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Psychosocial Distress: Cardiovascular Outcomes, Underlying Mechanisms, and Sex Differences

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Psychosocial Distress: Cardiovascular Outcomes, Underlying Mechanisms, and Sex Differences

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MPH in Epidemiology, Emory University, 2013

Advisor: Viola Vaccarino, MD, PhD

An abstract of
A dissertation submitted to the Faculty of the
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Abstract

Psychosocial Distress: Cardiovascular Outcomes, Underlying Mechanisms, and Sex Differences

By

Pratik M. Pimple

Background: While depression is a well-established risk factor for cardiovascular disease (CVD), results on post-traumatic stress disorders, anxiety, anger, hostility and perceived-stress are mixed. Even though these psychosocial phenotypes are correlated, previous studies have treated them as independent factors. Examining these factors together may provide new insights.

Objectives: This dissertation evaluated the association between an integrated indicator of chronic psychosocial distress and CVD outcomes, including future CVD events and mental stress-induced myocardial ischemia (MSIMI) with a specific focus on sex differences. To better understand vulnerability to MSIMI and the underlying pathophysiology, we also evaluated genetic determinants of MSIMI.

Methods: We used data from two observational studies at Emory University (N=950). For aim 1, we examined the association between the psychosocial distress indicator, created using latent-class analysis (LCA), and a composite CVD endpoint at 3 years of follow-up. For aim 2, we examined the association between the psychosocial distress indicator and MSIMI severity. For aim 3, we examined the association between a-priori selected 286 candidate-genes and MSIMI.

Results:
Aim 1: As compared to women in the lowest psychosocial distress class (LCA class-1), women in the highest class (LCA class-4) had 2.8-times the hazard of CVD events (95% CI: 1.2-6.6). No association was found in men.
Aim 2: As compared to women in the lowest psychosocial distress class, women in the highest class had 4.0-points higher summed rest score (95% CI: 0.2-7.7). This association was not observed in men. There was no association between psychosocial distress and MSIMI in either women or men.
Aim 3: Of 286 candidate-genes, the *FGF5* (Fibroblast Growth Factor-5) gene on chromosome 4 was associated with MSIMI at the Bonferroni-adjusted significant threshold ($P=4.7\times10^{-5}$).

Conclusion: A higher level of psychosocial distress is associated with the risk of cardiovascular events and higher resting perfusion abnormalities in women, but not in men. We also uncovered a signaling pathway related to tissue growth and repair and with links to the brain as being possibly involved in MSIMI. Overall, our findings suggest a prominence of the psychosocial sphere in CVD risk pathways especially for women.

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Chapter 1: Introduction, Study Background, Objectives & Hypotheses

This chapter describes in details study background, objective and hypotheses by each aims. The chapter includes a brief overview of the literature and describes the overarching goals of this dissertation, followed by the background, the main objectives and the study hypotheses for each of the three aims.

Brief Overview:

The prevalence of mental health disorders in the US population is growing steadily. In 2013, an estimated 18.1% of US adults aged 18 years or older had a diagnosed mental illness, of which 6.7% (15.7 million) had at least one major depressive episode.[1] There is a growing need to understand the impact of psychosocial health on observed physical outcomes, especially on cardiovascular disease (CVD). Psychiatric conditions and personality traits have received attention for their association with CVD risk. However, while depression is a fairly established risk factor for CVD,[2-4] results for other factors such as post-traumatic stress disorders (PTSD), anxiety, anger, perceived stress and hostility are mixed or limited, and more work is needed.[5]

The proposed mechanisms through which psychosocial stress affects cardiovascular health are multifactorial and can be grouped in two broad categories: 1) adverse health behaviors, and 2) acute or chronic biological consequences of altered sympathetic-parasympathetic balance on inflammation, platelet activation, vascular function and metabolism.[6-8] These factors can promote traditional CVD risk factors such as hypertension, obesity and insulin resistance, but can also more directly affect pathophysiological processes including atherosclerosis, thrombus formation, cardiac arrhythmias, and myocardial ischemia, potentially triggering acute coronary syndromes and cardiac death. This dissertation focused on the relatively understudied phenomenon of mental stress-induced myocardial ischemia (MSIMI),

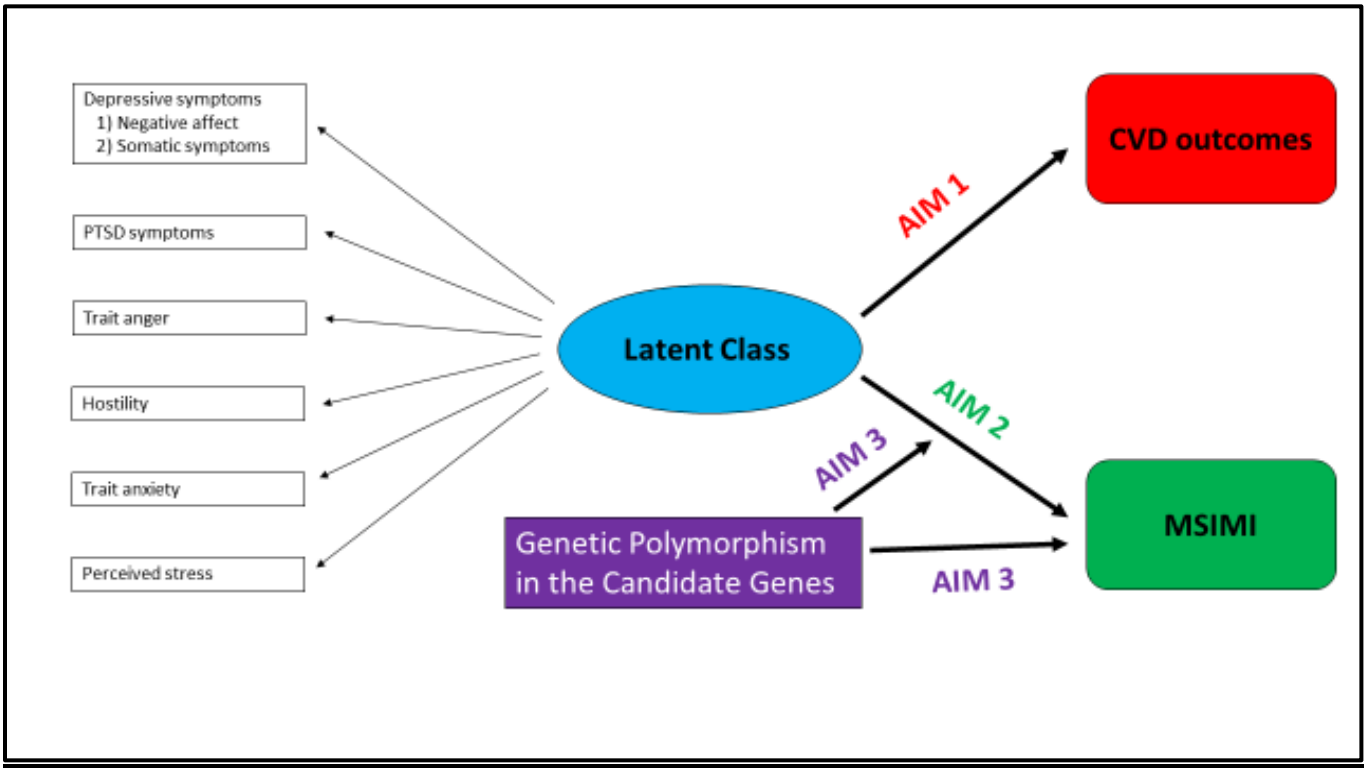
which reflects the acute effects of psychosocial stress on coronary perfusion. MSIMI is a transient myocardial ischemic response to a standardized laboratory-based mental stress challenge,[9] which can be induced in approximately one third of patients with CVD,[9] and is associated with adverse outcomes.[10] Recently, interest has grown in MSIMI as a metric that may index an individual's cardiovascular vulnerability to emotional stress.[11] As such, it should be informative as a pathophysiological mechanism, or intermediate outcome, to assess the effects of psychosocial distress on CVD.

Psychosocial phenotypes are likely inter-related, but most previous studies have treated them as independent factors. Examining these factors together as a comprehensive profile of psychosocial distress may provide new insights for our understanding of CVD risk and may help explain inconsistencies in previous research. Hence in aim 1 of this dissertation, we first examined whether a latent construct indexing "psychosocial distress" can be constructed using observed, correlated psychosocial symptom-scales (depressive symptoms, PTSD symptoms, anxiety, anger, hostility, and perceived stress) in individuals with pre-existing, stable coronary artery disease (CAD), and whether this latent construct is prospectively associated with adverse cardiovascular outcomes, assessed over three years of follow-up. In aim 2, we investigated whether the latent construct indexing psychosocial distress is positively associated with MSIMI. For both aims, we also conducted a sensitivity analysis using a summary score of psychosocial distress, based on a published method.[12]

Although physiological pathways such as altered sympathetic-parasympathetic balance and inflammatory response to stress have been studied in relation to MSIMI to a certain extent, the role of genetic predisposition and its possible interaction with psychosocial distress is virtually unknown. Identifying genetic variants which are associated with the MSIMI can

provide insight into mechanistic pathways responsible for this phenomenon and can also potentially provide new targets for devising preventive strategies and/or therapies. Hence in aim 3, we investigated the associations between genetic polymorphisms and MSIMI by 1) performing an exploratory genome-wide association analysis (GWAS); and 2) investigating association between a-priori defined set of candidate genes (those related to stress-response physiology and/or those with an established association with CAD) and MSIMI. We also tested for the interaction (on an additive scale) between our psychosocial distress index and a genetic risk score computed from a subset of these candidate genes using established methodology.[13,14]

Chapter 1, Figure 1: General Schema of Dissertation



Background: Association Between Psychosocial Distress Indicators & Cardiovascular Outcomes

Psychosocial health can be defined as a state of mental, emotional, social and spiritual well-being, and in contrast, mental health problems such as depression, post-traumatic stress disorder (PTSD), hostility, and anxiety can be viewed as a deviation from one's psychosocial health. The prevalence of mental health disorders in the US population is growing steadily. In 2013, an estimated 18.1% of US adults aged 18 years or older had a diagnosed mental illness, out of which 6.7% (15.7 million) had at least one major depressive episode.[1] Also, an estimated 3.5% of the US adult population had PSTD during the previous year[1,15] and about 3% of the total US adult population had generalized anxiety disorders.[1,15] There is a growing need for a better understanding of the impact of psychosocial health on observed physiological outcomes, especially the link between adverse psychosocial phenotypes and chronic diseases such as cardiovascular disease (CVD).

Types of Psychosocial Factors:

Psychosocial factors may be broadly divided into two major categories: 1) intrinsic factors (individual psychological characteristics), and 2) extrinsic/environmental factors (socioeconomic status (SES), discrimination).[16] Individual psychosocial characteristics may be further divided, for practical purposes, into 1) psychiatric disorders/symptoms (depressive symptoms, anxiety, PTSD), 2) personality trait (anger and hostility), and 3) perceived stress.

The link of adverse environmental psychosocial exposures such as low SES, early life trauma, and perceived discrimination, with CVD has been extensively investigated. For example, systematic reviews of literature have found that low SES is an important correlate of cardiovascular risk, including cardiovascular risk factors and incident CVD.[17,18] Early life trauma

is involved in the pathophysiology of mental disorders such as depression and PTSD[19,20] and is also associated with CVD. For example, in a retrospective cohort study of 17,337 adult Kaiser Health Plan members, exposure to severe childhood trauma was associated with 2.3 fold increased odds of ischemic heart disease.[21] Perceived discrimination has been associated with 28% higher risk of CVD in the Multi-Ethnic Study of Atherosclerosis study[22] and was found to be associated with adverse CVD risk factors such as hypertension,[23] smoking and poor sleep quality.[24]

Even though these exposures are important determinants of CVD risk, we decided to focus our attention only on intrinsic psychosocial factors, because 1) intrinsic psychosocial phenotypes such as depression and PTSD are often a consequence of external stressors, and thus may be more proximal risk determinants for CVD, and 2) intrinsic psychosocial phenotypes may be easier to modify as compared to external factors.

Association between psychiatric disorders/symptoms (Depression, PTSD, Anxiety) and CVD:

Of all the measured psychosocial health indicators, depression is one of the most studied. Recent reviews[3,4,25] have pointed out that more than 60 prospective studies have assessed the association between depression and future cardiac events or mortality. Depression is associated with incident CVD events in individuals without CVD at baseline[2,26-32] and is also associated with higher cardiovascular morbidity and mortality in individuals with pre-existing CVD.[3,4] In a comprehensive meta-analysis of 30 prospective cohort studies with 893,850 participants free of CVD at baseline, depression was associated with both incident myocardial infarction (pooled risk ratio: 1.30, 95% CI: 1.18-1.44) and incident coronary heart disease (pooled risk ratio: 1.30, 95% CI: 1.22-1.40).[2] An association between depression and future cardiovascular events was found in the majority of large U.S. based cohort studies with diverse populations, such as the

Framingham study[29], the Multi-Ethnic Study of Atherosclerosis[26], the CARDIA study[30], and a large retrospective cohort study of US veterans.[32] However, among patients with CVD, results have not been entirely consistent and effect sizes have varied,[4] and none of the clinical trials investigating the effect of antidepressant therapies on CVD outcomes among individuals with depression and significant CAD have found meaningful differences among treatment and control groups.[25]

Results for post-traumatic stress disorder (PTSD), and anxiety have also been inconsistent. PTSD is a disabling mental health disorder secondary to trauma exposure such as military combat, intimate partner violence, or natural disasters.[33] A meta-analysis of six studies (N= 402,274) in initially healthy individuals found that PTSD was associated with 27% higher CVD incidence [pooled hazard ratio (HR)= 1.27, 95% CI=1.08 - 1.49] independent of traditional CVD risk factors and depression, but this meta-analysis was limited by potential publication bias due to the small sample of included studies.[33]

Anxiety, measured using anxiety symptoms scales, was also found to be associated with future cardiovascular events in individuals with[34] and without[35,36] established CVD at baseline, but there is a substantial heterogeneity in results across studies. A meta-analysis of 37 studies (N= 1,565,699) in individuals initially free of CVD found that anxiety was associated with 41% higher incidence of CVD (HR= 1.52, 95% CI= 1.36-1.71), but individual study effect sizes ranged from 0.6 to 3.0, and the meta-analysis could not take into account the effect of co-occurring depression and other psychosocial variables.[35]

Association between Personality Traits (Anger, & Hostility) and CVD:

Psychosocial health is affected by personality traits which can influence a person's reaction to everyday stressors. Anger and hostility have long been considered potential

precipitants of acute myocardial infarction (MI) and significant risk factors for CVD, but results in the literature, again, are inconsistent.[37] A comprehensive meta-analysis of 44 prospective studies found that anger and/or hostility were associated with a modest increase in CVD risk in both initially healthy individuals (19% increase; HR: 1.19, 95% CI: 1.05 – 1.35) and in those with pre-existing CVD (24% increase; HR: 1.24, 95% CI: 1.08 – 1.42), but the effect sizes of individual studies ranged from 0.72 to 2.30 and more than 50% of included studies reported a weak or no association between anger/hostility and CVD events (hazard ratios below 1.20).[37]

Association between perceived general stress and CVD:

Perceived stress can be described as the degree to which situations in one's life are appraised as stressful, for example, how uncontrollable and overloaded respondents perceive their lives. Perceived stress was found to be modestly associated with incident CVD events, with a pooled 27% higher incidence (HR= 1.27, 95% CI= 1.12 - 1.45) in a meta-analysis of 6 prospective cohort studies (N= 118,696). Again, these results are limited by heterogeneity (effect sizes ranging from 1.0 to 1.6) and potential publication bias.[38]

Differential Effect of Sex

Given that women with CAD have a higher prevalence of psychosocial distress relative to men,[39] whether there is any effect modification by sex on the association between psychosocial distress and CVD events is of interest. While the overall relationship between some psychosocial factors like depression and future cardiovascular events is fairly established,[3,4,25] previous literature regarding sex-differences in the association between psychosocial distress and CVD is mixed.[2,40] However, two recent nationally representative studies, one from the US using the National Health and Nutrition Examination Survey,[41] and the second from Canada using the National Population Health Survey[42] have shown an increased effect of depression and other

related psychosocial factors on CVD in women, and not in men. Also, the large, 52-countries Interheart study found a differential effect on the impact of psychosocial distress on myocardial infarction by sex.[43] In this study, a composite measure of psychosocial symptoms yielded a 40% population attributable risk for acute myocardial infarction in women, while for men, the same attributable risk was only 25%.

Gaps in Literature:

Of several psychosocial phenotypes, depression has been most extensively studied, and found to be overall consistently associated with CVD, while results for other factors are mixed or limited.[5,44] Many of these psychosocial phenotypes share variance, but the majority of studies in the literature treat each psychosocial phenotype as an independent factor. Rarely have studies taken into account and integrated a persons' psychosocial profile in relation to CVD risk. One reason why an integrative approach may be important is that these psychosocial factors may share biological/behavioral substrates, explaining why they tend to correlate and cluster with each other.[45] Examining them together may provide new insights about specific psychosocial profiles that may be related to CVD. To the best of our knowledge, this is the first study to combine a wide-array of potentially interrelated psychosocial factors in patients with stable CAD through latent class analysis and to assess the association between such psychosocial profile with clinical cardiovascular outcomes as well as with the subclinical outcome of mental stress induced ischemia.

Aim 1: Psychosocial Distress and Future Cardiovascular Events: Objective & Hypothesis

Objectives:

The overall goal of this dissertation is to investigate: 1) whether a latent construct can distinctly define observed, inter-related psychosocial phenotype, indicating psychiatric conditions and/or personality traits (depressive symptoms, PTSD symptoms, anger, hostility, anxiety, and perceived stress) in individuals with pre-existing, stable CAD; and 2) whether a latent construct indexing greater psychosocial distress is significantly associated with adverse future cardiovascular events, as compared to a latent construct with low psychosocial distress. We will also investigate whether there is any effect measure modification by sex on this association between psychosocial distress and cardiovascular events

Hypothesis:

Patients with an adverse psychosocial profile indicative of elevated psychosocial distress, identified through latent class analysis, are at higher risk of future CVD events as compared to those with a more favorable psychosocial profile. We also hypothesize that this association will be stronger in women, as compared to men.

Background: Association Between Psychosocial Distress Indicators & Stress-Induced Ischemia

A laboratory-based mental stress challenge is an objective and standardized way of examining the effects of acute emotional factors on the cardiovascular system.[9] Mental stress-induced myocardial ischemia (MSIMI) is a transient myocardial ischemic response to this standardized mental stress challenge,[9] which can be induced in approximately one third to one half of patients with CVD.[9] MSIMI is analogous to conventional exercise or pharmacologically-

induced myocardial ischemia during standard cardiac clinical testing, except that the stressor used is psychological instead of conventional stress testing (exercise or pharmacological stress testing).[9] MSIMI is associated with a twofold increased risk of future cardiac events, which is similar to ischemia induced by conventional stress testing.[10] MSIMI, however, appears to be a unique phenomenon, since it occurs at lower levels of oxygen demand and is usually not related to severity of CAD.[9,46] Furthermore, MSIMI has been associated with myocardial ischemia measured in daily life ambulatory monitoring.[46,47] These features suggest that MSIMI is an expression of psychosocial burden, rather than CAD severity. Also, evaluation of myocardial ischemia with mental stress has considerably evolved over the years,[9] the current practice being to either use myocardial perfusion imaging (MPI) or echocardiography.

