71 (1.59-4.51)</td>
<td>0.0002</td>
<td>1.61 (0.99-2.62)</td>
<td>0.045</td>
</tr>
<tr>
<td>Sex stratified</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>181</td>
<td>3.02 (1.88-4.51)</td>
<td>&lt;0.0001</td>
<td>1.84 (1.11-3.09)</td>
<td>0.018</td>
</tr>
<tr>
<td>Female</td>
<td>123</td>
<td>2.95 (2.05-4.29)</td>
<td>&lt;0.0001</td>
<td>1.31 (0.88-2.00)</td>
<td>0.374</td>
</tr>
<tr>
<td>Race/ethnicity stratified</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>93</td>
<td>3.41 (2.08-5.60)</td>
<td>&lt;0.0001</td>
<td>1.65 (0.95-2.89)</td>
<td>0.075</td>
</tr>
<tr>
<td>Chinese</td>
<td>12</td>
<td>2.74 (0.82-9.17)</td>
<td>0.10</td>
<td>1.57 (0.40-6.12)</td>
<td>0.513</td>
</tr>
<tr>
<td>African American</td>
<td>69</td>
<td>2.38 (1.45-3.83)</td>
<td>0.0005</td>
<td>1.53 (0.89-2.63)</td>
<td>0.123</td>
</tr>
<tr>
<td>Hispanic</td>
<td>60</td>
<td>2.37 (1.24-4.67)</td>
<td>&lt;0.0001</td>
<td>1.29 (0.96-2.34)</td>
<td>0.061</td>
</tr>
</tbody>
</table>

Mean follow-up was 5.5 ± 2.4 years. *Multivariable model was adjusted for age, sex, race/ethnicity, body mass index, diabetes mellitus, systolic and diastolic blood pressure, total cholesterol, high-density lipoprotein, cigarette smoking status, blood pressure medications use, statin use, and incident atherosclerotic disease that occurred during the follow-up period.

Abbreviations as in Table 2.

 strokes, and ischemia. The heterogeneous pathophysiology of cerebrovascular disease/events makes predicting events using a single marker such as CAC in 1 of the pathophysiologic pathways unappealing. However, as evident from the present study and others, most cerebrovascular disease/events (approximately 85%, 152 of 180 strokes) are due to cerebral infarcts (21). With a few exceptions, such as cerebral infarcts from cardioembolic source, most of these cerebral infarcts may be due to in situ atherosclerosis, small-vessel disease including microatheroma, or embolism of plaques from extracranial vessels or the aorta; for all of these, CAC would be a good surrogate marker. Thus, despite the heterogeneous pathophysiologic physiology of cerebrovascular disease, CAC as a measure of atherosclerotic burden in the coronary bed can still be a good predictor and can be used to identify most subjects at risk for aggressive preventive therapy, such as statins.

Epidemiological and observational studies have shown a clear association between hypertension and incident CVE, suggesting that blood pressure control may be a good target for primary stroke prevention (22,23). Primary stroke prevention trials with upstream modification of blood pressure have shown a significant reduction in incident CVE (24–26). However, current data on the association between dyslipidemia and incident CVE are mixed (27–30). To date, no clinical trial data exist on the effects of lipid-lowering therapy on CVEs in asymptomatic individuals. However, a secondary analysis of primary prevention trials and long-term clinical trials, which evaluated CVE as a secondary outcome in patients with established coronary heart disease, showed a reduction in CVEs with statin therapy (31–33). Thus, CAC, a noninvasive test, can identify individuals for statin therapy with asymptomatic CHD but at high risk for CVE. Clinical trials primarily evaluating the effect of statins on CVEs in individuals without clinical cardiovascular disease but positive CAC are needed.

Although our study shows an improvement in discrimination by CAC over current risk factors, we caution the incorporation of CAC into primary...
stroke prevention strategies until concerns about ionizing radiation exposure (~1 mSv) are weighed and this approach has been deemed cost-effective. Our results also need to be replicated in other cohorts.

STUDY LIMITATIONS. The strengths of our study include its large sample size, multiethnic cohort, relatively long follow-up, and the use of adjudicated CVE. The limitations include the relatively small number of CVE, which limited our ability to make definitive inferences in subgroups formed by sex and race/ethnicity. MESA is an observational study, and thus, residual confounding may have influenced our results. MESA does not include other ethnic groups, such as American Indians, or Asian groups other than Chinese. In addition, the proportion of each ethnic group in MESA does not accurately reflect that of the U.S. population. Although our primary outcome, CVE, and its constituents were adjudicated, atrial fibrillation that occurred during follow-up were a combination of adjudicated; International Classification of Diseases, Ninth Revision code-derived; and self-reports. The FSRS includes prior CVD and atrial fibrillation and was derived for stroke prediction in individuals with and without these comorbidities. MESA participants were free of CVD and atrial fibrillation at baseline but should not affect the discriminative ability of the FSRS in this cohort (asymptomatic multiethnic cohort). Last, because the present study involved individuals without clinical cardiovascular disease at baseline, our results may not be applicable to other populations.

CONCLUSIONS

CAC was found to be an independent predictor of CVE, strokes, and TIA in a large multiethnic cohort. CAC also seems to have improved prediction over known risk factors for CVE, including atrial fibrillation and the FSRS.

ACKNOWLEDGMENTS The authors thank the investigators, staff, and participants of the MESA study for their valuable contributions. A full list of participating MESA investigators and institutions can be found at http://www.mesa-nhlbi.org. The authors also thank Karen P. Klein, MS, for editing this paper.

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REFERENCES

Chapter 3: Does Gender Modify the Relationship between Coronary Artery Calcium and incident Cerebrovascular Events

ABSTRACT

OBJECTIVE: The purpose of this study is to assess whether gender modifies the association between coronary artery calcium (CAC) score and ischemic stroke in an asymptomatic multiethnic cohort.

BACKGROUND: CAC is a well established marker of subclinical atherosclerosis, and an independent predictor of future coronary heart disease (CHD) events including myocardial infarct, as well as cerebrovascular disease (CVD) events such as stroke. CAC score ≥300 Agatston units has been identified by the American College of Cardiology (ACC) and American Heart Association (AHA) as a useful tool to aid in the determination of cardiovascular risk, specifically in patients whom have been identified as “intermediate risk” using traditional risk factors. CAC score ≥300 has also been shown to discriminate between women at increased risk of CVD events despite being classified as “low risk” by conventional risk prediction models. However, it is unclear if the association between CAC score and incident stroke is modified by gender.

