

**THE INTER-RELATED BIOMARKERS OF CARDIO-METABOLIC AND RENAL
DISEASE**

by

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Background:

Major chronic diseases such as cardiovascular disease, renal dysfunction and metabolic syndrome are having excessive impact on African Americans with higher prevalence rates, mortality and morbidity. But, relatively very few studies have been conducted among populations of African ancestry to evaluate the disease burden and etiology. This proposed study was built upon seven large, multigenerational families of African descent, residing on the island of Tobago, to investigate the genetic impact on major chronic diseases, and to evaluate the association of novel biochemical markers with subclinical cardiovascular measurements.

Methods:

Lipid profiles, subclinical cardiovascular measurements and renal function biomarkers were measured in 402 Afro-Caribbean individuals, aged 18 to 103 years, from 7 large, multi-generation pedigrees (average family size: 50; range: 19 to 96; 3535 relative pairs). Estimated GFR was calculated using the Modification of Diet in Renal Disease Study (MDRD) formula for standardized serum creatinine. Heritability (h^2) of cardio-metabolic renal traits was estimated employing maximum likelihood methods using SOLAR (Sequential Oligogenic Linkage Analysis Routines). Multivariate regression analysis was performed to assess the association

between renal function biomarkers and the subclinical cardiovascular measures, incorporating the effects of the relatedness of family members.

Results:

We determined that, among these Afro-Caribbeans of Tobago, renal function and metabolic syndrome related traits are all heritable phenotypes. The additive genetic effects accounted for 20-30% of the residual variation of several kidney function related traits. The heritability of each metabolic component ranged from 21% for large waist circumference to 46% for HDL-cholesterol ($P < 0.05$). Using the National Cholesterol Education Program Expert Panel (NCEP) and Treatment of High Blood Cholesterol in Adults (Adult Treatment panel III) (ATP III) definition, 18.6% (23.3% women and 11.8% men) of the participants had metabolic syndrome.

As a novel renal function biomarker, Cystatin C has heritability of 0.32 ± 0.1 ($P < 0.0001$), after adjusting for age, gender, hypertension, triglyceride and insulin level. Not only does cystatin C indicate renal function, but it was also significantly associated with ABI ($P = 0.02$) and PWV ($P = 0.04$) in this Afro-Caribbean population. Serum creatinine, microalbuminuria and eGFR were not found to be related to subclinical cardiovascular disease.

Public Health Significance:

This work was the first to estimate the heritability of renal function biomarkers and metabolic syndrome related traits among an African ancestry population living in the Caribbean. The significant finding of the association between serum Cystatin C level and subclinical cardiovascular disease suggest a promising biomarker for both renal function and cardiovascular

disease. If this result is confirmed, Cystatin C could be a useful prognostic tool in this high risk population.

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PREFACE

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1.0 INTRODUCTION

1.1 DISSERTATION OVERVIEW AND OBJECTIVES

Cardiovascular disease (CVD) is defined as the disease condition of the heart and blood vessels. It is the leading cause of death in United States, nearly 2600 Americans die as a result of CVD every day. Actually, the pathological process starts many years before the life-threatening events occur. Studies show strong evidence that the incidence of CVD is substantially increased for people with subclinical cardiovascular disease compared to those without subclinical disease (1). The impact of ethnicity and race on subclinical CVD has been identified. African Americans have significantly lower amounts of coronary artery and carotid artery calcified plaque relative to whites despite having increased carotid inter media-wall thickness (IMT) and blood pressure(2). Both pulse wave velocity (PWV) and ankle-brachial index (ABI) are well-established, non-invasive and easily performed measurements for subclinical CVD. High PWV assesses the arterial stiffness, which is a significant and independent predictor of CVD. ABI is an indicator for peripheral arterial disease (PAD) which increases individuals' risk for heart attack or stroke.

Metabolic syndrome (MetSyn), previously called insulin resistance syndrome, is characterized as clusters of risk factors including abdominal obesity, hypertension, dysglycemia and dyslipidemia (characteristically, increased triglyceride level and reduced high-density lipoprotein (HDL)). The prevalence of MetSyn among U.S. adults is high and increasing. High blood pressure, excessive waist circumference, and hypertriglyceridemia accounted for much of the increasing prevalence rate(3). Studies have found that MetSyn is a useful predictor for

cardiovascular disease and diabetes (4). The Atherosclerosis Risk in Communities Study (ARIC) studied prospectively the link between MetSyn and CKD. They found that among participants with MetSyn, the risk of CKD increased by 50%; the more traits of the syndrome, the higher risk for developing chronic kidney disease(5).

Chronic kidney disease (CKD) is a worldwide public health problem, with dramatically increased incidence and prevalence. It is a highly costly disease due to the number of patients who progress to kidney failure requiring dialysis or transplantation. Although, the mortality rate of cardiovascular disease and all-cause death are significantly higher among African Americans than those for Caucasians(6, 7), black individuals with serious CKD or end stage renal disease (ESRD) have consistently shown a survival advantage compared to white individuals(8). It has been hypothesized that black individuals who live long enough to reach the serious stage of renal disease are healthier compared to white individuals(8). Substantial evidence has shown the potential links between CKD and CVD. It has been reported that reduced renal function is not only related to multiple traditional cardiovascular risk factors such as hypertension, dyslipidemia, diabetes and hypertrophy, but also related to nontraditional factors such as inflammation and oxidative stress(9). Community based studies have identified kidney disease as an additional risk factor for the development of CVD(10).

The heritability of these complex diseases has been documented. The Northern Manhattan Family study, recruiting over eight hundred individuals from Hispanic Caribbean families, has documented the heritability of MetSyn to be 24%, with significant heritability for lipid/glucose/obesity (44%) and hypertension (20%)(11). The heritability of ankle brachial index (ABI) is about 27%, which has been estimated in a Caucasian population, the Framingham Offspring Study (12). However, such finding has not been replicated in various ethnicity groups,

especially among African American populations, which are at higher risk for cardiovascular and renal disorders.

Overall, this proposed research is to add the body of knowledge for the contributing factors, including biochemical markers, which increase the risk for major complex diseases. Cystatin C is not only a novel biomarker for renal disease, but also shows its association with cardiovascular system. This research seeks to investigate the association between renal measures both the traditional biomarker – serum creatinine, and the novel one – cystatin C, and subclinical cardiovascular disease among an African Caribbean population. Figure 1 is the proposed biological model for this project.

Proposed Biological Model

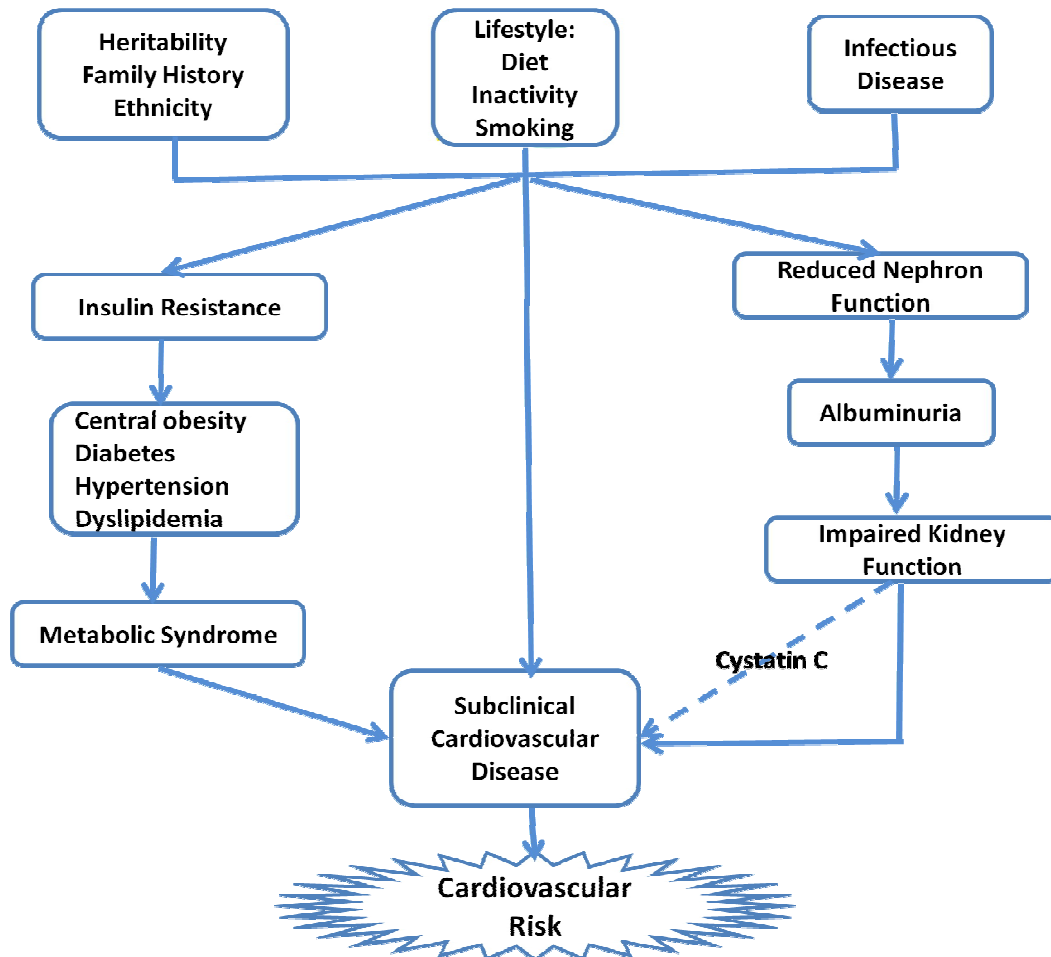


Figure 1.1 Proposed Biological Model for the Dissertation Research Proposal

1.1.1 Specific Aims

The objective of this project was achieved by three research studies which were built upon an existing collection of Afro-Caribbean family members who participate in the Tobago Family Health Study.

Specific Aim 1: to estimate the heritability of renal dysfunction in Tobago multigenerational families.

Specific Aim 2: to estimate the heritability of metabolic syndrome and its components in Tobago multigenerational families.

Specific Aim 3: to determine the association of renal function biomarkers with subclinical cardiovascular disease in Tobago multigenerational families.

1.2 EPIDEMIOLOGY

1.2.1 Cardiovascular Disease (CVD)

Cardiovascular Disease (CVD) is defined as dysfunctional conditions of the heart, arteries, and veins that supply oxygen to vital life-sustaining areas of the body. CVD includes various forms: high blood pressure, coronary heart disease, heart failure and stroke. According to American Heart Association (AHA) statistics, 16,000,000 people alive today have a history of heart attack, angina pectoris or both. CVD, primarily heart disease and stroke, is the Nation's leading killer for both men and women among all racial and ethnic groups(13). Almost 1 million Americans die of CVD each year, which adds up to 42% of all deaths. CVD costs the nation \$274 billion each year, including health expenditures and lost productivity. The burden continues to grow as the population ages. Worldwide, various forms of CVD make up an estimated 16.7 million - or 29.2% of total global deaths according to World Health Report 2003. Heart disease and stroke make up more than 50% of the deaths and disability, killing more than 12 million people each year, however, it can be prevented simply by reducing major risk factors such as high blood pressure, high cholesterol, obesity and smoking (WHO report). Heart disease is no longer only a disease condition of the developed world: about 80% of all CVD deaths worldwide took place in developing countries, which also accounted for 86% of the global CVD disease burden. It is estimated that by 2010, CVD will be the leading cause of death in developing countries (WHO report).

In the U.S., the death rate of heart disease has been decreasing since the 1960's. The mortality rate of the major form of CVD, coronary heart disease (CHD), declined from 1990 (558,291 deaths, NHLBI) to 2004 (481,458 deaths, CDC), the age adjusted death rate declined

about one third, from 224.3 to 154 per 100,000 population with CHD. But it is still the leading cause of death in the U.S. The impact of ethnicity and race on heart disease is substantial. On one hand, the incidence and prevalence rate are the higher among black population compared to whites in both gender groups. The incidence rate is 540 per 100,000 and the prevalence rate is 262.0 per 100,000 among U.S. black males, while, the incidence rate is 450 per 100,000 and the prevalence rate is 228.4 per 100,000 among the U.S. white males. CHD is less prevalent in females compared to males, the incidence and prevalence rate for black females is higher than white females (American Heart Association, NHLBI website). On the other hand, CHD mortality has declined faster in whites than in blacks, particularly for men. The underlying explanation of the disparities between blacks and whites in CHD prevalence and mortality rate is complicated, not easily untangled. It could be attributable to a net of individual risk factors, social economic status (SES) and the availability, acceptability and utilization of medical services. Compared to whites, blacks had higher a level of risk factors; for example, in previous epidemiological studies, blacks were found to have higher blood pressure(14) and glucose levels as well as higher prevalence of obesity and diabetes(15). Further, distinct racial differences are observed among the SES indicators. Blacks have been reported to be likely to have a lower school education, and decreased household income strata, and to work in the blue collar sector (16).

The pathological process in the cardiovascular system starts many years before the life-threatening events occur. Studies show strong evidence that the incidence of CVD is substantially increased for people with subclinical cardiovascular disease compared to those without subclinical disease (1). Racial difference is observed. African Americans have significantly lower amounts of coronary artery and carotid artery calcified plaque relative to whites despite having increased carotid intima-to-media wall thickness (IMT), measured by B-

mode Ultrasound examination and blood pressure(2). In contrast, African Americans were found to show greater arterial stiffness than Caucasians; such disparities occurred early in life (17). Arterial stiffness is the major component of cardiovascular disease. Increases in arterial stiffness increase the central systolic and pulse pressure, and decrease the perfusion pressure through the coronary arteries, therefore increasing the risk of coronary heart disease, myocardial infarction, and stroke. Pulse wave velocity is a well-established technique for assessing aortic stiffness, arteriosclerosis (18-20) or atherosclerosis (21-24) by measuring the velocity of the pulse wave between the carotid and femoral peripheral artery sites. The velocity of the pulse wave along an artery increases with increasing stiffness of that artery.

1.2.2 Peripheral Vascular Disease (PVD)

Peripheral vascular disease (PVD) is a slow and progressive circulation disorder, which involves diseases in any of the blood vessels outside of the heart vessels - the arteries, veins, or lymphatic vessels. When PVD occurs in the arteries outside the heart, it is referred to as peripheral arterial disease (PAD). PVD is caused by atherosclerosis, which is a progress of building up of fatty material within the vessels. Of the peripheral arteries, legs are most often affected. Other arteries frequently affected by atherosclerosis include those supplying blood to the kidneys or arms.

PVD is a very common condition in the U.S. It affects about 8 million Americans, about half are asymptomatic. PVD increases with age dramatically, affecting about 12 to 20 percent of the population by age 65. Diagnosis is critical, as people with PAD have much higher risk for heart attack or stroke. Detection can be relatively easy and inexpensive, based either on subjectively supplied historical information or upon physical examination. The ankle-brachial

index (ABI), the ratio of the ankle to brachial systolic blood pressure, is used to assess individuals with peripheral arterial disease. A ratio <0.90 indicates the presence of flow-limiting arterial disease affecting the limb(25). The ABI serves as a marker for increased risk for systemic vascular disease. It is a simple and noninvasive test that can be performed in the office or clinic setting. Epidemiology of risk factors for PVD is similar to coronary heart disease. Compared to women, men are slightly more likely to have PVD. Peripheral vascular disease is more common in smokers, and the combination of diabetes and smoking almost always results in more severe disease. Higher prevalence of lower extremity PAD in black populations has been documented, but the higher risk cannot be fully explained by differences in traditional cardiovascular risk factors (26). A recent study indicated that vitamin D deficiency may explain nearly one third of the excess risk of PAD in U.S. black compared with white adults (27).

1.2.3 **Chronic Kidney Disease (CKD)**

Chronic kidney disease (CKD), also known as **chronic renal disease**, is a progressive loss of renal function over a period of months or years through five stages. Each stage is a progression through an abnormally low and deteriorating glomerular filtration rate, which is usually estimated indirectly by the creatinine level in blood serum(28). Chronic kidney disease is a worldwide public health problem, with dramatically increasing incidence and prevalence. It is a highly costly disease, due to the number of patients with kidney disease who progress to kidney failure requiring dialysis or transplantation. Worldwide prevalence of kidney failure varies based on the availability and access to health resources, especially kidney replacement therapy, but is growing in most countries.

CKD is becoming a public health problem in the United States. The U.S. Renal Data System has declared that the number of persons with kidney failure requiring dialysis therapy or transplantation increased dramatically from 340,000 in 1999 to 398,000 in 2000, and a projected 651,000 patients in 2010(29). The estimated prevalence of CKD in the U.S. adult population was 11% (19.2 million), based on the National Health and Nutrition Examination Survey (NHANES) (30). For the analysis of NHANES III data, glomerular filtration rate (GFR) was estimated from serum creatinine concentration using a prediction equation derived from the Modification of Diet in Renal Disease (MDRD) Study, as demonstrated in Table 1.1 (31). An estimated 5.9 million individuals (3.3%) had stage 1 (persistent albuminuria with a normal GFR), 5.3 million (3.0%) had stage 2 (persistent albuminuria, GFR of 60 to 89 mL/min/1.73m²), 7.6 million (4.3%) had stage 3 (GFR, 30 to 59 mL/min/1.73 m²), 400,000 individuals (0.2%) had stage 4 (GFR, 15 to 29 mL/min/1.73 m²), and 300,000 individuals (0.2%) had stage 5, or kidney failure. For stage 1 and 2, kidney damage, assessed by spot albumin-to creatinine ratio > 17 mg/g (men) or >25 mg/g (women) based on one occasion, was 5.9% and 4.0% respectively, or 3.3% and 3.0% based on two measurements. The estimated population from stages 1-4 came from NHANES III (1984-1994) and stage 5 came from USRDS (1998). There are not yet projections for future prevalence of CKD.

Table 1.1 Prevalence of Stage of Chronic Kidney Disease and Levels of Kidney Function in the U.S.

Stages of CKD			Levels of Kidney Function		
	N(1000's)*	(%)	GFR (mL/min/1.73m ²)	N(1000's)*	(%)**
1	10,500 ^a 5,900	5.9 ^a 3.3	≥90	114,000	64.3
2	7,100 ^a 5,300	4.0 ^a 3.0	60-89	55,300	31.2
3	7,600	4.3	30-59	7,600	4.3
4	400	0.2	15-29	400	0.2
5	300	0.2	<15 (or dialysis)	300	0.2

* Data for stage 1-4 from NHANESIII (1988-1994). Population was 177 million with age ≥ 20 years. Data for stage 5 from USRDS (1998), includes approximately 230,000 patients treated by dialysis, and assumes 70,000 additional patients not on dialysis. Percentages total >100% are because NHANESIII may not have included patients on dialysis. GFR estimated from serum creatinine using MDRD study equation based on age, gender, race and calibration for serum creatinine.

** The reason for the percentage >100% is that NHANES III may not have included patients on dialysis. The GFR estimation was based on MDRD equation(32), using serum creatinine.

^a For stage 1 and 2, kidney damage was assessed by spot albumin-to creatinine ratio > 17 mg/g (men) or >25 mg/g (women) on one occasion (larger prevalence estimate) or on two measurements (smaller prevalence estimate).

