BIOMARKERS FOR THE PREDICTION OF INCIDENT CORONARY HEART DISEASE IN THE MESA COHORT

by

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Abstract

Introduction

Heart disease is the leading cause of death in the United States. Biomarkers have been integral to the advancement of preventive cardiology. They have been used to understand the mechanisms of heart disease, and have gone from merely being used in the diagnosis of disease to being important in predicting risk.

This study examined the association between biomarkers and cardiovascular events, and sought to identify which biomarkers would be best improve predictions of such events. Heterogeneity by sex and race on these associations were also examined.

Methods

In a prospective multi-ethnic study of men and women ages 45 to 84 years old without baseline clinical cardiovascular disease, the associations between eight biomarkers and incident coronary heart disease (CHD) were determined by Cox Regression. Tests for heterogeneity by race and by sex were performed. Models containing traditional risk factors were compared to those containing biomarkers to assess the discriminatory and predictive powers of adding biomarkers to the model.

Results

Among 5914 participants, the incident rate of CHD was 7.0 cases per 1000 person-years. After adjustment for the traditional risk factors of heart disease, three biomarkers were found to have significant associations with incident CHD –mean Agatston calcium score (adjusted hazard ratio = 1.0009 per one Agatston unit increase, 95% C.I.: 1.0006 - 1.001), urinary albumin (adjusted hazard ratio = 1.0025 per 1 mg/dL urinary albumin increase, 95% C.I: 1.0003 - 1.0048), and fibrinogen (adjusted hazard ratio = 1.003 per 1mg/dL fibrinogen increase, 95% C.I.: 1.001 –

1.004). Generally, the addition of biomarkers to traditional risk factor models improved model fit and the predictive power of the models, with the inclusion of mean Agatston calcium score and fibrinogen antigen showing the greatest improvement in model prediction.

Conclusions

In a cohort characterized by ethnic diversity, increasing coronary calcium, fibrinogen, and urinary albumin were found to be significantly associated with incident CHD. Prognostic models for CHD containing biomarkers had better predictive power than models containing only traditional risk factors. Therefore, when developing models for predicting heart disease, researchers may want to consider including biomarkers as they improve the discrimination afforded by current heart disease risk factors.

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Introduction

Background and Significance

Heart disease is the leading cause of death in the United States. In fact, cardiovascular disease causes more deaths than cancer, chronic lower respiratory diseases, and accidents combined. Each year, about 610 000 people die from heart disease in the United States. Coronary heart disease (CHD) is the most common type of heart disease, killing over 370 000 people annually. Heart disease, with its high morbidity and costs, is a tremendous burden on the healthcare system. Cardiovascular disease and stroke have estimated direct and indirect costs of about $315 billion, and this figure increases every year (Centers for Disease Control and Prevention, 2015) (Ahmad, et al., 2014) (Johns Hopkins Medicine, n.d.).

Biomarkers have served as tools for diagnosing and estimating the prognosis of heart failure in patients with cardiovascular disease, and have also been used to predict cardiovascular risk in patients without cardiovascular disease. A biomarker is a characteristic that can be objectively measured and evaluated as an indicator of a biological, pathogenic, or pharmacological processes or responses. To be useful clinically, biomarkers need to be accurate, reproducible, and standardised. They have to be of reasonable costs, and have high sensitivities, specificities, and short turnaround times (Vasan, 2006) (Vondrakova, Malek, Ost'adal, Kruger, & Neuzil, 2013). Several biomarkers are now considered standard predictors, and new ones continue to be developed, but evidence about new markers has not been substantial (Bayes - Genis, Zhang, & Ky, 2015). New markers, however, could provide more information about cardiovascular disease development and treatment, and reduce the burden on the health care system (Rollins, 2012) (Vasan, 2006).

A review published by Ahmad et al discusses the clinical importance of novel biomarkers (Ahmad, Fiuzat, Felker, & O'Connor, 2012). Several novel biomarkers have been identified,

which allow for the measurement of various molecular processes involved in the pathophysiology of chronic heart failure. Ahmad et al suggest that using biomarkers in clinical practice could result in improved diagnosis, risk assessment, and treatment of patients with chronic heart failure. Using a multi-marker strategy for parallel evaluation of traditional and novel biomarkers would help in better classifying heart failure on a molecular basis, and allow for therapies to be tailored accordingly (Ahmad, Fiuzat, Felker, & O'Connor, 2012).

Heart disease has been considered to be a male disease. However, heart disease is the leading cause of death in both men and women (Centers for Disease Control and Prevention, 2015). Yet, most of the existing clinical standards for heart disease are based on male pathophysiology and outcomes (Regitz-Zagrosek, 2011) (Taylor, Vallejo-Giraldo, Schaible, Zakeri, & Miller, 2011). In their 2007 update of their previously published Evidence-Based Guidelines for Cardiovascular Disease Prevention in Women, the American Heart Association suggested that the role of female sex in modifying the predictive value of new biomarkers and measures of subclinical cardiovascular disease be addressed in future research (Banka, et al., 2007).

Objective

To determine the association of traditional and emerging biomarkers with incident CHD, assess how well these biomarkers predict CHD and to identify which biomarkers are the strongest predictors of CHD. The effect of sex and race on these associations will also be examined.

Methods

Study Design and Participants

The study was conducted with participants of the Multi-Ethnic Study of Atherosclerosis (MESA). MESA is a cohort study funded by the National Heart, Lung, Blood Institute (NHLBI) to investigate the characteristics of subclinical cardiovascular disease (disease detected non-invasively before it has produced clinical signs and symptoms), and the risk factors that predict its progression to clinically overt cardiovascular disease.

The MESA cohort enrolled 6,814 men and women ages 45-84 years old from six U.S. communities: Forsyth County, NC (NWU); Northern Manhattan and the Bronx, NY (COL); Baltimore City and Baltimore County, MD (JHU); St. Paul, MN (UMN); Chicago, IL (WFU); and Los Angeles, CA (UCLA). MESA participants are non-Hispanic white, Hispanic, African-American, or Asian (of Chinese origin).

Subjects were excluded from the study if they had known cardiovascular disease (physician diagnosis of heart attack, stroke, TIA, heart failure, or angina, current atrial fibrillation, any cardiovascular procedures), pregnancy, active cancer treatment, weight >300 lbs, serious medical conditions that precluded long term participation, nursing home residence, cognitive inability, inability to speak English, Spanish, Cantonese, or Mandarin, plans to leave the community within five years, and those who had a chest CT within the past year.

The baseline visit took place from July 2000 through July 2002. During this visit, MESA participants responded to questionnaires, and underwent the following procedures: electrocardiogram (ECG), ankle-brachial index, cardiac non-contrast CT and MRI scanning, carotid ultrasound, brachial flow-mediated dilation (FMD) and arterial wave-form measures. Three follow-up exams were carried out every 17-20 months, and a fifth exam was conducted in April 2010 – January 2012. In addition, participants were contacted every 9 to 12 months

throughout the study to assess clinical morbidity and mortality. Additional details and protocols are available on the MESA website, www.mesa-nhlbi.org, and in Bild et al (MESA Coordinating Center, University of Washington, Seattle, 2015) (Bild, et al., 2002).

Variables

Biomarkers were identified by first reviewing the literature for biomarkers that have been shown, or could be used to predict future cardiovascular events. Vasan presented a detailed review of heart disease biomarkers (circulating, structural, functional, and genomic) that had been previously analyzed, and also described the weight of evidence linking these biomarkers to heart disease based on whether they were evaluated prospectively, whether they added predictive power to the Framingham Study Risk Score, and how well they tracked with disease treatment (Vasan, 2006). Liquori et al also reviewed established and candidate biomarkers, and described the use of these markers in clinical practice in terms of whether they were diagnostic or prognostic of heart failure, and whether they were used to guide therapy in heart failure (Liquori, Christenson, Collinson, & deFilippi, 2014).

The biomarkers chosen for analysis were the ones that were measured in MESA, and had been identified in these reviews. These biomarkers were C-reactive protein (CRP), tumour necrosis factor (TNF) α, matrix metalloproteinases (MMP) 3 and 9, low-density lipoprotein (LDL) particle number, soluble intercellular adhesion molecule (sICAM-1), interleukins (IL) 2 and 6, oxidised LDL, homocysteine, mean Agatston calcium score (as a measure of coronary calcium), ankle-brachial index (ABI), urine albumin, fibrinogen, D-dimer, plasminogen activator inhibitor 1 (PAI-1), and von Willebrand Factor (vWF), and Cystatin-C.

Cystatin-C was not used in this analysis as the MESA Data Committee advised that the data for this biomarker should not be used for longitudinal analysis as calibration efforts are still in progress. TNF α, MMP 3, MMP 9, LDL particle number, oxidised LDL, sICAM-1, IL-2, PAI-1, and vWF were only measured in a subset of 1,000 participants randomly selected from the first

5,030 participants enrolled (MESA 1000) (Keller, Katz, Cushman, Fried, & Shilpak, 2008). There was missing data for these biomarkers (50% - 80%), and thus, they were not used in the analysis to prevent loss of power.

The biomarkers used in the analysis were ABI, CRP, IL-6, mean Agatston calcium score, urine albumin, D-dimer, fibrinogen, and total homocysteine. The traditional CVD risk factors included age, race/ethnicity, sex, study site location, body mass index, smoking status (never, former, current), the average number of cigarettes smoked per day, systolic blood pressure (SBP), diabetes, family history of heart attacks (parents, siblings, or children), education (less than or completed high school), and high levels of LDL cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride levels. These were not studied as main predictors, but included in the models as they are known risk factors of CHD which should be adjusted for when determining the associations for the biomarkers.