Published literature on the association between indicators of psychosocial distress and MSIMI have provided conflicting results.[48-54] In some studies, depression was associated with MSIMI, irrespective of whether ischemia was measured using perfusion imaging[50,54] or echocardiography.[48] On the other hand, depression was not associated with MSIMI in the Psychophysiological Investigation of Myocardial Ischemia (PIMI) Study.[52] Anger and/or hostility were associated with MSIMI in two studies using nuclear imaging techniques,[49,53] but this association was non-significant in another study where ischemia was measured using echocardiography.[48] Neither anxiety[48,52] nor perceived stress[48] were found to be associated with MSIMI in published literature, and no studies were found on the association between PTSD symptoms and MSIMI.

Thus, even though it is a reasonable inference that person's psychosocial profile should predict his/her cardiovascular responses to an acute emotional stimulus, results in the literature are conflicting.[51] Previous studies investigating association between psychosocial factors and

MSIMI have similarly treated each psychosocial phenotype as an independent exposure, and no studies have taken into account and integrated persons' psychosocial profile more broadly. Analyzing the inter-related patterns of these complex phenotypes through LCA might provide more insight on the relationship between the psychosocial profile of an individual and myocardial ischemia with mental stress. Also, similar to aim 1, exploring the effect modification by sex on the association between MSIMI and psychosocial distress is important, given that women with coronary artery disease have a higher prevalence of psychosocial distress relative to men,[39] as well as a higher prevalence of MSIMI.[55,56]

Aim 2: Psychosocial Distress & MSIMI: Objective & Hypothesis

Objectives:

The overall goal of this aim is to investigate whether a latent construct indexing greater psychosocial distress is significantly associated with higher prevalence of myocardial perfusion abnormalities in resting condition and with mental stress, as compared to a latent class with low psychosocial distress.

Hypothesis:

Patients with an adverse psychosocial profile indicative of elevated psychosocial distress, identified through latent class analysis, have a higher prevalence of myocardial perfusion defects at rest, as well as a higher prevalence of inducible perfusion defects with mental stress (indicative of myocardial ischemia) as compared to patients with a more favorable psychosocial profile.

Background: Genetic Determinants of Stress-Induced Ischemia

Mental stress-induced myocardial ischemia (MSIMI) is a transient myocardial ischemic response to a standardized mental stress challenge.[9] MSIMI is analogous to conventional exercise or pharmacologically-induced myocardial ischemia during standard cardiac clinical testing, except that the stressor used is a standardized, laboratory based psychological stress challenge.[9] MSIMI has a similar prognostic value compared to conventional physical stress ischemia with approximately a two-fold increased risk of future cardiac events.[10] However, it appears to differ from ischemia induced by conventional testing, in that it occurs at lower levels of oxygen demand, and is usually not associated with severity of coronary artery disease (CAD).[9,46,57]

Several stress-related mechanisms have been postulated for MSIMI, including 1) hyper-activation of cerebral regions responsible for emotions, memory and sympathetic activation,[58,59] 2) imbalance in sympathetic-parasympathetic stimulation in response to stress,[60,61] 3) hyperactive response of inflammatory systems to stress,[60-62] and 4) endothelial dysfunction and/or microvascular disease.[57,61] However, the role of genetic predisposition in the occurrence of MSIMI and its possible interaction with psychosocial distress have not been studied in detail. Identifying genetic polymorphisms which are associated with the MSIMI can provide further insights into mechanistic pathways responsible for this phenomenon and can also potentially provide new targets for devising preventive strategies and/or therapies. To the best of our knowledge, only one study by Hassan et al[63] has investigated the association between genetic variants and MSIMI. These investigators examined five single nucleotide polymorphisms (SNPs) of β1-adrenergic receptors (ADRB1) and β2-adrenergic receptors (ADRB2) and MSIMI in a small sample (N= 148) of patients with stable CAD. Polymorphisms in these genes alter the

effects of epinephrine on cardiac and vasculature physiology and these polymorphisms have been associated with CVD.[64] Hassan et al. found a significant association between a variant of the ADRB1 gene (rs1801252: substitution of major allele adenine by guanine) and MSIMI at significance level of 0.05, but no other genes involved in stress-response pathways were analyzed and there was no adjustment for multiple testing and population stratification (the most important confounder for genetic association studies). Hence, in our study of patients with stable CAD, we investigated the associations between genetic polymorphisms and MSIMI by 1) performing an exploratory genome-wide association analysis (GWAS); and 2) investigating association between a-priori defined set of candidate genes (those related to stress-response physiology and/or those with an established association with CAD) and MSIMI.

We also computed a genetic risk score based on the 169 gene loci which were found to be significantly associated with CAD in four recently published genome-wide association studies.[65-69] Computing a genetic risk score provides a meaningful way of summarizing the risk of CVD imparted by these 169 gene loci, and this method has been has been extensively used for CVD risk prediction.[13,70,71]

As psychosocial distress is known to modulate stress-response physiology, we hypothesize that genetic variation in the candidate genes can modify the association between psychosocial distress and MSIMI. Hence, we also tested for the interaction (on an additive scale) between our psychosocial distress variable (similarly constructed as part of the other dissertation aims) and a genetic risk score computed from the candidate genes which are significantly associated with CAD (169 gene loci).

Aim 3: Genetic Determinants of Stress-Induced Ischemia: Objective & Hypothesis

Objectives:

The overall goal of this aim is three-fold. First, we investigated whether any genetic variants (SNPs) in the entire genome is significantly associated with mental stress-induced myocardial ischemia at the GWAS P-value threshold (hypothesis generation). Second, we investigated whether the genetic variants which can lead to structural changes in the proteins coded by pre-defined candidate genes are associated with MSIMI (hypothesis testing). Also, we explored whether these genetic variations in the candidate genes modify the association between an indicator of psychosocial distress derived through latent class analysis and MSIMI.

Hypothesis:

We expect that patients with risk alleles in functional SNPs of stress-response reactivity genes (for example, subjects with at least one risk allele for a SNP in genes ADRB1 and/or ADRB2) or in functional SNPs of genes related to CVD, have a higher likelihood of developing ischemia with mental stress, as compared to subjects who are homozygous for the corresponding non-risk allele. We also expect that some of these genetic variants will interact with psychosocial distress in predicting MSIMI, i.e., the relationship between psychosocial distress and MSIMI will vary according to the genotypes.

Chapter 2: Methods

This chapter provides specific details on methodology organized by aims. The chapter includes an overview of the methods, exposure, outcome and adjustment factor assessment, rationale and specifics of LCA methodology as well as detailed analysis plans and conceptual DAG schematics for each aim.

Brief Overview

In aim 1, we performed latent class analysis[72,73] of the seven psychosocial phenotypes scales (somatic and cognitive depressive symptoms, PTSD symptoms, anxiety, anger, hostility, and perceived stress), measured at baseline using self-reported, validated scales, in 950 individuals with pre-existing CAD. Cardiovascular outcomes (CVD mortality or non-fatal myocardial infarction) were assessed during a 36-month in-person and telephone follow-up, and we performed Cox proportional hazard regression to investigate the association between the categorical latent construct indicating psychosocial status and future cardiovascular events. For aim 2, myocardial perfusion data were obtained at rest, and with mental stress at the baseline visit using two single-photon emission computed tomography (SPECT) imaging studies. The difference in severity of perfusion abnormalities between mental stress and at rest was used as a measure of ischemia severity with mental stress. Multivariable linear regression was performed to investigate the association between the categorical latent construct indicating psychosocial distress as exposure and perfusion defects at rest and with mental stress as outcomes. For aim 3, genomic DNA was collected at baseline and Illumina's MEGA chip was used for genotyping. Candidate genes were selected 1) based on their role in the stress response, with focus on genes associated with hemodynamic, coronary and/or peripheral vascular response to stress; and 2) based on established associations with coronary artery disease. We performed multivariable

linear regression to investigate the association between each SNP of the entire genome and the inducible ischemia score with mental stress (difference in perfusion defect score with mental stress and rest, treated as continuous variable), adjusting for age, sex and population stratification indicators. We also tested for the interaction between the genetic risk score computed from a sub-set of our candidate genes (genes with established associations with CAD), and the psychosocial distress variable (derived using latent class analysis).

In the next sections, we will first describe the data source for all three dissertation aims, then discuss in details the main exposure and outcomes assessment for this dissertation, and at the end, describe the statistical analysis plan.

Data Source and Population characteristics:

This dissertation used data from two studies: Mental Stress Ischemia: Prognosis and Genetic Influences Study, or MIPS (N = 636) and Myocardial Infarction and Mental Stress Study, or MIMS (N = 314). There were important differences in the inclusion criteria of individuals between the two studies, but both these studies had same data collection protocol.

For MIPS (N = 636),[74] study participants were included in the study if they were between the ages of 18 and 80 years and had documented history of CAD during their lifetime. Subjects must have had satisfied at least one of the following five criteria: 1) angiographically proven CAD including at least 1 major vessel with evidence of disease; 2) prior myocardial infarction (>3 months); 3) abnormal coronary intravascular ultrasound for at least 1 vessel; 4) previous bypass surgery or post percutaneous intervention (> 1 year); and/or 5); positive nuclear scan or exercise stress test. For MIMS (N = 314), individuals were included if they were between the ages of 18 and 60 years, and had documented history of myocardial infarction within the previous 8 months. Thus, both studies recruited individuals with significant CAD, but MIMS

required individuals to have had a myocardial infarction while MIPS included a broader population of stable CAD patients. Another important distinction between these two studies is that MIMS recruited 50% men and 50% women (since it aimed at studying sex differences), while MIPS did not have any planned sex distribution (**Chapter 2, table 1**).

Exclusion criteria for both studies were overall similar and included: 1) history of unstable angina or acute MI within the past week, 2) severe comorbid medical or psychiatric disorder that could interfere with study assessments or results, such as cancer, renal failure, current alcohol or substance abuse or schizophrenia, 3) uncontrolled hypertension and/or deemed to be unfit to withhold anti-ischemic medications by study cardiologist, 4) weight over 400 lbs. (due to weight bearing limits of the nuclear stress test equipment), and 5) pregnancy or breastfeeding (**Chapter 2, table 1**).

Both the studies were approved by Emory University Institutional Review Board, and written informed consent was obtained from all participants.

Chapter 2, Table 1: Study Design Similarities & Differences Between MIPS and MIMS Studies

Characteristics	**MIPS Study**	**MIMS Study**
Sample size	636	314
Age range	30 to 80 years	18 to 60 years
Sex ratio (M/F)	70:30	50:50
Inclusion criteria	Significant history of CAD during their lifetime: 1) Angiographically proven disease including at least 1 major vessel with evidence of disease 2) Prior myocardial infarction (>3 months)	Documented history of myocardial infarction within the previous 8 months

	3) Abnormal coronary intravascular ultrasound for at least 1 vessel 4) Previous bypass surgery or post percutaneous intervention (> 1 year) 5) Positive nuclear scan or exercise stress test)	
Common exclusion criteria	1) History of unstable angina or acute MI within the past week 2) Severe comorbid medical or psychiatric disorder that could interfere with study results, such as cancer, renal failure, current alcohol or substance abuse or schizophrenia 3) Uncontrolled hypertension and/or deemed to be unfit to withhold anti-ischemic medications by study cardiologist 4) Weight over 400 lbs 5) Pregnancy or breastfeeding	
Exclusion criteria differences	Individuals with inflammatory diseases (like rheumatoid arthritis, lupus), on dialysis, or having any organ transplant excluded	No such exclusion criteria

Exposure Assessment: Composite Psychosocial Distress Indicator (Aim 1, & 2)

To assess psychosocial status, we administered following instruments to our study subjects at the baseline visit:

1. The **Beck Depression Inventory (BDI)**[75] is a self-administered 21-item scale which provides a continuous measure of depressive symptoms, with excellent internal consistency (Chronbach's alpha = 0.91).[76] We used the BDI scale as two separate subscales: negative affect (8 items) and somatic symptoms (13 items),[75] since several studies have found that these symptom dimensions differ in their association with incident CVD events, with somatic symptoms showing a much more robust association.[77,78] Each item of the scale is scored on a Likert-scale (0 indicating no symptoms to 3 indicating severe symptoms), and total score (range: 0 to 63) is

derived by adding the individual scores of each item, with a higher total score indicating greater depressive symptoms.

2. PTSD symptoms were measured using the **PTSD Checklist (civilian version),** a self-reported scale comprising of 17 Likert-scale items. Each item ranges from 1 to 5 (with a total score ranging from 17 to 85), with excellent internal consistency (Chronbach's alpha = 0.94).[79,80] This scale is used for screening individuals for PTSD, for diagnosing PTSD, and for monitoring symptom change during and after treatment. We used the civilian version of the scale, which is not limited to military-related events like combat.
3. Anxiety was assessed using **State-Trait Anxiety Inventory (STAI),**[81] a 40-item Likert-scale designed to assess both acute (current state) and chronic levels of anxiety (anxiety trait). Both scales have excellent internal consistency (Chronbach's alpha ranging from 0.86 to 0.95). Each item ranges from 1 to 4, with a total score ranging from 20 to 80 (a higher score indicates greater anxiety). We used the trait-anxiety scale as our indicator of chronic anxiety for latent class analysis.
4. Anger was assessed using the **Spielberger's State-Trait Anger Expression Inventory (STAXI-2)**, a 57 item questionnaire which measures the following anger dimensions: state-anger (intensity of anger at a particular time), trait-anger (disposition to experience angry feelings as a trait), and anger expression, including anger-out, anger-in, and anger-control.[82,83] Similar to STAI, each item ranges from 1 to 4, with a greater score indicating more anger. All scales have good internal consistency and validity.[82] Similar to anxiety, for this analysis, we used the trait-anger as our measure of choice.

5. The **Cook-Medley Hostility Scale (CMHS),**[84] a 50 item true/false self-reported validated questionnaire, was used to measure hostility. A total score is derived by summing up response to all 50 questions (range = 0 to 50), with a higher score indicating higher hostility.
6. The **Perceived Stress Scale (PSS)** assesses the perception of different every-day life stressors (for e.g., financial stress, occupational stress, relationship stress, parental stress, and stress within friendships), through a validated 10 item self-administered questionnaire, with Chronbach's alpha = 0.84.[85] Each item is scored on a Likert-scale of 0-4, and a total score is derived by summing responses to all items. The total score ranges from 0 to 40, with higher scores indicating greater perceived stress.

Observed phenotypes which informed the latent class analysis (LCA) include depressive symptoms (negative affect and somatic symptoms), PTSD symptoms, anxiety (anxiety trait), anger (anger trait), hostility and perceived stress, for a total of seven scales.

Latent Class Analysis:

Rationale for needing the latent variable and selection of latent class analysis:

Each of the measured psychosocial factors mentioned above (depressive symptoms, PTSD symptoms, anxiety, anger symptoms, hostility and perceived stress) are associated with poor health behaviors, but are not all established cardiovascular risk factors, with the possible exception of depressive symptoms. Even though these individual phenotypes index different dimensions of subjective distress, they are also highly related to each other and may share common substrates (both psychological and physiological). Integration of these phenotypes could better describe one's overall psychosocial status, and could provide more insight into a possible relationship with CVD, compared to investigating the association of these factors with

CVD independently. While we cannot measure a person's overall psychosocial status directly, we can deduce this construct using latent class analysis (LCA).[72,73]

A latent construct is a variable that is not directly observed or measured and therefore must be constructed through the observation of related variables.[72,73] LCA is a type of latent variable model based on the assumption that observed indicator variables are associated with each other because of an underlying unobserved factor, instead of being directly related with each other.[73] Many researchers have explored combinations of two or more psychosocial phenotypes through latent class analysis or related structural equation modelling methods, but none of these studies have investigated the association between an integrated psychosocial construct and CVD. Also, these studies either examined various symptom profiles within specific domains, such as depressive symptoms scales,[86-92] PTSD symptoms scales,[93-101] or anxiety symptoms scales,[102-104] or combined two or more of these scales like depression and anxiety, or PTSD symptoms and anxiety.[105-124] Ours was the first study seeking to explain the underlying correlation between observed multidimensional symptoms of emotional distress, including depression, PTSD, anxiety, anger, hostility and perceived stress, through a latent construct.

While traditional regression analysis can only utilize information from observed data, LCA creates a categorical latent variable based on designated observed indicators. LCA is one of several established statistical techniques based on structural equation modelling, including also factor analysis, latent class growth analysis, and cluster analysis. Among these, we chose latent class analysis as the method of choice due to 1) flexibility of using both continuous and categorical observed variables, 2) probabilistic approach of assigning individuals to different latent classes instead of deterministic approach, 3) use of maximum likelihood estimation (MLE)

to identify the latent variable, and 4) established MLE based criteria which can aid in deciding the number of latent classes which fit better to the observed data.[72,73]

LCA and factor analysis are analogous techniques, but LCA assumes that the underlying latent class is a categorical variable, i.e., that there exist distinct, qualitative differences between the latent groups of individuals, while factor analysis assumes that the underlying latent variable is a continuous variable, i.e., that individuals quantitatively differ from each other but lie on the same continuum.[72] We chose LCA over factor analysis, as we postulate that our study subjects can be indeed classified in different latent classes which will be qualitatively different in their psychosocial status.

Standard LCA also assumes that the distribution of the observed variables is multinomial. Because our observed variables are on a continuous scale, we used a variation of LCA called mixed-model latent cluster analysis,[73] which allows incorporation of both continuous and categorical observed variables. In the next sections, we have first briefly explained the basic concepts of LCA, and then elaborated on mixed-model latent cluster analysis.

Basic concepts of latent class analysis:

LCA requires distributional assumptions for both observed and latent variables.[72,73] For categorical observed variables, the distribution is assumed to be binomial (for two categories) or multinomial (for more than 2 categories). For latent variables, the distribution is assumed to be binomial or multinomial, based on the number of classes which fit the data best. Another important assumption of LCA is conditional independence, meaning that observed variables are assumed to be independent of each other when conditioned on the latent class variable.[72,73]

Two important aspects of LCA include the number of classes for the latent variable and the relative size of them. The number of classes can be determined by an a priori hypothesis, or using statistical tests of fit that can inform an appropriate number of classes (discussed later in *section "Data Analysis Plan"*). The relative size gives an idea of how much of the study population is categorized in each latent class.

The fundamental parameters of interest for LCA are the latent class probability and the item-response probability.[72,73] The latent class probability is the probability of being in a latent class "x" of a latent variable X for an individual, expressed as P(X= x). The sum of latent class probabilities over all classes should equal one. The item-response probability can be defined as the probability of observing a response pattern in the observed variable, given a latent class (conditional probability). For example, an item-response probability of observing a response "y" in a categorical variable Y, conditional on latent class "x" of variable X is expressed as P(Y= y|X= x). This conditional probability describes the relationship between the latent variable and observed variables and indicates how likely or unlikely an observation is to be in a latent class.[72,73] The number of item-response probabilities is the same as the number of unique combinations of observed variables. For example, if we have 3 dichotomous observed exposure variables (A, B, C), we will have 8 (2*2*2) unique combinations of observed variables, giving rise to 8 item-response probabilities. These item-response probabilities are important for understanding the characteristics of subjects in each latent class. For example, if a latent class has lowest item-response probability for variable A, subjects in that class can be deduced to have the lowest exposure levels of variable A.