Adapted from: K/DOQI Clinical Practice Guidelines for Chronic Kidney Disease: Evaluation, Classification, and Stratification

Although, the mortality rate of cardiovascular disease and all-cause death are significantly higher among African Americans than those for Caucasians(6, 7), black individuals with serious CKD or end stage renal disease (ESRD) have consistently shown a survival advantage compared to white individuals(8). It has been hypothesized that black individuals who live long enough to reach the serious stage of renal disease are healthier compared to white individuals(8). In contrast, higher mortality rates among younger black individuals could be attributed to factors other than biological differences, such as education, socioeconomic status and access-to-healthcare.

1.2.4 Metabolic Syndrome (MetSyn)

Metabolic syndrome (MetSyn) previously called insulin resistance syndrome or syndrome X, is characterized as clusters of risk factors including abdominal obesity, hypertension, dysglycemia and dyslipidemia (characteristically, increased triglyceride level and reduced high-density lipoprotein [HDL]). Metabolic syndrome and insulin resistance syndrome are two terms that have been used to characterize some of the abnormalities associated with insulin resistance and to recognize them as risk factors for future disease. (33) There are many published versions of the definition for MetSyn, varying combinations of above listed risk factors. Literature review indicates at least five published definitions (listed in Table 2), as proposed by the World Health Organization (WHO), National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) , European Group for the Study of Insulin Resistance (EGIR), American Association of Clinical Endocrinologists(AACE), and, most recently, the International Diabetes Federation (IDF) (34-39). The ATP III definition will be used in this proposal due to its wide acceptance, which facilitates the comparability of our findings to other

studies such as NHANES III. Six components of metabolic syndrome are included: abdominal obesity, atherogenic dyslipidemia, raised blood pressure, insulin resistance \pm glucose intolerance, proinflammatory state and/or prothrombotic state(34).

Rather than a single disorder or disease, MetSyn is relatively a new term, which has been used to describe a cluster of disorders that together increase the risk for diabetes and heart disease (4). Data from NHANES III (Third national Health and Nutrition Examination Survey) revealed the overall age-adjusted prevalence for MetSyn based on ATP III definition in the United States was about 24%, with the prevalence for each individual components being 38.6% for increased waist circumferences, 30.0% for hyper-triglyceridemia, 37.1% for lower HDL, 34% for hypertension and 12.6% for dysglycemia(40). However, variation in prevalence of metabolic syndrome across population is influenced by the use of different definitions, as well as by differences in distributions of individual metabolic traits across population. Different patterns of metabolic risk factor prevalence do exist across ethnic groups / populations.

The difference in prevalence of MetSyn components by race, age and gender has been documented. Based on ATP III definition, Mexican Americans had the highest age-adjusted prevalence of the metabolic syndrome (31.9%) compared to Caucasians (23.8%) and African Americans (21.6%). The prevalence increased with age, from 6.7% among young adults (20-29 years old) to 43.5% among aged 60-69 years and 42% among 70 and above decades. Among Caucasians, men and women have similar prevalence (24% for men vs. 23.4% for women). But, among African Americans, women had about 57% higher prevalence than men and among Mexican Americans, women had about a 26% higher prevalence than men(40).

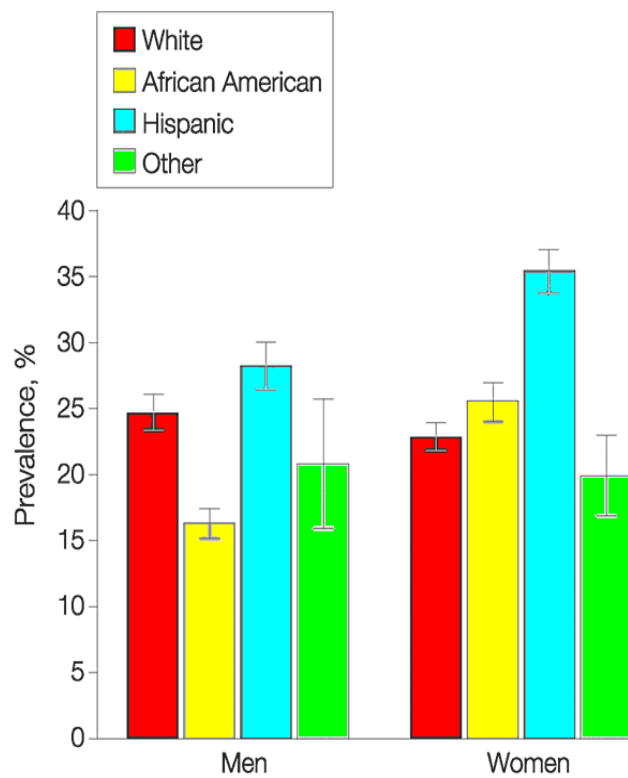


Figure 1.2 Age-Adjusted Prevalence of the Metabolic Syndrome Among 8814 US Adults Aged at Least 20 Years, by Sex and Race or Ethnicity, NHANES III, 1988-1994

Data are presented as: percentage (SE) (the figure was adapted from the reference (40))

The prevalence of metabolic syndrome is high among U.S. adults and increasing. The age-adjusted prevalence for MetSyn (using ATP III definition) was 24.1% (40) in NHANES III (1988-1994) and 27.0%(3) in NHANES III 1999-2000 respectively. The age-adjusted prevalence increased by 23.5% among women and 2.2% among men. For each individual component, high blood pressure, waist circumference and hypertriglyceridemia accounted for much of the increased prevalence rate, especially among women (3). The similar trend of increased prevalence rate for MetSyn was found in European countries (41, 42). The epidemic in childhood obesity results in a continuing increased prevalence of metabolic syndrome, and increased the risk for heart disease and diabetes as well (43). Thus, it is becoming a public health problem in the U. S. and worldwide.

Table 1.2 Comparison of Metabolic Syndrome

	WHO(44) (1998)	EGIR (36) (1999)	NCEP (34) (2001)	AACE(37) (2003)	IDF (45) (2006)
Minimum Criteria for Diagnosis	Impaired glucose tolerance or diabetes and/or insulin resistance and 2 other factors	Presence of fasting hyperinsulinaemia factors (the highest 25%) and 2 of the other factors	Three or more of the following factors	No defined number of risk factors is specified; diagnosis is left to clinical judgment	Central obesity and 2 other factors
Glucose /Insulin abnormality	Type 2 diabetes, impaired fasting glucose (FBG ≥ 6.1 mmol/l), impaired glucose tolerance (2hPPG ≥ 7.8 mmol/l) or lowest 25% for hyperinsulinemic euglycemia clamp-glucose uptake	Insulin resistance (highest quartile of population) and impaired fasting glucose (FBG ≥ 6.1 mmol/L)	Impaired fasting glucose (FBG ≥ 6.1 mmol/L)	2-Hour post glucose challenge >140 mg/dL or Fasting glucose Between 110 and 126 mg/dL	Fasting glucose ≥ 5.6 mmol/L or previous diagnosis of impaired glucose tolerance or diabetes
Obesity / Central Adiposity	Waist: hip ratio >0.9 (m) >0.85 (f) and/or BMI >30 kg/m ²	Waist circumference >94 cm (m) >80 cm (f)	Waist circumference >102 cm (m) >88 cm (f)	BMI ≥ 25 kg/m ²	Waist circumference ≥ 94 cm (European M), ≥ 80 cm (F); ≥ 90 cm (Asian M) ≥ 80 cm (F)
Increased triglyceride	TG >1.7 mmol/L	TG >2 mmol/L	TG ≥ 150 mg/dL (1.69 mmol/L)	≥ 150 mg/dL (1.69 mmol/L)	TG ≥ 1.7 mmol/L
Decreased HDLc	HDL <35 mg/dL (0.9 mmol/L)(m) <39 mg/dL (1.0 mmol/L)(f)	HDL < 1 mmol/L or on treatment	HDL < 40 mg/dL (1.04 mmol/L) (m) <50 mg/dL (1.29 mmol/L)(f)	<40 mg/dL (1.04 mmol/L) (m) <50 mg/dL (1.29 mmol/L) (f)	<1.04 mmol/L (m) <1.29 mmol/L(f)
Hypertension	Blood pressure $\geq 140/ 90$	Blood pressure $\geq 140/ 90$ or on treatment	Blood pressure $\geq 130/ \geq 85$ mmHg	Blood pressure $\geq 130/85$ mm Hg	SBP ≥ 130 or DBP ≥ 85 or treatment
Others	Microalbuminuria (≥ 20 ug/min albumin excretion rate or albumin:creatinine ratio ≥ 30 mg/g)	-	-	Family history of or high-risk ethnic group for diabetes, hypertension, CVD. Polycystic ovarian syndrome, sedentary lifestyle, aging	-

1.3 RISK FACTORS

1.3.1 Cardiovascular Disease

Cardiovascular disease is identified as a multifactorial disease with risk factors that cluster and interact in an individual to determine the risk level. To date, no single essential factor has been identified; multiple interrelated factors have been demonstrated to be associated with the risk of CVD. Extensive clinical and epidemiological studies have identified several risk factors that increase the risk of coronary heart disease and stroke. Individuals with certain characteristics are at higher risk for CVD compared to others. Above all, older age is a factor that contributes to disease and cannot be changed. For example, the mortality of CHD increases with age; 82% of people who die from CHD are 65 or older (AHA). Being a male gender is another unchangeable risk factor for CHD. Men have greater risk of heart attack than women, and tend to develop disease in an earlier age (46, 47). However, after menopause, the risk for CHD in women is increased, though the mortality rate is still lower than men (48-51). Heart disease has been found to be heritable, and clustered in families. Family history of CVD is not only a strong risk factor, but synergistic with other cardiovascular risk factors for facilitating disease development (52). Introduction of heritability will be discussed in the following section.

Except for those inherent personal characteristics, CVD is related to many modifiable, treatable or controllable factors which could be changed by lifestyle or medication. Since 1948 the Framingham longitudinal study has been committed to identify the common factors for CVD; and it has become a leader in the development and dissemination of multivariable statistical models to estimate the risk of coronary heart disease. As early as the 1960s, three important articles were published to identify five major risk factors for CHD in Framingham study (53-55).

In 1961, researchers found that cigarette smoking increased the risk of heart disease, the mortality rate of smokers was 2-3 times higher than non-smokers(53). Hypertension and hypercholesterol have been demonstrated to precede the development of CHD and associated with subsequent CHD development. High blood pressure increases the blood load and increases the risk for CHD 2.6-fold in men 40-60 years of age and 6-fold in women of the same age(54). Clearly, many clinic and epidemiological studies have demonstrated that hypertension, interrelated with lipids abnormalities and smoking, leads to vascular pathology and to disease development, which is also etiologically implicated by the epidemic of cardiovascular disease in developed countries decades ago(56).

Physical inactivity and obesity were also found to be associated with CHD risk(55). Regular moderate-to-vigorous physical activity helps prevent heart and blood vessel disease and relates to adequate collateral circulation (57). People who have excess body fat are more likely to develop heart disease and stroke even if they have no other risk factors (57). Excessive weight increases the heart's work and tends to raise the level of blood pressure, cholesterol and triglycerides(58), which contributes to another risk factor - diabetes. In contrast, physical activity can reduce the risk for type 2 diabetes by reducing insulin resistance and decreasing blood sugar level. In the long term, physical activity may reduce body weight and prevent adipose deposition. The increased demand of physical activity requires an increased glucose uptake by muscle cells, resulting in a reduced level of hyperglycemia(57).

Diabetes seriously increases the risk of developing cardiovascular disease. Diabetes and glucose tolerance as a risk factor for CHD have been intensively under research for decades (59-63). It has been reported that three-quarters of people with diabetes die of cardiac disorder or blood vessel disease (AHA report). There are other contributing factors that are associated with

increased risk for CHD, but the significance has not yet been precisely determined, such as stress and alcohol consumption.

1.3.2 Peripheral Arterial Disease

The prevalence of PAD steadily increases with age. With increasing life expectancy the prevalence of PAD is increasing. PAD is a manifestation of diffuse and severe atherosclerosis. It is a strong predictor of cardiovascular disease and other atherosclerotic disorders such as coronary artery disease (CAD) and cerebrovascular disease (CVD) (64).

Cigarette smoking, diabetes mellitus and advancing age are the critical risk factors. Smokers may have four-fold risk to develop PAD than nonsmokers. Physical inactivity is another risk factor of PAD that can be controlled. Studies showed that physical activity increases the distance that people with PAD can walk without pain and also helps decrease the risk of heart attack or stroke. In addition, high blood pressure and high cholesterol level contribute to build-up plaque, decreasing the blood flow, which eventually results in peripheral atherosclerosis. Thus, monitoring and controlling cholesterol level and blood pressure are essential to prevent and treat PAD patients (65).

1.3.3 Chronic Kidney Disease

The most common risk factors for chronic kidney disease (CKD) include diabetes, hypertension, cardiovascular disease(66), and a family history of CKD (67). Though obesity is a risk factor for incident diabetes, as well as cardiovascular disease, the interplay between the obesity and CKD has not been extensively evaluated. Recently, reports from the Cardiovascular

Health Study demonstrated a significant relationship between waist-to-hip ratio, but not body mass index (BMI), and incident CKD in a generalizable US cohort; and a significant relationship between waist-to hip ratio, but not BMI, with subsequent cardiovascular events and mortality among individuals with CKD(68). Moreover, findings from the Hisayama Study suggested that metabolic syndrome is a significant risk factor for the development of CKD in the general Japanese population (69, 70). But, such finding was not replicated in a study among a U.S. African American population, in which metabolic syndrome was found to be significantly associated with proteinuria in hypertensive African Americans, but not independently related to the progression of CKD(71).

In addition, age is also a risk factor for CKD. 11% of individuals older than 65 years without hypertension and diabetes suffered stage 3 or worse CKD(30) compared to 0.2% in the age group 20 to 39 years in the NHANESIII study. Further, the Census Bureau estimates that the proportion of individuals aged more than 65 years will increase from 12.6% (35 million in 2000) to 16.5% (54 million in 2030). CKD is bringing a huge economic burden to the society. The important risk factors are listed in Table 1.3. Major outcomes of CKD include progression to renal failure, development of complications of impaired kidney function and increased risk for cardiovascular system disorders (32, 72).

Table 1.3 Major Risk Factors and Contributing Factors for CHD, CKD and MetSyn

Coronary Heart Disease	Chronic Kidney Disease	Metabolic Syndrome
Major risk factors that can't be changed		
Increasing age	Older age	Age
Gender (male)	---	---
Heredity (including Race)	Family history of CKD	Race
Major factors that can be modified, treated or controlled by lifestyle changes or medication		
Obesity and overweight	---	Abdominal obesity
High blood cholesterol	High level of proteinuria	High Triglycerides, Low HDL cholesterol
High blood pressure	High blood pressure	High Blood pressure
Diabetes mellitus	Diabetes	High Fasting glucose
Physical inactivity	Autoimmune diseases, Systemic infections, Urinary tract infections, Urinary stones, Lower urinary tract obstruction,	---
Tobacco smoke	drug toxicity	---
Contributing factors		
Stress	Smoking	
Alcohol		

Major risk factors are those that research has shown **significantly increase the risk** of heart and blood vessel (cardiovascular) disease.

Contributing factors are **associated with increased risk** of cardiovascular disease, but their significance and prevalence haven't yet been precisely determined.

1.3.4 **Diabetes**

The clinical risk factors for type 2 diabetes include the following conditions: age, obesity, physical inactivity, race/geographical location, family history of diabetes and previous gestational diabetes. The major risk factors have been known for several years, such as obesity, sedentary lifestyle and genetic predisposition. These have been consistently implicated in among all studies in various populations.

Substantial evidence supported that obesity and weight gain were associated with increased risk of diabetes(73-75), and that weight loss reduced the risk of overweight people to develop diabetes(75). Obesity is commonly measured by body mass index (BMI), which averages the mass of an individual over an estimate of body area, in epidemiologic studies. However, body surface and measures of abdominal adiposity such as BMI and waist circumference, do not capture the relative contribution of subcutaneous and visceral fat to the total measure(76). Not only the amount of adipose tissue an individual has contributes to disease, but also where the fat is deposited. The most important distinction is whether it is deposited around/within the abdomen, typically seen in males, or along the hips and thighs, which is typically observed in females. To determine if individuals have an increased risk for disease due to fat distribution, waist to hip ratio has been used, with a ratio of greater than 1.0 in men and 0.8 in women putting individuals at higher risk respectively.

1.4 CYSTATIN C

Cystatin C is a non-glycated basic protein with a small molecular weight of 13.3 Kda. It belongs to the super family of cystatins, and it functions as a cysteine proteinase inhibitor (77). Unlike creatinine, which is a by-product of muscle cells and affected by many other factors than kidney disease, cystatin C is produced by all nucleated cells and excreted into the bloodstream at a constant level without impact of age, gender, race and muscle mass (78, 79).

1.4.1 Biomarker for Kidney Function

Glomerular filtration rate (GFR) is the gold standard measurement for kidney function, and widely accepted as the best overall test of kidney dysfunction. It is determined by the clearance of inulin or iothalamate, which is believed to be an ideal filtration marker since it is excreted from the body only through kidneys and fully excreted via glomerular filtration. However, the direct measurement of these clearances is cumbersome, requiring considerable time, money and is not easily implemented in daily clinic practice(80). To circumvent these drawbacks, the series of equations based on a traditional biomarker- serum creatinine provide the estimation of kidney function and has been widely used. The independent international Kidney Disease Improving Global Outcome organization (K/DOQI) provides two recommendations for detecting CKD: (1) eGFR as the best estimation of kidney dysfunction based on the simplified equation derived from the Modification of Diet in Renal Disease Study(MDRD): $GFR [mL \cdot min^{-1} \cdot (1.73 m^2)^{-1}] = 175 \times (serum\ creatinine [mg/dL]^{-1.154} \times age [years]^{-0.203} [\times 0.742, \text{ if female}] [\times 1.212, \text{ if African American}](81);$ and (2) determination of proteinuria, preferably microalbuminuria, corrected for urine creatinine (77). However, using serum creatinine as a

measurement to estimate GFR has several drawbacks, including the 'creatinine-blind range', which limits the utility of serum creatinine in detection of slight to moderate renal dysfunction (77) and some technical issues in assay measurements(77). Furthermore, serum creatinine is affected by other factors such as muscle mass, dietary intake, aging, and race(82). Due to these inconsistencies in serum creatinine estimation, the National Kidney Foundation concluded that serum creatinine should not be solely used to assess the level of renal function (83).

Cystatin C is an alternative measurement of kidney function and a large number of studies have shown that estimated glomerular filtration rate (eGFR) based on Cystatin C is superior to such estimation based on multiple serum creatinine measures (84). Numerous studies found that cystatin C levels were more accurate to predict GFR in various clinical presentations, especially in mild to moderate kidney disease patients (with a relatively higher eGFR)(85). Vikas et al conducted a meta-analysis and showed that cystatin C level is a significantly better biomarker of kidney function; they detected higher correlation coefficients between GFR and the estimated GFR based on cystatin C than serum creatinine(82). Zaharan et al collected 14 studies in kidney transplant patients and 29 studies among patients with native kidney disease to compare the performances of cystatin C vs. creatinine. The results demonstrated that 70% of studies performed on transplantation favored cystatin C, and 85% studies performed in native renal diseases showed a superiority of cystatin C based eGFR equations (86). Anders Grubb et al found a simple cystatin C based prediction equation for eGFR using only cystatin C concentration in mg/l and a prepubertal factor, if applicable, which is believed to perform equally well or better than the simplified MDRD formula (87).