Current smoking was defined as having smoked a cigarette in the last 30 days. Diabetes was defined as use of diabetes medications or fasting glucose equal to or greater than 126 mg/dL. Ankle-brachial index was categorized as follows - <0.9, 0.9 – 1.3, and >1.3. These cut-offs were chosen because ABI values of <0.9 and >1.3 have been identified in the literature as abnormal (Tison, et al., 2011). To measure systolic blood pressure, subjects were allowed to rest for 5 minutes in a seated position, after which blood pressure was measured 3 times at 2-minute intervals. An appropriate-size cuff and an automated oscillometric device (Dinamap Monitor Pro 100, GE Healthcare, Milwaukee, WI) were used. The average of the second and third measurements were used to determine SBP (MESA Coordinating Center, University of Washington., 2001) (Carson, et al., 2011).

Biomarker concentrations were determined by blood tests (HDL-cholesterol, triglycerides, fibrinogen, CRP, IL-6, total homocysteine, and D-dimer), urine tests (urinary albumin), and computed topography (coronary calcium).

Laboratory assays were measured using venous blood obtained after a 12- hour fast. HDL-cholesterol was measured in EDTA plasma using the cholesterol oxidase cholesterol method after precipitation of non-HDL-cholesterol with magnesium/dextran (Roche Diagnostics, Indianapolis, IN). Triglyceride was measured in EDTA plasma using Triglyceride GB reagent on the Roche COBAS FARA centrifugal analyser (Roche Diagnostics). LDL cholesterol was estimated by the Friedewald equation in individuals with triglyceride values <400 mg/dL (Yeboah, et al., 2009). Fibrinogen and CRP was measured using the BNII nephelometer (N High Sensitivity CRP; Dade Behring Inc., Deerfield, IL). IL-6 was measured by ultrasensitive enzyme-linked immunosorbent assay (ELISA) (Quantikine HS Human IL-6 Immunoassay; R&D Systems, Minneapolis, MN). Urinary albumin was determined using the Array 360 CE Protein Analyzer (Beckman Instruments, Inc., Drea, CA). Total homocysteine was measured by a fluorescence polarization immunoassay (IMx Homocysteine Assay, Oslo, Norway) using the IMx Analyzer (Abbott Diagnostics, Abbott Park, IL). Fibrin fragment D-dimer was measured using an immuno-turbidimetric assay (Liatest D-DI; Parsippany, NJ). Average analytical coefficients of variation across several control samples for these analytes ranged from 2.0% to 13.0% (MESA Coordinating Center, University of Washington, 2014).

Computed tomography (CT) scanning of the chest was performed using either ECG-triggered electron-beam computed tomography scanner (WFU, UCLA, COL) (Imatron C-150, Imatron, San Francisco) or using prospectively ECG- triggered scan acquisition with a multi-detector computed tomography system acquiring four simultaneous 2.5 mm slices for each cardiac cycle in a sequential or axial scan mode (JHU, NWU, UMN) (Lightspeed, General Electric, Waukesha, WI or Siemens, Volume Zoom, Erlangen, Germany). Each participant was scanned twice, and calcium scores among scanning centres and between participants were adjusted using a standard calcium phantom scanned simultaneously with the participant. The average of the two Agatston scores was used in all analyses. Additional details and protocols on the CT scanning are available in Takasu et al (Takasu, et al., 2009).

For the ABI, systolic blood pressure was measured in both the left and right brachial, dorsalis pedis, and posterior tibial arteries using a hand-held Doppler instrument with a 5-mHz probe. The ABI was calculated for both the left and right sides as maximum systolic blood pressure in the posterior tibial artery and dorsalis pedis, divided by the average of the left and right brachial pressures (Yeboah, et al, 2009). Additional information can be found on the MESA website, www.mesa-nhlbi.org.

Primary Outcome and Outcome Ascertainment

The primary outcome was all cause CHD, which was a composite outcome of either myocardial infarction (MI), resuscitated cardiac arrest, definite angina, probable angina (if followed by revascularisation), or CHD death.

At intervals of 9 – 12 months, a telephone interviewer inquired about interim hospital admissions, cardiovascular diagnoses, and deaths. Copies of death certificates and medical records for hospitalization and outpatient cardiovascular diagnoses were sent to an adjudication committee. The adjudication committee also conducted next of kin interviews for out-of-hospital cardiovascular deaths for verification. Records were obtained for 98% of reported hospitalized CVD events. Two physicians independently classified and assigned incidence dates. For disagreements, a full mortality and morbidity review committee made the final classification (Malik, et al., 2011).

Angina required documentation of chest pain or angina equivalent, and evidence of reversible myocardial ischemia, obstructive coronary artery disease, or a positive stress test. Myocardial infarction required information about chest pain, cardiac enzymes, and electrocardiograms. Resuscitated cardiac arrest was defined as full arrest (asystole or ventricular fibrillation and pulseless) and subsequent successful cardio-pulmonary resuscitation (including cardioversion) in persons in whom there we no clear-cut non-cardiac cause, and who lived 24 hours after resuscitation.

CHD death was classified as either definite fatal MI (an in-hospital death that met the criteria for MI, or an out-of hospital death with a documented MI within the previous 28 days), definite fatal CHD (which did not qualify as a definite fatal MI, and chest pain with the previous 72 hours or history of CHD), or possible fatal CHD (did not qualify as either of the previous two categories, and the underlying cause of death included ICD-10 codes I20-I25, I46, I51.6, R96, or R98-99) in the absence of known non-.atherosclerotic or non-cardiac cause of death.

Statistical Analysis

Data were analysed using Stata (Stata 13.0, StataCorp, College Station, TX) (StataCorp, 2013). The analyses performed were based on the recommendations for reporting novel risk markers published by Hlatyky et al. In addition to reporting the usual measures of association, these authors also recommend reporting the discrimination power of the new marker by the C-index for a model with established risk factors (Hlatky, et al., 2009).

The covariates of interest were the 8 biomarkers identified from the literature, as well as the traditional risk factors of heart disease. To be included in the analysis, participants had to have complete information on all covariates. There was missing data for current cigarette smoking status (N=22), systolic blood pressure (N=3), ABI (N=79), LDL cholesterol (N=113), HDL cholesterol (N=26), triglycerides (N=23), education (N=23), urinary albumin (N=39), family history (N=419), total homocysteine (N=20), IL-6 (N=192), fibrinogen antigen (N=47), CRP (N=52), D-dimer (N=45), and CHD outcome (N=5).

Differences in baseline characteristics and baseline biomarker concentrations were compared using the Pearson's Chi- squared test for categorical variables, or the Wilcoxon rank-sum test for continuous variables. All tests were two-tailed, and a level of significance of 0.05 was used. Boxplots were created to examine the distributions of the continuous variables, and correlation coefficients were calculated between continuous variables. The continuous variables were assessed for normality using the Shapiro-Wilk test.

Survival analysis techniques were used to analyse the data. Censored events were those where the participants did not have incident CHD during follow-up, so that their last observed follow-up time is less than their time to CHD (right censoring). Censored events included losses to follow-up, end of follow-up (administrative censoring) and deaths other than those defined above. Kaplan-Meier curves were created to compare the cumulative survival of CHD for the categorical variables. Log rank tests were used to test for statistical significance between cumulative survival proportions. The Cox Proportional Hazards Regression model was used to calculate hazard ratios and determine if any of the covariates were significant predictors of cardiovascular events. To test for heterogeneity of the covariates by race and sex, multiplicative interaction terms between the covariates and sex, and race, were generated. The statistically significant interaction terms were included in the multivariable models. Hazard ratios stratified by sex and by race were also presented. The Mantel-Haenszel log-rank test was used to determine whether the stratified estimates differed significantly. Clog-log plots were used to test the proportional hazard assumption among the categorical variables. Assumption of proportionality for all of the models was tested using Schoenfeld residuals, which was found to be statistically nonsignificant.

To look at predictive power of adding biomarkers, the Akaike Information Criterion (AIC), Somer's D, and Harrell's C statistics of a model containing only traditional risk factors were compared to those of models which included the traditional risk factors as well as the biomarkers (Newson, 2010).

Results

Baseline Characteristics

Table 1 compares the baseline demographic characteristics between those who had incident CHD, and those who did not, while Table 2 compares the biomarker levels between both groups. Both tables also show the p-values for the t-test of differences in the mean. Both boxplots (Figures 1, 2, and 3 in the appendix) and Shapiro-Wilks tests for normality (Table 12) showed that none of the continuous covariates were normally distributed. As such, the medians and interquartile ranges (IQR) were reported in Tables 1 and 2.

Table 1 shows that persons who had incident CHD differed significantly from those who did not by all covariates except BMI, study site, average number of cigarettes smoked per day, and education. Generally, persons who had incident CHD were older white males with higher systolic blood pressure, were either former or current smokers, and had diabetes and a family history of heart attacks compared to persons who did not have incident CHD.

Table 2 shows that persons who had incident CHD had significantly different levels of all the biomarkers except for LDL cholesterol, which was higher in the incident CHD group, but not significantly so. Generally, those who had incident CHD had ABIs less than 0.9, significantly higher CRP, IL-2, mean Agatston calcium score, urinary albumin, fibrinogen antigen, D-dimer, total homocysteine, and triglycerides, and significantly lower HDL cholesterol.

Table 1: Demographic Characteristics at baseline, Multi-Ethnic Study of Atherosclerosis, 2000-2002.