Multiplication of latent class probability (P(X= x)) and item-response probability (P(Y= y|X= x)) gives us the joint probability of X and Y [P(X=x & Y=y) = P(Y=y|X=x)* P(X= x)].

When we apply this for all the combinations of observed variables and latent classes, we get the equation for the joint distribution of all the observed variables and the latent class variable. Let us say we have three observed categorical variables A, B, C, with respectively i, j and k levels in each categorical variable, and we have a latent variable X with x levels. Then, the joint equation will be:

$$P(A_i B_j C_k X_x) = P(A_i|X_x) * P(B_j|X_x) * P(C_K|X_x) * P(X_x) \quad \text{Equation (1)}$$

This equation will support the conditional independence assumption, stating that observed variables are independent of each other, conditional on the latent variable.

Latent class probabilities and item-response probabilities from the above equation are estimated using maximum likelihood estimation via the Expectation-Maximization (EM) algorithm.[72,73] This algorithm calculates the likelihood function based on the above joint equation using either probabilistic or log-linear parameterizations and finds the values of all the parameters of the model, which will maximize the likelihood.

Extension of latent class analysis: Mixed-model cluster analysis[73]

In the previous section, we discussed the traditional LCA, where observed variables are assumed to have multinomial distribution and the unobserved latent class is also assumed to have multinomial distribution. Based on the same principles, LCA can be extended to a method called mixed-model cluster analysis, where observed variables can be both continuous and categorical.[73]

Let us consider "J" observed variables (denoted as "$Y = (Y_1, Y_2, \ldots, Y_J$") and a latent variable with "K" classes. Then, the joint distribution of observed and latent variables can be defined in general terms as:

$$f(Y|\theta) = \sum_{K=1}^{K} \pi_k \prod_{J=1}^{J} f_k\ (Y|\theta_{JK}) \qquad \text{Equation (2)}$$

In the above equation, θ represents the computed conditional parameter for each observed variable (Y_J), according to each latent group "K". For example, if y_1 is a dichotomous variable (yes/no), then θ_{11} will represent the probability of having a yes response for the observed variable y_1 in the latent class "1" (conditional probability, analogous to the item-class probability discussed above). Similarly, if y_2 is a continuous variable, θ_{21} will represent the conditional mean of variable y_2, given the latent class is "1"). Π_K in the above equation represents the prior probability of belonging to the latent class "K", i.e. it is the latent class probability explained in section "*Basic concepts of latent class analysis"*.

The distributional assumptions are analogous to what we discussed in the above sections. If the observed variable is categorical, a binomial or multinomial distribution is assumed, while if the observed variable is continuous, either univariate normal, gamma, student or log-normal distributions can be assumed, according to each latent class. Also, as explained above, conditional independence, postulating that observed variables are independent of each other when conditioned on the latent class variable, is also required for the analysis. A special point about the conditional independence assumption for this method is that we can relax the requirement of this assumption by assuming multivariate normal distribution for the set of continuous variables with model defined variance and covariance, instead of assuming univariate normal distribution.[73]

Exposure Assessment: Genetic Determinants (Aim 3)

Genomic DNA (gDNA) was extracted from blood or saliva samples of study participants. Each gDNA sample was quantified using the PicoGreen assay, standardized to 50ng/mL, and

processed following the standard Illumina protocol including hybridization, incubation and scanning. We used Illumina's new MEGA chip, which is optimized for genome-wide association studies in multi-ethnic populations. The MEGA chip directly measures 1.7 million genetic markers, including improved genome-wide coverage of non-European ancestry, exonic content of over 400,000 markers, more than 17,000 variants relevant to clinical and pharmacogenetic studies, and an additional 23,000 variants selected for functional, immunological, oncological, ancestry, forensic, and other common and rare diseases.

Participants were excluded if they had an overall SNP call rate (ratio of measured SNPs per participant over the total number of SNPs in the dataset) < 95% or sex mismatch between genotypic and phenotypic measurements. In addition, individual SNPs were excluded from the analyses if they belonged to non-autosomal chromosome, missing rate greater than 5%, ethnicity-specific Hardy-Weinberg Equilibrium (HWE) p-value less than 0.0001 or a minor allele frequency (MAF) less than 0.05.[125] Using the measured SNPs, we performed genome-wide imputation using 1000 Genomes (Phase 3) panel as the reference. This imputation was performed in Michigan Imputation Server,[126] and after the quality control (MAF $\geq$ 0.05; removal of duplicate, non-SNP or monomorphic sites; imputation $R^2 \geq 0.50$), 5,504,202 SNPs were analyzed.

Candidate genes were selected based on following criteria:

1) Genes associated with <u>stress response</u>: We used a systems biology-pathway driven approach for selection of candidate genes. Body systems informed this selection are:
    i. Autonomic (sympathetic/parasympathetic) nervous system: The autonomic nervous system plays a major role in the regulation of the cardiovascular

system, and specifically cardiovascular responses to stress, through the complex interplay of sympathetic and parasympathetic nerve stimulation.[127]

ii. Renin-Angiotensin-Aldosterone System: The renin-angiotensin-aldosterone system influences vascular reactivity and water and sodium balance, and plays a major role in blood pressure regulation.[128]

iii. Inflammation and immunity: Many of the pathological changes in the vascular system leading to CAD and acute coronary syndromes are known to be largely driven by chronic or acute inflammatory/immune processes.[129,130]

iv. Endothelium: The vascular endothelium is responsible for the control of vascular smooth muscle function via production of vasoactive substances such as nitric oxide (NO), a potent vasodilator, and endothelins (END-1 and END-2), potent vasoconstrictors.[131,132] It is also important for vascular injury repair and for preventing thrombus formation and atherosclerosis progression.[133]

2) All genes found to be <u>associated with cardiovascular disease</u> in recently published genome-wide association studies.[65-69]

Based on these two inclusion criteria, we curated a list of 286 (35 stress-related, 251 CAD-related) candidate genes for our analyses (details in Chapter 5).

<u>Outcome Assessment: Future Cardiovascular Events (Aim 1)</u>

For assessment of future cardiovascular events, each person was followed for maximum of 36 months after the baseline visit. Individuals were examined in-person at 12 and 24 months in the Emory University clinic and queried by telephone at 6 months, and 36 months. At each follow-up time, research staff queried participants regarding the occurrence of hospitalizations, cardiac procedures and/or cardiac events. If individuals reported such events, study coordinators

contacted their physicians or admitting hospitals and obtained medical records for those hospitalizations. If the patient did not come back for the in-person clinic visit, information was obtained by telephone regarding their current status and occurrence of intervening events. For all patients lost to follow-up, a National Death Index search was made, in addition to Social Security and Medicare list searches. For patients who died, a member of their immediate family was interviewed by telephone about the cause of death. We also queried electronic health records to capture any missed cardiovascular events.

Our main endpoint in this study was a composite outcome of cardiovascular death (cardiac death or death due to stroke or congestive heart failure), cardiac arrest, non-fatal myocardial infarction, non-fatal stroke, congestive heart failure or unstable angina. All of the events were adjudicated by a team of study cardiologist.

Outcome Assessment: Mental-Stress Induced Myocardial Ischemia (Aim 2 & 3)

Mental stress was induced by a standardized social stressor using a public speaking task, as previously described.[134,135] Briefly, each individual was asked to imagine a real-life stressful situation, and to make up a realistic story around this scenario. They were given two minutes to plan the story and three minutes to present it in front of a video camera and a small audience wearing white coats. Individuals were told that their speech would be evaluated by the laboratory staff for content, quality and duration. We conducted continuous blood pressure and heart rate monitoring during the resting stage (every 5 minutes) and during mental stress (every 1 minute).

Each study participants underwent two single-photon emission tomography (SPECT) imaging studies; at rest, and with mental stress, with ^{99m}Tc-Sestamibi, at the dose of 10-14 mCi for rest imaging and 30-40 mCi for stress imaging, based on weight. SPECT images were interpreted using accepted methodology by two experienced readers blind to patients' data using

a 17-segment model. Disagreements between the two readers were resolved by consensus and a third reader if needed. Each myocardial segment was scored from 0 (no abnormality) to 4 (absent perfusion), and summed scores were calculated in a conventional fashion, yielding a summed stress scores (SSS) for mental stress, and a summed rest score (SRS) for rest, each with a theoretical range of 0 to 68. A summed difference score (SDS) was calculated for mental stress by subtracting the SRS from the SSS. The SDS is a semi-quantitative measure of the number and severity of reversible (ischemic) myocardial perfusion defects.[136]

$$Mental\ Stress\ SDS = Mental\ Stress\ Summed\ Score\ (SSS) - Summed\ Rest\ Score(SRS)$$

Adjustment Factors

For aims 1 and 2, we adjusted for *a priori* chosen covariates, which included sociodemographic characteristics (age, sex, race, education less than or equal to high school education), and traditional cardiovascular risk-factors risk factors & severity indicators (smoking status, hypertension, dyslipidemia, diabetes, BMI, and history of previous revascularization). We used validated instruments to collect demographic, behavioral, social and health status data. Age was calculated at the date of enrollment into the study by subtracting the date of birth from the date of enrollment. Race was self-reported and classified as African-American vs non-African-American. Socio-economic status was assessed using educational level (categorized as ≤ high school or > high school graduation); smoking status was also self-reported and was categorized into current smokers or non-smokers. Hypertension, hyperlipidemia, and diabetes were ascertained by research staff during the clinic visit through a detailed medical history. Angiographic data and left ventricular ejection fraction were obtained from the most recent coronary angiogram documented in the patient's medical record. CAD severity was quantified using a cut-off of 70% blockage in any major arteries.

For aim 3 (genetic association analyses), we only adjusted for age, sex and indicators of population stratification. Principal components of independent ancestry genomic markers were calculated to represent population stratification across and within each race/ethnicity.[125]

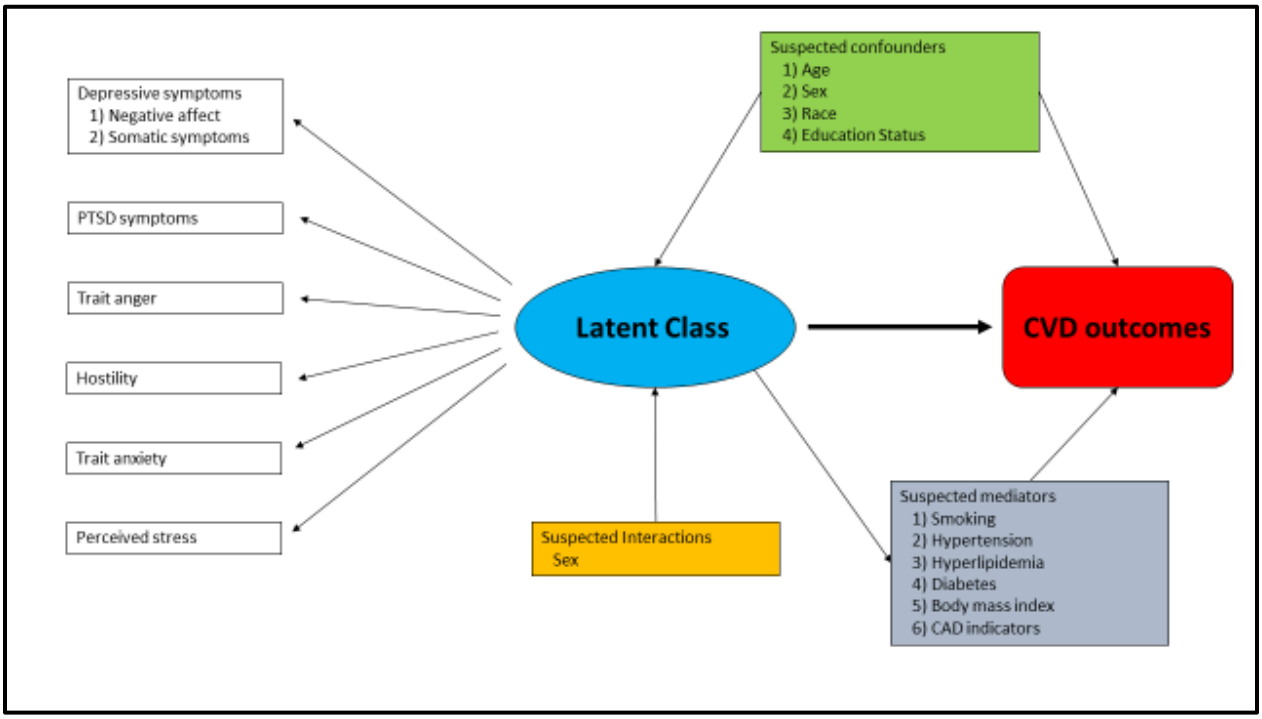
Data Analysis Plans

LCA was carried out using Latent Gold software.[137] The seven psychosocial symptom scales mentioned in the above section (somatic and cognitive depressive symptoms, PTSD symptoms, anxiety, anger, hostility, and perceived stress) were used as the loading factors. In order to assess the proper fit of the model (i.e., the minimum number of latent classes needed to get the best fit), we used established criteria, including Bayesian information criteria, entropy, the bootstrap likelihood ratio test, and the Integrated Classification Likelihood criteria.[72,73]

For aim 1, we performed multivariable Cox proportional hazard regression models with composite CVD events (cardiovascular death, cardiac arrest, non-fatal myocardial infarction, non-fatal stroke, congestive heart failure or unstable angina) as outcomes, and the psychosocial distress indicators (LCA-derived psychosocial distress indicator) as the main predictor variable. For theses analyses, the end of the follow-up was considered as either the end-date of the study (follow-up to 36 months) for patients who do not experience any endpoints, or the date of occurrence of study endpoints or death or loss to follow-up. We also checked whether the proportional hazard assumption is met in each of our individual models. We adjusted for *a priori* chosen covariates, which included sociodemographic characteristics (age, sex, race, education less than or equal to high school education). We decided not to adjust for CAD risk factors and severity indicators (smoking status, hypertension, dyslipidemia, diabetes, BMI, previous revascularization, and summed rest score), given that it is questionable whether these variables are confounders or mediators of our associations of interest. Psychosocial factors may increase

the risk of cardiovascular risk factors such as hypertension and diabetes, which in turn may act as mediators for future CVD events. Another possibility, however, is that higher cardiovascular risk status worsens the current psychosocial status of the individual, therefore confounding the association (**See chapter 2, figure 1, DAG for aim I**). In separate models, we also explored sex as effect modifier for the association between psychosocial distress and CVD events. We used SAS version 9.3 (Cary, NC) for the analysis, with an alpha level of 0.05 for statistical significance.

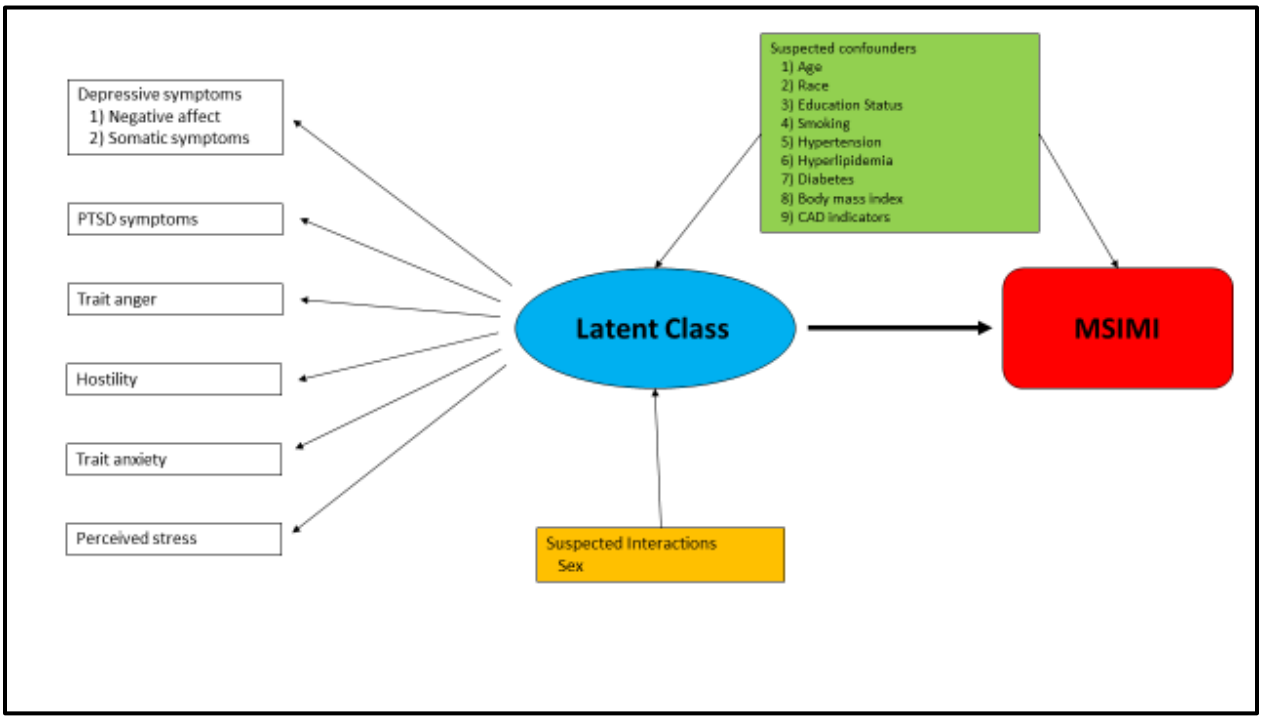
Chapter 2, Figure 1: Directed Acyclic Graph (DAG) for Aim I



In aim 2, we performed two separate multivariable linear regression models with resting myocardial perfusion defects (SRS), and ischemia with mental stress (mental stress SDS) as outcomes, and the psychosocial distress LCA categorical variable as the main predictor variable. Since the SDS for mental stress had a skewed distribution, while the SSS was approximately normally distributed, we used the SSS score as dependent variables while adjusting for the rest

score (SRS). Because of the mathematical relationship between these scores, the coefficient from a model with SSS as dependent variable, adjusted for SRS, is identical to that from a model where the dependent variable is the SDS. This strategy allowed us to obtain less biased standard errors and p values. We adjusted for a priori chosen covariates, including sociodemographic characteristics (age, sex, race, education less than or equal to high school education), CAD risk factors and severity indicators which might be on the pathway between stress and disease (smoking status, hypertension, dyslipidemia, diabetes, BMI, and previous revascularization) (**See chapter 2, figure 2, DAG for aim II**). In separate models, we explored sex as effect modifier for the association between psychosocial distress and perfusion measures. We used SAS version 9.3 (Cary, NC) for the analysis, with an alpha level of 0.05 for statistical significance.