1.4.2 **Biomarker for Cardiovascular Risk**

Cystatin C reflects inflammation, and is found to be related to C-reactive protein, which accounts for the association with cardiovascular disease (88). Many studies searched for such a relationship, and compared the performance between cystatin C and serum creatinine (89-93). Among these, some studies have examined the racial difference in association. Bibinns-Domingo et al conducted a community based study, of 1124 black and 1676 white adults. They found a stronger association between kidney dysfunction and heart failure among blacks than among the white population when using cystatin C as a biomarker (94).

Cystatin C was also found to be related to subclinical measurements for CVD. Results from Cardiovascular Health Study (CHS) indicated that elevated concentration of cystatin C was an independent predictor for incident PAD among elderly patients. The hazard ratio for participants with highest cystatin C (> 1.27 mg/L) was as high as 2.5 [95% confidence interval, 1.2 – 5.1] compared to those whose cystatin C level < 0.90 mg/L(95). Further, cystatin C was found to be related to arterial stiffness; a positive relationship between baPVW and cystatin C has been found independently of serum creatinine and eGFR among both men and women in a general Korean population(96). It will be meaningful to conduct such an association study among participants of African descent, since no similar studies have been reported among African Americans who are at much higher risk for cardiovascular disease. The proposed research will address this gap.

1.5 HERITABILITY

Heredity, as a risk factor, has been studied more extensively recently. Although the environmental factors have been established for decades, more recently, a number of genes have been reported to be associated with heart disease, stroke and high blood pressure in large population-based studies. A Danish twin study, including 1209 monozygotic and dizygotic twins, has found a substantial genetic influence on individual susceptibility to CHD after controlling for smoking and body mass index(97). Another large scale long term twin study conducted in Sweden, has also found a moderately large genetic variation in susceptibility to death from CHD. The heritability was 0.57 (95% CI, 0.45–0.69) amongst male twins, and 0.38 (0.26–0.50) amongst female twins(98). Regarding the subclinical cardiovascular measurement, the heritability of ankle brachial index (ABI), estimated in a Caucasian population, the Framingham Offspring Study, was about 27% (12). However, such findings have not been replicated in various ethnicity groups, especially among African American population, who were at higher risk for cardiovascular and renal disorders. Pulse wave velocity (PWV), which is a measurement of arterial stiffness, has also been confirmed to be heritable. The estimated heritability of radial and foot PWV was 0.43 and 0.53 respectively, without a detected difference between racial and gender groups(99).

Further, it has been established that genetic factors play a role in the progression of kidney disease; GFR and urinary albumin excretion (UAE) are known to be heritable. A recent genome-wide association study for kidney function has found that four SNPs were highly associated with serum cystatin C levels in whites (100). Although inheritance is a recognized determinant of cystatin C and kidney dysfunction, little is known about the genetic contributions to renal dysfunction in families of African descent. In addition to its relationship with renal

function, cystatin C has been reported to be associated with CVD risk factors among participants without chronic kidney disease. Cystatin C was a significant heritable trait with the multivariable-adjusted heritability of 0.35 ($p < 0.001$) in Framingham Offspring Study(101). It will be worthwhile to test the heritability of cystatin C in African Tobago population to inform future genetic studies to identify the loci related to kidney dysfunction in African individuals.

Metabolic syndrome, together with all its components, has been reported to be heritable. The Northern Manhattan Family study has documented the heritability of MetSyn, using ATP III definition itself, to be 24%, with significant heritability for lipid/glucose/obesity (44%) and hypertension (20%) among Caribbean-Hispanic families (11). Evidence for a genetic basis to type 2 diabetes includes family clustering of insulin sensitivity(102). Twin studies provided further evidence for genetic factors contributing to type 2 diabetes(103). There are many potential 'diabetic genes', such as candidate genes involving in regulating the secretion and action of insulin which may contribute to the disease development as reviewed by Permutt et al(104). However, no convincingly significant variations have been found among those genes investigated.

Furthermore, genetic association studies, by using case-control, cohort or family-based studies, relate genetic factors to heritable phenotypes. For example, ADIPOQ is one of the adipokine candidate genes. Its SNP276 has been found to be related to the reduced risk of obesity among Taiwanese individuals(105). And the adiponectin gene I164T polymorphism could be a stronger determinant of abdominal obesity with lower serum adiponectin levels among Japanese women(106). WNK4 (PRKWNK4) genes cause familial hypertension known as pseudohypoaldosteronism type II, which has been found to be involved in the development of

hypertension in Japanese general population(107). Thus, the variations associated with individual component phenotype could underlie an association with metabolic syndrome.

Although genetics play a critical role in the development of metabolic syndrome, genetic studies thus far provided conflicting association rather than consistent evidence. The apparent heritability of metabolic syndrome reflects the heritability of the individual risk factors included in the definition of metabolic syndrome. The dominance of each risk factor in the designation of metabolic syndrome differs across the ethnic/gender population, which explains the observed inconsistent heritability. The context-dependent factors, such as ethnicity, diet and gender could confound the association and affect the pathogenesis of the metabolic syndrome(108).

1.6 TOBAGO HEALTH STUDY POPULATION

Tobago is the smaller of the two main islands that make up the Republic of Trinidad and Tobago. It is located in the southern Caribbean Sea, northeast of the island of Trinidad and southeast of Grenada.

Tobago has a land area of 300 km² (116 mi²), and is approximately 42 kilometres (26 Miles) long and 10 kilometres (6 miles) wide. It is located at latitude 11° 9' N, longitude 60° 40' W, slightly north of Trinidad. The population is 54,084 (2000). The capital is Scarborough, with a population of about 17,000. While Trinidad is multiethnic, the population of Tobago is primarily of African descent, although with a growing proportion of Trinidadians of East Indian descent and Europeans. Tobago population is at high risk of chronic diseases such as hypertension, diabetes, cardiovascular disease and impaired kidney function. WHO statistics for Trinidad & Tobago document the highest rate of death due to CVD of any country outside the

former Soviet block. Furthermore, they live in a relatively isolated island with less westernized life style, are physically active, less fat intake and less medication intervention. Their genetic background is relatively homogenous compared to African Americans. A previous study based on ancestry informative markers estimated that older residents are 94% of West African origin (109). This population provides a special opportunity to look at the genetic impact of West African descent on the complex diseases.

The Tobago Family Health Study was initiated in 2003 by Dr. Joseph Zmuda as Principal Investigator. To be eligible, a proband must have been Afro-Caribbean, have had a spouse who was willing to participate in the study and have at least six living offspring and/or siblings aged 18+ years who were residing in Tobago. To date, 471 individuals, aged 18-103 years, are enrolled in this study. They belong to seven, multi-generation Afro-Caribbean families (mean family size 51 individuals) on the Tobago Island (Figure 1.3). Among these individuals, we have the 3535 relative pairs, including 361 parent-offspring, 495 full sibling, 101 grandparent-grandchildren, 1137 avuncular, 61 half sibs and 1380 cousins. The complete data for current analysis was available in 402 individuals. Written informed consent was obtained from every participant, using forms and procedures approved by the Tobago Division of Health and Social Services, and the University of Pittsburgh, Institutional Review Boards.

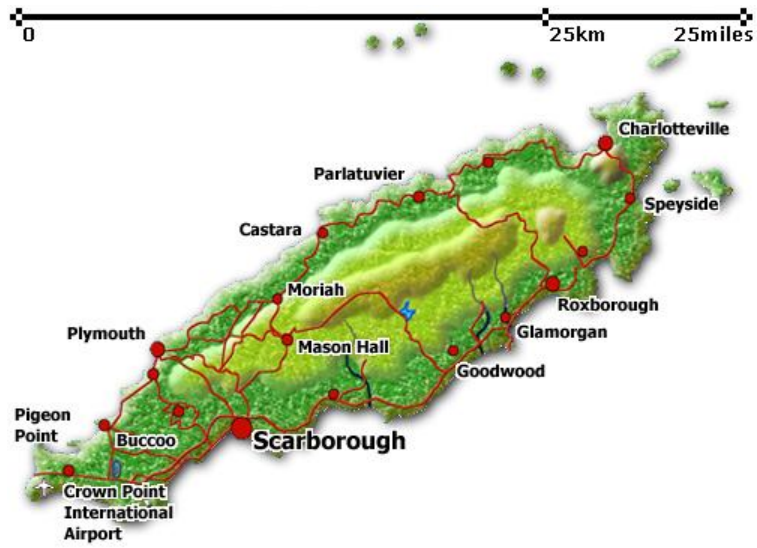


Figure 1.3 Map of Tobago Island

(The figure was adopted from the reference (110))

1.7 SUMMARY

Familial aggregation of major complex diseases is well known, and cardiovascular disease and chronic kidney disease have been reported to be heritable in previous studies(111). As a novel renal function biomarker, cystatin C was reported to be 35% heritable in the Framingham Offspring Study. The Northern Manhattan Family study has documented that the heritability of MetSyn was 24%, with significant heritability for lipid/glucose/obesity (44%) and hypertension (20%)(11). No previous study of metabolic syndrome or chronic kidney disease was published among African Caribbean populations. The proposed research will be the first to investigate the heritability estimation among multigenerational African Caribbean families, who are 94% of West African origin (109). The project will provide first hand evidence of disease occurrence as well as the heritability of metabolic traits among African Caribbeans which will benefit future studies.

In addition to estimating heritability, we are proposing to explore the association between renal function and subclinical cardiovascular disease, which process in the cardiovascular system starts many years before life-threatening events occur. With current techniques, subclinical CVD can be measured noninvasively and sensitively by the following laboratory examinations. (1) Pulse wave velocity (PWV) is a well established technique for evaluating arterial stiffness, or arteriosclerosis (18-20) and also is a marker for atherosclerosis (21-24). (2) Carotid intima-to medial wall thickness (IMT), a common assessment for atherosclerosis, is measured by B-mode Ultrasound examination of the carotid arteries. (3) Ankle brachial index (ABI), evaluated by a simple ratio of systolic blood pressure over the ankle and arm is a simple assessment for peripheral arterial disease.

Cystatin C is a novel biochemical marker for kidney function and has been reported to be superior to serum creatinine in estimating GFR(84). In addition, elevated cystatin C level is related to baPWV (96), peripheral arterial disease(95) and cardiovascular disease in absence of impaired kidney function(101). However, no previous study has comprehensively studied the association of renal function, with subclinical cardiovascular disease assessed by three measurements, in a population of African descent. The proposed study will fill the gap and become the first to conduct such association study among African descendants who are regarded to reflect the general healthy population on the Island of Tobago.

2.0 HERITABILITY OF KIDNEY FUNCTION BIOMARKERS IN AFRO-CARIBBEAN FAMILIES

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2.1 ABSTRACT

Objectives: Kidney disease is a prevalent clinical and public health problem among individuals of African ancestry. We assessed the prevalence and heritability of kidney function in families of Afro-Caribbean ancestry. **Design and Methods:** Serum creatinine and urinary microalbumin were measured in 402 adults from 7 large, multi-generation pedigrees (average family size: 50; range: 19 to 96; 3535 relative pairs) comprising a total of 402 Afro-Caribbean individuals, aged 18 to 103 years. Estimated glomerular filtration rate (eGFR, MDRD formula) and age-specific prevalence of decreased eGFR were calculated and compared to African Americans in the U.S. Heritability (h^2) was estimated with maximum likelihood methods using SOLAR (Sequential Oligogenic Linkage Analysis Routines). **Results:** The age standardized mean serum creatinine was 1.03 ± 0.01 mg/dL for Afro-Caribbean men and 0.82 ± 0.01 mg/dL for Afro-Caribbean women. Creatinine was significantly higher among older, diabetic and hypertensive participants. The eGFR was 100.6 ± 23.9 mL/min per 1.73 m^2 in men and 96.7 ± 27.4 in women, decreased across age groups and was significantly lower in diabetic, hypertensive and obese participants. The age standardized prevalence of decreased eGFR ($\text{eGFR} < 60 [\text{mL} \cdot \text{min}^{-1} \cdot (1.73 \text{ m}^2)^{-1}]$) was 8.9% among Afro-Caribbeans. Additive genetic effects accounted for: 28% of the variation in serum creatinine ($P < 0.0001$) adjusted for age, sex, anti-hypertensive treatment and low density lipoprotein cholesterol; 22% ($P < 0.0001$) of the variation in urinary albumin, adjusted for gender, and systolic blood pressure (SBP); and 19% ($P = 0.0007$) of the variation in urinary albumin to creatinine ratio, adjusted for gender, fasting glucose and SBP. **Conclusion:** A high prevalence of decreased eGFR was found in this relatively young sample of Afro-Caribbeans, which was associated with a high prevalence of hypertension and diabetes. Additive genetic effects accounted for a significant fraction of residual variation in several indices of kidney function.

Additional research is needed to identify the genetic factors which contribute to the high risk of kidney dysfunction in this Afro-Caribbean population.

Keywords: serum creatinine, renal dysfunction, heritability, Afro Caribbean, age standardized prevalence

2.2 INTRODUCTION

Chronic kidney disease (CKD) is a major public health problem, and is particularly prevalent among populations of African ancestry. Kidney dysfunction is associated with considerable morbidity including hypertension(112, 113), type 2 diabetes(114-116), cardiovascular disease(117), and increased risk of death(117). According to the NHANES III Survey, 5.5 million Americans have impaired kidney function, and more than 650,000 patients in the U.S. alone may require dialysis in 2010(83). Comparisons between African Americans and Caucasians indicate that the incidence of end stage renal disease (ESRD) related to diabetes is four times higher than that of Caucasians, and the prevalence of ESRD due to hypertension is two-fold higher than that of Caucasians(118). Chronic renal failure continues to be a major issue among African ancestry individuals living in other geographic regions such as the Caribbean (119), as it is internationally(120).

In 2002, the Kidney Disease Outcomes Quality Initiative (K/DOQI) of the National Kidney Foundation (NKF) defined CKD as either kidney damage, as evidenced by albuminuria, or by a glomerular filtration rate (GFR) below 60 ml/min/1.73 m² for ≥ 3 months irrespective of the cause (121). Serum creatinine is the most widely used measure of the presence and progression of CKD(122). The Modification of Diet in Renal Disease Study (MDRD) and the

Cockcroft Gault (CG) equations are the most frequently used assessments of the prevalence of CKD(123). These indirect estimates of GFR are critically dependent on the calibration and validation of the serum creatinine assay(124). The MDRD equation may outperform the CG equation (125) and has been applied in many epidemiological studies to investigate disease burden, such as Third National Health and Nutrition Examination Survey (NHANES III) in the U.S(81). However, serum creatinine concentration is influenced by many factors, including skeletal muscle mass, dietary intake, secretion by renal tubules and by some medications (126).

Previous epidemiological studies have reported that CKD may cluster in families, suggesting a genetic predisposition(127). CKD related traits such as elevated serum creatinine, serum creatinine clearance rate, eGFR and albuminuria are all highly heritable (128-130) in European ancestry individuals. Genome-wide linkage scans have detected chromosomal regions that may harbor CKD susceptibility loci (111). Other recent studies have identified *MYH9* as a possible candidate gene for CKD among African Americans (131, 132). *TCF7L2* alleles have also been related to reduced kidney function and CKD progression in both Caucasian and African ancestry individuals (133). However, the higher prevalence of CKD among persons of lower socio-economic status suggests that environmental factors may also contribute to this disease(127). Thus, a complex interplay between genetic and environmental factors plays a role in CKD.

The Afro-Caribbean population of Tobago is predominantly of West African origin. Previous studies using ancestry informative molecular markers have documented lower admixture (6% non-African) in this population compared with African Americans(109). In Trinidad and Tobago, 20 % of renal failure occurs among the working age population (119). Studies in other populations of African descent have documented an elevated prevalence of risk

factors for CKD such as diabetes mellitus and hypertension(119). However, to our knowledge, there is no estimation of the prevalence of decreased eGFR and albuminuria in African ancestry populations living in the Caribbean. The purpose of this study was to estimate the prevalence and heritability of renal impairment among Afro-Caribbeans living in Tobago.

2.3 METHODS

2.3.1 Study Population

The current study was conducted in 402 individuals aged 18 – 103 years (mean age 42 years) belonging to seven, multi-generation Afro-Caribbean families (mean family size 51 individuals) on the Tobago Island (99). Briefly, to be eligible, a proband must have been Afro-Caribbean, have had a spouse who was willing to participate in the study and have at least six living offspring and/or siblings aged 18+ years who were residing in Tobago. Participants were recruited without regard to health status. Among these individuals, there are 3,535 relative pairs, including 361 parent-offspring, 495 full sibling, 101 grandparent-grandchildren, 1137 avuncular, 61 half sib and 1380 cousin pairs. Complete data on kidney function measures for current analysis was available in 402 individuals. Written informed consent was obtained from every participant, using forms and procedures approved by the Tobago Division of Health and Social Services, and the University of Pittsburgh, Institutional Review Boards.

2.3.2 Covariate Measurements

Information on demographic characteristics, medical history, and lifestyle habits was obtained by questionnaire and interview by trained and certified staff. Race/ethnicity was based on self-declaration, and participants provided detailed information on the ethnic origin of their parents and grandparents. Respondents were assigned to an ethnic group if they reported that all four grandparents belonged to that group. Subjects were classified as current smokers (yes/no). Participants who had smoked <100 cigarettes in their lifetime were considered nonsmokers. Information on alcohol consumption was obtained by questionnaire and expressed as drinks per week. Subjects were asked if they walk for exercise (yes/no). Physical activity was also assessed as a continuous variable by the number of minutes walked, and physical inactivity as the hours spent watching television per week. Participants were asked to bring prescription medications to the clinic for verification. Current use was defined as use within the preceding 30 days. A study-specific medication dictionary was used to categorize the type of medication from product brand and generic names obtained from the medication containers. Blood pressure was measured three times using an automated blood pressure machine (Omron model HEM-705CP, Illinois). The average systolic blood pressure and diastolic blood pressure was calculated based on the average of second and third measurements. Metabolic traits, including triglyceride, total serum cholesterol level, low density cholesterol (LDL), high density cholesterol (HDL), fasting insulin and fasting glucose, were measured with standard protocols.

2.3.3 Serum and Urine Sample Collection

Blood samples were obtained by venipuncture in the morning after a 12-hour fast. Whole blood sat at room temperature for a minimum of 20 min to clot before centrifugation. A spot urine sample was also collected. Aliquots of serum and urine were frozen at -20°C locally and shipped on dry ice to the University of Pittsburgh by express courier within a month where the samples were stored at -80 °C.

2.3.4 Laboratory Measurements

Serum creatinine was quantitatively determined by the VITROS CREA Slide method. The sample was diluted with VITROS 7% BSA. Standards, serum controls and duplicate samples were run with each assay, which were traceable to a Gas Chromatography Isotope Dilution Mass Spectrometry (GC/IDMS) method and National Institute of Standards and Technology (NIST) SRM®914 creatinine standard reference material. Creatinine values traceable to this standard, and the results, are referred to as standardized creatinine values. Urine samples were diluted with 1:1 reagent-grade water, and urinary creatinine was analyzed as above.