Variables	No CHD N = 5521	CHD N = 420	p – value[1]
Age (years), Median (IQR)	61.0 (53.0, 69.0)	67.0 (59.0, 74.5)	**< 0.001**
Age distribution (years) (N, %)			
45 - 54	1700 (30.8)	52 (12.4)	**< 0.001**
55 - 64	1558 (28.2)	104 (24.8)	
65 - 74	1561 (28.3)	159 (37.9)	
75 - 84	702 (12.7)	105 (25.0)	
Sex (N, %)			
Female	2996 (54.3)	148 (35.2)	**< 0.001**
Male	2525 (45.7)	272 (64.8)	
Race/ Ethnicity (N, %)			
White	2123 (38.5)	193 (46.0)	**0.016**
African American	1509 (27.3)	106 (25.2)	
Hispanic	1219 (22.1)	83 (19.8)	
Chinese	670 (12.1)	38 (9.0)	
Study Site (N, %)			
UCLA	1069 (19.4)	74 (17.6)	0.10
WFU	832 (15.1)	83 (19.8)	
COL	912 (16.5)	58 (13.8)	
JHU	864 (15.6)	60 (14.3)	
UMN	868 (15.7)	73 (17.4)	
NWU	976 (17.7)	72 (17.1)	
BMI (kg/m^2), Median (IQR)	27.5 (24.5, 31.0)	28.0 (24.9, 31.5)	0.07
Systolic blood pressure (mmHg), Median (IQR)	122 (110.0, 138.5)	132.5 (118.5, 147.0)	**<0.001**
Cigarette Smoking Status (N, %)			
Never	2837 (51.4)	175 (41.7)	**<0.001**
Former	1999 (36.2)	184 (43.8)	
Current	685 (12.4)	61 (14.5)	
Average number of cigarettes smoked per day, Median (IQR)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.15
Diabetes (N, %)			
No	5039 (91.3)	338 (80.5)	**< 0.001**
Yes	482 (8.7)	82 (19.5)	
Family History of Heart Attack (N, %)			
No	3234 (58.6)	184 (43.8)	**< 0.001**
Yes	2284 (41.4)	236 (56.2)	
Highest Level of Education Completed (N, %)			
Less than High School	597 (10.8)	42 (10.0)	0.31
At least High School Completed	4924 (89.2)	378 (90.0)	

1 – Pearson's chi- squared test was used for the categorical variables, while the Wilcoxon rank-sum test was used for the continuous variables. Bolded p-values indicate statistically significant differences between both groups.

Table 2: Biomarker Concentrations at baseline, Multi-Ethnic Study of Atherosclerosis, 2000-2002.

Variables[1]	No CHD N = 5521	CHD N = 420	p - value[2]
Ankle - brachial index (N, %)			
< 0.9	165 (3.0)	34 (8.1)	**<0.001**
0.9 - 1.3	5163 (93.5)	374 (89.0)	
> 1.3	193 (3.5)	12 (2.9)	
C-reactive protein (mg/L)	1.9 (0.8, 4.1)	2.2 (0.9, 4.5)	**0.023**
Interleukin-6 (pg/mL)	1.2 (0.8, 1.8)	1.5 (0.9, 2.1)	**<0.001**
Mean Agatston Calcium Score	0.0 (0.0, 60.2)	185.9 (20.3, 553.7)	**<0.001**
Urinary albumin (mg/dL)	0.6 (0.3, 1.2)	0.8 (0.4, 2.0)	**<0.001**
Fibrinogen Antigen (mg/dL)	336.0 (293.0, 386.0)	351.5 (311.0, 398.5)	**<0.001**
D-dimer (µg/mL)	0.2 (0.1, 0.4)	0.3 (0.2, 0.5)	**<0.001**
Total Homocysteine (µmol/L)	8.6 (7.2, 10.3)	9.4 (7.9, 11.6)	**<0.001**
LDL Cholesterol (mg/dL)	116.0 (96.0, 136.0)	118.5 (95.0, 141.0)	0.31
HDL Cholesterol (mg/dL)	49.0 (41.0, 59.0)	45.0 (38.0, 55.0)	**<0.001**
Triglycerides (mg/dL)	109.0 (77.0, 157.0)	119.5 (88.0, 173.0)	**<0.001**

1 – Medians and interquartile ranges (in parentheses) of biomarker concentrations are presented.
2 – Pearson's chi- squared test was used for the categorical variable, while the Wilcoxon rank-sum test was used for the continuous variables. Bolded figures indicate a significant difference in biomarker concentration between both groups.

Survival and Regression Analyses

The average follow-up time was 10.1 years (range: 0.1 - 12.5 years). CHD events were experienced by 420 persons, and the total person-time corresponded to 60 020 person-years of follow-up. The overall incidence rate of CHD in the cohort was 7.0 events per 1 000 person-years (95% C.I.: 6.4 – 7.70).

Table 3 shows the incidence rates of CHD by age, sex, and race/ethnicity. As expected, the rates of CHD increased with age. In each age group, men had higher rates of CHD than women. In women, white and African American women had the highest rates of CHD in each age group except for the oldest group. In men, whites and Hispanics had the highest rates in each age group, except for the 65 – 74 group. Overall, the highest rates of CHD were found in Hispanic men aged 75-84 years.

Table 3: Incidence Rates by Age, Sex, and Race/ Ethnicity, Multi-Ethnic Study of Atherosclerosis.

Age	Sex	Race/ Ethnicity	CHD Events	Person-years	Incidence rate, CHD Events per 1 000 person-years
45 - 54	Female	White	7	4000	1.8
		African American	7	2734	2.6
		Hispanic	2	2369	0.8
		Chinese	0	1169	0.0
	Male	White	18	3333	5.4
		African American	8	2190	3.7
		Hispanic	9	2106	4.3
		Chinese	1	1117	0.9
55 - 64	Female	White	21	3655	5.7
		African American	8	2633	3.0
		Hispanic	5	2016	2.5
		Chinese	1	1129	0.9
	Male	White	29	3085	9.4
		African American	16	1974	8.1
		Hispanic	17	1807	9.4
		Chinese	7	985	7.1
65 - 74	Female	White	23	3454	6.7
		African American	16	2658	6.0
		Hispanic	10	1871	5.3
		Chinese	6	1054	5.7
	Male	White	52	3355	15.5
		African American	28	2033	13.8
		Hispanic	18	1414	12.7
		Chinese	6	917	6.5
75 - 84	Female	White	15	1627	9.2
		African American	10	948	10.6
		Hispanic	8	743	10.8
		Chinese	9	505	17.8
	Male	White	28	1395	20.1
		African American	13	735	17.7
		Hispanic	14	609	23.0
		Chinese	8	400	20.0

Table 4 shows the results of univariate Cox regression. The univariate analyses show that all the biomarkers are statistically significantly associated with incident CHD except for high ABI (p-value = 0.573), and CRP (p-value = 0.091).

Table 4: Hazard Ratios from Univariate Cox Regression, Multi-Ethnic Study of Atherosclerosis

Biomarker	Hazard Ratio (95% C.I.)
Ankle - brachial index	
< 0.9	3.03 (2.13 - 4.30)
0.9 - 1.3	1.00
> 1.3	0.85 (0.48 - 1.51)
C-reactive protein (mg/L)	1.014 (0.998 - 1.031)
Interleukin-6 (pg/mL)	1.16 (1.08 - 1.24)
Mean Agatston Calcium Score	1.00073 (1.00065 - 1.00081)
Urinary albumin (mg/dL)	1.005 (1.003 - 1.007)
Fibrinogen Antigen (mg/dL)	1.004 (1.002 - 1.005)
D-dimer (µg/mL)	1.11 (1.04 - 1.19)
Total Homocysteine (µmol/L)	1.03 (1.02 - 1.04)

Table 5 shows the p-values for the tests of heterogeneity (interaction) between the covariates (biomarkers and traditional risk factors) and sex and race with regard to the hazard of CHD. The only significant interaction terms were those between mean Agatston calcium score and sex, triglycerides and sex, age and race/ethnicity, and education and race/ethnicity.

Table 5: P-values for Tests for Heterogeneity, Multi-Ethnic Study of Atherosclerosis

Variable	Interaction Term p – value[1]	
	Sex	Race
Age (years)	0.512	**0.036**
Sex	-	0.821
BMI	0.427	0.415
Study Site	0.520	0.275
Cigarette Smoking Status	0.415	0.746
Average no. of cigarettes smoked per day	0.675	0.647
Diabetes	0.576	0.232
Family History of Heart Attack	0.376	0.085
Education	0.199	**0.013**
Ankle - brachial index	0.827	0.835
C-reactive protein (mg/L)	0.682	0.417
Interleukin-6 (pg/mL)	0.521	0.999
Mean Agatston Calcium Score	**0.037**	0.203
Urinary albumin (mg/dL)	0.901	0.683
Fibrinogen Antigen (mg/dL)	0.617	0.420
D-dimer (µg/mL)	0.117	0.385
Total Homocysteine (µmol/L)	0.652	0.327
Systolic blood pressure (mmHg)	0.666	0.646
LDL Cholesterol (mg/dL)	0.106	0.881
HDL Cholesterol (mg/dL)	0.706	0.849
Triglycerides (mg/dL)	**0.043**	0.255

1 – Bolded figures indicate statistically significant interaction terms.

Table 6 shows the results of the multivariable regression. After adjustment for the traditional risk factors of heart disease and statistically significant interaction terms, the only statistically significant associations were observed with mean Agatston calcium score (p-value = <0.001), urinary albumin (p-value = 0.026), and fibrinogen antigen (p-value = 0.001). A one Agatston unit increase in mean Agatston calcium score increased the hazard of CHD by 0.09%. An increase of 1 mg/L urinary albumin increased the hazard of CHD by 0.25%. An increase of 1 mg/L fibrinogen antigen increased the hazard of CHD by 0.3%.