Chapter 2, Figure 2: Directed Acyclic Graph (DAG) for Aim II



For both aims 1 and 2, to verify the robustness of our LCA findings, we conducted a sensitivity analysis calculating **a composite score of observed psychosocial scales using Z-**

score transformation. We converted each psychosocial symptom-scale into a Z-score variable by subtracting the mean of each scale from each individual's reported score, and then dividing this by the standard deviation of each scale. We then summed these individual Z-scores (total 7 Z-scores, corresponding to 7 symptom-scales) to derive the composite Summed-Z score and also divided our study participants into quartiles of the summed Z-score. A similar method was recently used by Blumenthal et al[12] as an outcome for their randomized controlled trial of assessing the effect of cardiac rehabilitation alone vs. cardiac rehabilitation + validated stress management training among individuals with pre-existing coronary artery disease. They found that stress management training when added to traditional cardiac rehabilitation significantly decreased this global score of psychosocial status.[12]

For aim 3, we performed both genome-wide association analysis (GWAS), and candidate gene approach, for the association between genetic variants and mental stress-induced myocardial ischemia. We mainly focussed on candidate genes approach due to limited sample size to assess genome-wide associations between genetic variants and MSIMI. However, given our availability of both phenotypic and genomic data, we did explore the genome-wide associations with MSIMI as a secondary analysis, which can generate new hypotheses for future genetic studies of MSIMI. The main distinctions between these two analytical approaches are described below:

Chapter 2, Table 2: Distinction Between Candidate Gene Approach and Genome Wide Association Analysis

	Candidate Gene Analysis	**Genome Wide Association Analysis**
Definition	Investigates the association between specific genetic variants and disease/trait under study	Investigates the association between genetic variants from the entire genome and disease/trait under study
Gene selection criteria	A-priori criteria of gene selection, based on prior knowledge about the disease/trait in question and trait-related genetic associations Selection criteria depend on the investigator	Whole genome is sequenced and analyzed, so there are no selection criteria required for genes
P value cut-off	Multiple testing corrected threshold 0.05 or 0.05/Number of genes studied (for gene-based inference)	Genome-wide significance threshold for common SNPs: $5^* 10^{-8}$
Sample size	Does not require a large sample size	Requires large sample size to have enough power for identifying associations with moderate effects
Genotypes under study	Based on existing knowledge about the disease/trait, without coverage of inter-genic regions.	Not based on a-priory knowledge. Can help in hypothesis generating discoveries, as entire genome is investigated

For GWAS analysis, we performed multivariable linear regression models with ischemia with mental stress (mental stress SDS) as outcome, and each SNP as the main predictor variable, adjusting for resting perfusion defect (SRS), age, sex and indicators of population stratification. Indicators of population stratification were created by principal component analysis using R, and first 10 principal components were adjusted for in the analysis. Since the SDS for mental stress

had a skewed distribution, while the SSS was approximately normally distributed, we used the SSS score as dependent variables while adjusting for the rest score (SRS). GWAS analysis was conducted using RVTEST,[138] and R; and meta-analysis of individual study results was conducted using METAL.[139] For candidate gene analysis, summary results from individual, non-synonymous SNPs (genetic variants which lead to direct structural change in coded protein of the gene) were aggregated to give gene-based test of significance using a web-based tool.[140] Gene-based analysis was performed using snp-wise test, where sum of –(log of individual SNP P-value) is used as summary statistic for the gene-level analysis.[141] For GWAS analysis, we used P-value threshold of $5*10^{-8}$; while for candidate-gene analysis, Bonferroni adjusted P-value threshold of was used.

In order to assess the interaction effect by psychosocial stress, we first computed a summary genetic risk score[13,14] based on the candidate genes described in earlier sections. Briefly, for each candidate gene, we assigned a score based on presence of risk alleles for each participants (0 if both alleles are reference alleles, 1 if one risk allele present, 2 if both alleles are risk alleles). We then calculated the unweighted genetic risk score as the sum of the number of risk alleles of all individual SNPs. We also calculated the weighted genetic risk score, the weights being published *log (odds ratio)* for the association between risk loci and CAD. We then performed multivariable linear regression with SDS for mental stress as the outcome, the computed genetic risk score as the exposure, and the psychosocial distress (LCA derived) variable as part of the interaction term.

Chapter 3: ***Psychological Distress and Future Cardiovascular Events***

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Key Points:

Question: Is chronic psychological distress associated with future cardiovascular disease (CVD) events, and is this association sex-specific?

Findings: In this prospective cohort-study of 662 individuals with stable coronary artery disease, women reporting high psychological distress symptoms had an adjusted 2.6 times higher hazard of CVD events as compared to women with low symptoms (95% CI=1.0-6.9); while there was no such association observed in men.

Meanings: Consideration should be given to incorporating psychological distress measures in the assessment of patients with coronary disease, especially women.

Abstract

Importance: Higher symptom levels of a variety of measures of emotional distress, like depression, anxiety or perceived stress, have been associated with cardiovascular disease (CVD), especially among women. However, their cumulative effects have rarely been examined.

Objective: To investigate the association between a composite measure of psychological distress, a summation index of individual symptom scales, and incident cardiovascular events, and to assess effect modification by sex.

Design: Prospective cohort study with average follow up of 2.8 years.

Setting: Emory University (single referral center).

Participants: 662 individuals with stable coronary artery disease (CAD).

Exposure: Composite score of psychological distress derived through summation of Z-transformed psychological distress symptom scales (depression, posttraumatic stress, anxiety, anger, hostility, and perceived-stress). Quartiles of increasing severity of psychological distress score were also calculated.

Main Outcome: Incidence of cardiovascular events (a composite endpoint of cardiovascular death, cardiac arrest, non-fatal myocardial infarction, non-fatal stroke, heart failure, or unstable angina), assessed through in-person interviews and medical chart abstraction and independently adjudicated.

Results: The mean age was 63 years (standard deviation, 9 years), 185 (28%) were women, and 197 (30%) African-Americans. During the follow up, 120 (18%) subjects developed CVD events. In the overall population, there was no association between the psychological distress measure and CVD events, but there was a sex-based interaction (P= 0.001). In women, higher

psychological distress was associated with a higher incidence CVD events; each standard deviation increase in the composite-score of psychological distress was associated with 1.6 times hazard of CVD events (95% CI: 1.2-2.1). Women in the highest psychological distress symptoms quartile had an adjusted 2.6 times higher hazard of CVD events as compared to women with low symptoms (95% CI: 1.0-6.9). No such association was found in men.

Conclusions and Relevance: Among patients with CAD, higher psychological distress is associated with future cardiovascular events in women only. These findings suggest that psychological status is an important dimension in the risk assessment of women with CAD.

Introduction:

The prevalence of mental health disorders in the US population is growing steadily. In 2013, an estimated 18.1% of US adults aged 18 years or older had a diagnosed mental illness, out of which 6.7% (15.7 million) had at least one major depressive episode.[1,15] In addition to significantly contributing to disability and health care costs, psychological disturbances have been increasingly associated with physical health consequences, especially cardiovascular disease (CVD).[142] For example, the association between depression, or depressive symptoms, and cardiac events or mortality is well established, both in individuals without coronary artery disease (CAD) at baseline, and among those with established CAD.[4,25] Similar associations have been reported for other psychological factors, including post-traumatic stress disorder (PTSD),[33] anxiety,[35] anger, hostility[37] and perceived stress,[38] but results remain either mixed or limited.

The majority of studies investigating the association between psychological factors and CVD have treated each psychological phenotype as an independent exposure, and none have taken into account and integrated individual psychological attributes more broadly. One reason why an integrative approach may be useful is that psychological factors may share biological or behavioral substrates, explaining why they tend to correlate with each other.[45] Examining them together may provide new insights onto specific psychological profiles that are relevant for cardiovascular risk.

In the current study, we investigated the association between a composite measure of psychological distress, derived using a summation score of individual symptoms-scales, and future incident cardiovascular events, in 695 individuals with pre-existing, stable CAD. Our composite measure integrated symptoms of depression, PTSD, anxiety, anger, hostility, and perceived stress. A similar composite measure of psychological distress was recently developed

in stable CAD patients and found to be modifiable.[12] We also explored a possible effect modification by sex, given emerging data suggesting that women may be more vulnerable to the effects of psychological stress on CVD risk than men.[40]

Methods:

Study Sample:

Between June 2011 and October 2014, we enrolled 695 individuals with stable CAD from Emory University-affiliated hospitals and clinics for the Mental Stress Ischemia: Prognosis and Genetic Influences Study. This research was approved by the Emory University Institutional Review Board, and all participants provided informed consent. A detailed protocol with inclusion and exclusion criteria has been previously described.[135] Briefly, subjects between ages 30 to 80 years of age were enrolled if they had significant history of CAD during their lifetime (prior myocardial infarction, bypass surgery or percutaneous intervention, positive nuclear scan/exercise stress test, angiographically proven major coronary vessel disease, or abnormal coronary ultrasound). Subjects were excluded if they had history of unstable angina or acute MI within the previous week of enrollment, severe comorbid medical or psychiatric disorders, uncontrolled hypertension, pregnancy or breastfeeding, chronic inflammatory disorders, organ transplant or were receiving dialysis.

Cardiovascular Events Assessment:

Each person was followed for maximum of 36 months after the baseline visit. Individuals were examined in-person at 12 and 24 months in the Emory University clinic and queried by telephone at 6 months and 36 months. At each follow-up time, research staff queried participants regarding the occurrence of hospitalizations, cardiac procedures and/or cardiac events. If

individuals reported such events, study coordinators contacted their physicians or admitting hospitals and obtained medical records for those hospitalizations. If the patient did not come back for the in-person clinic visit, information was obtained by telephone regarding their current status and occurrence of intervening events. For all patients lost to follow-up, a National Death Index search was made, in addition to Social Security and Medicare list searches. For patients who died, a member of their immediate family was interviewed by telephone about the cause of death. We also queried electronic health records to capture any missed cardiovascular events.

Our main endpoint in this study was a composite outcome of cardiovascular death (cardiac death or death due to stroke or congestive heart failure), cardiac arrest, non-fatal myocardial infarction, non-fatal stroke, congestive heart failure or unstable angina. All of the events were adjudicated by a team of study cardiologist (AS, PR, AQ).

Assessment of Psychological Distress:

Our global distress measure integrated established symptoms scales measuring psychological characteristics or symptoms with known association with cardiovascular disease and that were previously used in a composite measure developed by Blumenthal el at.[12] These included symptoms of depression, anxiety, anger, and perceived general stress. To these we added symptoms of posttraumatic stress disorder (PTSD) and hostility, given their recognized importance for cardiovascular risk.[37,143]

We assessed depressive symptoms using the Beck Depression Inventory (BDI-II), a 21-item self-administered scale.[75] Since symptom dimensions of the BDI may differ in their association with cardiovascular outcomes,[77] we calculated two separate subscales: negative affect (8 items) and somatic symptoms (13 items).[75] PTSD symptoms were assessed using the PTSD Symptom Checklist (PCL), civilian version, a 17-item scale.[79] Trait anxiety was measured

with the State-Trait Anxiety Inventory (STAI).[144] To measure trait anger symptoms we used the Spielberger's State-Trait Anger Expression Inventory (STAXI);[82] to measure hostility we administered the Cook-Medley Hostility Scale (CMHS),[84] and to assess general perceived stress we used the Perceived Stress Scale.[85]

These scales were standardized and combined in a composite psychological distress index as described under statistical analysis. We also performed a cluster analysis using latent class analysis (LCA),[72,73] where we constructed a categorical latent variable through observed related variables, using structural equation modeling.[72,73]

Other Study Measures:

We used validated instruments to collect demographic, behavioral, social and health status data. Race was self-reported and classified as African-American vs non-African-American. Socio-economic status was assessed using educational level (categorized as ≤ high school or > high school graduation); smoking status was categorized into current smokers or non-smokers. Hypertension, hyperlipidemia, and diabetes were ascertained by research staff during the clinic visit through a detailed medical history. Angiographic data and left ventricular ejection fraction were obtained from the most recent coronary angiogram documented in the patient's medical record. CAD severity was quantified using a cut-off of 70% blockage in any major arteries.

Statistical Analysis:

We converted each psychological symptom-scale into a Z-score variable by subtracting the mean of each scale from each individual's reported score, and then dividing this by the standard deviation of each scale. We then summed these individual Z-scores (a total of seven Z-

scores, corresponding to seven symptom-scales) to derive a composite psychological distress index. We compared subject characteristics according to quartiles of the psychological distress index, using either ANOVA test for normally distributed variables or the chi-square test for categorical variables. We also compared baseline characteristics according to the presence of CVD events and by sex. For our main analysis, we performed multivariable Cox proportional hazard regression models with the composite CVD endpoint as the outcome, and the psychological distress indicator as the main predictor variable. For theses analyses, the end of the follow-up was considered as either the end-date of the study (follow-up to 36 months) for patients who did not experience the endpoint, or the date of occurrence of the study endpoint, or death, or loss to follow-up. We also checked whether the proportional hazard assumption was met in each of our individual models. We adjusted for *a priori* chosen covariates, which included sociodemographic characteristics (age, sex, race, education less than or equal to high school education). We decided not to adjust for CAD risk factors and severity indicators (smoking status, hypertension, dyslipidemia, diabetes, BMI, history of MI, previous revascularization, and history of congestive heart failure) in our primary analysis, given that it is questionable whether these variables are confounders or mediators of our associations of interest. In addition, there were no differences by psychological distress level or by sex in most of these variables. However, we ran a final model with these variables included, to examine if results changed. We performed LCA using Latent Gold software,[137] and model fit (i.e., the minimum number of latent classes needed to get the best fit) was assessed using established criteria.[72,73] We performed similar analyses as above with LCA-derived categorical variable too. In separate models, we explored sex as effect modifier for the association between psychological distress and CVD

events. We used SAS version 9.3 (Cary, NC) for the analysis, with an alpha level of 0.05 for statistical significance.

Results:

Sample Characteristics

Thirty-three subjects out of the total of 695 had missing information on either exposure or outcome, leaving an analytical sample size of 662. The mean age of the study population was 63 years (standard deviation, 9 years), 185 (28%) were women, 197 (30%) were African-Americans, and 167 (25%) had less than or equal to high school education (**chapter 3, table 1**). As expected, the prevalence of cardiovascular risk factors was high in this population, including hypertension (76%), dyslipidemia (82%), and type 2 diabetes (32%). Furthermore, 37% had a previous myocardial infarction (MI), and 77% had a previous revascularization procedure.

The population mean of the composite psychological distress index was 0 (standard deviation = 5.3). Subjects with higher psychological distress (quartile 4= high symptoms) were younger (59 years in quartile 4 vs. 66 years in quartile 1), more likely to be female and African-American **(chapter 3, table 1).** Among lifestyle and medical history factors, only BMI and current smoking were significantly different according to psychological distress level, with patients in higher symptom quartiles showing greater BMI and a higher prevalence of smoking. None of the CAD severity indicators like history of MI, heart failure, previous revascularization and significant CAD stenosis were statistically different according to psychological distress level. Medication use was similar across the groups, except for beta-blockers and anti-depressants, which were more common in higher symptoms quartiles. When patient characteristics were examined by sex, women were more likely to be African-American, and had a higher ejection fraction, while all other cardiovascular risk factors and severity indicators were

similar between men and women. Among medications, women had lower prescriptions for ACE inhibitors and higher prescriptions for anti-depressants **(chapter 3, E-table 1).**

Subjects were followed for 2.8 years on an average. In total, 120 (18%) subjects had cardiovascular events during follow-up and women tended to have a higher incidence of CVD events than men (21% vs. 17%) **(chapter 3, table 2).** When patient characteristics were examined according to whether they developed the CVD endpoint **(chapter 3, E-table 2)**, patients who experienced the CVD endpoint were more likely to be African-American and less educated, as compared to patients without CVD events. Patients with CVD events also more often had diabetes, a higher BMI, higher prevalence of heart failure, a lower ejection fraction, and a higher use of antidepressant medications.

LCA classified the study population into 4 classes, with excellent gradation of symptoms across classes **(chapter 3, E-figure 1)**. The choice of a 4-class solution was based on established criteria (AIC, BIC, and ICL-BIC), with specific emphasis on finding the class solution with the lowest values of these indices **(chapter 3, E-table 3).** We also considered differences in within-class psychological distress differentiation for each solution, in order to have meaningful and parsimonious classes.

Association with CVD outcomes

Out of 120 (18%) observed events over the span of 3 years, the majority were hospitalization for unstable angina (N=71, 11%), followed by non-fatal myocardial infarction and congestive heart failure (4% each) **(chapter 3, table 2)**.

In the overall sample, there was no association between the psychological distress indicator (either the summed Z-score or quartiles of summed Z-score) and future CVD outcomes

(chapter 3, table 3, figure 1). However, a significant interaction by sex was noted (p= 0.001 for sex* summed Z-score interaction). In women, higher psychological distress was associated with a higher risk of CVD events: each standard deviation increase in the summed Z-score was associated with 1.58 times hazard of CVD events (95% CI: 1.21 to 2.07). Women with higher psychological distress (quartile 4) had an estimated 33% events at the end of 3 years, as compared to 13% events in women with low distress (quartile 1) **(chapter 3, figure 1)**. After adjusting for sociodemographic characteristics, as compared to quartile 1, quartile 4 was associated with an adjusted 2.58 times higher hazard of cardiovascular events (95% CI: 1.00 to 6.94) among women **(table 4)**. Among men, there were no differences in cardiovascular outcomes by psychological distress level, and the interaction between sex and psychological distress quartiles was significant (p=0.03). Analysis done with LCA-derived classes showed similar results **(chapter 3, table 4, E-figure 2).** In a separate analysis, we also adjusted for CAD risk factors and severity indicators, and even though the overall association was attenuated, sex-differences in the association between psychological distress and CVD events continued to be observed (p= 0.004 for sex* summed Z-score interaction). When we performed these analyses using individual scales instead of the summed Z-score variable, we found similar results **(chapter 3, E-table 4).**

Discussion:

In individuals with pre-existing, stable CAD, women with higher psychological distress, defined as a composite measure of psychological symptom scales (depression, PTSD, anxiety, anger, hostility, and perceived-stress) showed significantly higher incidence of CVD events, while there was no such association found in men. The sex difference in the association was

robust to the adjustment of sociodemographic factors and even traditional cardiovascular risk factors and clinical disease severity indicators.

While the overall relationship between psychological distress indicators like depression and future cardiovascular events is fairly established,[3,4,25] previous literature regarding sex-differences in the association between psychological distress and cardiovascular disease is mixed.[2,40] However, two recent nationally representative studies, one from the US using the National Health and Nutrition Examination Survey,[41] and second from Canada using the National Population Health Survey[42] have shown an association of depression and other psychological factors with CVD in women and not in men. Also, the large, 52-countries Interheart study found a differential effect of the impact of psychological distress on myocardial infarction by sex.[43] In this study, a composite measure of psychological symptoms yielded a 40% population attributable risk for acute myocardial infarction in women, while for men, the same attributable risk was only 25%. Our results of an association between a composite psychological distress measure and future cardiovascular events in women further add to this evolving literature, and highlight the potential advantage of measuring an individual's psychological distress as a whole.