Albumin in urine was measured using a turbidimetric procedure on the Olympus AU400 using reagents provided by Olympus America, Inc. (Center Valley, PA). 10 µl of urine was incubated with goat anti-human albumin antibody for 5 minutes at room temperature. The resulting turbidity was measured at 340/800 nm. The procedure was linear from 0.5 – 30 mg/dL. Blanks, calibrators and control pools were run simultaneously with all samples. The intra- and inter-assay coefficients of variation were below 2.5% and 5.1%, respectively.

2.3.5 Disease Definition

Hypertension was defined as diastolic blood pressure ≥ 90 mmHg, systolic blood pressure ≥ 140 mmHg, or currently taking blood pressure medication (134). Diabetes was defined as fasting glucose level ≥ 126 mg/dl or currently taking diabetes medication (135). Obesity was defined as BMI ≥ 30 kg/m². Chronic kidney disease (CKD) was defined as eGFR based on creatinine (eGFR < 60 mL/min per 1.73 m²) (83). The spot albumin-to-creatinine ratio (ACR) was calculated and reported in milligrams per gram. According to the American Diabetes Association (ADA) (136) and National Kidney Foundation (137), albuminuria was defined as ACR ≥ 30 mg/g; microalbuminuria: 30-299 mg/g and macroalbuminuria: ≥ 300 mg/g.

2.3.6 Statistical Methods

Before analysis, the distributions of serum creatinine, urinary albumin and urinary creatinine were assessed for normality. Outliers (defined as ± 4 SD) were removed from further analyses. No more than four values were removed for a single variable. The simplified Modification of Diet in Renal Disease Study (MDRD) equation was used to estimate glomerular filtration rate based on serum creatinine: $GFR [mL \cdot min^{-1} \cdot (1.73 m^2)^{-1}] = 175 \times (\text{serum creatinine } [mg/dL]^{-1.154} \times \text{age } [years]^{-0.203} [\times 0.742, \text{ if female}] [\times 1.212, \text{ if African American}]$ (81). Age was included in years and serum creatinine in mg/dl.

The age-specific prevalence of decreased eGFR and the age-specific standardized serum creatinine concentration for African Americans in the U.S. were calculated from the NHANES III 2005-2006 publicly available dataset (http://www.cdc.gov/nchs/nhanes/nhanes2005-2006/quex05_06.html). Serum creatinine concentration was standardized using an established

formula: Standardized creatinine (mg/dL) = $-0.016 + 0.978 * (\text{NHANES 05-06 uncalibrated serum creatinine, mg/dL})$ (138). We compared the age-adjusted prevalence of impaired kidney function in the Afro-Caribbeans with that observed in African Americans in NHANES III by direct age standardization with the U.S 2000 standard population.(139). The age-adjusted mean concentration of serum creatinine was calculated using standard age groups based on the U.S. 2000 standard population, accounting for the unequal probability of sampling or non-response(140). The analyses were performed in SAS (SAS Institute Inc., SAS® 9.2).

The heritability of renal function measures was assessed in SOLAR (Sequential Oligogenic Linkage Analysis Routines), after removing the effects of significant covariates. The covariates were evaluated first using multivariate linear regression modeling at a significance level of <0.10 . Second, potential covariates were evaluated using a variance component approach implemented in SOLAR, which accounted for the effects of relatedness of family members. Briefly, the variance components approach involves partitioning the variance of a quantitative trait into components attributable to individual-specific covariates, an additive genetic (polygenic) component and a residual non-measured environmental component. The significance of a particular independent variable (e.g., fasting glucose) was assessed by the likelihood ratio test, which compared the likelihood of a full model (e.g., age, BMI, and fasting glucose) to that of a nested model (e.g., age and BMI only, with the glucose effect constrained to be zero). Residual heritability was calculated as the proportion of the total phenotypic variance explained by additive genetic effects after accounting for covariates.

2.4 RESULTS

2.4.1 Characteristics of Study Participants

Characteristics of the 402 family members are shown in Table 1. BMI and obesity were higher ($p < 0.001$) in women than men, but waist circumference was similar. Prevalence of cigarette smoking and alcohol consumption was higher among men than women. The prevalence of hypertension and diabetes was similar in men and women.

2.4.2 Serum Creatinine by Age and Gender

Serum creatinine was not normally distributed. Among all family members aged 18 years and older, the median serum creatinine level was lower in women (0.81 mg/dL) than in men (1.01 mg/dL). The mean concentration of serum creatinine increased with age in both men and women (Table 2). Serum creatinine levels were significantly elevated in persons 40 years or older among both genders compared with those aged less than 40 years.

The age-adjusted standardized mean serum creatinine level was 1.03 ± 0.01 mg/dL for Afro-Caribbean men and 0.82 ± 0.01 mg/dL for Afro-Caribbean women. The age-adjusted calibrated serum creatinine level for African American men was 1.14 ± 0.04 mg/dL and 0.83 ± 0.02 mg/dL for African American women in the U.S. NHANES III (138). The mean serum creatinine level was adjusted for age groups based on the U.S. census standard population for the year 2000 (141). The comparison between Afro-Caribbeans and the U.S. black population indicates that Afro-Caribbean men have lower creatinine levels than U.S. blacks; but Afro-Caribbean women have higher levels than U.S. black women (data not shown).

2.4.3 Estimated GFR by Age and Gender

The estimated GFR calculated using the simplified MDRD equation was $98.2 \text{ [mL} \cdot \text{min}^{-1} \cdot (1.73 \text{ m}^2)^{-1}]$ (SD: 26.13) in the total sample (Table 2). Men had higher eGFR compared to women except in the youngest age group (<20 years). Altogether, 27 participants had eGFR less than $60 \text{ [mL} \cdot \text{min}^{-1} \cdot (1.73 \text{ m}^2)^{-1}]$; 24 (19 women and 5 men) had eGFR between 30 and $60 \text{ [mL} \cdot \text{min}^{-1} \cdot (1.73 \text{ m}^2)^{-1}]$; and 3 women had eGFR less than $30 \text{ [mL} \cdot \text{min}^{-1} \cdot (1.73 \text{ m}^2)^{-1}]$.

2.4.4 Prevalence of Decreased eGFR and Albuminuria

The prevalence of decreased eGFR increased with age among Afro-Caribbean men and women. For example, the prevalence of decreased eGFR was 0.6% among Afro-Caribbeans aged 20 to 39 years, 2.1% among those aged 40 to 59 years, 23.8% among those 60 to 70 years and 41.9% among those who were older than 70 years (Table 3). The age-standardized prevalence of decreased eGFR among Afro-Caribbeans in Tobago was $8.9\% \pm 1.4\%$, which was higher than that in U.S African American population, estimated in NHANES III ($6.9\% \pm 0.8$) (Table 3), although this difference did not reach statistical significance.

Afro-Caribbean women had significantly higher urinary albumin and ACR compared to Afro-Caribbean men ($p < 0.0001$). Table 4 shows the age-specific distribution of ACR. The prevalence of albuminuria (both micro- and macro- albuminuria) increased with age, and was higher in women compared to men in each corresponding age group. Overall, the age standardized prevalence of albuminuria was lower in Afro-Caribbeans than African Americans NHANES III in 2005-2006 ($9.2\% \pm 1.5$ vs. $14.5\% \pm 1.2$) (Table 4). However, Afro-Caribbean

women aged 60 and above appeared to have worse kidney damage compared to African American women of similar age.

2.4.5 Heritability of Kidney Function

Serum creatinine, urinary albumin and urinary albumin to creatinine ratio were all significantly heritable in these Afro-Caribbean families (Table 5). Age, sex, anti-hypertensive medication use (yes/no), serum LDL-C level, diabetes status (yes/no) and smoking status (yes/no) were considered as potential covariates of serum creatinine. After accounting for family relatedness, diabetes ($p=0.23$) and smoking status ($p=0.08$) were not significantly correlated with serum creatinine. The residual heritability of serum creatinine was 0.28 ± 0.10 ($P<0.0001$), simultaneously adjusting for age, sex, anti-hypertensive treatment and serum LDL-C (all covariates had $P<0.01$). Sex, fasting glucose and systolic blood pressure were considered as potential covariates for urinary albumin. The residual heritability of urinary albumin was 22% ($P=0.0006$) after simultaneously accounting for sex and systolic blood pressure. The additive genetic effects accounted for 19% ($p=0.0007$) of the urinary albumin to creatinine ratio, after adjusting for sex, glucose and systolic blood pressure. Body mass index and alcohol consumption were not related to any of the renal function measures.

2.5 DISCUSSION

The age standardized prevalence of decreased eGFR was 8.9% (± 1.4) among Afro-Caribbeans and 6.9% (± 0.8) among African Americans. A higher proportion of Afro-Caribbeans had decreased eGFR compared to African Americans, but the difference did not reach statistical significance due to the relatively small sample of Afro-Caribbeans. Kidney function decreased in a similar pattern among African Americans and Afro-Caribbeans until they reached age of 60. After age 60, Afro-Caribbeans had worse kidney function especially among women.

As reported previously (142), we found increased age and female gender were significantly and positively related to CKD. No participants had low eGFR before age 20 years whereas kidney function was dramatically decreased after age 40 years. As in the U.S., 11% of individuals older than 65 years without hypertension or diabetes had stage 3 or higher CKD(30) compared to 0.2% in the age group 20 to 39 years. Kidney damage, as reflected by microalbuminuria or macroalbuminuria, was also more prevalent among the older age groups. Compared to men, decreased kidney function was much more prevalent among women. The underlying reasons accounting for this gender difference are unclear. A possible explanation could be the higher prevalence of hypertension and diabetes among Afro-Caribbean women compared to men aged 60 and older in Tobago (data not shown). Both hypertension and diabetes are associated with decreased renal function and albuminuria (142).

In addition to reflecting creatinine excretion by the kidneys, serum creatinine is affected by other factors such as muscle mass(143), dietary intake, aging, secretion by renal tubules(144), and race(82). Differences among clinical laboratories in the calibration of serum creatinine may account for as much as a 20% error in the eGFR, especially among individuals with mild impaired kidney function(145). Due to these inconsistencies in serum creatinine measurement,

the National Kidney Foundation concluded that serum creatinine should not be solely used to assess the level of renal function (83). Current guidelines for detecting CKD recommend the use of eGFR from serum creatinine measurements using prediction equations such as MDRD and Cockcroft–Gault (145). Compared to the Cockcroft-Gault equation, MDRD has been reported to be more accurate and precise. The MDRD equation was developed in a large dataset (n>1000) that included both black and white individuals and was validated in an additional 500 patients(146). However, in addition to assumptions of similar muscle mass between populations, using serum creatinine to estimate GFR has several technical issues (77), which limits its use for detecting slight to moderate renal dysfunction (77). Also, there is concern regarding the application of the MDRD equation to different populations. Population-specific correction factors for CKD may be more appropriate. For example, the original MDRD equation has been modified with different coefficients when applied to the Japanese or Chinese population (147, 148). Even though MDRD has a correction coefficient for the African American population, the adjustment for gender and ethnicity might not be sufficient for Afro-Caribbeans. Body composition studies have found a higher proportion of lean muscle mass to total body mass in Afro-Caribbean men compared with U.S. men(149). Thus, the MDRD formula may not be appropriate for use in this population with high muscle mass, since the MDRD formula may overestimate impaired kidney function in Afro-Caribbean men, but not in women.

Our data suggest that the age adjusted mean standardized serum creatinine level in Afro Caribbean men was lower than the African American men, but higher in Afro-Caribbean women than the African American women. As in other populations, higher mean serum creatinine level was observed in men compared to women in each age group, which may be attributable to greater muscle mass in men. Ethnic differences in creatinine distribution have been reported in

the U. S. previously; non-Hispanic blacks have higher levels, and Mexican Americans have lower levels compared to Caucasians (126). However, the underlying reasons for such differences among ethnic groups are unknown. The differences may be the result of physiological differences such as muscle mass, renal tubular structure, or the different consequences of hypertension and diabetes.

Our analyses revealed a significant heritability of renal function measures in multi-generational Afro-Caribbean families. There are limited data on the heritability of renal function in blacks. Our findings support a significant genetic contribution to renal function in Afro-Caribbeans for serum creatinine, urinary albumin and the albumin to creatinine ratio. The heritability of serum creatinine in the Framingham Heart Study offspring cohort, in which 1224 Caucasian individuals from 330 families were analyzed, was 0.29 (128). A highly significant heritability for kidney function has also been found in diabetic(130), hypertensive (150, 151) and Hispanic individuals (152). Furthermore, previous genome wide linkage scans have found suggestive evidence (LOD score > 2) for loci linked to kidney function on chromosomes 2 (153), 3 (128) and 4(128). Thus, our significant heritability estimates of serum creatinine, eGFR and urinary albumin in families of African ancestry confirm a familial aggregation of renal function and justify further analyses aimed at understanding the genetic determinants of renal function in this high risk ethnic group.

Our study has potential limitations. Firstly, our definition of CKD relied on eGFR, rather than direct measurements of GFR. Equations for estimating GFR have limited precision and accuracy compared with directly measured GFR, especially among persons with mild kidney impairment. Our sample size was also relatively small. In addition, we estimated eGFR and prevalence of CKD from seven multigenerational families. The probands and family members

were recruited without regard to their health status and all participants were ambulatory. Therefore, we may have underestimated the prevalence of impaired kidney function in this population. Nonetheless, the data provide preliminary estimates of the prevalence of impaired kidney function in this Afro-Caribbean population.

In conclusion, among these African ancestry families, the serum concentration of creatinine and the age standardized prevalence of decreased eGFR were higher than in the African American population. The significant heritability of serum creatinine, eGFR and urinary albumin indicate a familial aggregation of these traits and justify future efforts to understand the genetic determinants of renal dysfunction in Afro-Caribbeans.

Table 2.1 Demographic Characteristics of Afro-Caribbean Family Members

Characteristics	Men	Women
N	156	246
Age (Mean \pm SD)	42.1 \pm 16.9	42.6 \pm 17.3
Anthropometric (Mean \pm SD)		
BMI	26.7 \pm 5.1	29.2 \pm 7.0 ***
Height	177.2 \pm 7.3	166.6 \pm 6.5 ***
Weight	84.1 \pm 17.9	81.1 \pm 19.6
Waist	90.2 \pm 12.7	89.8 \pm 17.3
Lifestyle Characteristics		
Current Smoking N (%)	17 (11.0)	1 (0.4) *
Alcohol consumption N (% >1 drink per day)	44 (28.4)	5 (2.0)
Walking per week (mins)	50.3 \pm 69.9	47.0 \pm 116.5
Medical Conditions: N (%)		
Hypertension †	46 (30.1)	68 (28.3)
Obesity ††	31 (20.0)	109 (44.7) ***
Diabetes †††	17 (11.0)	42 (17.4)
Kidney Function Measurements: (Median [Range])		
Serum Creatinine (mg/dL)	1.01 [0.5-1.8]	0.81 [0.5-2.1] ***
Urinary Albumin (mg/dL)	0.3 [0.1-47]	0.8 [0.1-128] ***
Albumin-to-Creatinine Ratio (mg/mmol)	1.3 [0.2-215]	3.8 [0.2-540] ***
Estimated GFR based on MDRD equation	100.8 [44.1-229.6]	95.4 [27.9-192.6] ***

* Comparison by gender is statistically significant * p<0.05, ** p<0.01, *** p<0.001;

† Hypertension was defined as a diastolic blood pressure \geq 90 mmHg, systolic blood pressure \geq 140 mmHg, or currently taking blood pressure medication.

†† Diabetes was defined as fasting glucose level \geq 126 mg/dl or currently taking diabetes medication.

††† Obesity was defined as BMI \geq 30.

Table 2.2 Serum Creatinine and Estimated GFR in Aro-Caribbean Family Members by Age

Age (years)	Men			Women		
	N	Creatinine (mg/dL)	MDRD_eGFR	N	Creatinine (mg/dL)	MDRD_eGFR
Overall	155	1.04±0.18	106.6±23.9	243	0.86±0.24	96.7±27.4
<20	10	0.95±0.09	126.6±14.3	14	0.72±0.10	130.5±24.5
20-39	61	0.98±0.13	113.4±22.6	99	0.77±0.13	111.9±21.9
40-59	54	1.06±0.14	92.3±14.9	88	0.84±0.15	89.5±15.4
60-69	17	1.10±0.23	85.51±19.4	24	1.05±0.28	66.4±22.6
70+	13	1.24±0.30	73.0±18.4	18	1.27±0.49	58.3±24.8

* **Estimated GFR using MDRD equation:** $\text{GFR [mL} \cdot \text{min}^{-1} \cdot (1.73 \text{ m}^2)^{-1}] = 175 \cdot \text{standardized Serum creatinine}^{-1.154} \cdot \text{age}^{-0.203} \cdot 1.212 \text{ [if black]} \cdot 0.742 \text{ [if female]} (81)$.

Table 2.3 Comparisons of Age Standardized Decreased eGFR between Afro-Caribbeans and the U.S. Population (NHANES III)

	Afro-Caribbean Families						African American (NHANES III 2005-2006) ²					
	Overall		Male		Female		Overall		Male		Female	
	N	%	N	%	N	%	N	%	N	%	N	%
Age standardized CKD prevalence ³												
Total	395	8.9	---	---	---	---	1108	6.9	---	---	---	---
Age Group												
<20	24	0	10	0	14	0	193	0	102	0	91	0
20-39	160	0.6	61	0	99	1.0	366	0.8	168	1.2	198	0.5
40-59	142	2.1	53	0	89	3.4	325	3.0	152	2.6	173	3.3
60-69	42	23.8	17	11.8	25	32.0	138	18.3	73	20.7	65	15.6
70+	31	41.9	13	23.1	18	55.6	86	25.9	49	19.7	37	32.7

¹. Estimated GFR, calculated by MDRD equation: $GFR [mL \cdot min^{-1} \cdot (1.73 m^2)^{-1}] = 175 * \text{standardized Serum creatinine}^{-1.154} * \text{age}^{-0.203} * 1.212 [\text{if black}] * 0.742 [\text{if female}]$. Decreased eGFR was defined as eGFR < 60 mL/min per 1.73 m².

². The disease prevalence among the African American population was estimated from NHANES III 2005-2006. Serum creatinine concentration was standardized with the following formula: Standard creatinine (mg/dL) = $-0.016 + 0.978 * (\text{NHANES 05-06 uncalibrated serum creatinine, mg/dL})$ (138).

³. Direct Standardization calculates a weighted average of the region's age-specific prevalence where the weights represent the age-specific sizes of the standard population.

Table 2.4 Categories of Urinary Albumin-to-Creatinine Ratio by Age Group in Afro-Caribbean Families

	Afro-Caribbeans Families			African Americans in NHANES 2005-2006		
	N	Micro-albuminuria N (%)	Macro-albuminuria N (%)	N	Micro-albuminuria N (%)	Macro-albuminuria N (%)
Age Standardized ¹	402	9.2%		1260	14.5%	
Men						
<20	10	0	0	107	3 (2.8)	0 (0)
20-39	63	1 (1.6)	0	185	5 (2.7)	4 (2.2)
40-59	53	2 (3.8)	0	168	12 (7.1)	5 (3.0)
60-69	17	1(5.9)	0	95	24 (25.3)	7 (7.4)
70+	13	2 (15.4)	0	62	14 (22.6)	3 (4.8)
Women						
<20	14	2 (14.3)	0	98	13 (13.3)	0 (0)
20-39	100	5 (5.0)	0	218	22 (10.1)	1 (0.5)
40-59	89	6 (6.7)	2 (2.2)	193	26 (13.5)	5 (2.6)
60-69	25	4 (16.0)	2 (8.0)	80	13 (16.3)	5 (6.2)
70+	18	6 (33.3)	1 (5.6)	54	10 (18.5)	5 (9.3)

¹ Direct Standardization calculates a weighted average of the region's age-specific prevalence where the weights represent the age-specific sizes of the standard population.