Table 6: Hazard Ratios from Multivariable Cox Regression, Multi-Ethnic Study of Atherosclerosis

Covariates	Hazard Ratio (95% C.I.)[1]
Ankle - brachial index	
< 0.9	1.07 (0.73 - 1.56)
0.9 - 1.3	1.00
> 1.3	0.69 (0.38 - 1.23)
C reactive protein (mg/L)	1.005 (0.985 - 1.025)
Interleukin-6 (pg/mL)	1.01 (0.93 - 1.10)
Mean Agatston Calcium Score	1.0009 (1.0006 - 1.0013)
Urinary albumin (mg/dL)	1.0025 (1.0003 - 1.0048)
Fibrinogen Antigen (mg/dL)	1.003 (1.001 - 1.004)
D-dimer (µg/mL)	1.005 (0.985 - 1.025)
Total Homocysteine (µmol/L)	1.008 (0.982 - 1.034)

1 – Multivariable analyses adjusted for age, sex, race/ethnicity, the interaction between age and race, BMI, current smoking status, average number of cigarettes smoked per day, study site, systolic blood pressure, diabetes, family history of heart attack, education, the interaction between race and education, LDL cholesterol level, HDL cholesterol level, triglyceride level, the interaction between triglyceride levels and sex, and the interaction between mean Agatston calcium score and sex.

Tables 7 and 8 present adjusted hazard ratios stratified by sex and race/ethnicity respectively. In women, mean Agatston calcium score is the only biomarker that is significantly associated with incident CHD (p-value = <0.001. In men however, mean Agatston calcium score (p-value = <0.001) and fibrinogen antigen (p-value = 0.004) were both found to be significantly associated with incident CHD. The Mantel-Haenszel log-rank tests showed that there were statistically significant differences by sex in the estimates for ankle-brachial index, IL-6, mean Agatston calcium score, urinary albumin, and D-dimer.

Table 7: Hazard Ratios Stratified by Sex, Multi-Ethnic Study of Atherosclerosis

Covariates	**Hazard Ratio (95% C.I.)[1]**		**Mantel-Haenszel log-rank test**
	Female[1]	**Male[2]**	
Ankle-brachial index			
< 0.9	1.53 (0.87 - 2.68)	0.86 (0.50 - 1.49)	0.0471
0.9 – 1.3	1.00	1.00	
> 1.3	0	0.77 (0.43 - 1.39)	
C reactive protein (mg/L)	1.01 (0.98 - 1.04)	1.0001 (0.9712 - 1.0299)	0.4466
Interleukin-6 (pg/mL)	1.04 (0.91 - 1.18)	0.97 (0.86 - 1.10)	0.0263
Mean Agatston Calcium Score	1.0009 (1.0005 - 1.0012)	1.0005 (1.0004 - 1.0007)	< 0.001
Urinary albumin (mg/dL)	1.004 (0.999 - 1.008)	1.002 (0.999 - 1.005)	0.0377
Fibrinogen Antigen (mg/dL)	1.002 (0.999 - 1.005)	1.003 (1.001 - 1.005)	0.6011
D-dimer (µg/mL)	1.07 (0.95 - 1.21)	0.93 (0.81 - 1.06)	0.0033
Total Homocysteine (µmol/L)	1.0005 (0.9548 - 1.0483)	1.02 (0.99 - 1.05)	0.5026

1 – Multivariable analyses adjusted for age, race/ethnicity, the interaction between age and race, BMI, current smoking status, average number of cigarettes smoked per day, study site, systolic blood pressure, diabetes, family history of heart attack, education, the interaction between race and education, LDL cholesterol level, HDL cholesterol level, triglyceride level.

Table 8 shows that Mean Agatston calcium score was a statistically significant predictor of incident CHD in all four racial/ethnic groups. Stratification by race/ethnicity also revealed that some biomarkers were statistically significant in some groups, and not in others.

In whites, mean Agatston calcium score (p-value = <0.001), fibrinogen antigen (p-value = 0.023), and D-dimer (p-value = 0.020) were significantly associated with incident CHD. In African Americans, mean Agatston calcium score (p-value = <0.001) and fibrinogen antigen (p-value = 0.014) were significantly associated with incident CHD. In Hispanics, mean Agatston calcium score (p-value = <0.001) and total homocysteine (p-value = 0.041) were significantly associated with incident CHD. In the Chinese, only mean Agatston calcium score (p-value = <0.001) was significantly associated with incident CHD. The Mantel-Haenszel log-rank tests showed that there were statistically significant differences by race/ethnicity in the estimates mean Agatston calcium score and total homocysteine.

Table 8: Hazard Ratios Stratified by Race/Ethnicity, Multi-Ethnic Study of Atherosclerosis

Covariates	Hazard Ratios (95% C.I.)[1]				Mantel-Haenszel log-rank test
	White	African American	Hispanic	Chinese	
Ankle - brachial index					
< 0.9	1.11 (0.57 - 2.17)	0.78 (0.40 - 1.54)	1.39 (0.56 - 3.44)	2.95 (0.69 - 12.56)	0.1195
0.9 - 1.3	1.00	1.00	1.00	1.00	
> 1.3	0.60 (0.26 - 1.37)	0.61 (0.14 - 2.58)	0.97 (0.34 - 2.74)	0 -	
C reactive protein (mg/L)	0.998 (0.963 - 1.035)	0.992 (0.956 - 1.03)	1.03 (0.99 - 1.07)	1.05 (0.96 - 1.15)	0.9191
Interleukin-6 (pg/mL)	0.99 (0.87 - 1.13)	1.11 (0.94 - 1.30)	0.90 (0.72 - 1.12)	0.94 (0.65 - 1.36)	0.7761
Mean Agatston Calcium Score	1.001 (1.0005 - 1.002)	1.0007 (1.0004 - 1.0009)	1.0005 (1.0002 - 1.0008)	1.001 (1.0008 - 1.002)	< 0.001
Urinary albumin (mg/dL)	0.98 (0.95 - 1.02)	1.004 (1.000 - 1.01)	1.003 (0.999 - 1.006)	0.96 (0.91 - 1.01)	0.3565
Fibrinogen Antigen (mg/dL)	1.003 (1.0004 - 1.005)	1.004 (1.001 - 1.007)	1.0009 (0.997 - 1.004)	0.999 (0.992 - 1.006)	0.9807
D-dimer (µg/mL)	1.31 (1.04 - 1.65)	1.02 (0.87 - 1.20)	1.03 (0.83 - 1.26)	0.85 (0.29 - 2.51)	0.3335
Total Homocysteine (µmol/L)	0.997 (0.956 - 1.040)	0.997 (0.946 - 1.052)	1.06 (1.003 - 1.13)	1.04 (0.91 - 1.19)	0.0057

1 – Multivariable analyses adjusted for age, sex, current smoking status, average number of cigarettes smoked per day, study site, systolic blood pressure, diabetes, family history of heart attack, education, LDL cholesterol level, HDL cholesterol level, triglyceride level, the interaction between triglyceride levels and sex, and the interaction between mean Agatston calcium score and sex.

Table 9 shows the change in Somer's D and Harrell's C when biomarkers were added to a model which included the traditional risk factors of CHD. With the exception of ABI and D-dimer, generally adding biomarkers to the model improved model fit and predictive power. When ABI and D-dimer were added to the model, the Somer's D and Harrell's were unchanged. The addition of mean Agatston Calcium score had the biggest improvement on prediction (0.0267 increase in Harrell's C), and this model also had the best fit (lowest AIC). The addition of fibrinogen antigen also had a fair improvement of prediction (0.004 increase in Harrell's C), and improved model fit. The other markers only marginally improved model fit and prediction.

Table 9: Comparison of Model Fit and Predictive Power between Traditional Risk Factor and Biomarker Models

Model	Somer's D	Harrell's C	AIC
Traditional risk factor[1]	0.4838	0.7419	6917.896
Traditional risk factor + Ankle - brachial index	0.4838	0.7419	6919.878
Traditional risk factor + C-reactive protein	0.4861	0.7430	6916.211
Traditional risk factor + Interleukin-6	0.4855	0.7428	6915.822
Traditional risk factor + Mean Agatston Calcium Score	0.5372	0.7686	6848.023
Traditional risk factor + Urinary albumin	0.4843	0.7422	6915.062
Traditional risk factor + Fibrinogen Antigen	0.4918	0.7459	6901.282
Traditional risk factor + D-dimer	0.4838	0.7419	6917.090
Traditional risk factor + Total Homocysteine	0.4843	0.7422	6918.987

1 – Model included age, race/ethnicity, sex, study site, BMI, cigarette smoking status, diabetes status, education, family history, systolic blood pressure, LDL cholesterol, HDL cholesterol, and triglycerides.

Discussion

Among a multi-ethnic asymptomatic population without clinical cardiovascular disease, the associations between consolidated and emerging biomarkers of heart disease and incident CHD were examined by both univariate and multivariable analyses. The effect of including these biomarkers in models containing the traditional risk factors of heart disease was also examined to assess whether the biomarkers improved the predictive power of these models.

The results reported show that mean Agatston calcium score, urinary albumin, and fibrinogen antigen were significantly associated with increased hazard of incident CHD after adjustment for traditional cardiovascular risk factors. The Mantel-Haenszel log-rank tests showed that there were statistically significant differences by sex in the estimates for ankle-brachial index, IL-6, mean Agatston calcium score, urinary albumin, and D-dimer. There were also statistically significant differences by race/ethnicity in the estimates mean Agatston calcium score and total homocysteine. The addition of biomarkers to a model containing traditional risk was found to improve model fit and prediction.