The proposed mechanisms through which psychological distress affects cardiovascular disease are multifactorial and can be grouped in two broad categories of behavioral factors (increased smoking, unhealthy diet, sedentary lifestyle, and medication non-adherence) and biological mechanisms, mainly through autonomic nervous system dysfunction leading to lower heart-rate variability, increased sympathetic nervous system activation and inflammatory activity, as well as endothelial and platelet abnormalities.[44] Women have been shown to be more prone to the postulated ill-effects of psychological stress on biological mechanisms like

increased inflammatory activity,[145] increased platelet activation,[146] and lower heart-rate variability.[147] However, the effect of these potential mediators on the association between psychological distress and CVD events needs to be explored further.

Potentially, the association between psychological distress and future CVD events among women with pre-existing CAD could be due to "reverse-causation", i.e. higher baseline CVD burden can lead to higher psychological symptoms, and to more future events. However, there were no sex differences in these factors, and the sex-differences in the association between psychological distress and future CVD events persisted even after adjusting for validated indicators of CAD severity like history of MI and heart failure.

Our study has several strengths. To the best of our knowledge, this is the first study to investigate the association between a comprehensive measure of psychological distress and future cardiovascular events. Our study population was well characterized clinically, and with thorough exposure assessment of psychological factors across multiple domains. Also, cardiovascular events and causes of death adjudicated by experienced cardiologists using an established protocol. Our study however is not without limitations. Measurement bias for the exposure (psychological distress) is an important issue, as all these factors are self-reported, and can be an explanation for the lack of association between distress and CVD events in men, as men might under-report depressive symptoms, as compared to women.[148] Also, as we studied individuals with established coronary artery disease, we cannot exclude a possible collider bias,[149] and traditional confounding adjustment may not be sufficient to correct for this bias. Finally, the number of events for specific CVD events was small, precluding the ability to analyze these events separately.

In conclusion, we found that, among CAD patients, a higher level of psychological distress, measured as a composite measure of a variety of symptom scales, is associated with higher cardiovascular events in women, but not in men. These findings suggest that the value of a regular assessment of psychological measures in cardiovascular practices, especially for women, should be considered. Equally important should be the exploration of treatment modalities for ameliorating psychological distress in patients with CAD, especially among women, including holistic approaches like meditation or relaxation techniques in addition to traditional medical approaches.[150]

Chapter 3, Table 1: Descriptive Characteristics of the Study Population According to Quartiles of Psychological Distress Score (Summed Z-score)

Variables	Quartile 1 (low Symptoms) (N= 167)	Quartile 2 (Mild Symptoms) (N= 166)	Quartile 3 (Moderate Symptoms) (N= 164)	Quartile 4 (High Symptoms) (N= 165)	Total Population (N= 662)
Demographic Factors					
Age, Mean (SD)[a]	66 (8)	65 (8)	62 (9)	59 (9)	63 (9)
Women, N (%)[a]	39 (23%)	38 (23%)	47 (29%)	61 (37%)	185 (28%)
African-American, N (%)[a]	38 (23%)	39 (23%)	49 (30%)	71 (43%)	197 (30%)
Education ≤ high-school, N (%)	28 (17%)	39 (23%)	47 (29%)	53 (32%)	167 (25%)
Lifestyle Factors and Medical History					
Current smokers, N (%)[a]	11 (7%)	21 (13%)	28 (17%)	34 (21%)	94 (14%)
Hypertension, N (%)	123 (74%)	131 (79%)	124 (76%)	127 (77%)	505 (76%)
Dyslipidemia, N (%)	130 (78%)	143 (86%)	133 (81%)	135 (82%)	541 (82%)
Diabetes, N (%)	45 (27%)	55 (33%)	56 (34%)	58 (35%)	214 (32%)
BMI, Mean (SD)[a]	28 (4)	30 (5)	30 (6)	31 (6)	30 (5)
Previous MI, N (%)	62 (37%)	54 (32%)	59 (35%)	72 (44%)	247 (37%)
History of heart failure, N (%)	16 (10%)	22 (13%)	22 (13%)	32 (19%)	92 (14%)

Previous revascularization, N (%)	127 (76%)	123 (74%)	128 (78%)	131 (79%)	509 (77%)
Ejection fraction in %, Mean (SD)	68 (14)	69 (14)	67 (13)	69 (14)	69 (14)
CAD ≥ 70% stenosis, N (%)[b]	133 (85%)	129 (88%)	113 (81%)	112 (83%)	487 (84%)
Current Medications					
Statins, N (%)	140 (84%)	140 (84%)	146 (89%)	139 (85%)	565 (86%)
Beta-blockers, N (%)[a]	106 (64%)	125 (75%)	129 (79%)	132 (80%)	492 (74%)
ACE-inhibitors, N (%)	79 (47%)	75 (45%)	67 (41%)	78 (48%)	299 (45%)
Aspirin, N (%)	146 (87%)	145 (87%)	144 (87%)	134 (81%)	569 (86%)
Anti-depressants, N (%)[a]	16 (10%)	27 (16%)	47 (28%)	61 (37%)	151 (23%)
Anxiolytics, N (%)	8 (5%)	13 (8%)	18 (11%)	16 (10%)	55 (8%)

SD: Standard Deviation; MI: Myocardial Infarction; BMI: Body Mass Index; CAD: Coronary Artery Disease;

[a] P value < 0.05

[b] CAD severity based on coronary angiography results prior to revascularization procedures (if any); 85 observations missing

Chapter 3, Table 2: Numbers and Percentages of Patients Who Developed Cardiovascular Outcomes, According to Sex

Variables	Men (N= 477)	Women (N= 185)	Total Population (N= 662)
Total CVD Events, N (%)[a]	82 (17%)	38 (21%)	120 (18%)
CV death, N (%)	8 (2%)	1 (1%)	9 (1%)
Cardiac arrest, N (%)	5 (1%)	1 (1%)	6 (1%)
MI, N (%)	18 (4%)	8 (4%)	26 (4%)
Stroke, N (%)	7 (1%)	3 (2%)	10 (2%)
CHF, N (%)	19 (4%)	6 (3%)	25 (4%)
Unstable angina, N (%)	46 (10%)	25 (14%)	71 (11%)

CVD: Cardiovascular Disease; CV: Cardiovascular; MI: Myocardial Infarction; CHF: Congestive Heart Failure

[a] Each individual events do not sum up to total events due to overlap (one individual having multiple events during follow-up)

Chapter 3, Table 3: Association of Psychological Distress Indicators with Future Cardiovascular Outcomes in the Overall Population

Exposure Comparison	Full Sample: Unadjusted HR (95% CI)	Full Sample: Adjusted HR (95% CI)
Composite Psychological Distress Index (Summed Z-Scores)		
Continuous Z-score	1.19 (1.00 to 1.41)	1.09 (0.92 to 1.30)
Quartile 1 (Low) vs. 2 (Mild)	1.22 (0.73 to 2.06)	1.15 (0.68 to 1.95)
Quartile 1 (Low) vs. 3 (Moderate)	1.01 (0.59 to 1.75)	0.89 (0.51 to 1.54)
Quartile 1 (Low) vs. 4 (High)	1.53 (0.93 to 2.52)	1.20 (0.70 to 2.03)
Latent Class Analysis (Cluster Analysis)		
LCA class 1 (Low) vs. 2 (Mild)	0.49 (0.26 to 0.91)	0.52 (0.28 to 0.97)
LCA class 1 (Low) vs. 3 (Moderate)	0.72 (0.47 to 1.11)	0.66 (0.42 to 1.03)
LCA class 1(Low) vs. 4 (High)	1.38 (0.83 to 2.30)	1.12 (0.65 to 1.88)

HR: represents estimated increase in future CVD events hazard when comparing low symptoms (reference) to upper quartiles/LCA classes (mild, moderate and high symptoms)

Results adjusted for age, sex, race, and education status

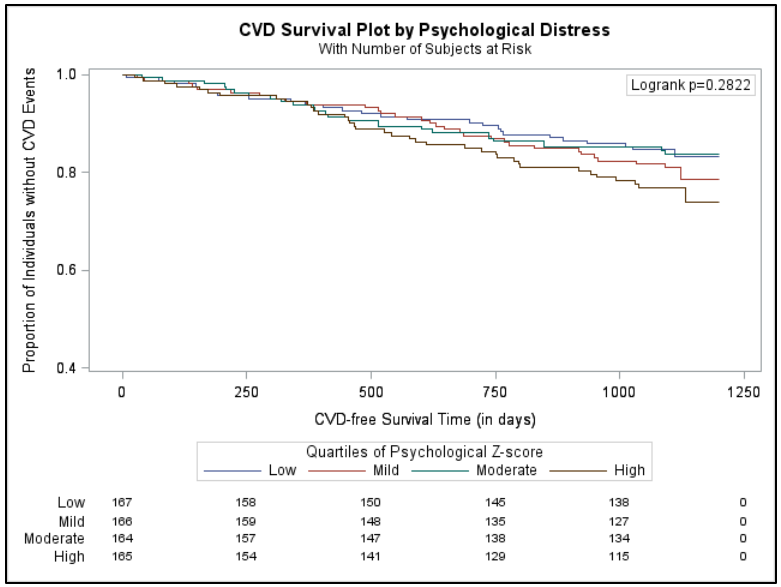
Chapter 3, Table 4: Association of Psychological Distress Indicators with Future Cardiovascular Outcomes, According to Sex

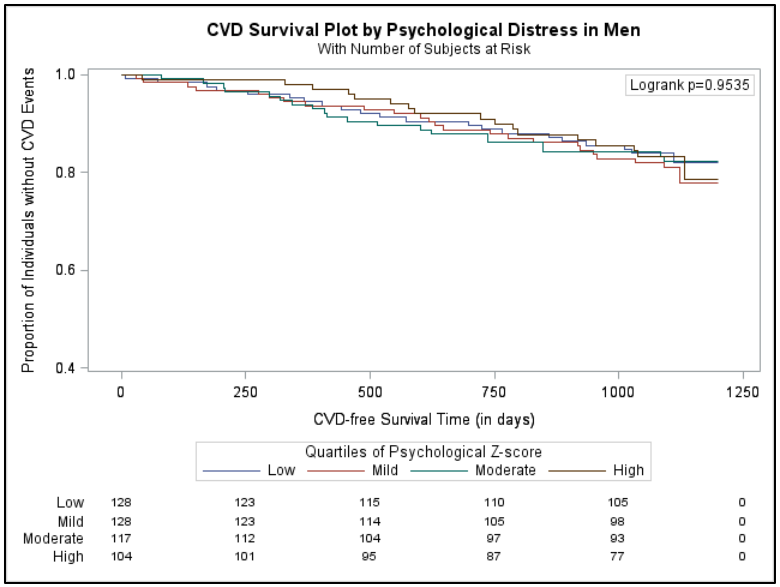
Exposure Comparison	Men: Unadjusted HR (95% CI)	Men: Adjusted HR (95% CI)	Women: Unadjusted HR (95% CI)	Women: Adjusted HR (95% CI)	P Value for Sex Interaction
Composite Psychological Distress Index (Summed Z-Scores)					
Continuous Z-score	0.97 (0.77 to 1.23)	0.88 (0.69 to 1.12)	1.66 (1.27 to 2.17)	1.58 (1.21 to 2.07)	0.001
Quartile 1 (Low) vs. 2 (Mild)	1.16 (0.65 to 2.09)	1.07 (0.59 to 1.92)	1.48 (0.47 to 4.67)	1.44 (0.45 to 4.53)	
Quartile 1 (Low) vs. 3 (Moderate)	1.04 (0.57 to 1.92)	0.93 (0.50 to 1.74)	0.98 (0.30 to 3.21)	0.84 (0.25 to 2.75)	0.03
Quartile 1 (Low) vs. 4 (High)	1.00 (0.52 to 1.90)	0.75 (0.39 to 1.47)	2.99 (1.12 to 7.95)	2.58 (1.00 to 6.94)	
Latent Class Analysis (Cluster Analysis)					
LCA class 1 (Low) vs. 2 (Mild)	0.36 (0.17 to 0.77)	0.36 (0.17 to 0.76)	1.24 (0.39 to 3.96)	1.70 (0.52 to 5.53)	
LCA class 1 (Low) vs. 3 (Moderate)	0.62 (0.37 to 1.05)	0.57 (0.33 to 0.97)	1.10 (0.48 to 2.54)	1.06 (0.45 to 2.48)	0.009
LCA class 1(Low) vs. 4 (High)	0.83 (0.39 to 1.76)	0.60 (0.28 to 1.29)	2.87 (1.24 to 6.64)	2.84 (1.22 to 6.61)	

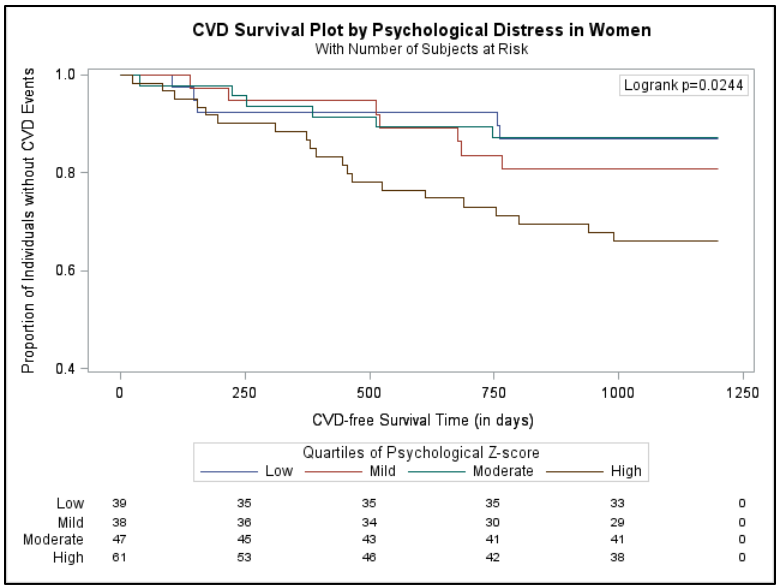
HR: represents estimated increase in future CVD events hazard when comparing low symptoms (reference) to upper quartiles/LCA classes (mild, moderate and high symptoms)

Results adjusted for age, sex, race, and education status

Chapter 3, Figure 1: Cardiovascular Survival by Psychological Distress: 1) Overall Population, 2) Men, 3) Women







Chapter 3, E-Table 1: Descriptive Characteristics of the Study Population According to Sex

Variables	Men (N= 477)	Women (N= 185)	Total Population (N= 662)
Demographic Factors			
Age, Mean (SD)	63 (9)	63 (9)	63 (9)
African-American, N (%)[a]	118 (25%)	79 (43%)	197 (30%)
Education ≤ high-school, N (%)	116 (24%)	51 (28%)	167 (25%)
Lifestyle Factors and Medical History			
Current smokers, N (%)	66 (14%)	28 (15%)	94 (14%)
Hypertension, N (%)	359 (75%)	146 (79%)	505 (76%)
Dyslipidemia, N (%)	397 (83%)	144 (78%)	541 (82%)
Diabetes, N (%)	144 (30%)	70 (38%)	214 (32%)
BMI, Mean (SD)	29 (5)	30 (6)	30 (5)
Previous MI, N (%)	176 (37%)	71 (38%)	247 (37%)
History of heart failure, N (%)	63 (13%)	29 (16%)	92 (14%)
Previous revascularization, N (%)	364 (76%)	145 (78%)	509 (77%)
Ejection fraction in %, Mean (SD)[a]	66 (12)	74 (14)	69 (14)
CAD ≥ 70% stenosis, N (%)[b]	356 (85%)	131 (82%)	487 (84%)

Current Medications			
Statins, N (%)	411 (86%)	154 (84%)	565 (86%)
Beta-blockers, N (%)	349 (73%)	143 (77%)	492 (74%)
ACE-inhibitors, N (%)[a]	234 (49%)	65 (35%)	299 (45%)
Aspirin, N (%)	416 (87%)	153 (83%)	569 (86%)
Anti-depressants, N (%)[a]	91 (19%)	60 (32%)	151 (23%)
Anxiolytics, N (%)	36 (8%)	19 (10%)	55 (8%)

SD: Standard Deviation; MI: Myocardial Infarction; BMI: Body Mass Index; CAD: Coronary Artery Disease

[1] P value < 0.05

[2] CAD severity based on coronary angiography results prior to revascularization procedures; 85 observations missing

Chapter 3, E-Table 2: Descriptive Characteristics of the Study Population According to Occurrence of CVD Events

Variables	No CVD Events (N= 542)	CVD Events (N= 120)	Total Population (N= 662)
Demographic Factors			
Age, Mean (SD)	63 (9)	62 (9)	63 (9)
Women. N (%)	147 (27%)	38 (32%)	185 (28%)
African-American, N (%)[a]	151 (28%)	46 (38%)	197 (30%)
Education ≤ high-school, N (%)[a]	119 (22%)	48 (40%)	167 (25%)
Lifestyle Factors and Medical History			
Current smokers, N (%)	77 (14%)	17 (14%)	94 (14%)
Hypertension, N (%)	408 (75%)	97 (81%)	505 (76%)
Dyslipidemia, N (%)	441 (81%)	100 (83%)	541 (82%)
Diabetes, N (%)[a]	163 (30%)	51 (42%)	214 (32%)
BMI, Mean (SD)[a]	29 (5)	31 (6)	30 (5)
Previous MI, N (%)	201 (37%)	46 (38%)	247 (37%)
History of heart failure, N (%)[a]	59 (11%)	33 (27%)	92 (14%)
Previous revascularization, N (%)	411 (76%)	98 (82%)	509 (77%)
Ejection fraction in %, Mean (SD)[a]	69 (13)	65 (16)	69 (14)

CAD ≥ 70% stenosis, N (%)[b]	391 (84%)	96 (88%)	487 (84%)
Current Medications			
Statins, N (%)	467 (86%)	98 (82%)	565 (86%)
Beta-blockers, N (%)	396 (73%)	96 (80%)	492 (74%)
ACE-inhibitors, N (%)	248 (46%)	51 (43%)	299 (45%)
Aspirin, N (%)	465 (86%)	104 (87%)	569 (86%)
Anti-depressants, N (%)[a]	114 (21%)	37 (31%)	151 (23%)
Anxiolytics, N (%)	42 (8%)	13 (11%)	55 (8%)

SD: Standard Deviation; MI: Myocardial Infarction; BMI: Body Mass Index; CAD: Coronary Artery Disease

a P value < 0.05

[b] CAD severity based on coronary angiography results prior to revascularization procedures; 85 observations missing

Chapter 3, E-Table 3: Model Fit Statistics for Latent Class Analysis

LCA Model	N	Log-Likelihood	AIC	BIC	ICL-BIC
1 Class	665	-15375	30778	30841	30841
2 Class	665	-13988	28035	28165	28254
3 Class	665	-13534	27157	27355	27505
4 Class	665	-12321	24759	25020	25144
5 Class	665	-12065	24274	24598	24780
6 Class	665	-12212	24600	24996	25245

LCA: Latent Class Analysis; N: Number of parameters in each model; AIC: Akaike Information Criterion; BIC: Bayesian Information Criterion; ICL-BIC: Integrated Completed Likelihood - Bayesian Information Criterion

Chapter 3, E-Table 4: Association of Individual Psychological Indicators with the CVD events Composite Endpoint, According to Sex