* Albuminuria was defined by ADA(136) and the National Kidney Foundation(137), as Normal Albumin creation ratio (ACR): <30mg/g; Albuminuria: ACR \geq 30 mg/g; Microalbuminuria: 30-299 mg/g; Macroalbuminuria: \geq 300 mg/g.

Table 2.5 Results of Heritability Estimation for Kidney Function

Trait	Covariate	Residual Heritability (hr ²)	Proportion of variance due to covariates
Serum Creatinine ¹	Age ^{***} , sex ^{***} , antihypertensive medication ^{**} , LDL ^{**}	0.28±0.10 ^{***}	0.46
Urinary Albumin ¹	Gender ^{***} , and Systolic blood pressure [*]	0.22±0.08 ^{**}	0.11
Urinary Albumin to creatinine ratio ¹	Gender ^{***} , fasting glucose ^{**} and Systolic blood pressure ^{**}	0.19±0.08 ^{**}	0.17

¹ Measurements of kidney function and BMI were log transformed;
Significance level: *P<0.05, ** P<0.01, ***P<0.0001,

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3.0 HERITABILITY ESTIMATION OF METABOLIC TRAITS IN AFRO-CARIBBEAN FAMILIES

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3.1 ABSTRACT

Background: The public health burden of metabolic syndrome is considerable. Metabolic syndrome is defined by a cluster of risk factors, elevated blood pressure, dyslipidemia, central obesity, and glucose intolerance, which increase cardiovascular disease and diabetes risk. African Americans are at higher risk for hypertension and diabetes, but lower risk for metabolic syndrome, compared to U.S. Caucasians. The aim of this study is to characterize the metabolic syndrome in a sample of Caribbean African-origin families and estimate the heritability of metabolic risk factors to better understand the underlying pathophysiological mechanisms.

Method: Components of metabolic syndrome: lipids, central obesity, fasting glucose and insulin and blood pressure were measured among 401 individuals aged 18 to 103 years (mean age: 42.4 ± 16.9 years) from seven multigenerational African Caribbean pedigrees (average family size: 50; range: 19 to 96; nearly 3500 relative pairs. Metabolic syndrome was defined according to the World Health Organization (WHO), International Diabetes Federation (IDF), and National Cholesterol Education Program Expert Panel and Treatment of High Blood Cholesterol in Adults (Adult Treatment panel III) (NCEP/ATP III) definitions. Heritability (h^2) was estimated with maximum likelihood methods. To obtain the structures underlying metabolic syndrome, factor analysis was performed in SAS (9.2). **Results:** Among these Afro-Caribbeans, 9.4% (11.3% women and 5.4% men), 18.7% (23.9% women and 10.7% men), and 25.3% (30% women and 18% men) had metabolic syndrome using WHO criterion, NCEP/ATP III and IDF definition, respectively. The heritability of each component ranged from 21% for large waist circumference to 46% for HDL-cholesterol ($P < 0.05$). Factor analysis of the components produced two major factors: factor 1, comprised of lipids, central obesity and fasting glucose and insulin, was 29% heritable ($p = 0.0001$), and factor 2, comprised of systolic and diastolic blood pressure, was 20%

heritable ($p=0.002$). **Conclusion:** Moderate and significant heritability of metabolic syndrome and each of its components was estimated among these African Caribbean families. Future studies are needed to identify underlying susceptibility genes for specific components of metabolic syndrome.

3.2 INTRODUCTION

Metabolic syndrome (MetSyn) is a clustering of risk factors, central obesity, hypertension, dyslipidemia, hyperinsulinemia and diabetes, which increase the risk of developing cardiovascular disease and diabetes. There are many published versions of the definition for MetSyn, with varying combinations of risk factors, as proposed by the World Health Organization (WHO), National Cholesterol Education Program Adult Treatment Panel III (NCEP/ATP III) , European Group for the Study of Insulin Resistance (EGIR), American Association of Clinical Endocrinologists (AACE), and the International Diabetes Federation (IDF)(34-39). The prevalence of metabolic syndrome is high among U.S. adults and increasing in both men and women. The age-adjusted prevalence for MetSyn (defined by ATP III) was 24.1% in NHANES III (1988-1994) (40), 27% in NHANES III 1999-2000(3) and 34% in NHANES 2003-2006 (154). High blood pressure, waist circumference and hypertriglyceridemia accounted for much of the increased prevalence rate (3). The prevalence of metabolic syndrome varies among ethnic groups. Mexican Americans have the highest age-adjusted prevalence of the metabolic syndrome (31.9%) compared to Caucasians (23.8%) and African Americans (21.6%) (40). MetSyn is the focus of intense research due to its association with adverse complications such as cardiovascular disease (4), stroke (155) and diabetes(156).

The lack of consensus on definition leads to the debates on whether there is genetic basis for MetSyn. Indeed, growing evidence from families and twin studies has yielded significant heritability estimations for the individual components of the metabolic syndrome such as obesity, insulin resistance, dyslipidemia and hypertension (157-160). Since metabolic syndrome is characterized by constellation of highly intercorrelated quantitative phenotypes, factor analysis and principle component approach have been used to simplify the syndrome and have potentially

provided new insights into the pathophysiology of the disease (161-164). The Northern Manhattan Family study investigated Caribbean-Hispanic families and demonstrated 24% heritability for metabolic syndrome itself, individual five components from 16 to 60% and two independent factors (44% for lipids/glucose/obesity, and 20% for blood pressure)(11).

While cardiovascular disease (CVD) rates specific to the Caribbean Africans residing in the island of Tobago are not available, the Trinidad & Tobago government has classified CVD as one of the five leading causes for death in all age groups in the combined population of the two islands. The Tobago population is predominantly of West African origin, with a low admixture rate (6% non-African) (109). With the advancing westernization of lifestyle, the Tobago population is exposed to high risk for obesity, hyperglycemia and hypertension, to categorize as metabolic syndrome. A previous study in Trinidad and in Tobago found that a higher prevalence rate of MetSyn were present among diabetic patients in both islands (165). However, no accurate estimation of metabolic syndrome among Afro-Caribbeans has been conducted. Our study will be the first to evaluate the heritability of metabolic syndrome related phenotypes among the Tobago population.

The aim of this study is to characterize the metabolic syndrome in a sample of Caribbean African-origin families, estimate the heritability of metabolic syndrome related components. In order to understand the genetic architecture of metabolic syndrome, factor analysis was performed to examine the existing pattern of correlations between metabolic measures and explain the underlying genetic background.

3.3 METHODS

3.3.1 Study Population

We began the Tobago Family Health Study on the island of Tobago to assess heritability and better understand the genetic and environmental factors which contribute to the metabolic phenotypes including subclinical cardiovascular disease, diabetes, obesity and chronic kidney disease. To be eligible, a proband must have been Afro-Caribbean, have had a spouse who was willing to participate in the study and have at least six living offspring and/or siblings aged 18+ years who were residing in Tobago. Recruited without regard to their health status, to date, 401 individuals have been enrolled into the study; the mean age was 43 years, ranging from 18 to 103 years. They belong to seven multigenerational families (mean family size 51 individuals) of Western African ancestry with the following relationship: 361 parent-offspring, 495 full siblings, 101 grandparent-grandchildren, 1,137 avuncular, 61 half-sibs, and 1,380 cousins (3,535 relative pairs). The population from Tobago is predominantly African origin with low 6% admixture, which has been confirmed from previous study using molecular markers(109). Metabolic traits measurements are available among 401 of 471 participants in this community-based sample of families. Written informed consent was obtained from each participant using a protocol and consent forms approved by the Institutional Review Boards of the University of Pittsburgh and the Tobago Division of Health and Social Services.

3.3.2 Data Collection

Height was recorded to the nearest 0.1 cm using a wall-mounted stadiometer. Weight was measured to the nearest 0.1kg without shoes on a balance-beam scale. Waist circumference was measured at the narrowest point of the waist using an inelastic fiberglass tape. Information on demographic characteristics (race/ethnicity), medical history, and lifestyle habits (smoking status, alcohol intake, oral contraceptive use, physical activity [minutes per week]) was obtained by questionnaire and interview by trained and certified clinical staff. Participants were asked to bring prescription and nonprescription medications to the clinic for verification. Race/ethnicity was self-declared. Detailed information on the ethnic origin of their parents and grandparents was provided by participants. Individuals were assigned to an ethnic group if they reported that all four grandparents belonged to that group. Blood pressure was measured three times using an automated blood pressure machine (Omron model HEM-705CP, Illinois); systolic blood pressure and diastolic blood pressure were calculated based on the average of the second and third measurements.

Diabetes was defined as a fasting glucose level greater or equal to 126mg/dL or currently taking anti diabetes medication. Hypertension was defined as diastolic blood pressure ≥ 90 mmHg, or systolic blood pressure ≥ 140 mmHg, or currently taking blood pressure medication.

3.3.3 Blood Sample Collection and Lab Measurements

A blood sample was obtained by venipuncture in the morning after 12 hours fasting. Whole blood was drawn into sterile red top (serum) tubes, which were left to clot at the room temperature for 20 minutes before centrifugation. Then serum was aliquotted into 1 mL

cryovials. The aliquots of serum samples were frozen, and shipped on dry ice to the University of Pittsburgh where they were stored in a -80 °C freezer.

Metabolic traits (serum glucose, insulin, triglycerides, HDL, microalbumin) were measured in the Heinz Nutrition Laboratory, University of Pittsburgh, using standard lab analytical procedures. The accuracy and precision of each technique was verified. Accuracy was estimated by comparing values obtained for the reference controls with their stated values. Precision was evaluated as the coefficient of variation both intra-assay and inter-assay. Briefly, serum glucose was quantitatively determined by an enzymic determination read at 340/380 nm(166). The coefficient of variation between runs was 1.8%. Insulin was measured using an RIA procedure developed by Linco Research, Inc. The coefficient of variation between runs was 10%. Triglycerides were determined enzymatically using reagents from Clinical Diagnostics(167). Coefficient of variation between runs was 1.7%. HDL cholesterol was determined after selective precipitation by heparin/manganese chloride and removal by centrifugation of very low density (VLDL) and low density lipoprotein (LDL)(168). The coefficient of variation between runs was 1.3%. Microalbumin in urine was measured using a turbidimetric procedure on the Olympus AU400 using reagents provided by Olympus America, Inc. The intra- and inter-assay coefficients of variation were below 2.5% and 5.1%, respectively.

3.3.4 Metabolic Syndrome Definition

World Health Organism (WHO) published the first official guidelines, followed by the definition from National Cholesterol Education Program Expert Panel and Treatment of High Blood Cholesterol in Adults (NCEP / ATP III) and the International Diabetes Federation (IDF). NECP/ATP III has been widely used in large epidemiological studies, such as the Third National

Health and Nutrition Examination Survey (NHANES III) in the U.S. Table 1 compares the diagnostic criteria for metabolic syndrome according to the three definitions, which differ in combinations of risk factors. According to WHO consultation group (169), 1) Insulin resistance was viewed as a required component for diagnosis; 2) A higher blood pressure cut point (140/90mmHg) was required, compared with the later definitions (ATP III and IDF define hypertension as blood pressure greater than 130/85 mmHg); 3) Increased waist: hip ratio (or BMI) rather than waist circumference, and microalbuminuria were listed as additional criteria in WHO definition. The requirement of objective evidence of insulin resistance should increase the power of WHO definition to predict diabetes compared with the later definitions. In terms of waist circumference, the ATP III definition introduces a uniform cut point of increased waist circumference, which fails to capture the elevated risk in Asian population at relatively lower levels of abdominal obesity. In contrast, the International Diabetes Federation developed ethnic specific definitions. The Asian population has a lower cut point for waist (men: 90cm, women 80cm) compared to Europids (men: 94cm, women 80cm). However, ethnic-specific definition has not been developed for other ethnic groups, e.g. population of African descent; thus, in U.S. clinics, the value used in ATP III definition (men: >102, women >88) is still used for clinical purposes.

Table 3.1 Diagnostic Criteria of Metabolic Syndrome Definitions: WHO, ATP III and IDF

	WHO (insulin resistance plus two of the following) (169)	ATP III (three of the following) (38)	IDF (central obesity plus two of the following) (35, 45)
Abdominal /central obesity			
	Waist to hip ratio:	Waist Circumference:	Waist Circumference: using ethnicity specific cut point*
Men	>0.90	>102 cm (40in)	>94cm
Women	>0.85	>88cm (33in)	80 cm
Hypertriglyceridemia			
	≥150 mg/dL (1.7 mmol/L)	≥150 mg/dL	≥150 mg/dL
Low HDL cholesterol			
Men	<35 mg/dL (0.9 mmol/L)	<40 mg/dL	<40 mg/dL
Women	<39 mg/dL (1.0mmol/L)	<50 mg/dL	<50mg/dL
High Blood Pressure			
	≥140/90 mmHg or use of antihypertensive medication	≥130/85 mmHg or anti-hypertensive medication usage	≥130/85 mmHg or use of antihypertensive medication
High fasting Glucose			
	Type 2 diabetes, impaired fasting glucose (FBG ≥ 6.1mmol/l), impaired glucose tolerance (2hPPG ≥ 7.8 mmol/l)	Impaired fasting glucose (FBG ≥ 6.1 mmol/L) or drug treatment for high glucose	FBG 5.6 mmol/L or previous diagnosis of impaired glucose tolerance or diabetes
Others	Microalbuminuria (albumin:creatinine ratio ≥30 mg/g)		

IDF: ethnicity specific cut point of waist circumference is used, Asian population is considered having a lower cut point (men >90, women >80 cm), however in the U.S. the same value as ATP III is used in clinic settings.

3.3.5 Statistical Analysis

Initially, the distributions of all metabolic traits were assessed for non-normality and data was transformed before analysis to reduce non normality. Outliers (defined as ± 4 SD) were removed. No more than four values were removed for a single variable.

All potential covariates for each trait were screened using linear/logistic regression analysis, ignoring the non-independence of subjects using SAS (9.2). The significance level was set as 0.10 at this screening stage. Subsequently, we evaluated each of the potential significant covariates using a variance component framework which takes into account of the correlations among family members. To estimate the heritability among metabolic syndrome and its components, we analyzed each component as dichotomized trait as defined in the ATP III. We also estimate heritability for quantitative data of each continuous component of this syndrome with adjustment for covariates and medications to reduce the misclassification of phenotypes. These analyses were performed using the program SOLAR (Sequential Oligogenic Linkage Analysis Routines, Copyright © 1995-2003, Version 2.1.4; Southwest Foundation for Biomedical Research, San Antonio, TXT). With this approach, the maximum-likelihood estimation is applied, incorporating significant covariate effects, additive genetic effects and residual error. The additive genetic effects and residual error are assumed to be normally distributed and to be mutually independent. Heritability is calculated as the proportion of phenotypic variance explained by additive genetic effects after accounting for covariates.

For factor analysis, the normally distributed, or otherwise, log transformed continuous traits were first adjusted for age, sex, age², and age² by sex, by multiple regression and the standardized residual were obtained. Blood pressures were adjusted for use of antihypertensive medication by adding 15mm Hg and 10mm Hg to SBP and DBP respectively (170). Image

factoring was used to extract the factors. The promax rotation, as a common orthogonal rotation option, was used to make the output more understandable (171). Factor numbers were determined by Eigen value-one criterion and the scree test, which is a plot of variance associated with each factor. A distinct break presents between the steep slope of the large factors and gradually trails off. It is also suggested by Meigs, JB et al that the number of factors equals or is less than the number of original variables divided by three(172). Items with loading > 0.40 were considered as large effect and entered the factor (173). Weighted factor scores were used, which was determined by the sum of the values of the phenotypes multiplied by standardized scoring coefficients for that factor. SAS (9.2) was used to perform factor analysis.

3.4 RESULTS

The Tobago Family Heath Study consisted of 401 individuals from seven multigenerational families who had enrolled to date with available metabolic traits measurements. The average family size was 50, ranging from 19 to 96. The mean age was 43.3 years (SD=16.9), similar among men and women, with a range from 18 to 103 years. Men accounted for 39% of the study subjects. Characteristics of metabolic syndrome and its components are shown in Table 3.2. Compared to women, Tobago men had higher systolic ($p<0.0001$) and diastolic ($p=0.03$) blood pressure and percentage of hypertension ($p=0.05$). Though Tobago women had higher fasting glucose level and the rate of diabetes, such trends did not reach statistical significance. Mean HDL-cholesterol level in Tobago was 40.0 ± 12.4 mg/dL (men: 39.4 mg/dL, women: 40.9 mg/dL). More than half of the participants had low HDL cholesterol (67.4%: men 50% vs. women 78.5%). According to the WHO definition, 9.4% Afro-Caribbean (11.3% women and 5.4% men) are suffering metabolic syndrome, which is the lowest proportion compared to the other definitions 18.7% (23.9% women and 10.7% men) by ATP III, and 25.3% (30% women and 18% men) by IDF (Table 3.2).

Table 3.3 shows the heritability estimation. When the components were treated as continuous variables, the residual heritability varied from 12% for fasting glucose to 46% for HDL-cholesterol after adjustment for age, sex and appropriate medication. When the metabolic traits were treated as discrete traits according to ATP III cut points, the estimated heritability varied from 21% for large waist circumference to 72% for high fasting glucose level.

The correlations between metabolic traits were then examined adjusting for age and sex. The partial correlation coefficients were shown in Table 3.4. All the components were significantly intercorrelated except for blood pressure and lipids profile. As expected, the

correlation between systolic blood pressure and diastolic blood pressure is very high, ($r=0.75$, $p<0.001$). Total cholesterol is also highly related to triglycerides ($r=0.41$, $p<0.001$). The factors and patterns of factor loading are shown in Table 3.5. Two significant factors were extracted from eight metabolic traits: total cholesterol, HDL, triglycerides, waist, fasting glucose, insulin and systolic and diastolic blood pressure. The first factor explained 55.8% of variance, comprised of lipids, central obesity and fasting glucose and insulin. The second factor accounted for 26.8% of variance, and was dominated by systolic and diastolic blood pressure. The heritability estimations for these two factors were both significant, 0.29 ($p<0.0001$) and 0.20 ($p=0.002$) respectively.

3.5 DISCUSSION

In this study, we found a significant heritability of metabolic syndrome related traits among Afro-Caribbean families. The estimated heritability ranged from 12% to 46% for fasting glucose to HDL cholesterol. To our knowledge, this is the first report of heritability of metabolic syndrome related traits among Afro-Caribbeans. Our results confirmed that genetic contributors play a role in the familial aggregation of metabolic syndrome. Using factor analyses approach, the heritability of factor one (comprised by central obesity, glucose and lipids) was 29% heritable ($P < 0.0001$) and factor two (comprised by blood pressure) was 20% heritable. Although the significant heritability estimated from both factor analysis and metabolic traits are not directly comparable because they comprise different traits, these results illustrated that there is an underlying genetic contributions to the metabolic syndrome.