Coronary calcium has been shown to be a sensitive marker of early stages of atherosclerosis in large prospective studies, and is a strong predictor of incident coronary heart disease. Increased deposition of calcium in the coronary arteries cause the vessels to harden and narrow over time, thus limiting the blood supply (and oxygen) to the heart (Detrano, et al., 2008) (Grossman, Ehrlich, Shemesh, Koren-Morag, & Grossman, 2015). An analysis by Polonsky et al found that adding coronary calcium scores to a prediction model based on traditional risk factors significantly improved the classification of risk (Polonksy, et al., 2010). In this study, the addition of mean Agatston calcium score had the biggest impact on prediction, and therefore, the results of this study support these findings.

Urinary albumin has been shown to be associated with atherogenic changes in the cardiovascular risk profile, and has been used to predict cardiovascular disease risk in patients with diabetes (Weir, 2004). The mechanism linking elevated urinary albumin (microalbuminuria) to increased cardiovascular risk is still unknown, but microalbuminuria has been suggested as a marker of endothelial dysfunction and hyperpermeability to macromolecules, which occurs early in atherogenesis (de Zeeuw, Parving, & Henning, 2006). In this study, while urinary albumin was found to be significantly associated with incident CHD, its inclusion in the risk factor model only marginally improved prediction. Therefore, the use of urinary albumin in predicting heart disease needs to be examined further.

Fibrinogen is a marker of inflammation, which is produced when proinflammatory cytokines from the vascular endothelium and from macrophages are secreted. Chronic inflammation has been linked to heart disease as it is involved in all stages of atherosclerosis. Fibrinogen may increase cardiovascular risk in several ways. It plays an important role in platelet aggregation, plasma viscosity, and fibrin formation. Fibrinogen is an acute-phase reactant that is increased in inflammatory states, and is involved in the formation of atherosclerotic plaque during the first stages of coronary artery disease (CAD) (Libby, 2006).

Several studies have reported strong associations between fibrinogen levels and cardiovascular outcomes. The Gothenburg study found that plasma fibrinogen levels were an independent risk factor for MI and stroke in univariate analyses. The Framingham study also reported that the risk of MI and stroke increased progressively with fibrinogen levels (Papageorgiou, Tousoulis, Siasos, & Stefanadis, 2010). A meta-analysis by the Emerging Risk Factors Collaboration group also found that including inflammatory biomarkers, namely CRP and fibrinogen, in prognostic models improved model prediction. They concluded that including inflammatory markers in models could prevent one additional cardiovascular event over a period of 10 years for every 400 to 500 persons at intermediate risk for a cardiovascular event who were screened (The Emerging Risk

Factors Collaboration, 2013). The analyses presented showed that fibrinogen was associated with incident CHD, and also improved the model prediction, and thus support these findings. Previous studies have found associations between other inflammatory markers like CRP and IL-6 and CHD. As such, it was expected that similar findings would have been observed in this analysis. One possible explanation could be that there could be interaction between fibrinogen and CRP, and IL-6, as all three markers are part of the inflammatory cascade that initiates plaque formation.

D-dimer was significantly higher individuals with incident CHD, and was significantly associated with CHD in the univariate analyses. However, when adjusted, the only association with CHD was found in whites. D-dimer is an end product of fibrinolysis and may promote the inflammatory cascade by activating neutrophils and monocytes, thus inducing the secretion of inflammatory cytokines (e.g. IL-6) (McDermott, et al., 2007). A study by Pieper et al found that African Americans had D-dimer levels 40% higher than those of whites, however in this analysis, there was no statistically significant interaction between race/ethnicity and D-dimer levels (Pieper, Rao, Currie, Harris, & Cohen, 2000). A meta-analysis performed by Danesh et al found that there was a significant association between D-dimer and CHD after adjustment for smoking, other classic risk factors for CHD, and indicators of socioeconomic status (Danesh, et al., 2001). While including D-dimer in the prediction model did not improve model prediction, its interaction with race/ethnicity should be examined further when evaluating its ability to predict cardiovascular risk.

Homocysteine was also found to be significantly higher in individuals CHD, and was significantly associated with CHD in the univariate analyses. However, when adjusted, the only significant association with CHD was observed in Hispanics. Studies by Selhub et al and Estrada et al have found that whites had the highest levels of homocysteine, followed by African Americans, and Hispanics (Estrada & Billett, 2001). In this analysis, there was no significant interaction between homocysteine and race/ethnicity, but there was a significant difference in the

hazard ratios for homocysteine by race. Homocysteine is an amino acid, and elevated levels of homocysteine in the blood are thought to make a person more prone to endothelial cell injury, leading to inflammation in the blood vessels which can in turn lead to atherogenesis. A meta-analysis performed by Boushey et al in 1995 showed that a 5μmol/L increase in homocysteine level increased the relative risk of CAD by the same amount as an increase of 20 mg/dL of total cholesterol. However, most of the studies were cross-sectional and case-control (Marinou, et al., 2005). A causal link between homocysteine and CHD has yet to be established (American Heart Association, 2014). The relationships between race/ethnicity and homocysteine should be considered further when evaluating homocysteine as a risk factor for CHD.

A secondary goal of this analysis was to assess the role of sex in the association between the biomarkers and CHD. Only one biomarker, mean Agatston calcium score, had a statistically significant interaction by sex. The sex stratified hazard ratios for mean Agatston calcium score were also significantly different; and women had a slightly higher hazard ratio as compared to men. Coronary calcium scanning was shown to have a significant contribution in accurately detecting CHD in asymptomatic women, in addition to traditional cardiovascular risk (Youssef & Budoff, 2012). The results of this study therefore support the role of Agatston score as a risk stratification tool for women.

Coronary calcium, fibrinogen, and urinary albumin were significantly higher among individuals with incident CHD, and significantly increased the hazard of incident CHD. Coronary calcium and fibrinogen were the only two biomarkers that markedly improved model prediction. These results, along with other findings identifying these biomarkers as independent risk factors of evidence, support the proposal that biomarkers be included prognostic models for coronary heart disease and be used in population screening to identify individuals at increased cardiovascular risk. The results of this study also support the use of sex specific prediction models (e.g. the Framingham model), and suggest that race/ethnicity specific models may be also useful in predicting cardiovascular risk.

Strengths

This study has many strengths. The cohort was large, providing adequate power, and multi-ethnic. MESA participants were also carefully characterised with respect to cardiovascular risk factors. All blood samples were analysed centrally at one laboratory. Most cohorts used in cardiovascular disease research have study populations that are predominantly male, but the MESA cohort was sex-balanced (52.8% female). Therefore, the results of the study are likely more externally valid. Because the MESA cohort includes persons of different ages and ethnicities from across the United States, the results of the study may be generalizable to urban middle aged and older Americans at risk for cardiovascular disease.

Limitations

There are several limitations to this study. There could be residual confounding present because of unknown or unmeasured confounders. The correlation coefficients (Table 11 in the appendix) indicate that there are weak to moderately positive relationships between some of the biomarkers. However, the interactions between biomarkers, and between biomarkers and the traditional risk factors of cardiovascular disease were not assessed.

There could also be selection bias in this analysis as participants were only included in the analysis if they had information on all covariates of interest. Persons with missing data could have been different to those with complete information. Furthermore, in some of the regression models, the average number of cigarettes smoked per day was found to be significantly associated with incident CHD. However, this was a self-reported variable and therefore was subject to information bias.

Another limitation is that some of the associations observed could be due to chance because of multiple comparison testing, which would have decreased the level of significance ($\alpha = 0.05$).

Public Health Impact

Coronary calcium, fibrinogen, and urinary albumin were found to be significantly associated with incident CHD. Coronary calcium is already used to detect CAD. However, more attention should be placed on the inflammatory markers, and their role in predicting CHD should be examined further.

Addition of biomarkers to regression models improved model fit and discrimination. Only 8 biomarkers were used in this study. However, newer research has identified over 60 biomarkers of interest (Rollins, 2012). Therefore, further research needs to be done. Existing cardiovascular cohort studies should add measurement of novel biomarkers to their study protocols, and new cohort studies should also be developed to look at these biomarkers.

While more research is needed to improve the body of evidence, when developing models for predicting heart disease, researchers may want to consider including biomarkers as they improve the discrimination afforded by current heart disease risk factors, and may have the potential to prevent additional cardiovascular events.