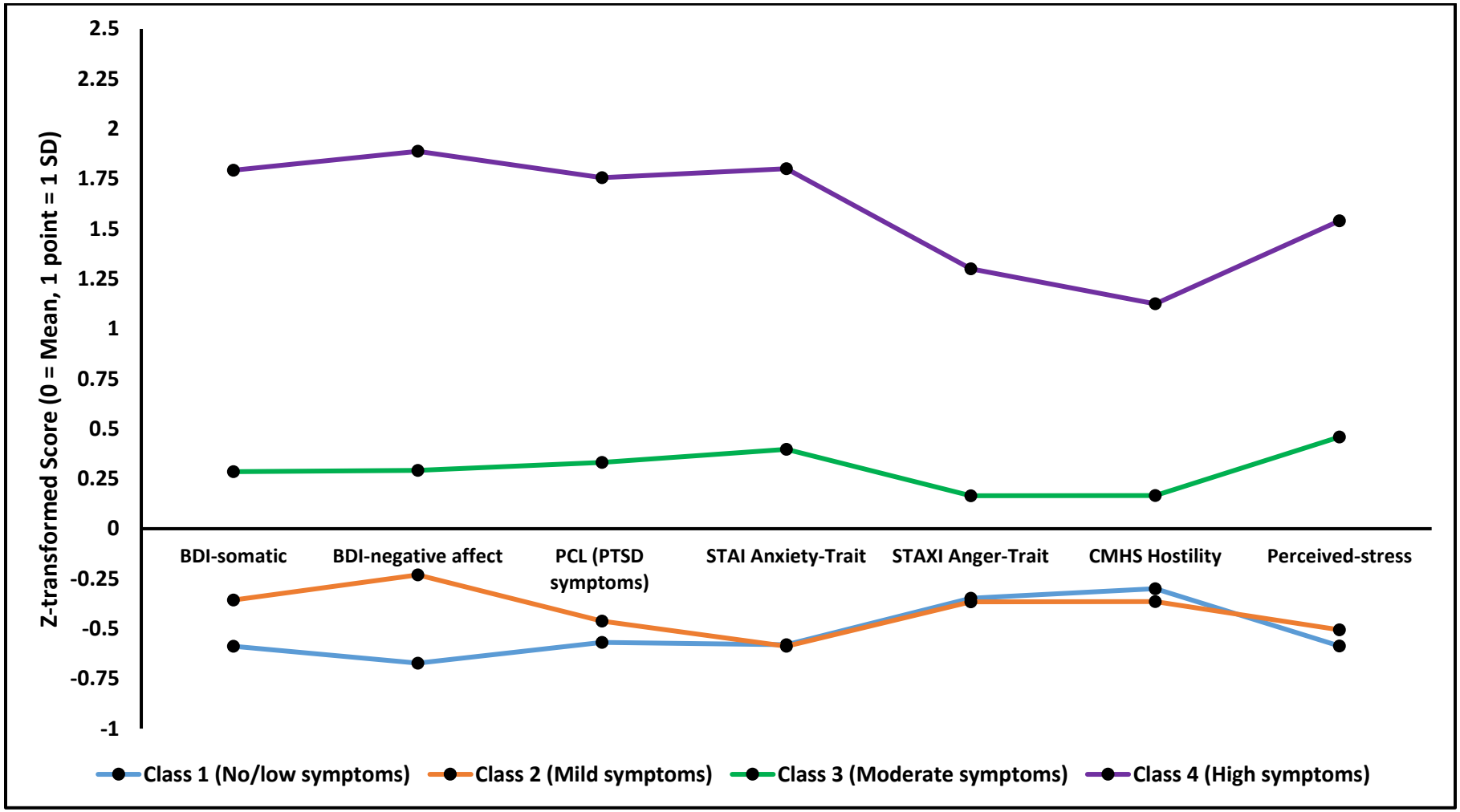
Exposure Comparison	Full Sample: Unadjusted HR (95% CI)	Full Sample: Adjusted HR (95% CI)	Men: Adjusted HR (95% CI)	Women: Adjusted HR (95% CI)	P Value for Sex Interaction
CVD Events (CV Death/Cardiac Arrest/MI/Stroke/CHF/UA)					
BDI-somatic score	1.21 (1.03 to 1.42)	1.12 (0.95 to 1.32)	0.95 (0.76 to 1.20)	1.48 (1.13 to 1.93)	0.01
BDI-negative affect score	1.15 (0.98 to 1.35)	1.08 (0.92 to 1.28)	0.92 (0.72 to 1.16)	1.45 (1.13 to 1.87)	0.007
PCL score	1.22 (1.05 to 1.43)	1.18 (1.00 to 1.38)	1.00 (0.79 to 1.26)	1.45 (1.15 to 1.83)	0.02
STAI Anxiety-Trait score	1.15 (0.97 to 1.37)	1.06 (0.87 to 1.27)	0.89 (0.70 to 1.12)	1.47 (1.09 to 1.98)	0.01
STAXI Anger-Trait score	1.09 (0.92 to 1.30)	1.07 (0.90 to 1.28)	0.98 (0.79 to 1.22)	1.29 (0.96 to 1.73)	0.14
CMHS Hostility score	1.11 (0.93 to 1.32)	1.00 (0.83 to 1.19)	0.84 (0.68 to 1.05)	1.43 (1.06 to 1.94)	0.005
Perceived-stress score	1.06 (0.89 to 1.26)	0.96 (0.80 to 1.17)	0.79 (0.62 to 1.01)	1.36 (1.00 to 1.84)	0.01

HR: represents estimated increased hazard in future CVD events with each standard deviation increase in the individual psychsocial scale

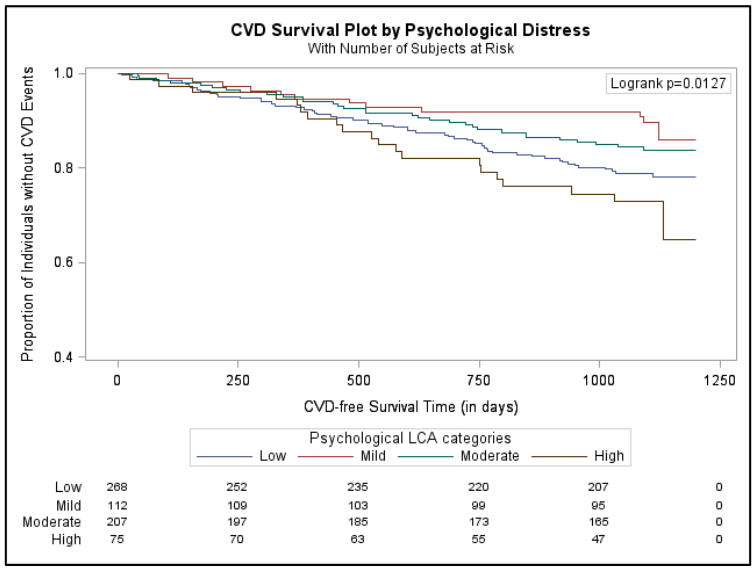
BDI: Beck Depression Inventory; PCL: PTSD Symptom Checklist (Civilian); STAI: State- Trait Anxiety Inventory; STAXI: State-Trait Anger Expression Inventory; CMHS: Cook-Medley Hostility Score

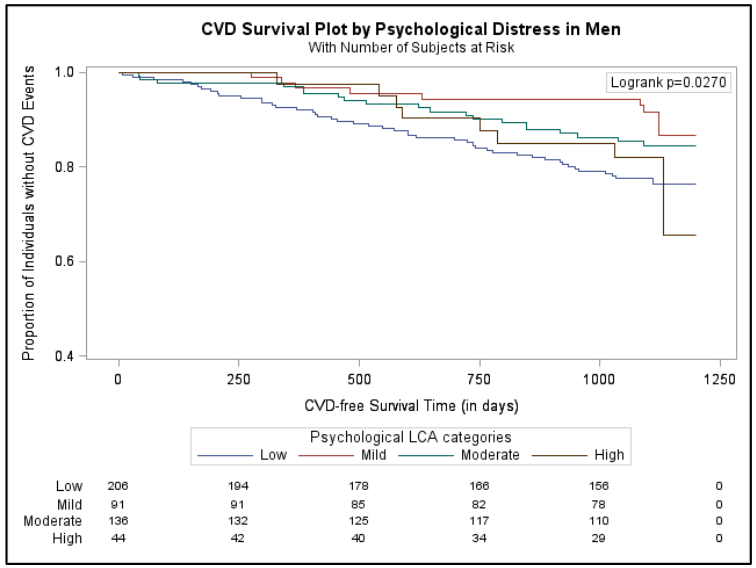
Results adjusted for age, sex, race, and education status

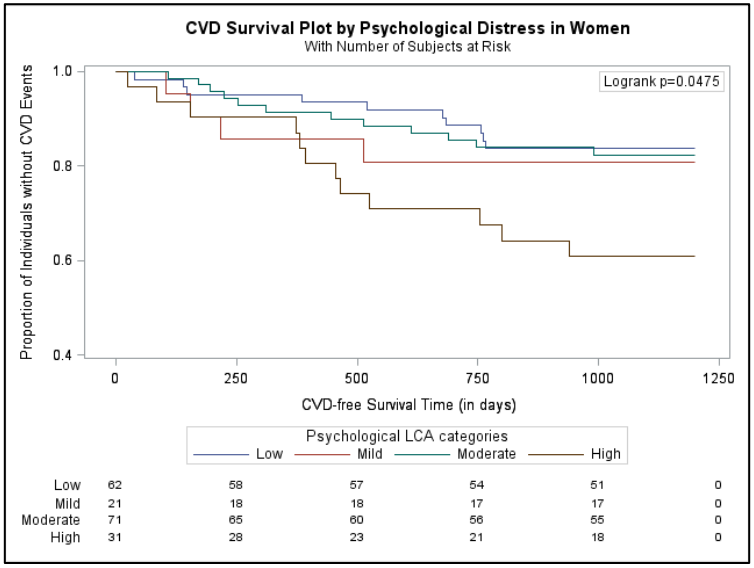
Chapter 3, E-Figure 1: Panel plot of the psychological distress LCA variable, according to individual scale levels. The graph shows the distribution of each latent class according to the individual psychological scale Z scores (scale score – sample mean/ sample SD).



Chapter 3, E-Figure 2: Cardiovascular Survival Curves by Psychological LCA Categories in 1) Overall Population, 2) Men, 3) Women







Chapter 4: *Psychosocial Distress and Stress Induced Myocardial Ischemia*

Short Title: Psychosocial Distress & MSIMI

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Access: All the authors had access to the data; and a significant contribution in either data collection and/or design, data analysis, and execution of the manuscript. All authors contributed to the writing of the manuscript.

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Key Words: Psychosocial stress, cardiovascular disease, ischemia, sex differences

Abstract

Background: Mental stress-induced myocardial ischemia (MSIMI) is a frequent phenomenon in patients with coronary artery disease (CAD). The link between an integrated measure of chronic psychosocial distress and MSIMI, and whether it differs by sex, has not been examined before.

Methods: We used latent class analysis (LCA) to derive a composite measure of psychosocial distress by integrating psychosocial symptom-scales (depression, posttraumatic stress, anxiety, anger, hostility, and perceived-stress) in 665 individuals with stable CAD. Subjects underwent myocardial perfusion imaging with mental stress (standardized public speaking task). Expert readers quantified perfusion defects at rest (summed rest score), with mental stress (summed stress score), and their difference (summed difference score), the latter being an index of inducible ischemia. Multivariable linear regression was used to adjust for sociodemographic and medical history factors.

Results: LCA characterized the study-population into four distinct classes, with incremental gradation in psychosocial symptomatology from class-1 (no/low symptoms) to class-4 (highest symptoms). In women, but not in men, as compared to LCA class 1, class 4 had an adjusted 4.0-points higher summed rest score (95% CI: 0.2-7.7). There was no association between the psychosocial distress latent variable and summed difference score in either women or men.

Conclusion: Higher psychosocial distress is associated with more resting perfusion defects in women and not in men, but is not associated with mental stress ischemia in either sex. These results suggest that, among women with CAD, chronic psychosocial distress may have enduring effects resulting in infarcts and fixed perfusion defects, rather than provoking ischemia acutely.

Introduction:

Mental stress-induced myocardial ischemia (MSIMI) is a transient myocardial ischemic response to mental stress,[9] which can be induced in patients with coronary artery disease (CAD) during a standardized mental stress challenge.[9] MSIMI is associated with a twofold increased risk of future cardiac events, which is similar to ischemia induced by conventional stress testing (exercise or pharmacological stress testing.[10] MSIMI, however, appears to be a unique phenomenon, since it occurs at lower levels of oxygen demand and is usually not related to severity of CAD.[9,46] Furthermore, MSIMI has been associated with myocardial ischemia measured in daily life ambulatory monitoring.[46,47] These features suggest that MSIMI is an expression of psychosocial burden, rather than CAD severity.

Published literature on the association between indicators of psychosocial distress and MSIMI have provided conflicting results.[48-54] In some studies, depression was associated with MSIMI, irrespective of whether ischemia was measured using perfusion imaging,[50,54] or echocardiography.[48] On the other hand, depression was not associated with MSIMI in the Psychophysiological Investigation of Myocardial Ischemia (PIMI) Study.[52] Anger and/or hostility were associated with MSIMI in two studies using nuclear imaging techniques,[49,53] but this association was non-significant in other studies that used echocardiography,[48] or perfusion imaging.[52] Neither anxiety,[48,52] nor perceived stress[48] were found to be associated with MSIMI in published literature.

Previous studies investigating the association between psychosocial factors and MSIMI treated each psychosocial phenotype as an independent exposure, and none have taken into account and integrated individual psychosocial attributes more broadly. One reason why an integrative approach may be useful is that psychosocial factors may share biological or

behavioral substrates, explaining why they tend to correlate and cluster with each other.[45] Examining them together may provide new insights onto specific psychosocial profiles that are relevant for MSIMI and cardiovascular risk.

In the current study, we investigated the association between a composite measure of psychosocial distress, derived using latent class analysis (LCA), and myocardial perfusion abnormalities at rest and with mental stress. Our composite measure integrated symptoms of depression, posttraumatic stress disorder (PTSD), anxiety, anger, hostility, and perceived stress. A similar composite measure of psychosocial distress was recently developed in stable CAD patients and found to be modifiable.[12] As women with CAD have a higher prevalence of psychosocial distress relative to men,[39] as well as a higher prevalence of MSIMI,[55,56] we explored whether the above associations differed by sex.

Methods:

Study Sample:

Between June 2011 and October 2014, we enrolled 695 individuals with stable CAD from Emory University-affiliated hospitals and clinics for the Mental Stress Ischemia: Prognosis and Genetic Influences Study. This research was approved by the Emory University Institutional Review Board, and all participants provided informed consent. A detailed protocol with inclusion and exclusion criteria has been previously described.[135] Briefly, subjects between ages 30 to 80 years of age were enrolled if they had significant history of CAD during their lifetime (prior myocardial infarction, bypass surgery or percutaneous intervention, positive nuclear scan/exercise stress test, angiographically proven major coronary vessel disease, or abnormal coronary ultrasound. Subjects were excluded if they had history of unstable angina or acute MI within the previous week of enrollment, severe comorbid medical or psychiatric disorders,

uncontrolled hypertension, pregnancy or breastfeeding, chronic inflammatory disorders, organ transplant or were receiving dialysis.

Stress Testing Procedures:

Mental stress was induced by a standardized social stressor using a public speaking task, as previously described.[134,135] Briefly, each individual was asked to imagine a real-life stressful situation, and to make up a realistic story around this scenario. They were given two minutes to plan the story and three minutes to present it in front of a video camera and a small audience wearing white coats. Individuals were told that their speech would be evaluated by the laboratory staff for content, quality and duration. We conducted continuous blood pressure and heart rate monitoring during the resting stage (every 5 minutes) and during mental stress (every 1 minute). We calculated the rate-pressure product as the mean systolic blood pressure times the mean heart rate at rest. Hemodynamic reactivity was calculated as the rate-pressure product during stress minus the rate-pressure product at rest.

Myocardial Perfusion Imaging:

Each study participants underwent two single-photon emission tomography (SPECT) imaging studies; at rest, and with mental stress, with ^{99m}Tc-Sestamibi, at the dose of 10-14 mCi for rest imaging and 30-40 mCi for stress imaging, based on weight.

SPECT images were interpreted using accepted methodology by two experienced readers blind to patients' data using a 17-segment model. Disagreements between the two readers were resolved by consensus and a third reader if needed. Each myocardial segment was scored from 0 (no abnormality) to 4 (absent perfusion), and summed scores were calculated in a conventional fashion, yielding a summed stress scores (SSS) for mental stress, and a summed rest score (SRS)

for rest, each with a theoretical range of 0 to 68. A summed difference score (SDS) was calculated for mental stress by subtracting the SRS from the SSS. The SDS is a semi-quantitative measure of the number and severity of reversible (ischemic) myocardial perfusion defects.[136]

Assessment of Psychosocial Distress:

Our global distress measure integrated intrinsic dimensions (i.e., psychological characteristics or symptoms) previously used in a composite measure developed by Blumenthal el at., which included symptoms of depression, anxiety, anger, and perceived general stress.[12] To these components we added symptoms of posttraumatic stress disorder (PTSD) and hostility, given their recognized importance for cardiovascular risk.[37,143]

We assessed depressive symptoms using the Beck Depression Inventory (BDI-II), a 21-item self-administered scale.[75] Since symptom dimensions of the BDI may differ in their association with cardiovascular outcomes,[77] we calculated two separate subscales: negative affect (8 items) and somatic symptoms (13 items).[75] PTSD symptoms were assessed using the PTSD Symptom Checklist (PCL), civilian version, a 17-item scale.[79] Trait anxiety was measured with the State-Trait Anxiety Inventory (STAI).[144] To measure trait anger symptoms we used the Spielberger's State-Trait Anger Expression Inventory (STAXI);[82] to measure hostility we administered the Cook-Medley Hostility Scale (CMHS),[84] and to assess general perceived stress we used the Perceived Stress Scale.[85]

Using the above symptom scales, we developed a latent psychosocial distress construct using latent class analysis (LCA).[72,73] A latent construct is a variable that is not directly observed or measured, but that is constructed through observed related variables.[72,73] LCA models are based on the assumption that observed indicator variables are associated with each other because of an underlying unobserved factor, rather than being directly related.[73] Using structural equation

modeling, LCA creates a categorical latent variable based on the designated observed indicators through maximum likelihood estimation.[72,73]

Other Study Measures:

We used validated instruments to collect demographic, behavioral, social and health status data. Angiographic data and left ventricular ejection fraction were obtained from the most recent coronary angiogram documented in the patient's medical record. CAD severity was quantified using a cut-off of 70% blockage in any major arteries. We also assessed a number of extrinsic psychosocial dimensions, i.e., those related to the social environment or other external exposures, which may cause psychosocial distress or affect its perception. These included exposure to traumatic events, which were measured using the Early Trauma Inventory, short form, for events before the age of 18, and the Lifetime Trauma Inventory (LTI) for events after the age of 18;[151] exposure to discrimination, assessed through the Everyday Discrimination Scale,[152] and perceived social support, assessed using the Multidimensional Scale of Perceived Social Support (MSPSS).[153]

Statistical Analysis:

LCA was carried out using Latent Gold software.[137] The seven psychosocial symptom scales mentioned above (somatic and cognitive depressive symptoms, PTSD symptoms, anxiety, anger, hostility, and perceived stress) were used as the loading factors. In order to assess the proper fit of the model (i.e., the minimum number of latent classes needed to get the best fit of the maximum likelihood), we used established criteria, including Bayesian information criteria, entropy, the bootstrap likelihood ratio test, and the Integrated Classification Likelihood criteria.[72,73]

We compared subject characteristics according to categories of the psychosocial distress LCA variable using either the ANOVA test for continuous, normally distributed variables or the chi-square test for categorical variables. We also examined whether the psychosocial distress LCA variable was associated with hemodynamic responses to stress, by comparing the change in rate-pressure product according to psychosocial distress class using linear regression models. For our main analyses, we performed two separate multivariable linear regression models with resting myocardial perfusion defects (SRS), and ischemia with mental stress (mental stress SDS) as outcomes, and the psychosocial distress LCA categorical variable as the main predictor variable. Since the SDS for mental stress had a skewed distribution, while the SSS was approximately normally distributed, we used the SSS score as dependent variables while adjusting for the rest score (SRS). Because of the mathematical relationship between these scores, the coefficient from a model with SSS as dependent variable, adjusted for SRS, is identical to that from a model where the dependent variable is the SDS. This strategy allowed us to obtain less biased standard errors and p values. We adjusted for a priori chosen covariates, including sociodemographic characteristics (age, sex, race, education less than or equal to high school education), CAD risk factors and severity indicators which might be on the pathway between stress and disease (smoking status, hypertension, dyslipidemia, diabetes, BMI, and previous revascularization). We did not consider CAD severity based on angiographic data due to missing values in 84 subjects, but considered the SRS as an indicator of severity of CAD. In separate models, we explored sex as effect modifier for the association between psychosocial distress and perfusion measures. We used SAS version 9.3 (Cary, NC) for the analysis, with an alpha level of 0.05 for statistical significance.