METHODS: CAC was measured during the baseline exam for participants in the Multi-Ethnic Study of Atherosclerosis (MESA) cohort (N= 6,814). MESA participants were followed for an average of 9.5 +/- 2.4 years and incident stroke was adjudicated by a MESA committee. Cox proportional hazards analyses were used to model CAC along with gender and a two-way interaction with gender to determine if CAC had differential
effects within the two gender groups and/or an overall effect on incident stroke after adjusting for gender. CAC was introduced into the models as a binary variable (present/absent) and (<300/≥300), and as a continuous variable. Likelihood ratio tests were utilized to test for model fit, and Wald chi-squared tests were used to test the statistical significance of all covariates and interaction terms.

**RESULTS:** During follow-up, 143 (2%) adjudicated ischemic strokes occurred. Across both genders, participants who experienced an incident stroke were more likely to have CAC present compared to participants who did not experience an incident stroke (women 64% vs 39% and men 82% vs 61%, p<0.001). The presence of CAC was a significant predictor of incident stroke in both women (HR: 3.3 [95% CI: 2.0 to 5.4], p<.0001) and men (HR: 3.4 [95% CI 1.9 to 6.2], p<.0001), however the risk associated with CAC attenuated after adjustment for Framingham Stroke Risk (FSR) score. Unlike CAC present/absent, after adjustment for FSR score, CAC score ≥300 was associated with an increased risk of incident stroke in both women (HR: 1.8 [95% CI: 1.0 to 3.3], p=0.0452) and men (HR: 1.6 [95% CI: 1.0 to 2.6], p=0.0452). [The interaction term describing the relationship between CAC score ≥300 and gender trended towards significance (p=0.0994) suggesting that males have a lower relative risk compared to females with CAC score ≥300]. When modeled as a continuous variable, a 100 Agatson unit increase in CAC score was associated with a 20% increase in the adjusted hazard rate (HR 1.2 [95% CI: 1.1 to 1.2], p<0.0001) for incident stroke. In the CAC as a continuous variable model, the interaction term between CAC and gender was significant (p=0.0006), suggesting that there is an increase in the risk of stroke afforded by CAC in women.
compared to men. After adjustment for the FSR score, the test for interaction was no longer significant (p=0.2374). A sensitivity analysis was performed using CAC score ≥100 and the interaction term between gender and CAC remained insignificant. 

**CONCLUSION:** CAC score ≥300 is an independent predictor of incident ischemic stroke in both men and women and this association remains significant even after adjusting for the FSR score. The initial tests for interaction between CAC and gender suggest that the hazard rate for CAC may be different between males and females, however after controlling for age, systolic blood pressure, anti-hypertension medication, diabetes, cigarette smoking, and LVH (no CVD or atrial fibrillation during the baseline exam), the differential risk afforded by CAC was no longer a significant contributor to the overall risk of stroke.

**BACKGROUND**

Despite numerous advances in medical therapies and improvements in preventative medicine, stroke remains a leading cause of morbidity and mortality in both the United States and around the world. According to the “Heart Disease and Stroke Statistics - 2015 Update” released by the American Heart Association (AHA), 795,000 people in the United States experience a new or recurrent stroke each year. First time stroke accounts for over 75% of these events. According to the Framingham Heart Study, the life-time risk of a person age 55-75 years is 1 in 5 for women (20-21%) and 1
in 6 in men (14-17%). The gender difference in the prevalence of stroke is substantial, with at least 55,000 more women than men experiencing a stroke each year over the last decade.

There are well described differences in the underlying biology of men and women which may, in part, relate to the variation in stroke risk. Examples include differences in anatomy, vascular physiology, and the presence of neuroprotective factors such as estrogen. There are also gender-specific co-morbidities which have been linked to an increased risk of stroke including migraine with aura, and a prior history of preeclampsia during pregnancy.

Recently the AHA and American Stroke Association (ASA) called for improvement in the prediction of stroke risk in women. The writing group noted that while most risk factors have an independent effect, there may be important interactions between individual factors that should be considered when attempting to predict risk and especially clinically, when trying to decide on an appropriate risk modification therapy.

The “Guidelines for the Prevention of Stroke in Women” published in 2014, commented on the lack of strong evidence-based stroke prevention measures specific to gender, and called for the development of female specific risk scores to help fill this discrepancy. The guidelines suggested the utilization of gender-specific risk factors or identification of factors more prevalent in women when constructing the stratified models. Finally, the writers suggested that future research should be aware and incorporate the complexities of risk factor interactions, specially as they relate to gender-specific factors.
CAC is a well described marker of subclinical atherosclerotic disease and is an independent predictor of atherosclerotic cardiovascular events (ASCVD) including coronary heart events and stroke.\textsuperscript{5,8,10} According to the ‘2013 American College of Cardiology (ACC)/AHA Guideline Statement on the Assessment of Cardiovascular Disease Risk’, CAC is the only known measure of subclinical disease which has been shown to provide additional risk information above that predicted by traditional risk factors.\textsuperscript{9} The guideline authors stated that if, after the initial risk assessment using traditional risk factors, an individual is determined to be at intermediate risk for future ASCVD events, then CAC score may be considered as an additional risk factor to help inform decisions regarding treatment (Recommendation IIb, Level of Evidence B).\textsuperscript{9} The authors also advocated that if a healthcare provider has uncertainty regarding the appropriateness of initiating pharmacological therapy, he or she can revise a person’s individual risk upward if they have a CAC score $\geq 300$ Agatston units or have a CAC score $\geq 75^{th}$ percentile based on age, sex and ethnicity.

Several large population studies have unveiled a relationship between CAC and age, with CAC increasing in both incidence and magnitude during the aging process.\textsuperscript{11,12,16} In one large population study, CAC was measured for over 35,000 participants ages 45-90. The authors concluded that while both genders experience an increase in prevalence of CAC with age, men consistently demonstrate CAC scores equal to women who are at least 15 years older.\textsuperscript{52}

Theories as to the clinical significance of CAC differences among men and women exist, including the potential that higher CAC scores in similar aged men and
women represent our ability to detect subclinical CAD earlier in men compared to women. In contrast, many researchers believe that the differences in CAC score represent an underlying relationship, such as biological interaction, between CAC score and gender which has yet to be identified. An interaction occurs when a relation between two variables is modified by another variable. A special consideration in the field of epidemiology is the concept of biological interaction, also referred to as effect modification, which occurs when the biologic effect of an exposure differs according to the presence or absence of another factor.

In response to the call by the AHA/ASA to improve clinical risk assessment tools predicting future stroke, and in light of the variation in CAC scores between genders, the goal of this study is to further characterize the relationship between CAC score and gender as it relates to the prediction of incident stroke in an asymptotic, multi-ethnic cohort free of cerebrovascular disease.