Rather than a clinical disease, metabolic syndrome by definition is a cluster of the intercorrelated risk factors. Variation in prevalence of metabolic syndrome across population is influenced by the use of different definitions, as well as by differences in distributions of individual metabolic traits across population. Ethnic differences in metabolic syndrome prevalence have been reported. As documented in NHANES survey data, African American men had a nearly 50% lower rate (13.9% vs. 20.8%, $P = 0.006$); African women had similar rate as Caucasians (20.9% vs. 22.9%, $P = 0.10$) using the ATP III definition (174). Different patterns of metabolic risk factor prevalence do exist across populations. For instance, low HDL cholesterol and central obesity are the most two important contributors among Caucasians, while, hypertension and central obesity are the top risk factors among African Americans, and central obesity and hyperglyceridemia are the major contributors for Mexican Americans (154). By gender, white and Mexican American men had the highest age adjusted prevalence rate of central

obesity, hypertriglycerides and low HDL cholesterol; African American men had the highest age adjusted prevalence of high blood pressure and Mexican American men had the highest age adjusted prevalence of hyperglycemia. Among women, Mexican Americans and African Americans had the highest age adjusted prevalence of abdominal obesity, African American women had the highest age adjusted blood pressure and Mexican American women had the highest age adjusted prevalence of hypertriglyceridemia, low HDL cholesterol and hyperglycemia (154). Furthermore, all these metabolic traits are correlated with each other. The degree of the correlation between these risk factors was also differentiating across ethnic groups. For example, the increased waist circumference significantly correlated with the increased risk for diabetes and hypertriglyceridemia among Hispanics compared to whites (Hispanic men 0.12 vs. white men 0.10 and 0.12 vs. 0.08 for women respectively); but it was closely related to hypertension among black population (175). Thus, in addition to the differences across definitions, no matter which definition is used, metabolic syndrome is a very heterogeneous phenotype across ethnic groups rather than a well defined clinical presentation. By saying that, both environmental and genetic factors are playing the role. Thus, comparison of the estimated heritability of metabolic traits as a single phenotype, i.e. metabolic syndrome, across population has little meaning.

According to NHANES, African Americans have a lower prevalence of metabolic syndrome than whites. Our study confirmed that the low rate of metabolic syndrome also exist among African ancestry from Caribbean island. The lower rate in blacks is partly ascribed to the unique features among blacks, namely the lower serum triglyceride level, higher HDL cholesterol when compared to whites. Despite these favorable lipids and lipoprotein profile, African population has high prevalence of central obesity and hypertension, resulting in higher

cardiovascular mortality and morbidity. Although the reasons are uncertain and appear to be multifactorial, blacks have higher serum insulin and insulin resistance than whites (176). Recently, Haffner et al showed that in addition to lower serum triglycerides, African Americans have larger LDL-particle size, which are more elastic and less atherogenic than whites (177). The paradox of favorable lipid profile and conversely the unfavorable hypertension in blacks calls into the question whether the current criteria of metabolic syndrome is valid among blacks. In our study, we found that African Caribbean population has low triglyceride level, but unlike African American, they possess the low HDL cholesterol, especially among Tobago women. The underlying reason is unclear and future studies are needed to confirm among other African ethnicity groups. In addition, we reported that Tobago women have much higher risk of metabolic syndrome compared to Tobago men. This could be attributed to psychological difference including hormone level or lifestyle factors such as eating habits, physical activity.

Moreover, the heritability estimation represents the proportion of total phenotypic variance which is attributable to additive genetic variations; the heritability of the same trait will differ between populations that differ in the distribution of life style risk factors for that trait. Reported from previous studies, the estimates of heritability ranged from 15% to 40% in blood pressure, 25-60% in BMI, 40-60% in HDL cholesterol and 25-30% in fasting glucose across different populations (178). Among families from North European origin, after adjusting for age sex and body mass index, the heritability estimates for fasting glucose, triglycerides and HDL cholesterol were 20%, 20% and 43% respectively(179). In Mexican families, estimated heritabilities are fasting glucose (18%) triglyceride (40%) and HDL-cholesterol (49%). In our study, the observed heritability is modestly high among these African Caribbean families: fasting glucose (36%), HDL-cholesterol (46%), triglyceride (18%), systolic BP (25%), diastolic BP

(31%) after adjusting for age and gender. These estimations are in the range of commonly reported heritability estimation from other populations. Participants who were currently taking medication for hypertension and diabetes were excluded from this analysis. Even though heritability estimation of individual components varied among different populations, the significant finding in our study, consistent with previous research, indicated that there are underlying genetic influences on all of the components. Accumulated evidence suggests that metabolic syndrome most likely results from the interplay between several genes and an affluent environment(180). Lin et al confirmed that genetic factors contributed to the familial aggregation of metabolic syndrome among Caribbean-Hispanic families; they found that HDL cholesterol had highest estimation of 60% heritability, while systolic blood pressure had the lowest one of 16% (11).

In order to better understand the structures of clustering of the metabolic traits, factor analysis or principle component analysis has emerged as a useful method to understand patterns underlying metabolic risk factors. As reviewed by Teran-Garcia et al, factor analysis or principle component analysis have been used by many investigators to demonstrate that common genes exert pleiotropic effects on these metabolic traits based on the assumption of the presence of common genetic and/or environmental factors underlying these components(180). In fact, as an argument against ‘common ground’ or a ‘yes/no’ metabolic syndrome, previous studies have broken the related metabolic traits into two(11, 163, 164), three (161, 162, 181, 182)or four (183-185) factors. In general, central obesity related traits, together with lipids, insulin and fasting glucose, fall into one factor distinguished from the measures related to blood pressure, which form a separate factor. We were able to produce two factors from seven metabolic syndrome related traits, with the similar patterns indicated by previous studies. The number of retrieved

factors obeyed the rule of equaling or less than the number of included traits divided by three. The failure of blood pressures to load with several other components on one factor was consistently reported by other studies. We found that factor 1 comprising of only lipids, glucose and central obesity explained majority of the variance (55.8%), and had heritability of 29% ($p=0.0001$), which was comparable to the heritability estimation among Japanese-American population(162). Another factor was found to be comprised of blood pressure, which explained for 26.8% of total variance and had 20% heritability ($p=0.001$). Very few studies have presented results from factor analysis among African Americans. Evidence from Miami Community Health Study showed that the identical factor patterns appear in black and white populations, but the small sample size (50 participants including 23 African Americans) limited their power to address the issue appropriately (186). Two principle components were used in the HERITAGE Family Study, which explained 55% of total variance in metabolic traits among 456 white and 217 black participants. Promising evidence for linkage has been found for both blacks and whites, suggesting the genetic basis for metabolic syndrome. However, the fact that quantitative linkage patterns are different from blacks to whites underlines the existence of different risk factor distribution across various ethnic populations. (164) Future studies are needed to identify the risk factor patterns of metabolic syndrome among African origin populations.

Our study was the first study of metabolic conducted syndrome conducted among non patient population in the island of Tobago. We have several strengths and limitations. We recruited participants from seven multigenerational families, with extended family members, which provide better power to detect genetic signals than nuclear families or sib-pair data. Such family structure will give more accuracy in heritability estimation. The relationship among individuals has been validated by genotyping data, so we minimized the possibility of inaccurate

reports of biological kinship. However, our study does have limitations. First, the sample size was relatively small; about 400 individuals have complete measurements on all metabolic trait measurements. Secondly, even though we recruited people without regarding to their health status, these participants are ambulatory. It is possible that we underestimated the disease burden in the general Afro-Caribbean population.

In summary, we report a moderate and significant heritability of metabolic syndrome and its components estimated among these African Caribbean families. Factor analysis illustrated two clusters of related risk factors with significant heritability. Further linkage studies or association studies are strongly needed to identify specific loci contributing to the susceptibility of metabolic syndrome.

Table 3.2 Characteristics of the Study Participants

	All, n=401	Women, n=245	Men, n=156	P value
Age (years)	42.4±16.9	42.6±17.3	42.1±16.9	0.75
Waist circumference (cm)	89.9±15.4	89.6±17.2	90.3±12.4	0.71
Height (cm)	170.7±8.6	166.5±6.4	177.4±7.1	<0.0001
Weight (kg)	82.3±19.1	80.8±19.7	84.5±17.8	0.08
Body mass Index (kg/m²)	28.2±6.5	29.2±7.1	26.8±5.1	<0.0001
Fasting glucose (mg/dL)	87.8±28.3	89.3±31.7	85.5±21.9	0.07
Triglycerides (mg/dL)	88.7±46.4	85.5±43.0	93.6±51.0	0.06
LDL-cholesterol (mg/dL)	131.3±40.1	134.0±41.7	127.2±37.4	0.08
HDL-cholesterol (mg/dL)	40.0±12.4	39.4±12.7	40.9±11.7	0.16
Systolic blood pressure (mmHg)	122.9±24.4	119±25.7	128.8±21.0	<0.0001
Diastolic blood pressure (mmHg)	76.3±12.9	75.1±13.1	78.1±12.4	0.03
Insulin (mIU/ml)	15.9±9.9	17.2±10.9	13.9±7.7	0.001
Hypertension (%)^a	35.6	31.7	41.8	0.08
Diabetes (%)^b	14.9	17.4	11.0	0.11
High triglycerides (%)^c	7.7	7.0	9.0	0.46
Low HDL cholesterol (%)^d	67.4	78.5	50.0	<0.0001
High waist circumference (%)^e	37.6	52.1	15.4	<0.0001
Metabolic Syndrome (%)				
WHO definition^f	9.4	11.3	5.4	0.05
ATP definition^g	18.7	23.9	10.7	0.001
IDF definition^h	25.3	30	18	0.008

^a Hypertension is defined as blood pressure \geq 130/85 mmHg or currently taking anti hypertensive medication

^b Diabetes is defined as fasting glucose \geq 126 mg/dL or currently taking anti diabetic medication

^c High triglycerides is defined as TG \geq 150 mg/dL

^d Low HDL cholesterol is defined as HDL <40 mg/dL (men), <50 mg/dL (women)

^e High waist circumference is defined as >102cm (men), > 88 cm (women)

^f WHO definition is impaired glucose tolerance or diabetes or insulin resistance and 2 of the other factors, including type 2 diabetes, BMI > 30 kg/m², TG > 150 mg/dL, HDL < 35 mg/dL (men) <39 mg/dL (women), Blood pressure \geq 140/90 mmHg and microalbuminuria

^g ATP definition is three or more of the following factors: impaired fasting glucose, increased waist circumference >102 (men) >88 (women), HDL <40 (men) <50 (women) and Blood pressure \geq 130/85 mmHg

^h IDF definition is central obesity and 2 other factors: fasting glucose \geq 110mg/dL, waist circumference \geq 94(European men) \geq 80 (European women) \geq 90 (Asian men) \geq 80 (Asian women), HDL <40 (men) <50 (women), BP \geq 130 / 85 mmHg.

Table 3.3 Heritability Estimation for Metabolic Syndrome (ATP III Definition) related Components Treated as Both Continuous and Discrete Traits

	H ² (SE)	P-value	Variance explained by covariates ^f
Waist			
cm ^a	0.19 (0.11)	0.02	0.08
Women >89, men >102 ^b	0.22 (0.20)	0.10	0.15
Fasting Glucose			
mmol/L ^{a c d}	0.36 (0.14)	0.004	0.05
≥6.1 ^{b d}	0.88 (0.47)	0.03	0.10
Triglycerides			
mmol/L ^{a c}	0.18 (0.10)	0.008	0.14
≥1.7 ^b	0.21 (0.25)	0.16	0.03
HDL cholesterol			
mmol/L ^a	0.46 (0.11)	<0.001	---
Women <1.3, men <1.04 ^b	0.36 (0.19)	0.002	0.07
Blood Pressure			
Systolic, ^{a c e}	0.25 (0.09)	0.0002	0.36
Diastolic, ^{a c e}	0.31 (0.10)	<0.0001	0.27
Systolic≥130 or diastolic ≥85 or anti hypertensive medication ^b	0.33 (0.20)	0.02	0.27

*<0.05, ** <0.0001

a – treated as continuous traits

b – treated as discrete traits

c – log transformation

d – participants with anti- diabetic medication were excluded from analysis

e – participants with anti-hypertensive medication were excluded from analysis

f – Proportion of variation explained by covariates of age and gender

Table 3.4 Partial Correlation Coefficient between Variables Used in Factor analysis (Controlling for Age and Gender)

	Waist	Triglycerides	Total cholesterol	HDL Cholesterol	LDL Cholesterol	Fasting Glucose	Fasting Insulin	Systolic BP	Diastolic BP
Waist	1.00								
Triglycerides ^a	0.28**	1.00							
Total Cholesterol ^a	0.13*	0.41**	1.00						
HDL Cholesterol	-0.14*	-0.16*	-0.13*	1.00					
LDL Cholesterol	0.11*	0.25**	0.92**	-0.43**	1.00				
Fasting Glucose ^{ac}	0.22**	0.17**	0.14*	-0.17*	0.17*	1.00			
Fasting Insulin ^{ac}	0.46**	0.34**	0.05	-0.12*	0.02	0.31**	1.00		
Systolic BP ^{a b}	0.13*	0.08	0.01	-0.05	0.03	0.19*	0.11*	1.00	
Diastolic BP ^{a b}	0.12*	0.09	0.1	-0.03	0.09	0.16*	0.05	0.75**	1.00

*P <0.05; **p<0.0001

^a log transformation to achieve normality

^b Adjusted for use of antihypertensive medication by adding 15mm Hg and 10mm Hg to SBP and DBP respectively

^c Exclude participants with anti diabetic medication

Table 3.5 Factor Analysis of Metabolic Syndrome Components, Variance Components and Heritability Estimation

	Factor 1 (lipids/central obesity/glucose)	Factor 2 (blood pressure)
Total Cholesterol ^a	0.47	0.02
HDL cholesterol ^a	-0.35	-0.06
Triglycerides ^a	0.76	0.03
Fasting Glucose ^a	0.41	0.12
Fasting Insulin ^a	0.71	-0.0002
Waist ^a	0.67	0.07
Systolic BP ^{a b}	0.08	0.95
Diastolic BP ^{a b}	0.10	0.94
Percent of variance (%)	55.8	26.8
Heritability	0.29 (p = 0.0001)	0.20 (p = 0.002)

^a adjusted for age, age², age²*sex and sex

^b Adjusted for use of antihypertensive medication by adding 15mm Hg and 10mm Hg to SBP and DBP respectively

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4.0 THE ASSOCIATION BETWEEN RENAL FUNCTION BIOMARKERS AND THE SUBCLINICAL CARDIOVASCULAR MEASURES IN AFRICAN CARIBBEAN FAMILIES IN TOBAGO

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4.1 ABSTRACT

Objectives: To test the hypothesis that decreased renal function may be related to subclinical cardiovascular disease (CVD) in this community-based sample of African ancestry families.

Design and Methods: 402 participants aged 18 to 103 years (male: 42.1 ± 16.9 (SD) years, female: 42.6 ± 17.3 (SD) years), from 7 large, multi-generation pedigrees (average family size: 50; range: 19 to 96; nearly 3500 relative pairs) were included in this study. Subclinical cardiovascular disease was examined by ankle brachial index (ABI), pulse wave velocity (PWV) and carotid intima-media thickness (IMT). Serum cystatin C and creatinine were measured using standard protocols. Estimated GFR was calculated using Modification of Diet in Renal Disease Study (MDRD) formula for standardized serum creatinine. Multivariate regression analysis was used to evaluate the association between subclinical CVD and renal function measures adjusted for conventional CVD risk factors. The variance component approach was implemented in SOLAR to assess the associations, incorporating the effects of the relatedness of family members.

Results: The mean \pm SD cystatin C was 0.62 ± 0.19 mg/L, serum creatinine, 0.93 ± 0.23 mg/dL, and microalbumin, 2.6 ± 9.3 mg/dL. Cystatin C was significantly related to ABI ($P=0.02$) and PWV ($P=0.04$), but not to IMT. Serum creatinine, microalbuminuria and eGFR were not significantly associated with these markers of subclinical cardiovascular disease. The heritability of serum cystatin C was 0.32 ± 0.1 ($P<0.0001$), after adjusting for age, gender, hypertension, triglyceride and insulin level.

Conclusion: Our data suggest that cystatin C is significantly heritable ($h^2=0.32$, $p<0.0001$) among these Afro-Caribbean families. In addition, we detected a significant association between peripheral arterial disease and arterial stiffness with cystatin C, but not with the other renal function measurements among these Afro-Caribbean families.

4.2 INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death in United States; nearly 2600 Americans die as a result of CVD every day. The pathological process starts many years before the life-threatening events occur. Studies show strong evidence that the incidence of CVD and mortality are substantially increased for people with subclinical cardiovascular disease compared to those without subclinical disease (1, 187-189). With the advances in technology, identifying and quantifying subclinical cardiovascular disease is noninvasive and sensitive, and these methods are being translated into clinic practice (190). B-mode ultrasound examination of the carotid arteries is the most commonly used measurement to monitor atherosclerosis as assessed by intima-media wall thickness (IMT). Lower-extremity peripheral arterial disease (PAD) can be detected noninvasively indicated by the ankle-brachial index (ABI), a ratio of systolic blood pressures in the lower and upper extremities. Pulse wave velocity (PWV) is an index of arterial stiffness that is now easily quantitated using a simple device developed for measurement of the brachial-ankle PWV. baPWV is a useful predictor of coronary artery diseases(191).

Cystatin C is an alternative marker of kidney function. Unlike the more commonly used marker, serum creatinine, which is a by-product of muscle cells and affected by many factors other than kidney disease, cystatin C is produced by all nucleated cells and is excreted into the bloodstream at a constant level without impact of age, gender, race and muscle mass (78, 79). Numerous studies found that cystatin C levels were more accurate to predict glomerular filtration rate (GFR) in various clinical presentations, especially in mild to moderate kidney disease patients (eGFR between the range of 60 to 90 [$\text{mL} \cdot \text{min}^{-1} \cdot (1.73 \text{ m}^2)^{-1}$]) (82, 86, 192). Cystatin C was also found to be a stronger predictor of subclinical coronary atherosclerosis (193), peripheral arterial disease(194), all-cause mortality, cardiovascular morbidity and heart failure

(195, 196) independent of its relationship to renal function. The underlying mechanisms accounting for such associations are not clear but may involve insulin resistance and inflammation (175, 197).

The impact of ethnicity and race on subclinical CVD is substantial. African Americans have significantly lower amounts of coronary artery and carotid artery calcified plaque relative to whites despite having increased carotid IMT and blood pressure(2). The Tobago population is approximately 94% of West African origin (109) and is at high risk of hypertension, diabetes, cardiovascular disease and impaired kidney function. No previous study has comprehensively studied the association of the renal function, assessed by both serum creatinine and cystatin C, with subclinical cardiovascular disease measurements in a population of African descent. The purpose of this study is to assess the association between renal function markers and subclinical coronary atherosclerosis, arterial stiffness and peripheral arterial disease in this African Caribbean population.