Results:

Sample Characteristics

Thirty subjects out of the total of 695 had missing information on either exposure or outcome, leaving an analytical sample size of 665. The mean age of the study population was 63 years (SD= 9 years), 185 (28%) were women, 198 (30%) were African-Americans, and 169 (25%) had less than or equal to high school education (**chapter 4, table 1**). As expected, the prevalence of cardiovascular risk factors was high in this population, including hypertension (76%), dyslipidemia (82%), and type 2 diabetes (32%). Furthermore, 37% had a previous myocardial infarction (MI), and 77% had a previous revascularization procedure. When patient characteristics were examined by sex **(chapter 4, supplementary table 1)**, women were more likely to be African-American, and had a higher BMI and a higher ejection fraction. Women also had higher levels of psychosocial factors (symptoms of depression, PTSD, anxiety and perceived stress) than men, and a higher use of antidepressant medications.

LCA classified the study population into 4 classes **(chapter 4, figure 1, supplementary table 2)**. Class 1 had 268 subjects, with no or low symptomatology, showing the lowest scores for all psychosocial scales, with the class level mean scores ranging from 0.5 to 0.75 standard deviations (SD) below the sample mean. Class 2 had 112 subjects, and showed similar psychosocial scale mean-scores as class 1, except for depressive symptoms. Class 3 had 208 subjects, with all scale mean-scores 0.25 to 0.5 SD above the mean. Finally, Class 4 had 77 subjects, showing the highest psychosocial burden, with scale mean-scores between 1.25 to 1.75 SD above the sample mean of each scale **(chapter 4, figure 1).** The choice of a 4-class solution was based on AIC, BIC, and ICL-BIC **(chapter 4, supplementary table 3)**. We also took into

account differences in within class characteristics for each solution, in order to have meaningful and parsimonious classes.

Subjects with higher psychosocial distress (higher latent class) were younger (57 years in class 4 vs. 65 years in class 1), more likely to be female, and African-American **(chapter 4, table 1).** Among lifestyle and medical history factors, only BMI and history of previous MI were significantly different according to psychosocial distress level, with higher symptom classes showing greater BMI and a higher frequency of history of MI. Medication use was similar between the groups, except for beta-blockers, anti-depressants and anxiolytics, which were more common in higher symptoms categories. As expected, higher classes of the psychosocial distress latent variable were associated with significantly more traumatic events, more reports of discrimination, and lower perceived social support **(chapter 4, table 1).**

Association Between Psychosocial Distress and Hemodynamic Changes with Stress

In the overall study sample, on average, systolic blood pressure increased with mental stress by 26 mmHg (SD, 16 mmHg) and heart rate increased by 11 beats/minute (SD, 9), with a change in rate-pressure product of 3,505 units (SD, 2,326).

Subjects with higher psychosocial distress showed a blunted hemodynamic response to mental stress, as shown by a decreasing rate-pressure product as psychosocial distress class increased (p=0.02 for trend), with no meaningful sex differences **(chapter 4, figure 2)**.

Association Between Psychosocial Distress and SPECT perfusion defects with Mental Stress

In the overall population, the summed rest score was 5.1 units (SD= 8.7); and the mean summed stress score with mental stress was 6.0 units (SD= 9.2). The mean summed difference score (SDS), quantifying inducible ischemia with mental stress, was 0.8 units (SD= 2.0).

While the psychosocial distress latent variable was not related to resting perfusion defects in the overall population **(chapter 4, table 2)**, there was an interaction by sex **(chapter 4, table 3, figure 3)**. In women, higher psychosocial distress was associated with a higher level of resting perfusion defects (SRS score). After adjusting for sociodemographic characteristics, CAD risk factors, and CAD severity indicators, as compared to LCA class 1 (no/low symptoms), class 4 (high symptoms) was associated with an adjusted 3.98-points higher summed rest score (95% CI: 0.22 to 7.73) among women. Women with higher psychosocial distress also showed more perfusion defects with mental stress **(chapter 4, figure 3)**, but this difference was driven by resting perfusion defects, since there was no difference in inducible ischemia (SDS score, **chapter 4, table 3**). Among men, there were no differences in resting or inducible perfusion defects by psychosocial distress class; the interaction between sex and psychosocial distress class was significant for resting perfusion (p=0.04). Even after adjusting for hemodynamic changes with mental stress (rate-pressure product), results did not change (data not shown). When we performed these analyses using individual scales instead of the latent variable, we found similar results **(chapter 4, table 4).**

Discussion:

Among individuals with stable CAD, we observed that psychosocial distress, defined as a composite measure of psychosocial symptom scales (depression, PTSD, anxiety, anger, hostility,

and perceived-stress) using latent class analysis, was not associated with MSIMI, overall and in sex-stratified analysis. However, women with higher psychosocial distress showed significantly higher resting perfusion defects, while there was no such association in men. These findings were independent of traditional CAD risk factors.

Although this is the first study examining the relationship between a composite measure of psychosocial distress and myocardial perfusion, previous investigations that evaluated individual psychosocial risk factors have provided conflicting results.[48-54] For example, depression was found to be associated with MSIMI in some studies,[48,50,54] but not in others.[52]

Higher hemodynamic response to stress is one of the postulated mechanism for MSIMI.[9] In a published study from the same cohort, a higher increase in rate-pressure product with mental stress, a measure of hemodynamic response to stress, was independently associated with MSIMI.[154] In our analysis, higher psychosocial distress was found to be associated with blunted hemodynamic response to the stresses, which is consistent with previous studies.[155] In addition to the potentially detrimental effects of increased cardiovascular reactivity, a blunted hemodynamic response to stress has increasingly gained recognition as being potentially unhealthy.[156] However, even after adjusting for rate-pressure product with mental stress, we found no association between psychosocial distress and MSIMI.

Another reason for a lack of association between psychosocial distress and MSIMI might be the presence of collider bias. Because all our subjects have a history of CAD, there could be selection bias affecting the results, and traditional confounding adjustment may not be sufficient to correct for this bias.[157]

Although our psychosocial distress composite variable was not associated with ischemia, it was related to a higher degree of resting perfusion abnormalities among women. Few previous

studies have examined this issue. Boyle et al found a significant association between resting wall-motion abnormalities and depression,[48] while Burg et al did not find a relationship between depression and resting myocardial perfusion.[50] However, these studies did not examine sex differences. Our results of a positive association between psychosocial distress and resting perfusion abnormalities in women may underline the influence of chronic stressors on CAD burden in this group. The fact that we found this association in women only, may underscore a vulnerability of women with CAD towards the chronic effects of stress on the cardiovascular system. Alternatively, due to the cross-sectional nature of our analyses, the psychosocial distress indicator could index distress that is a consequence of previous infarcts, especially among women. However, our results mirror previous findings of an association of depression with coronary artery disease burden and cardiovascular outcomes that was stronger in women than in men.[41,158] Furthermore, the large, 52-countries Interheart study found a differential effect of the impact of psychosocial distress on myocardial infarction by sex.[43] A composite measure of psychosocial symptoms yielded a 40% population attributable risk for acute myocardial infarction in women, while for men, the same attributable risk was only 25%.

Our study has several strengths. To the best of our knowledge, this is the first study to investigate the association between a comprehensive measure of psychosocial distress and myocardial ischemic responses to acute emotional stress using latent class analysis of multiple observed psychosocial phenotypes. This is also the largest study of mental stress ischemia using myocardial perfusion imaging, since previous studies had sample sizes below 500 subjects. Our study population was well characterized clinically, and with thorough exposure assessment of psychosocial factors across multiple domains. Myocardial perfusion imaging remains the state-

of-the-art method for ischemia assessment, and scans were read by experienced readers according to established protocols.

Our study does suffer from some limitations. A limitation of latent class analysis is that because the LCA variable is not directly observed, there is a possibility that our psychosocial distress construct was not a proper representation of true underlying distress. However, we believe that our latent class variable was a valid measure, since there was a clear separation of symptomatology levels across classes. Furthermore, we followed sound statistical methodology, and results for each observed psychosocial phenotype showed similar trends when analyzed separately. Another limitation is that the extent of ischemia with mental stress was relatively mild, which may have influenced our non-significant results. Finally, as this is a cross-sectional analysis, we cannot infer casualty based on these data, and it is not possible to ascertain whether some of the risk factors adjusted for in the analysis are mediators or confounders of the association between psychosocial status and myocardial perfusion.

In conclusion, we found that, among CAD patients, a higher level of psychosocial distress is not associated with ischemia provoked by mental stress, but it is associated with more resting perfusion abnormalities in women, but not in men, as well as a with blunted hemodynamic response to mental stress in both men and women. While the exact implications of these findings need to be evaluated in the context of future outcome studies, they suggest that chronic psychosocial distress, considered as a global measure, may affect the severity of CAD more than ischemia provoked by acute stress exposure, especially among women.

Chapter 4, Table 1: Descriptive Characteristics of the Study Population According to Latent Classes of Psychosocial Distress

Variables	Class 1 (No/low Symptoms) (N= 268)	Class 2 (Mild Symptoms) (N= 112)	Class 3 (Moderate Symptoms) (N= 208)	Class 4 (High Symptoms) (N= 77)	Total Population (N= 665)
Demographic Factors					
Age, Mean (SD)[1]	65 (8)	66 (8)	61 (9)	57 (9)	63 (9)
Women, N (%)[1]	62 (23%)	21 (19%)	71 (34%)	31 (40%)	185 (28%)
African-American, N (%)[1]	70 (26%)	21 (19%)	77 (37%)	30 (39%)	198 (30%)
Education ≤ high-school, N (%)	64 (24%)	21 (19%)	57 (27%)	27 (35%)	169 (25%)
Lifestyle Factors and Medical History					
Current smokers, N (%)	33 (12%)	8 (7%)	42 (20%)	11 (14%)	94 (14%)
Hypertension, N (%)	202 (75%)	87 (78%)	158 (76%)	61 (79%)	508 (76%)
Dyslipidemia, N (%)	220 (82%)	91 (81%)	166 (80%)	67 (87%)	544 (82%)
Diabetes, N (%)	83 (31%)	30 (27%)	76 (36%)	27 (35%)	216 (32%)
BMI, Mean (SD)[1]	29 (5)	29 (4)	30 (6)	32 (6)	30 (5)
Previous MI, N (%)[1]	91 (34%)	34 (30%)	93 (44%)	31 (40%)	249 (37%)
History of heart failure, N (%)	29 (11%)	15 (13%)	32 (15%)	17 (22%)	93 (14%)
Previous revascularization, N (%)	207 (77%)	85 (76%)	158 (76%)	60 (78%)	510 (77%)

Ejection fraction in %, Mean (SD)	70 (14)	67 (14)	68 (13)	69 (14)	69 (14)
CAD ≥ 70% stenosis, N (%)[2]	210 (86%)	84 (85%)	139 (82%)	55 (83%)	488 (84%)
Current Medications					
Statins, N (%)	225 (84%)	97 (87%)	183 (89%)	62 (81%)	567 (86%)
Beta-blockers, N (%)[1]	185 (69%)	82 (73%)	163 (79%)	65 (85%)	495 (75%)
ACE-inhibitors, N (%)	126 (47%)	52 (46%)	89 (43%)	35 (45%)	302 (46%)
Aspirin, N (%)[1]	233 (87%)	98 (87%)	186 (90%)	55 (71%)	572 (86%)
Anti-depressants, N (%)[1]	34 (13%)	20 (18%)	58 (28%)	40 (52%)	152 (22%)
Anxiolytics, N (%)[1]	12 (4%)	14 (13%)	21 (10%)	9 (12%)	56 (8%)
Extrinsic Psychosocial Factors					
ETI score, Mean (SD)[1]	5 (4)	6 (4)	8 (5)	10 (6)	7 (5)
LTI score, Mean (SD)[1]	16 (9)	18 (9)	20 (10)	23 (13)	18 (10)
EDS score, Mean (SD)[1]	13 (4)	13 (4)	16 (4)	20 (6)	15 (5)
Perceived-social support score, Mean (SD)[1, 3]	73 (12)	70 (11)	64 (15)	53 (17)	67 (15)

SD: Standard Deviation; MI: Myocardial Infarction; BMI: Body Mass Index; CAD: Coronary Artery Disease; ETI: Early Trauma Inventory (total score); LTI: Life-Traumatic Events (total score); EDS: Everyday Discrimination Scale (total score)

[1] P value < 0.05

[2] CAD severity based on coronary angiography results prior to revascularization procedures (if any); 86 observations missing

[3] For perceived social-support scale, a higher score indicates better social support

Chapter 4, Table 2: Association of Latent Classes of Psychosocial Distress with Perfusion Defect Severity at Rest (Summed Rest Score), and Induced Ischemia Severity (Summed Difference Score) with Mental Stress in the Overall Population

Exposure Comparison	Full Sample: Unadjusted β (95% CI)	Full Sample: Adjusted[1] β (95% CI)
Summed Rest Score (SRS)		
LCA class 1 vs. 2	0.31 (-1.62 to 2.23)	0.35 (-1.54 to 2.25)
LCA class 1 vs. 3	0.71 (-0.88 to 2.30)	0.80 (-0.78 to 2.39)
LCA class 1 vs. 4	0.73 (-1.49 to 2.95)	0.88 (-1.41 to 3.16)
Summed Difference Score (SDS)		
LCA class 1 vs. 2	0.01 (-0.44 to 0.45)	0.04 (-0.41 to 0.48)
LCA class 1 vs. 3	0.10 (-0.27 to 0.46)	0.04 (-0.34 to 0.41)
LCA class 1 vs. 4	-0.19 (-0.71 to 0.32)	-0.31 (-0.86 to 0.23)

β: represents estimated point increase in perfusion defect score (either SRS for rest, or SDS for stress) when comparing class 1 (reference) to class 2, 3 or 4

[1] Results adjusted for age, sex, race, education, history of smoking, hypertension, hyperlipidemia, diabetes, revascularization, body mass index, and summed rest score (for the summed difference score analysis only)

Chapter 4, Table 3: Association of Latent Classes of Psychosocial Distress with Perfusion Defect Severity at Rest (Summed Rest Score), and Induced Ischemia Severity (Summed Difference Score) with Mental Stress, According to Sex

Exposure Comparison	Men: Unadjusted β (95% CI)	Men: Adjusted[1] β (95% CI)	Women: Unadjusted β (95% CI)	Women: Adjusted[1] β (95% CI)	P Value for Sex Interaction
Summed Rest Score (SRS)					
LCA class 1 vs. 2	0.03 (-2.23 to 2.30)	-0.02 (-2.14 to 2.10)	0.71 (-2.76 to 4.19)	1.62 (-2.63 to 5.87)	0.50
LCA class 1 vs. 3	0.64 (-1.35 to 2.64)	0.41 (-1.45 to 2.28)	1.88 (-0.53 to 4.29)	1.98 (-0.99 to 4.96)	0.38
LCA class 1 vs. 4	-0.26 (-3.22 to 2.71)	-0.87 (-3.74 to 1.99)	3.61 (0.59 to 6.64)	3.98 (0.22 to 7.73)	0.04
Summed Difference Score (SDS)					
LCA class 1 vs. 2	-0.11 (-0.57 to 0.34)	-0.08 (-0.57 to 0.42)	0.50 (-0.70 to 1.70)	0.44 (-0.56 to 1.44)	0.37
LCA class 1 vs. 3	-0.14 (-0.53 to 0.27)	-0.15 (-0.59 to 0.29)	0.58 (-0.25 to 1.42)	0.52 (-0.18 to 1.22)	0.11
LCA class 1 vs. 4	-0.27 (-0.87 to 0.33)	-0.30 (-0.98 to 0.38)	-0.06 (-1.13 to 1.00)	-0.16 (-1.06 to 0.73)	0.81

β: represents estimated point increase in perfusion defect score (either SRS for rest, or SDS for stress) when comparing class 1 (reference) to class 2, 3 or 4

[1] Results adjusted for age, sex, race, education, history of smoking, hypertension, hyperlipidemia, diabetes, revascularization, body mass index, and summed rest score (for the summed difference score analysis only)

Chapter 4, Table 4: Association of Individual Psychosocial Indicators with Perfusion Defect Severity at Rest (Summed Rest Score), and Induced Ischemia Severity (Summed Difference Score), Overall & According to Sex