References

Appendices

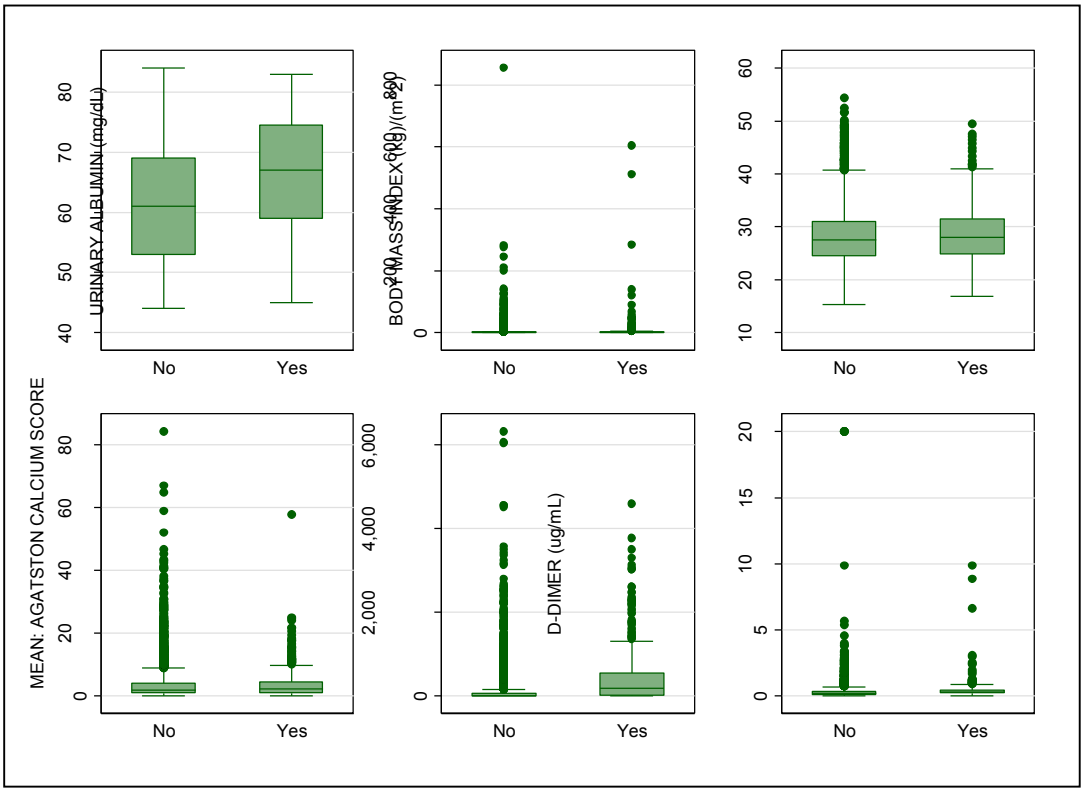

Figure 1: Boxplots of Age, Urinary Albumin, BMI, CRP, Mean Agatston Calcium Score, and D-dimer by Incident CHD.

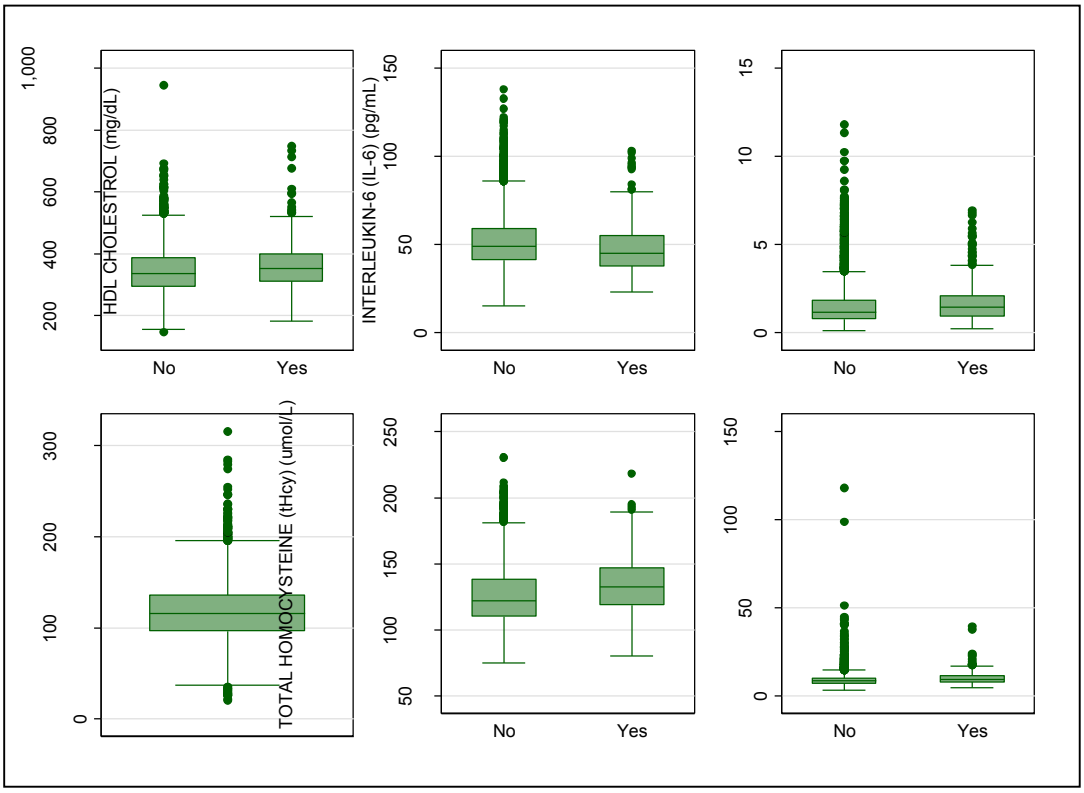

Figure 2: Boxplots of Fibrinogen, HDL Cholesterol, IL-6, LDL Cholesterol, and Total Homocysteine Concentrations, and Systolic Blood Pressure by incident CHD.

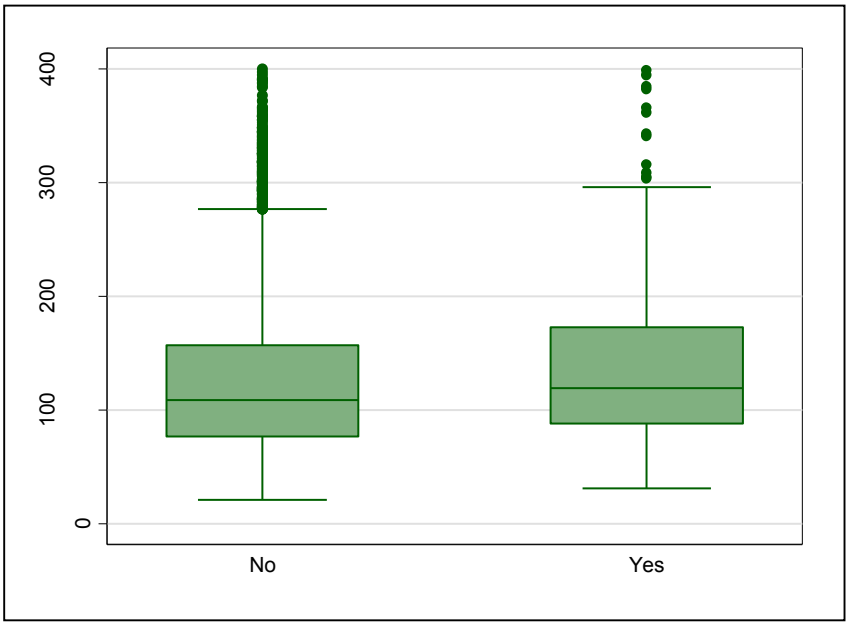

Figure 3: Boxplot of Triglyceride Concentration by incident CHD.

Table 10: Correlation Coefficients

	age1c	bmi1c	crp1	il61	agatpm1c	ualbumn1	fib1	ddimer1	hcytot1	sbp1c	ldl1	hdl1	trig1
age1c	1.0000												
bmi1c	-0.0916	1.0000											
crp1	-0.0012	0.2859	1.0000										
il61	0.1295	0.3009	0.4375	1.0000									
agatpm1c	0.2805	0.0025	-0.0321	0.0796	1.0000								
ualbumn1	0.0315	0.0494	0.0618	0.0576	0.0422	1.0000							
fib1	0.1641	0.2789	0.4278	0.3703	0.0404	0.1401	1.0000						
ddimer1	0.1382	0.0480	0.0717	0.1229	0.0798	0.0494	0.1221	1.0000					
hcytot1	0.2194	0.0214	-0.0293	0.1087	0.1265	0.0626	0.0503	0.0659	1.0000				
sbp1c	0.3629	0.1571	0.0970	0.1275	0.1063	0.1142	0.1282	0.0823	0.1205	1.0000			
ldl1	-0.0430	0.0372	-0.0056	-0.0567	-0.0180	0.0373	0.1304	-0.0072	-0.0336	0.0155	1.0000		
hdl1	0.0827	-0.2140	-0.0094	-0.1086	-0.0637	-0.0401	-0.0404	0.0080	-0.0677	0.0112	-0.0676	1.0000	
trig1	-0.0314	0.1421	0.0646	0.0299	0.0271	0.0564	0.0259	-0.0256	0.0182	0.0649	0.0576	-0.4085	1.0000

Table 11: Results of Shapiro-Wilks Tests for Normality of Continuous Variables

Variable	Obs	W	V	z	Prob>z
Age (years)	5941	0.9752	78.31	11.502	0.00000
BMI	5941	0.95872	130.362	12.847	0.00000
Average number of cigarettes smoked per day	5941	0.50794	1553.806	19.383	0.00000
C-reactive protein (mg/L)	5941	0.59932	1265.249	18.841	0.00000
Interleukin-6 (pg/mL)	5941	0.77816	700.527	17.282	0.00000
Mean Agatston Calcium Score	5941	0.49003	1610.342	19.477	0.00000
Urinary albumin (mg/dL)	5941	0.09532	2856.744	20.989	0.00000
Fibrinogen Antigen (mg/dL)	5941	0.97222	87.725	11.802	0.00000
D-dimer (µg/mL)	5941	0.26557	2319.149	20.44	0.00000
Total Homocysteine (µmol/L)	5941	0.64308	1127.062	18.536	0.00000
Systolic blood pressure (mmHg)	5941	0.96947	96.412	12.051	0.00000
LDL Cholesterol (mg/dL)	5941	0.98926	33.906	9.294	0.00000
Triglycerides (mg/dL)	5941	0.91073	281.884	14.881	0.00000
HDL Cholesterol (mg/dL)	5941	0.94025	188.686	13.822	0.00000