**METHODS**

**STUDY POPULATION AND DATA COLLECTION**

A detailed description of the study design for MESA has been published. In brief, MESA is a cohort study that began in July 2000 to investigate the prevalence, correlates, and progression of subclinical CVD. At baseline, the cohort included 6,814 women and men ages 45 to 84 years recruited from 6 U.S. communities (Baltimore, Maryland; Chicago, Illinois; Forsyth County, North Carolina; Los Angeles County,
California; northern Manhattan, New York; and St. Paul, Minnesota). MESA participants were 38% white, 28% black, 22% Hispanic, and 12% Chinese. Individuals with a history of physician-diagnosed myocardial infarction, angina, heart failure, stroke, or transient ischemic attack (TIA) or who had undergone an invasive procedure for CVD (coronary artery bypass graft, angioplasty, valve replacement, pacemaker placement, or other vascular surgeries) were excluded.

Demographics, medical history, and anthropometric and laboratory data for these analyses were obtained at the first MESA examination (July 2000 to August 2002). Current smoking was defined as having smoked a cigarette in the past 30 days. Diabetes mellitus was defined as fasting glucose ≥126 mg/100 dl or use of hypoglycemic medications. Use of antihypertensive and other medications was based on the review of prescribed medication containers. Resting blood pressure was measured 3 times in a seated position, and the average of the second and third readings was used. Hypertension was defined as a systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, or use of medication prescribed for hypertension. Body mass index was calculated as weight (kg)/height$^2$ (m$^2$). Total and high-density lipoprotein cholesterol were measured from blood samples obtained after a 12-h fast. Low-density lipoprotein cholesterol was estimated by the Friedewald equation.$^{49}$ The MESA study was approved by the institutional review boards of each study site, and written informed consent was obtained from all participants.
MEASUREMENT OF CAC SCORE

Details of the MESA computed tomography (CT) scan and interpretation methods have been reported by Carr et al.\textsuperscript{50} Scanning centers assessed CAC by noncontrast cardiac CT with either an electron-beam CT scanner (Chicago, Illinois; Los Angeles, California; and New York, New York field centers) or a multidetector CT system (Baltimore, Maryland; Forsyth County, North Carolina; and St. Paul, Minnesota field centers). Certified technologists scanned all participants twice over phantoms of known physical calcium concentration. A radiologist or cardiologist read all CT scans at a central reading center (Los Angeles Biomedical Research Institute at Harbor–University of California, Los Angeles, Torrance, California). Our research utilized a mean Agatston score which was derived from the 2 scans.\textsuperscript{51} Intraobserver and interobserver agreements were excellent (kappa= 0.93 and 0.90, respectively).

ASCERTAINMENT OF CVE

Incident stroke was adjudicated by a MESA committee that included cardiologists, physician epidemiologists, and neurologists. A detailed description of the adjudication process has been published.\textsuperscript{36} For the purpose of this study, we defined stroke as a fatal or nonfatal ischemic brain infarct event due to the underlying relationship between ischemic infarct and atherosclerotic disease. To improve the accuracy of this study, incident transient ischemic attacks (TIA) were not included in the analysis due to the subjective nature of a TIA diagnosis. In addition, due to the biological plausibility that
ischemic and hemorrhagic infarcts are a reflection of two distinct underlying disease processes, incident hemorrhagic infarcts were also not included in this analysis.

**STATISTICAL ANALYSIS**

Demographic and disease comorbidities were compared after stratification by gender and development of incident ischemic infarct over the follow-up period. Among participants with more than 1 adjudicated stroke event during the follow-up period, only the first event was used in this analysis. Stratified analysis were tested using the Student’s t test for continuous covariates and the Pearson Chi-Square test for categorical covariates.

CAC score was introduced into the models in several different forms including as a binary viable (absent/present) and as a continuous variable. CAC was also introduced as a binary variable based on the most recent ACC/AHA recommendations (CAC<300 Agatston units or ≥300 Agatston units) due to its ability to improve the assessment of CVD risk. Framingham Stroke Risk (FSR) score components were collected for each MESA participant (using baseline data only) and include the following: age, systolic blood pressure, diabetes mellitus, cigarette smoking, prior CVD, atrial fibrillation, left ventricular hypertrophy (ECG criteria), and blood pressure medications. A FSR score was calculated for each participant utilizing the gender-stratified algorithm reported by the Framingham Heart Study authors. Of note, no MESA participant had a prior CVD event or atrial fibrillation during the baseline examination. Cox proportional hazards analyses were used to model CAC along with gender (female=0) and a two-way interaction with gender to determine if CAC had differential effects within the two gender
groups and/or an overall effect on incident stroke after adjusting for gender. A second Cox proportional hazards model was produced for each measure of CAC incorporating covariates from the first model (CAC, gender and interaction term) and introducing the Framingham Stroke Risk (FSR) score as a continuous variable.

Individual hazard estimates of association stratified by gender when appropriate and the 95% Wald confidence limits are reported separately for each model. Proportional Hazards assumptions were checked for each covariate in the model to ensure the hazard estimates did not change over time. A 2-tailed value of $p < 0.05$ was considered significant in all analysis. All statistical analyses were performed using SAS version 9.2 (SAS Institute, Cary, North Carolina).

RESULTS

BASELINE DEMOGRAPHICS STRATIFIED BY GENDER

After a mean of 9.5 +/- 2.4 years, there were 143 adjudicated ischemic cerebrovascular infarcts (2%). Demographic characteristics stratified by gender are reported in Table 1. Compared to men, women were more likely to have a higher BMI (29 vs 28, $p<0.0001$), higher HDL (56 vs 45, $p<0.0001$) and were more likely be diagnosed with hypertension (47% vs 43%, $p=0.0018$). Men were more likely to smoke and have a diagnosis of diabetes compared to women (15% vs 12%, $p=0.0004$ and 14% vs 11%, $p=0.0018$ respectively). Men on average had a higher CAC score compared to women (224 vs. 77 Agatston units, $p<0.0001$). In addition, a greater proportion of men
than women had CAC present (61% vs 40%, p<0.0001) and CAC score ≥300 (18% vs. 7%, p<0.0001). Of note, women had a higher FSR score compared to men (6 vs 5, p<0.0001) however these scores correlate with a less than 5% risk of stroke over 10 years which according to the Framingham Heart Study is “low risk” for both genders.21

Ischemic infarcts were observed in 70 females (49%) and 73 males (51%). Demographics and comorbidities stratified by gender and incident ischemic infarct are presented in Table 2. Women who experienced an ischemic infarct were older (70 vs 62, p<0.0001), had a lower HDL (52 mg/dl vs 56 mg/dl, p=0.0256), and were more likely to have hypertension (77% vs. 46%, p<0.0001) compared to women who did not develop an ischemic infarct. Similarly, men who developed an ischemic infarct had worse cardiac profiles compared to men who did not experience an ischemic infarct (diabetes 34% vs 14%, p<0.0001 and hypertension 70% vs 42%, p<0.0001). Both women and men who developed an incident ischemic infarct were more likely to have CAC present (female 64% vs 39%, p<0.0001, male 82% vs 61%, p=0.0002) and CAC score ≥300 (female 23% vs 7%, p<0.0001, male 33% vs. 18%, p<0.0001) compared to men and women who did not experience an incident stroke.