4.3 METHODS

4.3.1 Subjects and Methods

This study was conducted in 402 individuals aged 18-103 years (mean age 42 years) belonging to seven, multi-generation Afro-Caribbean families (mean family size 51 individuals) on the Tobago Island. To be eligible, a proband must have been Afro-Caribbean, have had a spouse who was willing to participate in the study and have at least six living offspring and/or siblings aged 18+ years who were residing in Tobago. Among these individuals, we have the 3535 relative pairs, including 361 parent-offspring, 495 full sibling, 101 grandparent-grandchildren, 1137 avuncular, 61 half sibs and 1380 cousins. Complete data for the current analysis was available in 402 individuals. Written informed consent was obtained from each participant, using forms and procedures approved by the Tobago Division of Health and Social Services and the University of Pittsburgh, Institutional Review Boards.

4.3.2 Assessment of Subclinical Cardiovascular Disease (SCVD)

The common carotid artery (CCA) intima-media thickness was measured using B-mode ultrasonography with an Acuson Cypress portable ultrasound machine (Siemens Medical Solutions, Malvern, PA) on all participants returning for the Tobago Family Health Study ancillary carotid ultrasound scan in 2007. Both the near and far walls of the distal common carotid artery were captured for one centimeter proximal to the carotid bulb. IMT was obtained using semi-automated reading software (AMS system; Dr. Thomas Gustavsson, Sweden). This system detects and traces lines, with reader input, between the lumen-intima and media-adventitia

borders across the 1 cm segment. Then, the software generates one thickness measurement per pixel across this area, for about 140 measures in total. Mean IMT measures correspond to the mean IMT across all pixels of both the near and far wall of the common carotid artery on both the right and left arteries. All images were read centrally at the Department of Epidemiology's Ultrasound Research Laboratory (University of Pittsburgh, Pittsburgh, PA). Reproducibility analyses were conducted on 35 Tobago Family Health Study participants. The inter-sonographer intraclass correlation (ICC) and Inter-reader ICC were 0.97 and 0.99 for mean IMT respectively.

The ankle brachial index (ABI) and brachial-ankle pulse wave velocity (baPWV) measures were automatically generated using a noninvasive and automated waveform analyzer (VP1000, Omron Co., Komaki, Japan). Following 10 minutes of rest in a supine position, occlusion and monitoring cuffs were placed around both arms and ankles, following standardized placement procedures. The arm cuffs were placed on bare arms or over light clothing, and the ankle cuffs were placed on bare ankles. ECG electrodes were placed on both wrists and a phonocardiogram (PCG), which is a microphone for detecting heart sounds, was placed on the left edge of sternum. The cuffs were connected to a plethysmographic sensor that determined volume pulse waveforms and to an oscillometric pressure sensor that measured blood pressure. Volume waveforms for the arm (brachial artery) and ankle (tibial artery) were stored for a sampling time of 10 seconds with automatic gain analysis and quality adjustment. ABI was calculated by the ratio of the ankle systolic BP divided by the arm systolic BP, and the lower value of the ankle systolic BP was used for the calculation. An average of ABI, from the right and left sides, was used in our analyses.

The baPWV was calculated as (distance between arterial sites) divided by (time between the foot of the respective waveforms). The distance measure was calculated using height-based

formulas rather than the actual “above the body” distances and also corrects for the opposite direction of blood flow by subtracting the heart-to-brachial distance from the heart-to-tibia distance, as seen in the following equation (198): path length from the heart to the brachium (L_b) = $0.2195 \times \text{height of the patient (cm)} - 2.0734$; path length from the heart to ankle (L_a) = $0.8129 \times \text{height of the patient (cm)} + 12.328$. The baPWV was calculated by time-phase analysis, for the right and left sides, using waveforms of the respective brachial and ankle (tibial) arterial sites, from the following equation: $(L_a - L_b)/\text{time difference between the brachial and ankle waveform}$. An average of baPWV, from the right and left sides, was used in our analyses.

4.3.3 Laboratory Measurements

Blood samples were obtained by venipuncture in the morning after a 12-hour fast. Whole blood sat at room temperature for a minimum of 20 min to clot before centrifugation. A spot urine sample was also collected. Aliquots of serum and urine were frozen at -20°C locally and shipped on dry ice by express courier within a month, and later stored in -80°C freezers at the University of Pittsburgh.

Serum cystatin C was measured at the University of Vermont from frozen samples collected at the baseline study visit (2004), using a protocol similar to the Cardiovascular Health Study (CHS) (199). A BNII nephelometer (Dade Behring, Inc, Deerfield, Ill) was used with a particle-enhanced immunonephelometric assay (N Latex Cystatin C, Dade Behring, Inc). The assay range was from 14.6 to 549.0 nmol/L (0.195 to 7.330 mg/L); the reference range for young, healthy persons was reported to be 40 to 71 nmol/L (0.53 to 0.95 mg/L). The intra-assay coefficient of variation ranged from 2.0% to 2.8%, and the interassay coefficient of variation ranged from 2.3% to 3.1%.

Serum creatinine was quantitatively determined at the University of Vermont using the VITROS CREA Slide method. The sample was diluted with VITROS 7% BSA. Standards, serum controls and duplicate samples were run with each assay, which were traceable to a Gas Chromatography Isotope Dilution Mass Spectrometry (GC/IDMS) method and National Institute of Standards and Technology (NIST) SRM®914 creatinine standard reference material. Creatinine values traceable to this standard and the results are referred to as standardized creatinine values. Urine samples were diluted with 1:1 reagent-grade water, and urinary creatinine was analyzed as above. The coefficient of variation between runs is 6.0%.

Albumin in urine was measured at the University of Vermont using a turbidimetric procedure on the Olympus AU400 using reagents provided by Olympus America, Inc. (Center Valley, PA). The intra- and inter-assay coefficients of variation were below 2.5% and 5.1%, respectively. Metabolic traits, including triglyceride, total serum cholesterol level, low density cholesterol (LDL- calculated), high density cholesterol (HDL), fasting insulin and fasting glucose, were measured in Heinz Laboratory in the University of Pittsburgh using standard protocols.

4.3.4 Disease Definitions

Hypertension was defined as diastolic blood pressure ≥ 90 mmHg, systolic blood pressure ≥ 140 mmHg, or currently taking blood pressure medication(134). Diabetes was defined as fasting glucose level ≥ 126 mg/dl or currently taking diabetes medication(135). Obesity was defined as BMI ≥ 30 kg/m². The spot albumin-to-creatinine ratio (ACR) was calculated and reported in milligrams per gram. According to the American Diabetes Association (ADA) (136)

and the National Kidney Foundation(137) criteria, albuminuria was defined as ACR > 30 mg/g; microalbuminuria: 30-299 mg/g and macroalbuminuria: ≥ 300 mg/g.

4.3.5 Statistical Methods

Estimated GFR was calculated using the four-variable MDRD equation for standardized serum creatinine. The simplified Modification of Diet in Renal Disease Study (MDRD) equation was used to estimate glomerular filtration rate based on serum creatinine: $\text{GFR [mL} \cdot \text{min}^{-1} \cdot (1.73 \text{ m}^2)^{-1}] = 175 \times (\text{serum creatinine [mg/dL]}^{-1.154} \times \text{age [years]}^{-0.203} [\times 0.742, \text{ if female}] [\times 1.212, \text{ if African American}]$ (81). Age was included in years and serum creatinine in mg/dl.

Before analyses, all distributions were assessed. The natural log transformation was applied to achieve normality for subclinical cardiovascular measures (ABI, baPWV and carotid IMT), renal function measures (serum cystatin C, serum creatinine, microalbuminuria and eGFR), cholesterol, triglyceride, insulin and glucose. Outliers (± 4 S.D) were removed to achieve the normal distribution. The characteristics of individuals were compared using the χ^2 test (categorical variable) and ANOVA (continuous variables).

Multiple linear regression models were performed to evaluate potential influencing factors for the association of renal measures and subclinical cardiovascular disease, without taking into account of participant relatedness, at 0.10 significance level using SAS 9.2. The null model was built with age and gender. Subsequently, traditional risk factors of cardiovascular disease were added to the null model, including BMI, smoking status, diabetes mellitus, blood pressure stages, LDL-cholesterol, HDL-cholesterol and triglycerides. Then, each renal biomarker (serum cystatin C, creatinine, microalbuminuria and eGFR) was added into individual models to evaluate the relationship of each to subclinical cardiovascular disease. The significance level was

set at 0.05. The associations of these traits with renal measures were subsequently evaluated using a variance component approach as implemented in SOLAR, incorporating the effects of the relatedness of family members(200). Briefly, the variance components approach involves partitioning the variance of a quantitative trait into a component attributable to individual-specific covariates, an additive genetic (polygenic) component and a residual non-measured environmental component. The significance of particular independent variables (such as diabetes status) were assessed by the likelihood ratio test, which compares the likelihood of a full model (e.g. age, BMI and diabetes status) to that of a nested model (e.g. age, and BMI only, with the diabetes status effect constrained to be zero). The interactions were tested between renal biomarkers and gender, BMI and gender. The significance level was set at 0.05.

4.4 RESULTS

Among the 402 participants in the Tobago Health Study with renal function measures, the mean \pm SD cystatin C was 0.62 ± 0.19 mg/L, serum creatinine was 0.93 ± 0.23 mg/dL, and micro albumin was 2.6 ± 9.3 mg/dL. There was no difference in the mean serum concentration of Cystatin C between men and women ($p=0.24$). Table 4.1 shows the characteristics by tertiles of cystatin C. Higher concentrations of cystatin C were associated with older age, higher body mass index, triglycerides, fasting insulin, serum creatinine and lower estimated GFR. The prevalence of hypertension increased across ascending tertiles of cystatin C. The heritability of serum cystatin C was $32\% \pm 0.1$ ($P<0.0001$) after adjusting for age, gender, hypertension, triglyceride and insulin level. The heritability of other renal function measurements in this population has been reported previously (201).

Figure 4.1-4.3 displays the age adjusted means of subclinical cardiovascular disease measures. The mean \pm SD baPWV was 1422.3 ± 374.0 cm/s, somewhat higher in men than in women (1438.4 ± 321.1 vs. 1421.3 ± 403.8 , $P=0.34$); mean \pm SD ABI was 1.05 ± 0.10 , significantly higher in men than women (1.06 ± 0.09 vs. 1.04 ± 0.10 , $P=0.008$); mean \pm SD common carotid IMT was 0.69 ± 0.15 , higher (but not significantly) in men than women (0.71 ± 0.16 vs. 0.68 ± 0.14 , $P=0.09$). Subclinical CVD measures increased across age group in both men and women. After the age of 70, the levels of ABI decreased to 1.01 ± 0.12 . Among older men and women, 16% (n/N) and 14% (n/N), respectively, exhibited low ABI ($ABI < 0.9$) in Tobago families. Older women also had lower carotid IMT, but the sample size was relatively small.

Multiple regression analysis of the association between renal measures and ABI was performed and the results shown in table 4.2. The model with only age and gender explained 14% of the total variance in ABI. By adding traditional cardiovascular disease risk factors, including BMI, diabetes, hypertension, LDL cholesterol, HDL cholesterol, triglyceride and smoking, the model explained an additional 8% of the total variance in ABI, even though none of the traditional risk factors was individually significant. Subsequently, serum cystatin C, creatinine, microalbuminuria and eGFR were added into separate models along with the traditional risk factors. We found that only cystatin C was significantly related to ABI ($P=0.02$) and explained an additional one percent of variance in the total variance in ABI. None of the other renal measures was significantly associated with ABI adjusting for all the traditional cardiovascular disease risk factors.

Incorporating the effects of relatedness of family members, the association of renal measures with baPWV and carotid IMT was evaluated using the variance component approach

in SOLAR. As a basic model with only age and gender, 54% of total variance in baPWV was explained (Table 4.3). Adding renal measures into the individual models, Cystatin C was the most significant factor related to baPWV with P value = 0.0005, explaining eight more percent of total variance of baPWV. Serum creatinine was also significantly (P=0.04) related to baPWV but explained less variance (5% of total variance in baPWV). Furthermore, Table 4.4 shows the fully adjusted model, with hypertension, diabetes and BMI in the model additional to age and gender, only cystatin C was significantly related to baPWV (P=0.04). The proportion of variance due to covariates was 62%. Adding cystatin C into the model explained an additional 1% of variance. None of the other renal function measurements was significant. We did not find any significant results for the association between renal measures and carotid IMT (data not shown).

4.5 DISCUSSION

Participants in our study came from the island of Tobago, located in the Southern Caribbean Sea. Compared to African Americans, the Tobago population is a more homogeneous population of West African descent with only about 6% admixture with other ethnic groups (109). We found a significant heritability of cystatin C among these multigenerational Afro-Caribbean families. The heritability of serum cystatin C was $32\% \pm 0.1$ ($P < 0.0001$), after adjusting for age, gender, hypertension, triglyceride and insulin level, which is similar to the reports of Framingham Offspring Study ($h^2 = 0.35$, $p < 0.0001$) (101). Further, we detected a significant association between peripheral arterial disease and arterial stiffness with cystatin C, but not with the other renal function measurements among these Afro-Caribbean families.

Cystatin C is a small protein that is produced throughout the body by nucleated cells and freely filtered by the glomeruli. When it functions normally, the kidney reabsorbs cystatin C and degraded by the proximal tubular epithelial cells, maintaining normal levels of serum cystatin C (202). But, when kidney function deteriorates, serum cystatin C is elevated due to the reabsorption dysfunction. Such increase occurs as the GFR falls and is often detectable before a measurable decrease in the GFR (203). Numerous studies found that cystatin C levels were more accurate for predicting GFR in various clinical presentations, especially among mild to moderate kidney disease patients (with a relatively higher eGFR)(85). Serum cystatin C concentration is not significantly affected by muscle mass, gender, age, or race (78, 79). It has been demonstrated that there is no correlation between cystatin C and lean tissue mass in the children(204) and healthy adults(143), but a modest correlation in the older subjects(205). In contrast, serum creatinine is a by-product of muscle cells, and is affected by many other factors than kidney disease such as lean muscle mass. Furthermore, using serum creatinine as a measurement to estimate GFR has another drawback, called 'creatinine-blind range'. As serum creatinine increases in mild kidney impairment, creatinine clearance is little affected until the tubular secretion mechanism is saturated. Thus, the utility of serum creatinine in detection of slight to moderate renal dysfunction is limited (77). While there are growing data and literature providing support that serum cystatin C correlates with kidney disease better than serum creatinine, there is still a degree of uncertainty about when and how it should be used (85) (82).

Besides the problems attributable to creatinine metabolism, creatinine measurement is technically problematic. Several different methods have been applied with the Jaffé method being the most commonly used. This assay is hampered by interferences up to 20% non-creatinine chromogenes including acetic acid, acetone, pyruvate, glucose and ascorbic acid(196).

With the development of enzymatic assays, the problem of interference has been reduced but not eliminated (206). Low intra-assay specificity makes comparison of creatinine measurements across different laboratories difficult. Even the inter-assay coefficient of variation in serum creatinine is relatively high, 6.0%, compared to only 2.3% in serum cystatin C. Thus, cystatin C is a better marker, though this does not necessarily mean that it is biologically more relevant than serum creatinine.

Patients with decreased renal function are highly predisposed to atherosclerotic artery disease. Previous study reported a remarkably high prevalence of PAD in nondialyzed patients with CKD in NHANES III 1999-2000(207). Ankle brachial index is a simple, non-invasive, and reliable measurement to assess PAD. The presence of ABI lower than 0.9 has been found to be associated with an increased odds of having an estimated GFR<90 (OR: 1.54; 95% C.I.: 1.17-2.04); such relationship appeared to be stronger among African Americans than whites (OR=1.88 vs. 1.36) (195). Another study reported that cystatin C was a marker of peripheral atherosclerotic disease, apart from its relation to kidney function(194). Similarly, we found that only cystatin C was highly associated with ABI in this African Caribbean population. The relationship between renal insufficiency and PAD is possibly due to the pathogenic mechanisms associated with reduced glomerular filtration rate, such as alternative metabolism of calcium, phosphate, and parathyroid hormone, hyper-homocysteinemia, inflammatory alterations, or coagulation pathway(208).

We also found that arterial stiffness, measured by PWV, was inversely related to renal function independent of other risk factors(203). Elevated PWV is a marker of arterial stiffness and predicts cardiovascular events independent of age, gender and other traditional cardiovascular risk factors (209). Our finding indicated that the association between cystatin C

and baPWV was significant after adjusting for age, gender, hypertension, diabetes and BMI. In contrast, serum creatinine lost its significance in the fully adjusted model. These findings are consistent with previous reports. Cystatin C has been found to be associated with arterial stiffness in older adults(210), independent of serum creatinine and eGFR based on creatinine(96). However, the mechanisms underlying the relationship between the renal function and arterial stiffness are not fully understood. It is believed that renal failure is associated with structural or functional alterations exclusively located in small resistance arteries (211). Not only was the stiffness of small arteries, but the stiffness occurring in large arteries was independently associated with reduced renal function. The increased arterial stiffness leads to increased pulse pressure (systolic BP – diastolic BP), which leads to deterioration in kidney function, eventually resulting in decreased GFR. In addition, kidney damage may cause an increase in other risk factors for arterial stiffness such as hypertension, anemia or vascular calcification(212). Such vicious cycles promote each other. However, the cross sectional nature of our study does not allow us to distinguish the direction of this association.

The carotid IMT exam uses state-of-the-art ultrasound technology to measure the thickness of the first two layers of the carotid arteries located in the neck, where the plaque first develops. Abnormal, premature thickening of the arterial walls is an early sign of vascular disease. It has been found to be related to cardiovascular risk factors such as triglyceride, LDL, HDL and total cholesterol (213), and increased the risk of stroke and myocardial infarction(188, 214, 215). A previous study also reported that patients with chronic renal failure exhibited significantly increased carotid IMT after adjusting for traditional risk factors(216). However, there was no significant association of any renal function measures with carotid IMT in our study samples. The Multi-Ethnic Study of Atherosclerosis (MESA) Study recently published their null

findings of cystatin C and IMT among 6,557 ethnically diverse persons free of clinical cardiovascular disease, aged 45 to 84 years ambulatory adults, suggesting that the cystatin C level is not significantly related to carotid IMT (217). Thus, the association between cystatin C and cardiovascular risk is not likely to be explained through accelerated arterial atherosclerosis. In contrast to the significant association between IMT and renal dysfunction among end stage renal disease patients who have advanced CKD, early kidney disease does not appear to be associated with increased levels of atherosclerosis. Furthermore, previous studies found that microalbuminuria but not cystatin C was related to carotid IMT beyond traditional risk factors among middle aged adults (218). However, this association is less consistent, especially among low-risk populations (219).

Gender difference in the association between BMI, body composition and CKD, attributable to sex hormones has been reported before, though this association is not yet clear (220). Deterioration of renal function is more rapid in males than in females(221). Furthermore, it has been reported that high BMI is positively associated with CKD in males but not in females from a Singapore cohort (222). Tobago women are extremely obese in our study; the mean waist circumference was 89.6 ± 17.2 cm. However, a test of interaction term between BMI and gender was not statistically significant related to PWV measurement. Thus, the combined model was built up for analyzing the association between PWV and renal biomarkers.