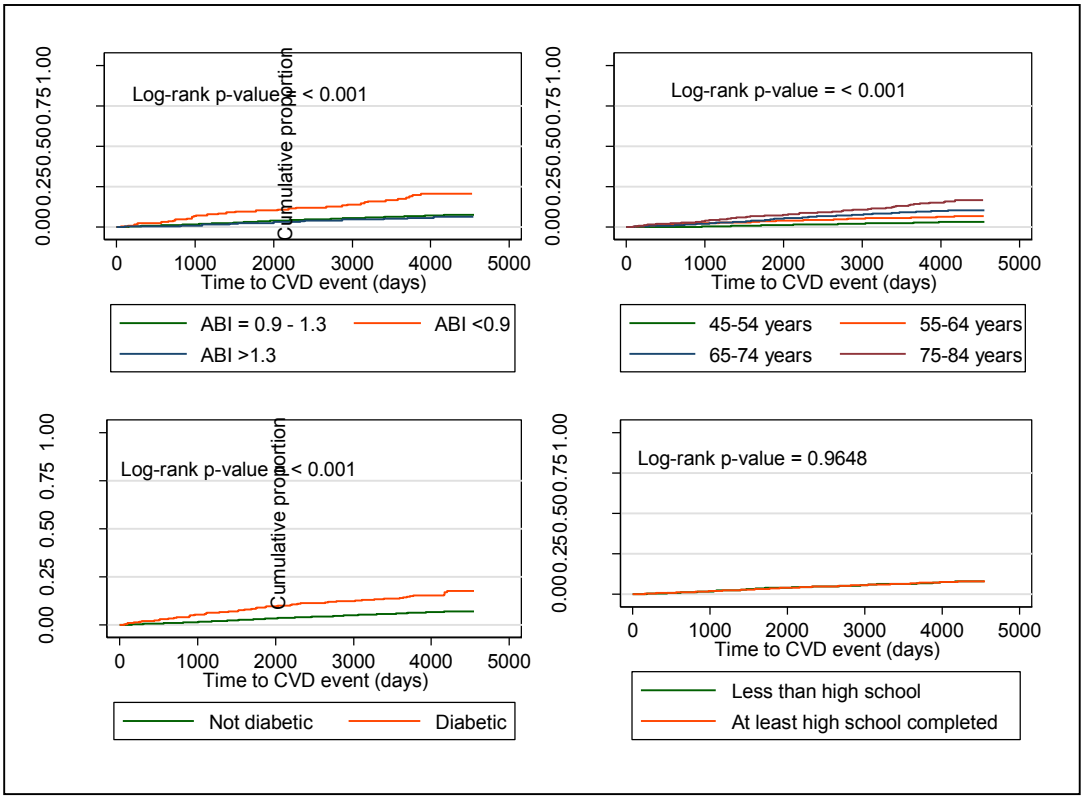

Figure 4: Kaplan Meier Curves and Log rank p-values for ABI, Age, Diabetes Status, and Education.

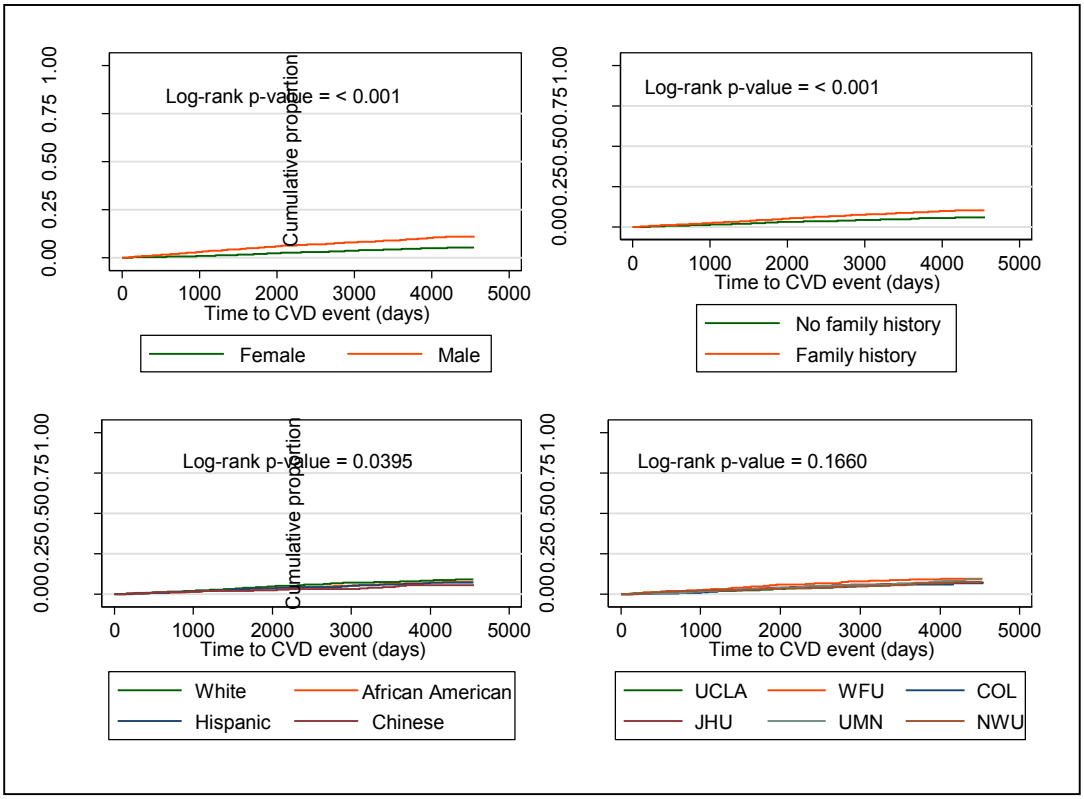

Figure 5: Kaplan Meier Curves and Log-Rank p-values for Sex, Family History of Heart Attack, Race, and Study Site.

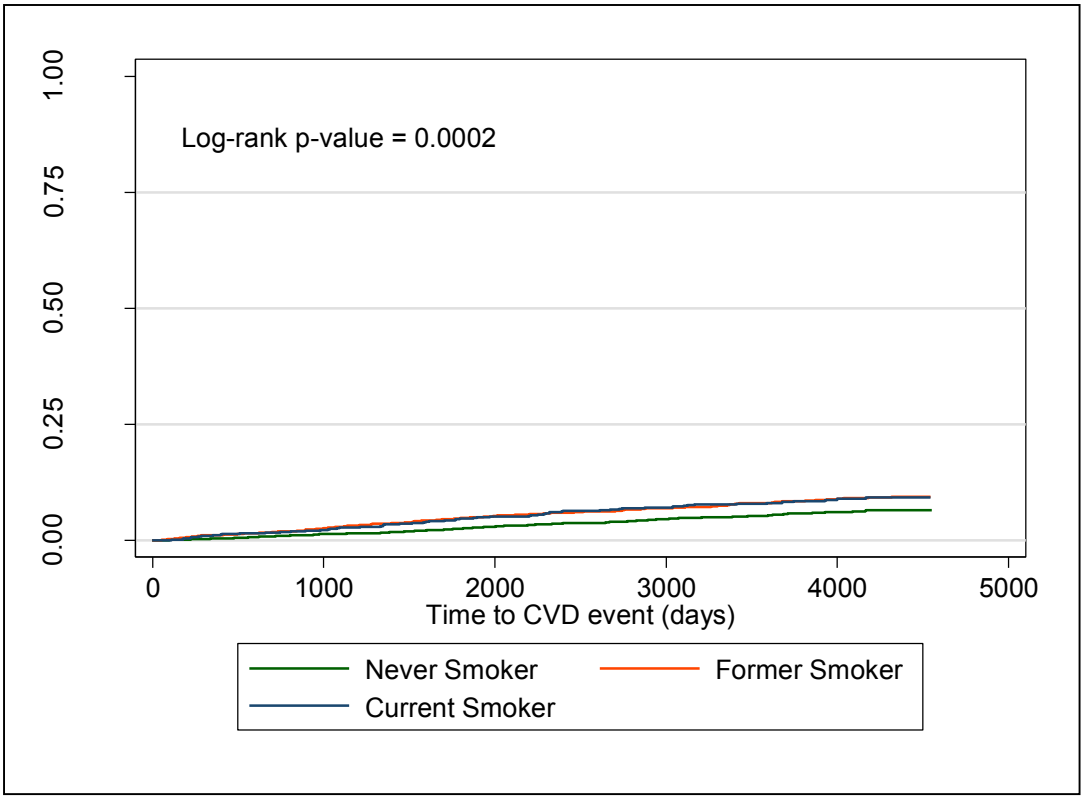

Figure 6: Kaplan Meier Curve and Log rank p-value for Current Smoking Status.