CAC AND PREDICTION OF ISCHEMIC INFARCT EVENT

Table 3 presents the predictive value of CAC (present/absent) for incident ischemic infarct introduced into two unique models. Model1 includes CAC (present/absent), gender, and an interaction term assessing for a difference between the effect of CAC within the two gender groups. Model2 included all existing variables listed in
Model 1 as well the FSR score. In Model 1, the presence of CAC was a significant predictor of incident stroke in both women (HR: 3.3 [95% CI: 2.0 to 5.4], p < .0001) and men (HR: 3.4 [95% CI: 1.9 to 6.2], p < .0001), however after adjustment for the Framingham Stroke Risk (FSR) score, this relationship was no longer significant (p = 0.3201). Interestingly, after the addition of FSR score to the model, male gender was associated with a significantly increased risk of incident stroke compared to the female gender (HR: 6.0 [95% CI 2.7 to 13.4], p < 0.0001). When CAC was introduced into the models as absent/present, there was no evidence that gender effected the relationship between CAC and incident infarct (test for interaction: Model 1 p = 0.9431 and Model 2 p = 0.2456).

Figure 1 plots the baseline survival function for males and females at the reference level of CAC <300. The reference curve of the survival function for females is slightly higher than the curve for males, suggesting that the overall survival experience (ie. not experiencing a stroke) is potentially better for females after controlling for CAC.

Table 4 reports the results of the next model which measured the predictive value of CAC for incident ischemic infarct according to the latest ACC/AHA recommendations (CAC<300 Agatston units or ≥300 Agatston units). When stratified by gender, females with a CAC score ≥300 were more than twice as likely to experience an ischemic infarct compared to males with a CAC score ≥300 (female HR: 5.3 [95% CI: 3.0 to 9.3] and male HR: 2.9 [95% CI: 1.8 to 4.7]). In contrast to the presence/absence of CAC, after adjustment for FSR score, CAC score ≥300 was associated with an increased risk of incident ischemic stroke in both women (HR: 1.8 [95% CI: 1.0 to 3.3], p = 0.0452) and
men (HR: 1.6 [95% CI: 1.0 to 2.6], p=0.0452). The hazard ratio for the interaction term
describing the relationship between CAC score ≥300 and gender (female = reference)
trended towards statistical significance (HR:0.5 [95% CI: 0.3 to 1.1), p=0.0994). While
CAC score ≥300 is associated with an increased risk of stroke in both genders, the
interaction results suggest that males have a lower associated risk when compared to
females. A visual representation of this relationship is available on Figure 2 and Figure 3
which plot the survival curve for females and males respectively at CAC <300 and CAC
score ≥300. The greater separation between curves in the female survival graph
compared to the male graph supports the idea that the effect of CAC≥300 is more severe
in females than in males. After adjustment for the FSR score, CAC score ≥300 was
associated with a HR of 1.8 in women (95% CI: 1.0 to 3.3) and 1.6 in men (95% CI: 1.0
to 2.6). In a second model adjusted for FSR score, the test for interaction was no longer
significant (p=0.7196).

Table 5 presents the results of Cox Proportional hazards models introducing CAC
as a continuous variable. CAC is a significant predictor of incident ischemic infarct (HR
per 100 unit increase: 1.2 [95% CI: 1.1 to 1.2], p<0.0001). As shown in Table 5, for
every 100 Agatston unit increase in CAC score, female gender is associated with a 10%
increased risk of incident stroke compared to the male gender at the same CAC score.
After addition of the FSR score to the model, CAC remains a significant predictor of
incident ischemic infarct although the strength of effect is lower (HR per 100 unit
increase: 1.1 [95% CI: 1.0 to 1.1], p=0.0053). Although gender was not a significant
predictor of stroke in the baseline model, after adjusting for FSR score, male gender was
associated with a significantly increased risk of incident stroke compared to female gender (HR: 9.8 [95% CI: 5.7 to 16.9], p<0.0001). Finally, Table 5 lists several gender-specific hazard ratios at increasing values of CAC. While the interaction term between CAC and gender is not significant in the full model (Model2), there appears to be a decreasing hazard associated with the male gender as CAC score increases.

Finally, Table 6 reports the results of a sensitivity analysis performed to assess for misclassification using CAC score ≥100 in models with and without the introduction of FSR score. The effect measure for interaction between gender and CAC remains below statistical significance in both models.

Proportional hazards assumptions were checked for all measures of CAC introduced into the models and there was no evidence of increase or decrease in trend overtime in the hazard associated with any of these covariates (ie. no violations were found). In addition, proportional hazards assumptions were checked for each covariate after adjusting for CAC and no violations were found. Linearity was checked for continuous covariates and there was no indication of violations.

**DISCUSSION**

A recent study discussed various methodologies used by researchers to address gender when producing stroke risk prediction models. The authors reported that of 796 risk prediction models related to stroke, only 33% included gender as a model covariate or stratified the model by gender to produce separate intercepts and parameter estimates.
Furthermore, in instances where the model predicted first stroke event, 35% of models did not include gender at all.\textsuperscript{59} 

According to data extracted from the Third National Health and Nutrition Examination Survey (NHANES III), 95% of women in the United States younger than age 70 are classified as “low risk” according to the Framingham 10-year coronary heart disease (CHD) risk score, one of the more established prediction models estimating the probability that an individual will experience a cardiac event within the next 10 years.\textsuperscript{66} Unfortunately, despite being classified as “low risk” at a young age, most women will ultimately die from cardiac disease. Some authors have suggested that much of the inaccuracy in current prediction models centers around the fact that using current models women do not reach the “intermediate” or “high risk” range until after age 70 despite having established risk factors. 

The underestimate of a woman’s true risk results in a lost opportunity to initiate medical therapies and ultimately fails to prevent the development of disease. Lakoski, et al. performed an analysis on the MESA cohort aimed at identifying a subset of the female participants deemed “low risk” that are actually at an increased of developing CHD. CAC $\geq 300$ was found to be highly predictive of future CHD and cardiovascular disease (CVD) events when compared to CAC $=0$.\textsuperscript{65} The authors suggested utilizing CAC score to better risk stratify women and ultimately improve the utilization of targeted preventative therapies. 