Our study has several limitations. Most important, as mentioned already, in any cross-sectional study, the direction of the association cannot be ascertained. A longitudinal study design is needed to establish a causal relationship between these renal function biomarkers and subclinical CVD or CVD. Secondly, real GFR was not directly measured in this study. Although the MDRD equation has gained widespread acceptance and is reported by most clinical

laboratories, the limitation of estimation of GFR based on serum creatinine is noted and the major limitation is imprecision among patients with mild renal dysfunction. The MDRD equation had no bias for severe kidney disease patients (eGFR <60 ml/min per 1.73 m²), but underestimated GFR for levels of eGFR between 60 and 119 ml/min per 1.73 m², and overestimated GFR for levels of eGFR >120 ml/min per 1.73 m²(223). It is also possible that there is residual confounding from other unmeasured factors that were not evaluated in this study. Third, the sample size was relatively small, which decreased the power to detect slight differences. The probands and family members were recruited without regard to health status, all participants were ambulatory, relatively young and healthy; thus the association of renal function biomarkers and subclinical CVD among these healthy families, so called “healthy family effect”, might have biased findings towards the null.

In conclusion, our data suggest that reduced kidney function, indicated by elevated serum cystatin C level was significantly related to arterial stiffness and peripheral arterial disease among these Afro-Caribbean families.

4.6 ACKNOWLEDGEMENT

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Table 4.1 Characteristics of Study Participants by Cystatin C level

	Male (156)			Female (246)			P Value _d
	Cystatin C Tertile			Cystatin C Tertile			
	1 :[0.35-0.54]	2: [0.55-0.64]	3: [0.65-1.52]	1: [0.29-0.54]	2: [0.55-0.64]	3: [0.65-1.81]	
Characteristics							
N	39	66	51	106	62	78	---
Age (years)	40.1±14.9	38.0±14.2	48.7±19.7	32.9±11.4	39.5±13.1	58.6±15.6	<0.0001
Current Smoker N(%)	6 (15.4)	5 (7.7)	6 (12)	1 (1)	0 (0)	0(0)	0.35
Hypertension N(%)	7 (18.4)	16 (24.6)	25 (50)	10 (9.4)	16 (26.2)	44 (59.5)	0.004
Diabetes N(%)	5 (12.8)	5 (7.7)	8 (15.7)	10 (9.5)	6 (9.7)	28 (36.8)	0.13
BMI (kg/m ²) ^a	25.3±5.1	26.9±4.9	27.8±5.2	25.9±6.1	30.9±6.2	32.4±7.0	<0.0001
Triglyceride (mg/dL)	78.0±23.0	93.9±53.0	105.0±60.6	67.5±24.1	89.9±37.7	106.8±55.3	0.0002
Cholesterol (mg/dL)	184.6±36.6	188.7±48.9	191.9±37.3	173.4±37.4	190.1±36.1	217.3±48.9	0.18
Insulin	12.9±6.7	14.2±8.6	14.3±7.3	15.2±8.6	18.4±11.5	19.2±12.8	0.04
Glucose	86.3±21.3	83.4±24.2	87.5±19.0	84.8±35.7	83.9±15.5	100.0±33.6	0.24
HDL (mg/dL)	39.2±11.7	42.2±11.9	40.5±11.6	39.6±11.3	37.8±12.4	40.4±14.7	0.76
Systolic BP (mmHg)	122.5±18.1	123.2±20.2	133.6±21.3	109.0±18.2	116.8±22.3	133.6±30.1	0.30
Diastolic BP (mmHg)	74.6±10.6	76.0±12.7	80.6±13.4	70.1±11.0	73.8±13.7	79.4±13.5	0.66
Serum Creatinine (mg/dL)	0.96±0.1	1.01±0.1	1.13±0.2	0.77±0.1	0.8±0.1	1.1±0.6	<0.0001
MDRD_eGFR ^b	109.7±26.3	103.5±18.2	90.3±25.2	117.8±24.9	108.0±24.1	78.6±28.9	<0.0001
AC ratio (mg/g) ^c	2.86±4.0	5.50±26.4	11.86±31.4	9.39±32.6	14.63±38.0	45.4±108.3	0.43

^a BMI: body mass index^b MDRD_eGFR: estimated GFR^c AC ratio: Urinary albumin creatinine ratio^d P value represents differences across cystatin C tertile, adjusted for age and gender, not taking into account sample relatedness.

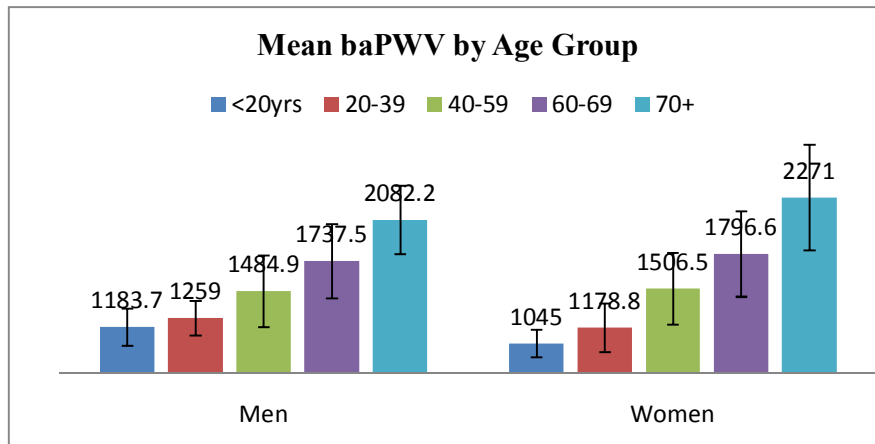


Figure 4.1 Age Specific baPWV^a by Age Group among Participants

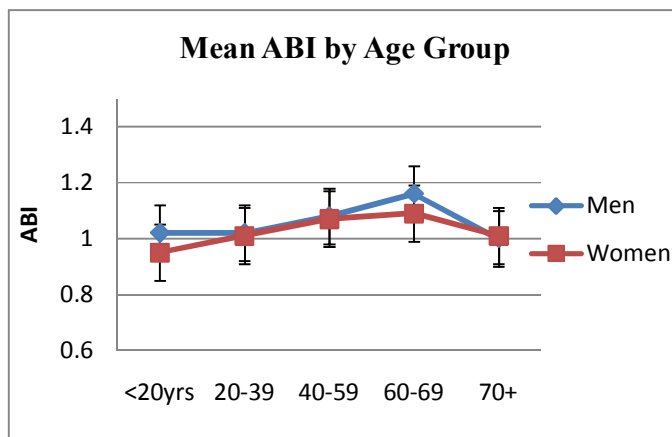


Figure 4.2 Age Specific ABI^b by Age Group among Participants

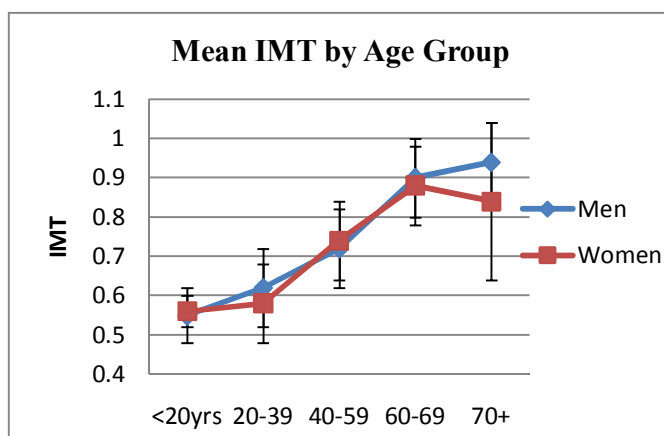


Figure 4.3 Age Specific IMT^c by Age Group among Participants

^a : baPWV: brachial ankle pulse wave velocity, an average of left and right sides

^b : ABI: ankle brachial index, average of left and right sides

^c : carotid IMT: carotid intima-media thickness, an average of left and right sides

Table 4.2 Multivariate Analysis of Traditional Cardiovascular Risk Factors, Serum Cystatin C, Serum creatinine, Microalbuminuria and eGFR on Ankle Brachial Index (ABI)

	Age- and gender-adjusted		+Traditional risk factors ^a		+Traditional risk factors +Cystatin		+Traditional risk factors +Creatinine		+Traditional risk factors +Microalbuminuria		+Traditional risk factors +eGFR	
Adjusted R^2	0.14 ^b		0.22		0.23		0.22		0.22		0.22	
	β (SE)	<i>P</i>	β (SE)	<i>P</i>	β (SE)	<i>P</i>	β (SE)	<i>P</i>	β (SE)	<i>P</i>	β (SE)	<i>P</i>
Age (years)	0.002 (0.0003)	<.0001	0.002 (0.0004)	<.0001	0.002 (0.0004)	<0.001	0.002 (0.0004)	<.0001	0.002 (0.0004)	<.0001	0.002 (0.0004)	<.0001
Gender	-0.03 (0.009)	0.002	-0.03 (0.01)	0.008	-0.03 (0.01)	0.003	-0.035 (0.01)	0.006	-0.02 (0.01)	0.01	-0.03 (0.01)	0.008
Serum cystatin C (mg/l)	-0.06 (0.02)	0.006			-0.06(0.03)	0.02						
serum creatinine (mg/l)	-0.05(0.03)	0.08					-0.03(0.03)	0.29				
Urinary albumin excretion (mg /g)	-0.001 (0.003)	0.76							0.0003 (0.003)	0.92		
eGFR [mL * min ⁻¹ *(1.73 m2) ⁻¹]	0.03(0.02)	0.15									0.02 (0.02)	0.35

^aAdjusted for traditional risk factors including age, gender, BMI, smoking status, diabetes mellitus, blood pressure stages, LDL-cholesterol, HDL-cholesterol and triglycerides. None of the traditional risk factors is significant.

^bAdjusted $R^2 = 0.14$, indicating the adjusted R^2 for the model with only age and gender

Table 4.3 The Association of baPWV (Age, Gender Adjusted) with Renal Measures

	Null Model		Serum cystatin C		Serum creatinine		Urinary albumin excretion		eGFR	
Proportion of variance due to covariates	0.54		0.62		0.59		0.59		0.59	
	β (SE)	P	β (SE)	P	β (SE)	P	β (SE)	P	β (SE)	P
Age (years)	0.01	<0.0001	0.01	<0.0001	0.01	<0.0001	0.01	<0.0001	0.01	<0.001
Gender	-0.03	0.05	-0.04	0.01	-0.02	0.29	-0.05	0.004	-0.05	0.005
Serum cystatin C (mg/l)			0.16	0.0005						
Serum creatinine (mg/l)					0.1	0.04				
Urinary albumin excretion (mg /g)							0.006	0.27		
eGFR [mL * min ⁻¹ *(1.73 m ²) ⁻¹]									-0.08	0.07

Table 4.4 The Association of baPWV (Age, Gender, Diabetes and Hypertension Adjusted) and Renal Measures

	Null Model		Serum cystatin C		Serum creatinine		Urinary albumin excretion		eGFR	
Proportion of variance due to covariates	0.62		0.63		0.62		0.62		0.62	
	β (SE)	P	β (SE)	P	β (SE)	P	β (SE)	P	β (SE)	P
Age (years)	0.009	<0.0001	0.008	<0.0001	0.009	<0.0001	0.009	<0.0001	0.009	<0.0001
Gender	-0.06	0.009	-0.06	0.01	-0.05	0.04	-0.06	0.01	-0.06	0.006
Diabetes (yes/no)	0.07	0.02	0.02	0.02	0.08	0.02	0.07	0.03	0.08	0.02
Hypertension (yes/no)	0.11	<0.0001	0.10	<0.0001	0.11	<0.0001	0.12	<0.0001	0.11	<0.0001
BMI (kg/m ²)	0.02	0.6	0.02	0.7	0.02	0.7	0.19	0.7	0.02	0.7
Serum cystatin C (mg/l)			0.09	0.04						
Serum creatinine (mg/l)					0.003	0.55				
Urinary albumin excretion (mg /g)							-0.002	0.71		
eGFR [mL * min ⁻¹ *(1.73 m ²) ⁻¹]									-0.03	0.67

5.0 GENERAL DISCUSSION

5.1 SUMMARY OF FINDINGS

Chronic disease is becoming a major public health concern in developing countries as well as in developed countries. With advancing westernization of lifestyle, Afro-Caribbeans are at greater risk of developing chronic kidney disease, metabolic syndrome or cardiovascular disease. However, in contrast to numerous studies conducted in the western world, very limited resources are available among these minority groups who are at higher risk of chronic diseases. This dissertation built upon the data collection from seven multigenerational families who reside in the island of Tobago, and comprehensively studied the following disease conditions – chronic kidney disease, metabolic syndrome and subclinical cardiovascular disease among this sample population of African origin. The Afro-Caribbeans who reside on the island of Tobago provide a valuable opportunity to conduct studies in a population with a relatively homogeneous genetic background of western African origin, with approximately 6% admixture with other ethnic groups (109).

In the effort to explain the genetic contribution to complex diseases such as CKD, CVD and metabolic syndrome related components, a large number of studies have been conducted for statistical associations with disease phenotypes among European origin populations, including candidate gene association studies, linkage analyses, and genomewide association studies.

However, even fundamental studies are sparse among minority populations. We are the first, to our knowledge, to report the heritability of renal function biomarkers and metabolic syndrome related components in an Afro-Caribbean population, confirming the existence of familial aggregation of diseases in this population.

Rather than a defined clinical presentation, metabolic syndrome is combination of medical disorders which increase the risk of developing cardiovascular disease and diabetes. The prevalence of metabolic syndrome has been increasing dramatically over the recent decades. We found a significant heritability of metabolic syndrome related traits among Afro-Caribbean families. The estimated heritability ranged from 12% to 46% for fasting glucose to HDL cholesterol. Furthermore, to better understand the structures of clustering of these inter-correlated metabolic traits, factor analysis was performed based on the assumption of the presence of common genetic and/or environmental factors underlying these components. We found that factor 1 comprised only of lipids, glucose and central obesity explained the majority of the variance (55.8%), and heritability was 29% ($p=0.0001$). Factor 2 comprised mainly of systolic and diastolic blood pressure was 20% heritable ($p=0.002$). Thus, with these significant genetic contributions to the metabolic traits, metabolic syndrome was heritable among these Afro-Caribbean family members.

As a traditional renal function biomarker, serum creatinine was found to be 28% heritable in our study. However using serum creatinine as a measurement to estimate GFR has several drawbacks. It is a by-product of muscle cells, being affected by many other factors other than renal function, and more and more investigators have argued about the accuracy of using a serum creatinine based equation to estimate GFR. Thus, we tested a novel biomarker of renal function - Cystatin C, among these participants. Cystatin C is a small protein and produced throughout the

body by nucleated cells. Levels are not significantly affected by muscle mass, gender, age, or race (78, 79). We reported the heritability of cystatin C was 32%, after adjusting for age, gender, hypertension, triglycerides and insulin level.

Patients with decreased renal function are highly predisposed to atherosclerotic artery disease. A previous study reported a remarkably high prevalence of PAD in nondialyzed patients with CKD in NHANES III 1999-2000(207). We further tested the association between renal biomarkers and subclinical cardiovascular measures including IMT, ABI and PWV. We found that only cystatin C was highly associated with ABI and PWV among this African Caribbean population, but not serum creatinine or estimated GFR. However, the mechanisms underlying the relationship between the renal function and subclinical cardiovascular disease are not fully understood. Due to the reduced glomerular filtration rate, renal insufficiency may result in alternative metabolism of calcium, phosphate, and parathyroid hormone, inflammatory alterations, or coagulation pathway, which might contribute to vascular abnormalities, eventually cause PAD. Furthermore, kidney disease may interact with not only small but also large arteries, increasing the risk for hypertension, anemia or vascular calcification, which eventually contributes to arterial stiffness.

In conclusion, our data suggest that both reduced kidney function and metabolic syndrome related components are significantly heritable among Afro-Caribbean families. The reduced renal function indicated by elevated serum cystatin C level was associated with subclinical cardiovascular disease, eventually may explain a proportion of the observed excessive cardiovascular risk among this population.

5.2 STRENGTHS AND LIMITATIONS

The study, the first to be conducted in this Afro-Caribbean population, provides heritability estimation of complex chronic diseases. We recruited participants from seven multigenerational families, with extended family members, which provide better power to detect genetic signals than parent-offspring or sib-pair data. Such family structure will give more accuracy in heritability estimation. The relationship among individuals has been validated by genotyping data, so we minimized the possibility of inaccurate reports of biological kinship. However we do have several limitations to address which might influence the findings: 1) the sample size was relatively small; 2) even though we recruited people without regard to their health status, these families were selected for large size, and participants were required to be ambulatory; thus a ‘healthy family effect’ cannot be excluded; 3) cross-sectional study design limits the ability to test the direction of the association.

5.3 FUTURE DIRECTIONS

Our significant heritability estimates of renal dysfunction and metabolic syndrome in families of African ancestry confirm a familial aggregation of these complex diseases and justify further analyses aimed at understanding the genetic determinants of renal function in this high risk ethnic group. Future studies are needed to identify underlying susceptibility genes or regions on the chromosome which contribute to the disease susceptibilities. Obtaining genotype data on markers across genome, by performing genomewide association study or genomewide linkage analyses, would enable the identification of genes contributing to the disease susceptibility.

Our findings could be extended to some additional measurements or calculation of GFR to better understand renal function among this population. The gold standard measurement of renal function is by measuring the clearance of injected inulin. Renal function is routinely estimated based on serum creatinine using the MDRD equation. The CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) has recently published as a new estimating equation for GFR (REF). The comparison of the accuracy between different equations could provide insight and suggestions for routine use in epidemiological studies and in the clinical setting.

The cross-sectional nature of this project limits our ability to test the direction of the association. Longitudinal data collection will bring the important insights of the disease causality. For example, the association between CKD and CVD remains as a question of ‘chicken’ or ‘egg’, which comes from first?

5.4 PUBLIC HEALTH SIGNIFICANCE

Through this project, we estimated the genetic impact of the following important chronic diseases conditions among African Caribbean population.

Chronic kidney disease (CKD) is a major, growing public health problem, and is particularly prevalent among populations of African descent. Higher prevalence of diabetes mellitus and hypertension account for part of this excess risk for kidney disease, and all three conditions increase risk of cardiovascular disease. In Trinidad and Tobago, 20 % renal failure occurs among the working –age population, which brings a huge economic burden.

Chronic cardiovascular disease (CVD) is the leading cause of death worldwide. Tobago population is at high risk of hypertension, diabetes, cardiovascular disease and impaired kidney

function. WHO statistics for Trinidad & Tobago document the highest rate of death due to CVD of any country outside the former Soviet Union. Morbidity of chronic renal disease and its reporting are complicated by the fact that the incidence of cardiovascular diseases, including morbid events, increases with the severity of renal disease. Indeed, the main cause of death in chronic renal disease is cardiovascular disease and not renal disease(224). This leads to the likely under-reporting of renal disease in the clinical setting and on death certificates.

Although inheritance is a recognized determinant of cardiovascular disease, metabolic syndrome, and kidney dysfunction, little is known about the genetic contributions to renal dysfunction in families of African descent. The results of this project provide the first comprehensive heritability estimation of important complex diseases among this Afro-Caribbean population. Not only did our findings confirm the familial aggregation of diseases, but also benefit future studies among this minority group of population for these important phenotypes. It is believed that this project contributes to a better understanding of genetic and environmental impact of disease susceptibility, leading to the possibility of better disease control and prevention in the Afro-Caribbean community.

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