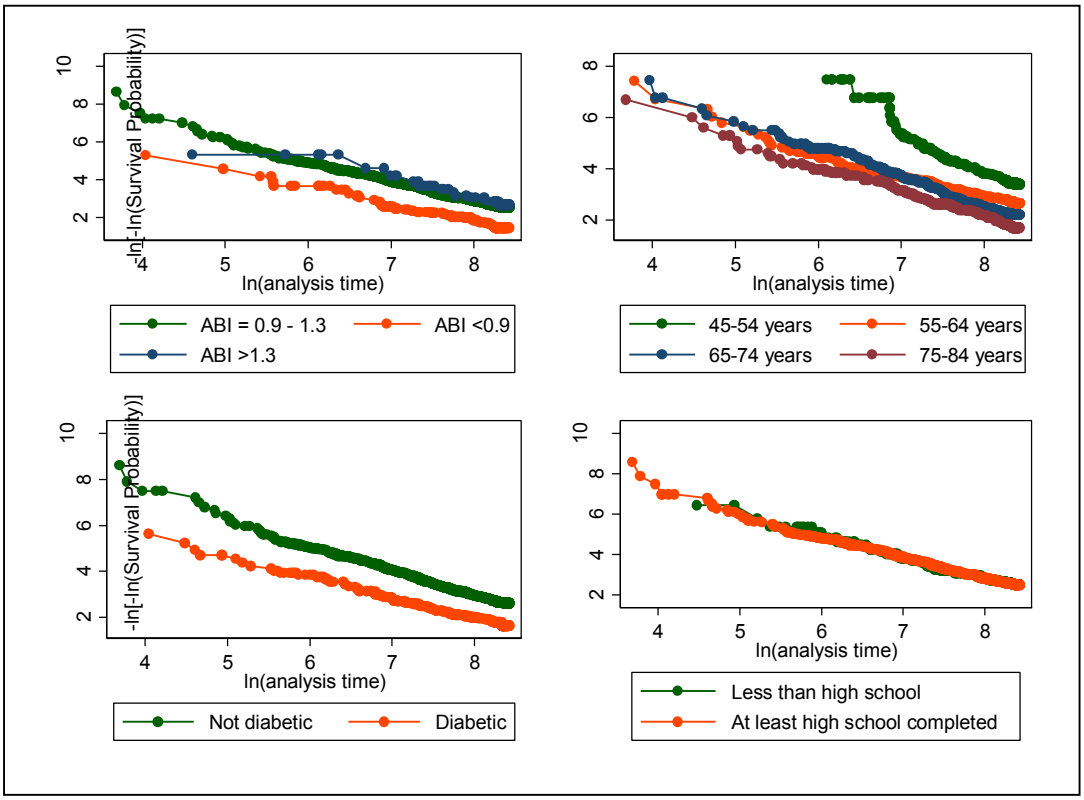

Figure 7: Clog-log plots for ABI, Age, Diabetes Status, and Education.

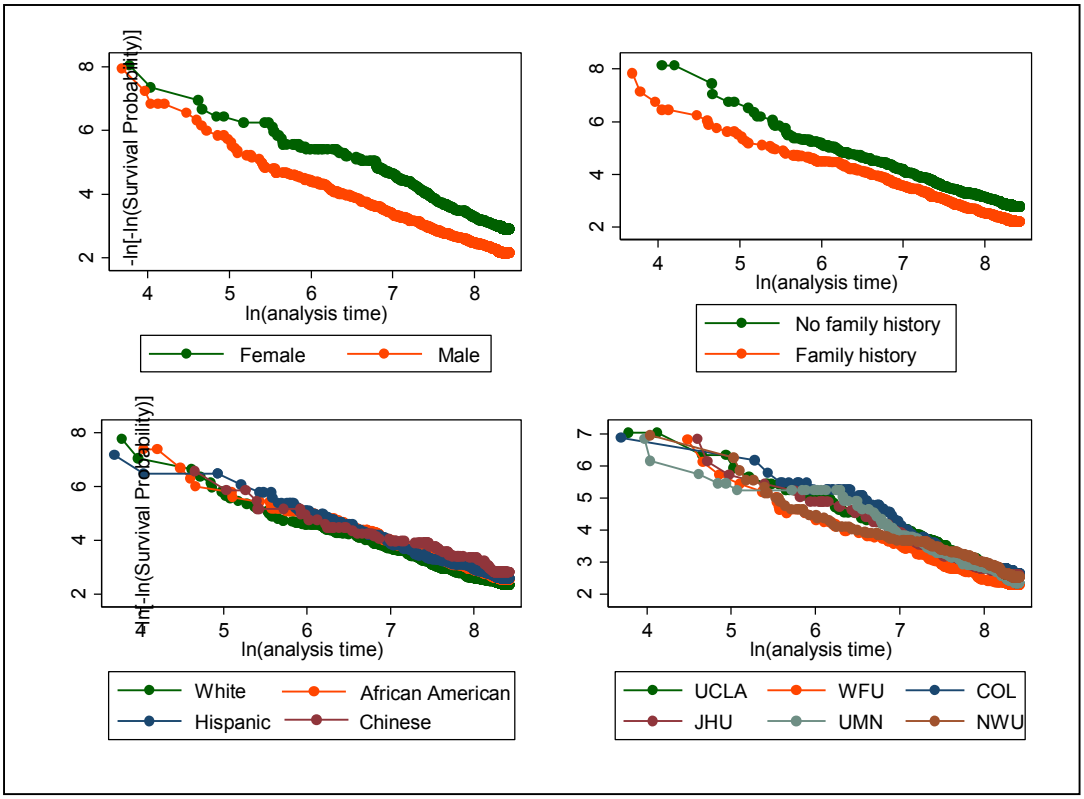

Figure 8: Clog-log plots for Sex, Family History of Heart Attack, Race, and Study Site.

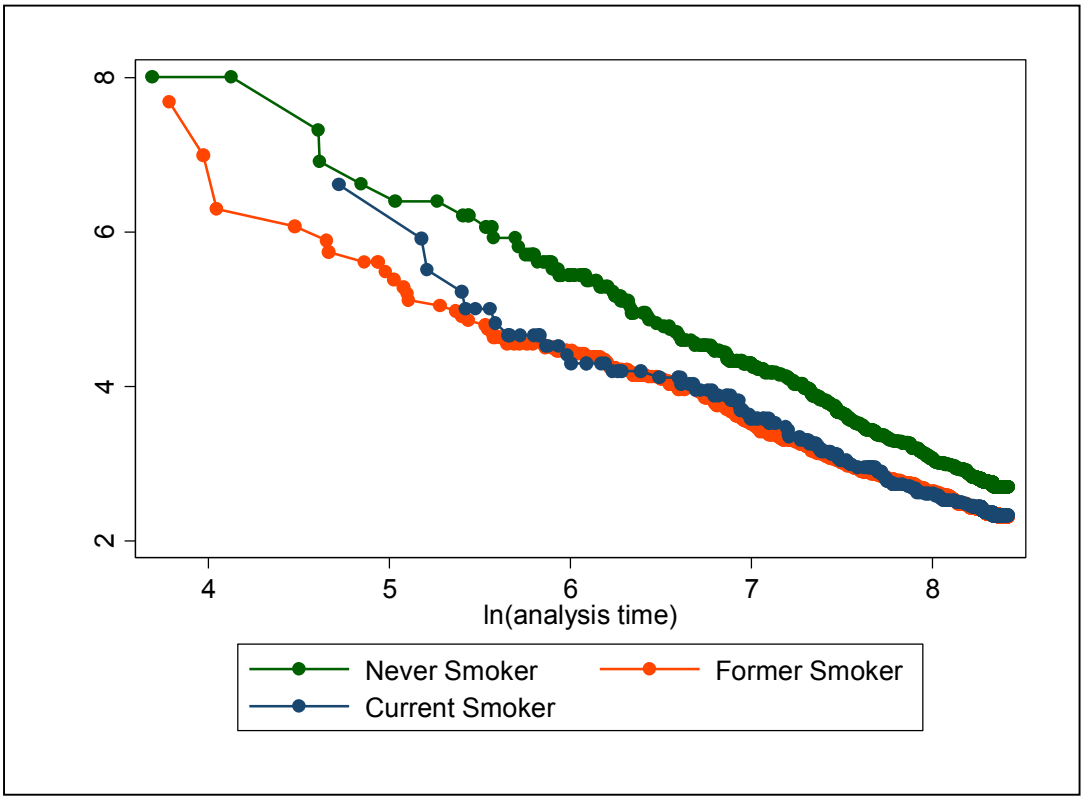

Figure 9: Clog-log plot for Current Smoking Status.

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Curriculum Vitae

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7th March 1991
San Fernando, Trinidad, West Indies

EDUCATION

Master of Health Science (MHS) in Epidemiology Expected May 2015
Concentration: General Epidemiology and Methodology
Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
Thesis: Biomarkers for the Prediction of Incident Coronary Heart Disease

Risk Science and Public Policy Certificate Expected May 2015
Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

Bachelor of Science (BS) in Molecular Bioscience and Biotechnology November 2012
Minor: Statistics
Concentration: Spanish Language and Culture
Rochester Institute of Technology, Rochester, NY

WORK EXPERIENCE

Graduate Trainee March 2013 – August 2013
Caribbean Industrial Research Institute (CARIRI), Trinidad, West Indies

- Analysed potable water samples by membrane filtration in the Food and General Microbiology Laboratory.
- Collected samples.
- Prepared media and reagents. Maintained stock.
- Trained new employees in potable water sample analysis.

TEACHING EXPERIENCE

Teaching Assistant March 2011 – May 2011
Rochester Institute of Technology, Rochester, NY

- Assisted in leading the Introduction to Biology III laboratory.
- Tutored students.
- Graded student work.

PROFESSIONAL DEVELOPMENT

Language Skills: Conversational Spanish

Computer Skills: Proficient in STATA (biostatistics computing software); ArcMap (mapping software); Crystal Ball (risk assessment software); Microsoft Office (Word, Excel, and PowerPoint); research search engines including PubMed

Training: ISO 17025, PLEA Pass Programme

Memberships: American Public Health Association

HONOURS AND AWARDS

Postgraduate Scholarship
Government of the Republic of Trinidad and Tobago — September 2013

Dean's List
Rochester Institute of Technology — September 2009 – November 2012

RIT Honours Programme
Rochester Institute of Technology — September 2009 – May 2012

Best Design of Experiments Project
School of Mathematical Sciences, Rochester Institute of Technology — May 2011

National Additional Scholarship in Mathematics
Government of the Republic of Trinidad and Tobago — September 2009

RIT International Scholarship
Rochester Institute of Technology — May 2009

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Faculty of Engineering, University of the West Indies, St. Augustine — February 2009