As a result of the analysis presented in this study, the association between gender, CAC score and risk of ischemic stroke has been better defined. There is evidence that the
risk of incident stroke associated with CAC is higher in females compared to males and this increase in associated risk holds true when CAC is studied as absent/present, score \( \geq 300 \) and CAC as a continuous variable. After addition of the FSR score there is no longer any suggestion of a significant association. The loss of significance does not mean that there is no difference between genders in the risk associated with CAC, but rather that after controlling for age, systolic blood pressure, anti-hypertension medication, diabetes, cigarette smoking, and LVH (no CVD or atrial fibrillation during the baseline exam), the differential effect of CAC by gender was no longer a significant contributor to the overall risk of stroke.

**STUDY LIMITATIONS**

The strengths of this study include the large cohort size, over 10 years of follow-up time, and the use of adjudicated events. One limitation to this study is the relatively small number of adjudicated events. In addition, the present study involved individuals without clinical cardiovascular disease at baseline, and therefore our results may not be applicable to other populations.

While the MESA study design was powered to detect a significant association for most main effect variables, the study was not powered to detect a significant difference for an interaction term, which requires a much larger sample size compared to the main effect variables to detect a significant effect. Due to the lack of power in our study it is plausible that effect modification between gender and CAC score does exist. Given the documented association between gender and risk of incident stroke, our analysis suggests
that at a minimum gender should be included as a variable in any prediction model estimating the risk of stroke, and if possible gender should be used to stratify risk model.

Our study utilized the FSR score as a continuous variable, however as published in the literature the original FSR score is designed to produce a gender stratified 10 year probability of stroke. Utilization of the risk score as a component measured allowed the integration of several well established stroke risk factors under a single linear variable, minimizing the risk of omission bias and maximizing the likelihood that a significant effect would be detected in the study.

While the models produced in this study used risk score and not the probability of stroke estimated by the FSR score, it should be noted that the mean FSR score for females was 6.63 (median 6.59, 25-75% interquartile range 5.77—7.41) which estimates a mean 10-year probability of stroke for women in the MESA cohort of 4% (3-4% interquartile range). For males the mean FSR score was 5.16 (median 5.13, 25-75% interquartile range 4.56-5.71) which estimates a mean 10-year probability of stroke for men in the MESA cohort as 5% (5% interquartile range). While there is a significant difference between the mean FSR score for females and males in the MESA Cohort (p value <.0001), due to the fact that a 10-year probability of stroke less than 5% is considered low risk for both genders, we did not feel a clinically significant bias was introduced into the models by our choice to use risk score rather than probability of stroke.
FUTURE STUDIES

CAC is a marker of subclinical atherosclerotic disease and normal ranges based on age and gender are available for the general population. Based on these ranges women typically develop CAC 10 to 15 years after their male counterparts. This study elicits a differential risk between genders, however further studies are needed to determine the etiology of this difference in risk. Potentially CAC measured in women at a relatively young age (compared to the population normal) is a surrogate marker of advanced underlying disease and will provide a method in the future to identify women at greater risk of future events based on the presence of this surrogate. Future studies aimed at identifying the best age to screen for CAC, the level of CAC associated with significant risk, and ultimately therapeutic options to minimize the risk of future events are needed.

CONCLUSION

CAC $\geq$300 Agatston units is an independent predictor of incident ischemic stroke in both men and women and this association remains statistically significant even after adjusting for the FSR score. Despite the absence of statistical significance after the addition of FSR score to the predictive models, this study provides evidence for effect modification by gender between CAC and the risk of incident stroke. Based on the results of this study, CAC in women is associated with a higher risk of incident stroke compared to their male counterparts. In the future, predictive studies incorporating CAC score
should consider producing stratified risk models in order to account for these potential interactions.
Table 1. Distribution of Demographics and Comorbidities in the MESA cohort stratified by gender

<table>
<thead>
<tr>
<th></th>
<th>Female (N=3601)</th>
<th>Male (N=3213)</th>
<th>p value (&lt;= 0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>62 ± 10</td>
<td>62 ± 10</td>
<td>0.8317</td>
</tr>
<tr>
<td>Race/Ethnicity (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>1363 (38)</td>
<td>1259 (39)</td>
<td>0.2886</td>
</tr>
<tr>
<td>Chinese</td>
<td>413 (12)</td>
<td>390 (12)</td>
<td>0.3924</td>
</tr>
<tr>
<td>Afr. American</td>
<td>1050 (29)</td>
<td>843 (26)</td>
<td>0.0072*</td>
</tr>
<tr>
<td>Hispanic</td>
<td>775 (22)</td>
<td>721 (22)</td>
<td>0.9141</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>29 ± 6</td>
<td>28 ± 5</td>
<td>&lt;.0001*</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>118 ± 32</td>
<td>117 ± 31</td>
<td>0.1818</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>56 ± 15</td>
<td>45 ± 12</td>
<td>&lt;.0001*</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>420 (12)</td>
<td>467 (15)</td>
<td>0.0004*</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>410 (11)</td>
<td>449 (14)</td>
<td>0.0013*</td>
</tr>
<tr>
<td>HTN (%)</td>
<td>1680 (47)</td>
<td>1378 (43)</td>
<td>0.0018*</td>
</tr>
<tr>
<td>CAC present (%)</td>
<td>1434 (40)</td>
<td>1964 (61)</td>
<td>&lt;.0001*</td>
</tr>
<tr>
<td>CAC (Agatston unit)</td>
<td>77 ± 242</td>
<td>224 ± 541</td>
<td>&lt;.0001*</td>
</tr>
<tr>
<td>CAC score ≥300 (%)</td>
<td>266 (7)</td>
<td>581 (18)</td>
<td>&lt;.0001*</td>
</tr>
<tr>
<td>FSR score</td>
<td>7 ± 1</td>
<td>5 ± 1</td>
<td>&lt;.0001*</td>
</tr>
</tbody>
</table>

Values are mean +/- SD or n (%). Mean follow-up was 9.5 +/- 2.4 years.
CAC present refers to CAC score >0.
BMI = Body Mass Index, HTN = hypertension, FSR = Framingham Stroke Risk score
Table 2. Distribution of Demographics and Comorbidities in MESA cohort participants based on the development of stroke during follow up and stratified by gender

<table>
<thead>
<tr>
<th></th>
<th>Female No Stroke (N=3531)</th>
<th>Female Stroke (N=70)</th>
<th>p value (a = .05)</th>
<th>Male No Stroke (N=3140)</th>
<th>Male Stroke (N=73)</th>
<th>p value (a = .05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>62 ± 10</td>
<td>70 ± 9</td>
<td>&lt;.0001*</td>
<td>62 ± 10</td>
<td>66 ± 10</td>
<td>0.0017*</td>
</tr>
<tr>
<td>Race/Ethnicity (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>1333 (38)</td>
<td>30 (43)</td>
<td>0.3831</td>
<td>1232 (39)</td>
<td>27 (37)</td>
<td>0.6971</td>
</tr>
<tr>
<td>Chinese</td>
<td>409 (12)</td>
<td>4 (6)</td>
<td>0.1270</td>
<td>386 (12)</td>
<td>4 (6)</td>
<td>0.0780</td>
</tr>
<tr>
<td>African American</td>
<td>1030 (29)</td>
<td>20 (29)</td>
<td>0.9130</td>
<td>820 (26)</td>
<td>23 (32)</td>
<td>0.3005</td>
</tr>
<tr>
<td>Hispanic</td>
<td>759 (22)</td>
<td>16 (23)</td>
<td>0.7837</td>
<td>702 (22)</td>
<td>19 (26)</td>
<td>0.4574</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29 ± 6</td>
<td>30 ± 6</td>
<td>0.0515</td>
<td>28 ± 5</td>
<td>29 ± 4</td>
<td>0.1010</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>118 ± 32</td>
<td>124 ± 27</td>
<td>0.0972</td>
<td>119 ± 30</td>
<td>117 ± 31</td>
<td>0.6223</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>56 ± 15</td>
<td>52 ± 12</td>
<td>0.0256*</td>
<td>45 ± 12</td>
<td>43 ± 11</td>
<td>0.2168</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>409 (12)</td>
<td>11 (16)</td>
<td>0.2863</td>
<td>457 (15)</td>
<td>10 (14)</td>
<td>0.8376</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>399 (11)</td>
<td>11 (16)</td>
<td>0.2496</td>
<td>424 (14)</td>
<td>25 (34)</td>
<td>&lt;.0001*</td>
</tr>
<tr>
<td>HTN (%)</td>
<td>1626 (46)</td>
<td>54 (77)</td>
<td>&lt;.0001*</td>
<td>1327 (42)</td>
<td>51 (70)</td>
<td>&lt;.0001*</td>
</tr>
<tr>
<td>CAC present (%)</td>
<td>1389 (39)</td>
<td>45 (64)</td>
<td>&lt;.0001*</td>
<td>1904 (61)</td>
<td>60 (82)</td>
<td>0.0002*</td>
</tr>
<tr>
<td>CAC (Agatston unit)</td>
<td>74 ± 237</td>
<td>209 ± 399</td>
<td>&lt;.0001*</td>
<td>220 ± 540</td>
<td>363 ± 549</td>
<td>0.0255*</td>
</tr>
<tr>
<td>CAC score ≥300 (%)</td>
<td>250 (7)</td>
<td>16 (23)</td>
<td>&lt;.0001*</td>
<td>557 (18)</td>
<td>24 (33)</td>
<td>&lt;.0001*</td>
</tr>
</tbody>
</table>

Values are mean ± SD or n (%). Mean follow-up was 9.5 ± 2.4 years. Abbreviations as per Table 1. CAC present refers to CAC score >0.
Table 3: Predictive value of Coronary Artery Calcium (absent vs. present) for incident stroke in the MESA cohort.

<table>
<thead>
<tr>
<th></th>
<th>Model 1⁺ Hazard Ratio (95% Wald CL)</th>
<th>p value (a=0.05)</th>
<th>Model 2++ Hazard Ratio (95% Wald CL)</th>
<th>p value (a=0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male CAC absent</td>
<td>1.0</td>
<td></td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Male CAC present</td>
<td>3.4 (1.9 to 6.2)</td>
<td>&lt;.0001⁺</td>
<td>2.1 (1.1 to 3.9)</td>
<td>0.3201</td>
</tr>
<tr>
<td>Female CAC absent</td>
<td>1.0 (0.5 to 1.9)</td>
<td>0.9656</td>
<td>6.0 (2.7 to 13.4)</td>
<td>&lt;.0001⁺</td>
</tr>
<tr>
<td>Female CAC present</td>
<td>3.3 (2.0 to 5.4)</td>
<td>&lt;.0001⁺</td>
<td>1.3 (0.8 to 2.2)</td>
<td>0.3201</td>
</tr>
<tr>
<td>Gender*CAC present</td>
<td>1.0 (0.5 to 2.2)</td>
<td>0.9431</td>
<td>1.6 (0.7 to 3.5)</td>
<td>0.2456</td>
</tr>
<tr>
<td>FSR score</td>
<td>—</td>
<td>—</td>
<td>3.1 (2.5 to 3.8)⁺</td>
<td>&lt;.0001⁺</td>
</tr>
</tbody>
</table>

Mean follow up of 9.5 +/-2.4 years. Abbreviations as in Table 1.
Reference group for model is Males CAC absent.
Model 1 = CAC (absent/present), gender (female/male), and an interaction term for gender*CAC.
⁺Likelihood Ratio chi squared for model fit = 47.13, p <.0001, Wald test for interaction 0.9431.
Model 2 = CAC (absent/present), gender (female/male), FSR score, and an interaction term for gender*CAC.
++Likelihood Ratio chi squared for model fit = 164.2, p<.0001, Wald test for interaction 0.2456.
⁺Hazard Ratio for FSR score = hazard associated with a 1 unit increase in the FSR score.
Table 4: Predictive Value of Coronary Artery Calcium score ≥300 for incident stroke in the MESA cohort.

<table>
<thead>
<tr>
<th></th>
<th>Model 1⁺</th>
<th></th>
<th>Model 2⁺⁺⁺</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hazard Ratio (95% Wald CL)</td>
<td>p value (a=0.05)</td>
<td>Hazard Ratio (95% Wald CL)</td>
<td>p value (a=0.05)</td>
</tr>
<tr>
<td>Male CAC &lt;300</td>
<td>1.0</td>
<td></td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Male CAC ≥300</td>
<td>2.9 (1.8 to 4.7)</td>
<td>&lt;.0001*</td>
<td>1.6 (1.0 to 2.6)</td>
<td>0.0452*</td>
</tr>
<tr>
<td>Female CAC &lt;300</td>
<td>1.3 (0.9 to 1.9)</td>
<td>0.2542</td>
<td>8.9 (5.1 to 15.7)</td>
<td>&lt;.0001*</td>
</tr>
<tr>
<td>Female CAC ≥300</td>
<td>5.3 (3.0 to 9.3)</td>
<td>&lt;.0001*</td>
<td>1.8 (1.0 to 3.3)</td>
<td>0.0452*</td>
</tr>
<tr>
<td>Gender*CAC</td>
<td>0.5 (0.3 to 1.1)</td>
<td>0.0994</td>
<td>0.9 (0.4 to 1.9)</td>
<td>0.7196</td>
</tr>
<tr>
<td>FSR score</td>
<td>—</td>
<td></td>
<td>3.1 (2.5 to 3.8)</td>
<td>&lt;.0001*</td>
</tr>
</tbody>
</table>

Mean follow up of 9.5 +/-2.4 years. Abbreviations as in Table 1.
Reference group for model is Males CAC <300.
Model 1 = CAC ≥300 (no/yes), gender (female/male), and an interaction term for gender*CAC.
⁺Likelihood Ratio chi squared for model fit = 42.35, p <.0001, Wald test for interaction 0.0994.
Model 2 = CAC ≥300 (no/yes), gender (female/male), FSR score, and an interaction term for gender*CAC.
⁺⁺⁺Likelihood Ratio chi squared for model fit = 164.4, p<.0001, Wald test for interaction 0.7196.
*Hazard Ratio for FSR score = hazard associated with a 1 unit increase in the FSR score.
Figure 1. Baseline survival function stratified by gender in the MESA cohort.

A reference curve of the survival function at the level of CAC <300 stratified by gender in the MESA (Multi-Ethnic Study of Atherosclerosis) cohort. (F=female, M=male).
Figure 2. Survival function for females according to CAC score in the MESA cohort.

A curve of the survival function based on CAC score (<300 or ≥300) for females in the MESA (Multi-Ethnic Study of Atherosclerosis) cohort.
Figure 3. Survival function for males according to CAC score in the MESA cohort.

A curve of the survival function based on CAC score (<300 or ≥300) for males in the MESA (Multi-Ethnic Study of Atherosclerosis) cohort.
Table 5: Predictive value of Coronary Artery Calcium score as a continuous covariate for incident stroke in the MESA cohort.

<table>
<thead>
<tr>
<th></th>
<th>Model 1†</th>
<th>Model 2‡‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hazard Ratio (95% Wald CL)</td>
<td>p value (a=0.05)</td>
</tr>
<tr>
<td>CAC ≥ 300</td>
<td>1.2 (1.1 to 1.2)</td>
<td>&lt;.0001*</td>
</tr>
<tr>
<td>Female CAC ≥ 300</td>
<td>1.2 (1.1 to 1.2)</td>
<td></td>
</tr>
<tr>
<td>Male CAC ≥ 300</td>
<td>1.1 (1.0 to 1.1)</td>
<td></td>
</tr>
<tr>
<td>Gender **</td>
<td>1.3 (0.9 to 1.9)</td>
<td>0.1217</td>
</tr>
<tr>
<td>Female CAC = 100</td>
<td>0.8 (0.6 to 1.2)</td>
<td>9.4 (5.5 to 16.0)</td>
</tr>
<tr>
<td>Female CAC = 200</td>
<td>0.9 (0.6 to 1.2)</td>
<td>8.9 (5.3 to 15.3)</td>
</tr>
<tr>
<td>Female CAC = 300</td>
<td>1.0 (0.7 to 1.4)</td>
<td>8.6 (5.0 to 14.7)</td>
</tr>
<tr>
<td>Female CAC = 400</td>
<td>1.1 (0.8 to 1.5)</td>
<td>8.2 (4.7 to 14.4)</td>
</tr>
<tr>
<td>Female CAC = 500</td>
<td>1.2 (0.8 to 1.7)</td>
<td>7.9 (4.4 to 14.1)</td>
</tr>
<tr>
<td>Gender*CAC</td>
<td>0.99 (0.99 to 1.0)</td>
<td>0.0006*</td>
</tr>
<tr>
<td>FSR score*</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

Mean follow up of 9.5 +/-2.4 years. Abbreviations as in Table 1. Model 1 = CAC score (continuous variable), gender (female/male), and an interaction term for gender*CAC. † Likelihood Ratio chi squared for model fit = 32.33, p < .0001, Wald test for interaction 0.0006. Model 2 = CAC ≥ 300 (no/yes), gender (female/male), FSR score, and an interaction term for gender*CAC. ‡‡ Likelihood Ratio chi squared for model fit = 162.07, p < .0001, Wald test for interaction 0.2437. *Hazard Ratio for FSR score is equal to the hazard associated with a 1 unit increase in FSR score. †† Hazard Ratio for CAC = hazard associated with a 100 unit increase in CAC score. ‡‡ Stratified Hazard Ratio for CAC = hazard according to gender associated with a 100 unit increase in CAC. **Reference group for model is Male gender.
Table 6: Sensitivity Analysis: Predictive value of Coronary Artery Calcium score ≥100 for incident stroke in the MESA cohort.

<table>
<thead>
<tr>
<th></th>
<th>Model 1⁺</th>
<th></th>
<th>Model 2++⁺⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hazard Ratio (95% Wald CL)</td>
<td>p value (α=0.05)</td>
<td>Hazard Ratio (95% Wald CL)</td>
</tr>
<tr>
<td>Male CAC &lt;100</td>
<td>1.0</td>
<td></td>
<td>1.0</td>
</tr>
<tr>
<td>Male CAC ≥100</td>
<td>3.3 (2.1 to 5.2)</td>
<td>&lt;.0001*</td>
<td>1.8 (1.1 to 2.9)</td>
</tr>
<tr>
<td>Female CAC &lt;100</td>
<td>1.1 (0.7 to 1.7)</td>
<td>0.7628</td>
<td>7.4 (4.0 to 13.7)</td>
</tr>
<tr>
<td>Female CAC ≥100</td>
<td>3.7 (2.2 to 6.0)</td>
<td>&lt;.0001*</td>
<td>1.4 (0.8 to 2.3)</td>
</tr>
<tr>
<td>Gender*CAC</td>
<td>0.9 (0.5 to 1.8)</td>
<td>0.7459</td>
<td>1.3 (0.7 to 2.6)</td>
</tr>
<tr>
<td>FSR score</td>
<td>—</td>
<td>—</td>
<td>3.1 (2.5 to 3.8)</td>
</tr>
</tbody>
</table>

Mean follow up of 9.5 +/-2.4 years. Abbreviations as in Table 1.
Reference group for model is Males CAC <100.
Model 1 = CAC ≥100 (no/yes), gender (female/male), and an interaction term for gender*CAC.
⁺Likelihood Ratio chi squared for model fit = 49.47, p <.0001, Wald test for interaction 0.7459.
Model 2 = CAC ≥100 (no/yes), gender (female/male), FSR score, and an interaction term for gender*CAC.
++⁺⁺Likelihood Ratio chi squared for model fit = 165.06, p<.0001, Wald test for interaction 0.4380.
*Hazard Ratio for FSR score = hazard associated with a 1 unit increase in the FSR score.
REFERENCES


Ashleigh Anne Owen, M.D.

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EDUCATION
Wake Forest Baptist Medical Center, Winston-Salem, NC 2012-2016
- Clinical Cardiology Fellowship Training Program
- Clinical Cardiovascular T32 Research Training Program
- Masters of Science in Clinical and Population Translational Sciences
- US Ten Day Seminar on Epidemiology and Prevention of Cardiovascular Disease

Duke University Hospital, Durham, NC 2009-2012
- Internal Medicine Residency Training Program

Medical University of South Carolina, Charleston, SC 2005-2009
- Doctor of Medicine; GPA 3.55

Clemson University, Clemson, SC 2000-2004
- Bachelor of Science in Biochemistry, magna cum laude
- Minor in Business Administration
- Calhoun Honor’s College; Cumulative GPA 3.80/4.0; Biochemistry GPA 3.89/4.0

South Iredell High School, Statesville, NC 1996-2000
- Honor Speaker Class of 2000, class rank 3/366

BOARD CERTIFICATION
American Board Internal Medicine 2013

LICENSURE & CREDENTIALS
North Carolina Medical Board (#158284) 2012-2016

WORK EXPERIENCE
Forsyth Medical Center, Novant Inpatient Care Specialists, Winston Salem, NC 2012-present
- Moonlighting physician with a large hospitalist team; responsibilities include providing initial evaluation and care to patients being admitted to the medical and critical care floors.
Forsyth Medical Center, Palliative Care, Winston Salem, NC  
2013-present
Gained substantial experience providing palliative care medicine for patients with chronic life-limiting illnesses in the hospital setting. Worked on interdisciplinary team to educate patients and family members on ways to improve patient wellbeing, provide direction for patients completing advanced directives and provided end-of-life care to terminally ill patients.

Duke University Hospital, Inpatient Oncology Hospitalist, Durham, NC  
2011-2012
Moonlighting physician caring for patients admitted on the Oncology, Hematology and Bone Marrow Transplant Units of a large tertiary care hospital.

Sexual Assault Forensic Examiner (SAFE) Team, Charleston, SC  
2005-2009
Executive Coordinator of a volunteer organization responsible for the forensic exam of sexual assault victims in Charleston, SC and the surrounding five counties. Responsibilities included logistic coordinating between examiners, victims, police and advocates; designing emergency evaluation and victim advocate protocols; recruiting and training; monthly scheduling; weekly call and designated 24hr back-up for emergency support.

RESEARCH
Department of Cardiovascular Medicine
Wake Forest University School of Medicine, Winston Salem, NC  
2013-2016
Assessed the predictive value of coronary artery calcium (CAC) score for cerebrovascular events (CVE) in participants enrolled in the MESA (Multi-Ethnic Study of Atherosclerosis) cohort. Verified that CAC is an independent predictor of CVE, strokes, and TIAs and improves the discrimination afforded by current stroke risk factors for incident CVE.

Department of Medicine
Duke University School of Medicine, Durham, NC  
2010-2012
Worked with the Medtronic Foundation and the North Carolina HeartRescue Cardiac Arrest (RACE CARS) System to increase overall out-of-hospital cardiac arrest survival rates by 50% over five years in the geographic areas supported by the program. Assisted in the development of a statewide system of cardiac arrest management involving the entire chain of care including community education, implementation of sub-specialized “cardiac arrest” paramedics who initiate hypothermia in the field. Implemented a prototype educational program in 12 North Carolina public middle schools located in Iredell County.
Department of Medicine and Department of Surgery
Duke University School of Medicine, Durham, NC 2010-2012

Performed a retrospective analysis of data collected over a 10 year period on patients who were enrolled in the extended criteria cardiac transplant (ECCT) program. Compared independent clinical variables associated with improved survival in ECCT patients. Determined serum creatinine is an independent predictor of survival in all transplant recipients and recommended that renal function be incorporated in to risk-stratification for patients undergoing ECCT.

Cardiothoracic Surgery Department, Medical University of South Carolina
Ralph H Johnson Veterans Affairs Medical Center, Charleston, SC 2007-2008

Performed a comprehensive literature review on 200 scientific articles related to matrix metalloproteinases and their unique role in heart failure and the cardiovascular remodeling process. Compared changes in the heart’s extracellular matrix in three distinct disease states known to be associated with the development of heart failure including myocardial infarct, hypertension and dilated cardiomyopathy.

Medical University of South Carolina
Neuroscience Department, Charleston, SC 2006

Designed and optimized a Western Blot assay for amyloid protein precursor (APP) to measure the inhibition of amyloid-b peptide, a pathogenic protein linked to plaque formation in the brain of patients with Alzheimer’s disease. Utilized the above assay during Phase 2 of a double-blinded, randomized pharmaceutical trial aimed at slowing progression of Alzheimer’s disease.

Clemson University
Biochemistry Department, Clemson, SC 2003-2004

Received NSF grant to characterize a unique Ap3A hydrolase responsible for the internalization of adenosine dinucleotides into bovine endothelial cells. Developed a thesis describing a possible mechanism for Ap3A hydrolase as an extracellular signal responsible for producing nitric oxide during stress-induced in vivo vasoregulation of coronary vasculature.

PUBLICATIONS


**PROFESSIONAL AFFILIATIONS & EXPERIENCES**
Sudden Cardiac Death Ancillary Study ARIC Cohort Adjudication Committee, 2013-present
North Carolina Medical Society, 2012-present
American College of Cardiology, 2012-present
American Heart Association, 2010-present
American College of Physicians, 2009-present

**HONORS & AWARDS**
T32 Clinical Cardiovascular Research Grant, 2012-2014
Barton F. Haynes Resident Research Award, Duke University Hospital, 2011-2012
H. Rawling Pratt-Thomas Service & Leadership Award, MUSC College of Medicine, 2009
Medical University of South Carolina Women’s Club Scholarship, 2008
Medical University of South Carolina Volunteer of the Year, 2007-2008
Duke Energy National Scholar, 2000-2004
Philip Prince National Scholar, 2000-2004
Omicron Delta Kappa National Honor Society, 2002-2004
Alpha Epsilon Delta Premedical Honor Society, 2002-2004
Golden Key International Honor Society, 2002-2004
North Carolina High School Journalist of the Year, 2000
COMMUNITY SERVICE
Volunteer physician for high school athlete annual physicals, Statesville, NC, 2015-2016
RACECARS in the Classroom, CPR in North Carolina middle schools, Mooresville, NC, 2011
Volunteer at Pet Helpers, Charleston, SC, 2008-2009
Speaker for Women in Charge, Moultrie Middle School, Mt Pleasant, SC, 2008
Campus Ministries, free medical clinic for the homeless, Charleston, SC, 2006-2008
CARES Clinic, free medical clinic for the uninsured, Charleston, SC, 2007-2009

PRESENTATIONS


APPENDIX


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