Exposure Comparison	Full Sample: Unadjusted β (95% CI)	Full Sample: Adjusted β (95% CI)	Men: Adjusted β (95% CI)	Women: Adjusted β (95% CI)	P Value for Sex Interaction
Summed Rest Score (SRS)					
BDI-somatic score	0.10 (-0.56 to 0.77)	0.18 (-0.50 to 0.86)	-0.17 (-0.99 to 0.64)	0.89 (-0.26 to 2.05)	0.13
BDI-negative affect score	0.14 (-0.53 to 0.81)	0.09 (-0.59 to 0.77)	-0.25 (-1.05 to 0.55)	0.95 (-0.32 to 2.23)	0.11
PCL score	-0.24 (-0.91 to 0.42)	-0.16 (-0.84 to 0.52)	-0.55 (-1.39 to 0.28)	0.60 (-0.55 to 1.75)	0.10
STAI Anxiety-Trait score	0.41 (-0.25 to 1.08)	0.30 (-0.39 to 0.99)	-0.23 (-1.04 to 0.57)	1.61 (0.37 to 2.84)	0.01
STAXI Anger-Trait score	0.02 (-0.64 to 0.68)	0.08 (-0.59 to 0.74)	-0.20 (-0.98 to 0.57)	0.84 (-0.42 to 2.10)	0.17
CMHS Hostility score	0.23 (-0.44 to 0.89)	-0.02 (-0.72 to 0.67)	-0.32 (-1.13 to 0.48)	0.80 (-0.48 to 2.08)	0.14
Perceived-stress score	0.23 (-0.43 to 0.90)	0.19 (-0.51 to 0.88)	-0.40 (-1.23 to 0.44)	1.38 (0.19 to 2.55)	0.02
Summed Difference Score (SDS)					
BDI-somatic score	-0.02 (-0.17 to 0.13)	-0.06 (-0.23 to 0.10)	-0.09 (-0.29 to 0.10)	-0.01 (-0.28 to 0.28)	0.60
BDI-negative affect score	-0.08 (-0.24 to 0.08)	-0.12 (-0.28 to 0.04)	-0.13 (-0.32 to 0.06)	-0.08 (-0.38 to 0.23)	0.77
PCL score	-0.08 (-0.23 to 0.08)	-0.11 (-0.27 to 0.05)	-0.07 (-0.26 to 0.13)	-0.19 (-0.46 to 0.09)	0.52
STAI Anxiety-Trait score	-0.14 (-0.30 to 0.01)	-0.21 (-0.37 to -0.05)	-0.19 (-0.38 to -0.01)	-0.25 (-0.55 to 0.04)	0.73
STAXI Anger-Trait score	-0.17 (-0.32 to -0.01)	-0.18 (-0.34 to -0.03)	-0.20 (-0.38 to -0.02)	-0.15 (-0.44 to 0.15)	0.78

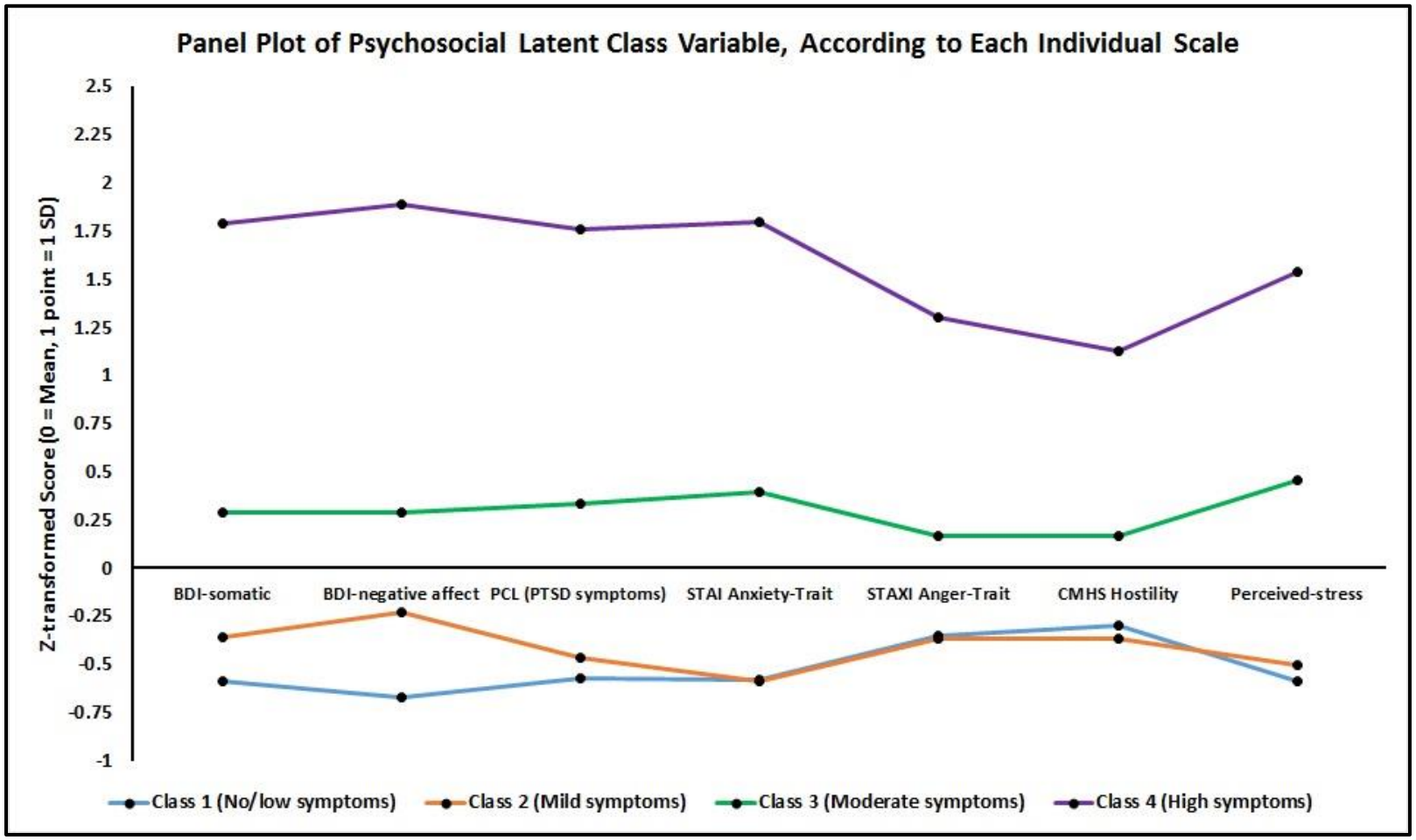
CMHS Hostility score	-0.19 (-0.34 to -0.04)	-0.24 (-0.40 to -0.08)	-0.16 (-0.34 to 0.03)	-0.44 (-0.74 to -0.14)	0.11
Perceived-stress score	-0.05 (-0.21 to 0.11)	-0.12 (-0.29 to 0.05)	-0.14 (-0.34 to 0.06)	-0.08 (-0.36 to 0.21)	0.71

β: represents estimated point increase in perfusion defect score (either SRS for rest, or SDS for stress) with 1 standard deviation increase in each psychosocial scale

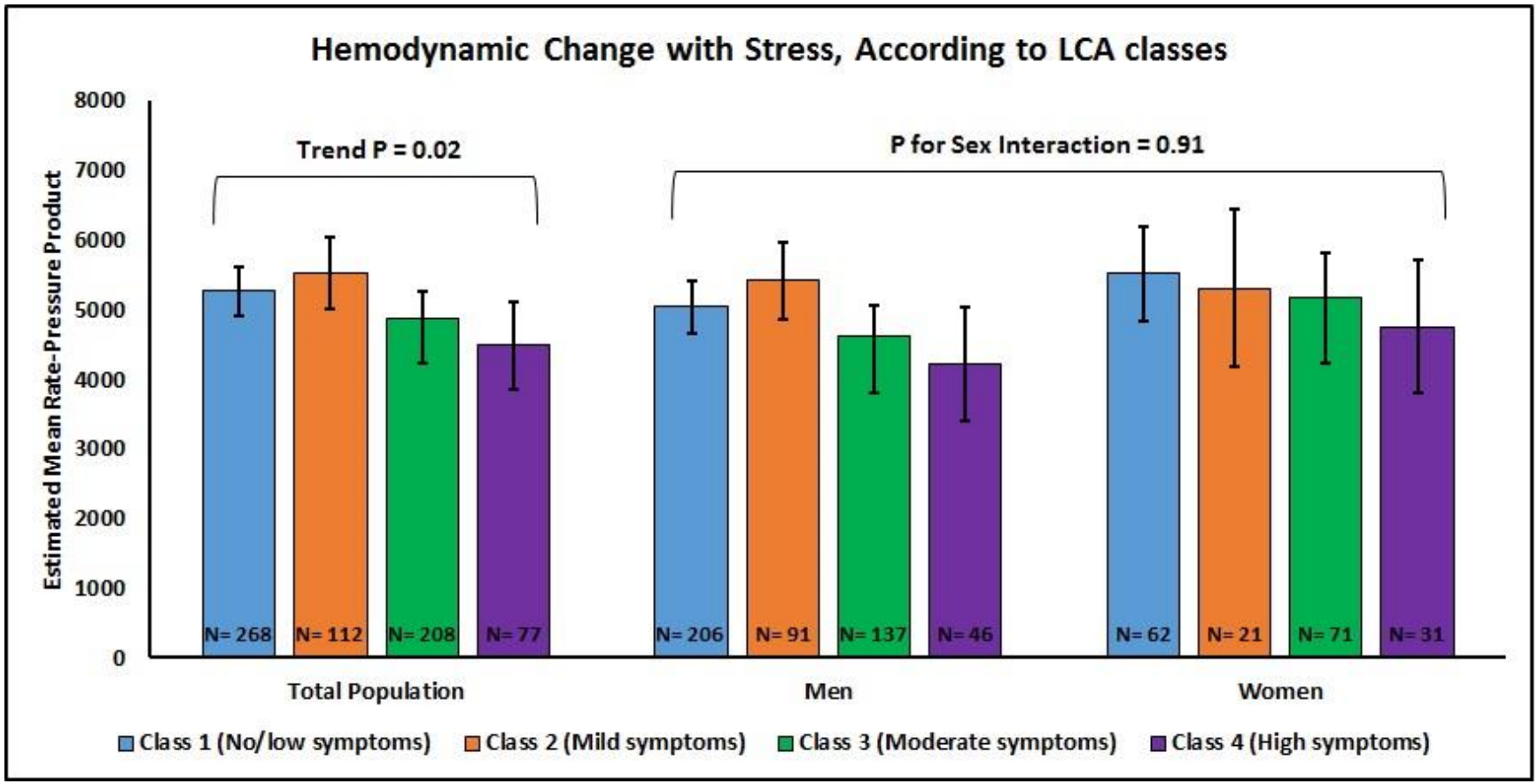
BDI: Beck Depression Inventory; PCL: PTSD Symptom Checklist (Civilian); STAI: State- Trait Anxiety Inventory; STAXI: State-Trait Anger Expression Inventory; CMHS: Cook-Medley Hostility Score

[1] Results adjusted for age, sex, race, education, history of smoking, hypertension, hyperlipidemia, diabetes, and revascularization, body mass index, and summed rest score (for the summed difference score analysis only)

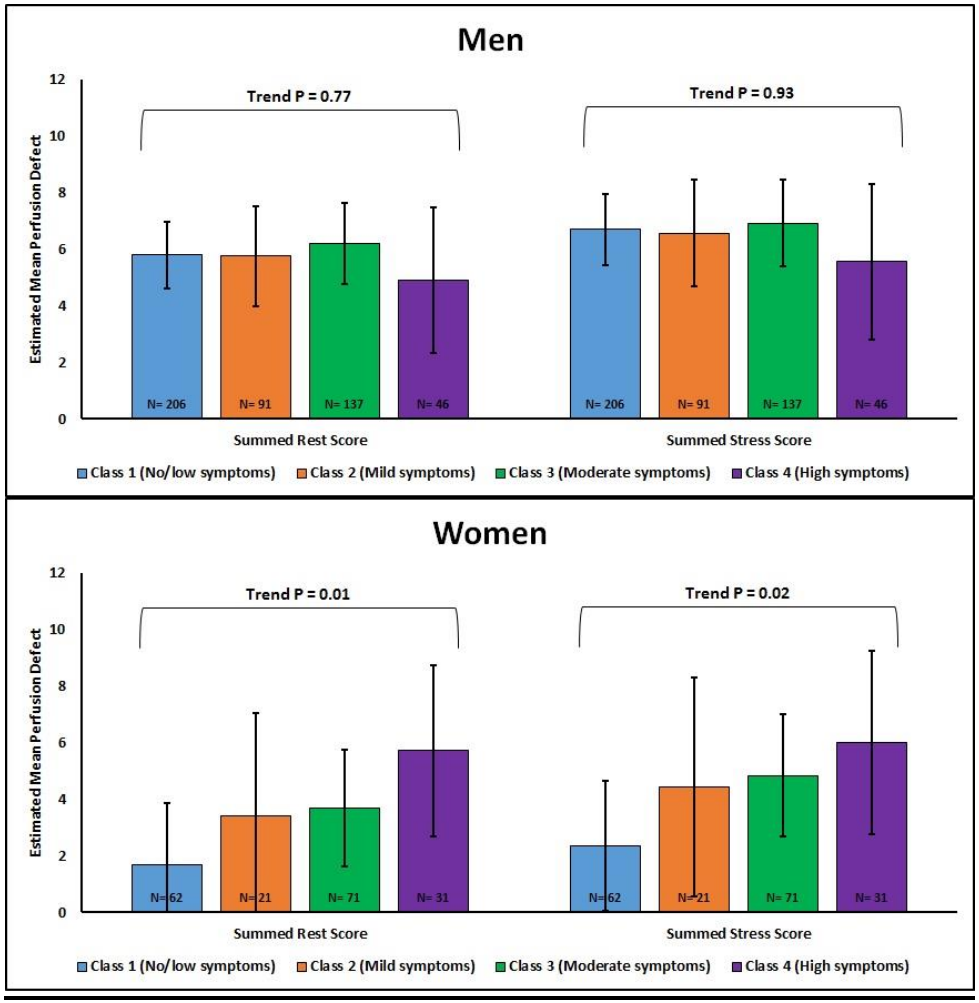
Chapter 4, Figure 1: Panel plot of the psychosocial distress LCA variable, according to individual scale levels. The graph shows the distribution of each latent class according to the individual psychosocial scale Z scores (scale score – sample mean/ sample SD).



Chapter 4, Figure 2: Hemodynamic Change with Stress (Rate-Pressure Product Difference), Stratified by Sex, According to Psychosocial Distress Latent Classes. Estimates are adjusted for age, sex, race, education, smoking, and body mass index



Chapter 4, Figure 3: Perfusion Defect Severity (Mean Summed Scores) at Rest and with Stress, Stratified by Sex, According to Psychosocial Distress Latent Classes. **<u>In women, but not in men, higher psychosocial distress was associated with more perfusion defects (denoting abnormal myocardial perfusion), which were already present at rest</u>**



Chapter 4, Supplementary Table 1: Descriptive Characteristics of the Study Population According to Sex

Variables	Men (N= 480)	Women (N= 185)	Total Population (N= 665)
Demographic Factors			
Age, Mean (SD)	63 (9)	63 (9)	63 (9)
African-American, N (%)[1]	119 (25%)	79 (43%)	198 (30%)
Education ≤ high-school, N (%)	118 (25%)	51 (28%)	169 (25%)
Lifestyle Factors and Medical History			
Current smokers, N (%)	66 (14%)	28 (15%)	94 (14%)
Hypertension, N (%)	362 (75%)	146 (79%)	508 (76%)
Dyslipidemia, N (%)	400 (83%)	144 (78%)	544 (82%)
Diabetes, N (%)	146 (30%)	70 (38%)	216 (33%)
BMI, Mean (SD)[1]	29 (5)	30 (6)	30 (5)
Previous MI, N (%)	178 (37%)	71 (38%)	249 (37%)
History of heart failure, N (%)	64 (13%)	29 (16%)	93 (14%)
Previous revascularization, N (%)	365 (76%)	145 (78%)	510 (77%)
Ejection fraction in %, Mean (SD) [1]	66 (12)	74 (14)	69 (14)
CAD ≥ 70% stenosis, N (%)[2]	357 (85%)	131 (82%)	488 (84%)

Current Medications			
Statins, N (%)	413 (86%)	154 (84%)	567 (86%)
Beta-blockers, N (%)	352 (73%)	143 (77%)	495 (75%)
ACE-inhibitors, N (%)[1]	237 (49%)	65 (35%)	302 (46%)
Aspirin, N (%)	419 (87%)	153 (83%)	572 (86%)
Anti-depressants, N (%)[1]	92 (19%)	60 (32%)	152 (23%)
Anxiolytics, N (%)	37 (8%)	19 (10%)	56 (8%)
Intrinsic Psychosocial Factors			
BDI-somatic score, Mean (SD)[1]	6 (5)	8 (6)	6 (6)
BDI-negative affect score, Mean (SD)	2 (3)	3 (3)	2 (3)
PCL score, Mean (SD)[1]	26 (10)	30 (12)	27 (11)
STAI Anxiety-Trait score, Mean (SD)[1]	33 (10)	35 (11)	33 (11)
STAXI Anger-Trait score, Mean (SD)	15 (4)	15 (4)	15 (4)
CMHS Hostility score, Mean (SD)	16 (8)	16 (8)	16 (8)
Perceived-stress score, Mean (SD)[1]	12 (7)	14 (8)	12 (8)
Extrinsic Psychosocial Factors			
ETI score, Mean (SD)	6 (5)	7 (5)	7 (5)
LTI score, Mean (SD)	18 (10)	18 (9)	18 (10)

EDS score, Mean (SD)	15 (5)	15 (4)	15 (5)
Perceived-social support score, Mean (SD)[3]	67 (14)	67 (16)	67 (15)

SD: Standard Deviation; MI: Myocardial Infarction; BMI: Body Mass Index; CAD: Coronary Artery Disease; BDI: Beck Depression Inventory; PCL: PTSD Symptom Checklist (Civilian); STAI: State- Trait Anxiety Inventory; STAXI: State-Trait Anger Expression Inventory; CMHS: Cook-Medley Hostility Score; ETI: Early Trauma Inventory (total score); LTI: Life-Traumatic Events (total score); EDS: Everyday Discrimination Scale (total score)

[1] P value < 0.05

[2] CAD severity based on coronary angiography results prior to revascularization procedures; 86 observations missing

[3] For perceived social-support scale, higher score indicates better social support

Chapter 4, Supplementary Table 2: Individual Psychosocial Factors of the Study Population According to Latent Classes

Variables	Class 1 (No/low Symptoms) (N= 268)	Class 2 (Mild Symptoms) (N= 112)	Class 3 (Moderate Symptoms) (N= 208)	Class 4 (High Symptoms) (N= 77)	Total Population (N= 665)
Intrinsic Psychosocial Factors					
BDI-somatic score, Mean (SD)	3 (3)	4 (3)	8 (4)	16 (7)	6 (6)
BDI-negative affect score, Mean (SD)	0 (0)	2 (1)	3 (2)	9 (5)	2 (3)
PCL score, Mean (SD)	20 (4)	22 (4)	30 (9)	46 (14)	27 (11)
STAI Anxiety-Trait score, Mean (SD)	27 (6)	27 (4)	37 (7)	52 (8)	33 (10)
STAXI Anger-Trait score, Mean (SD)	13 (3)	13 (2)	15 (4)	20 (6)	15 (4)
CMHS Hostility score, Mean (SD)	14 (8)	13 (6)	17 (8)	25 (7)	16 (8)
Perceived-stress score, Mean (SD)	8 (5)	9 (4)	16 (6)	24 (6)	12 (8)

SD: Standard Deviation; BDI: Beck Depression Inventory; PCL: PTSD Symptom Checklist (Civilian); STAI: State- Trait Anxiety Inventory; STAXI: State-Trait Anger Expression Inventory; CMHS: Cook-Medley Hostility Score

Chapter 4, Supplementary Table 3: Model Fit Statistics for Latent Class Analysis

LCA Model	N	Log-Likelihood	AIC	BIC	ICL-BIC
1 Class	665	-15375	30778	30841	30841
2 Class	665	-13988	28035	28165	28254
3 Class	665	-13534	27157	27355	27505
4 Class	665	-12321	24759	25020	25144
5 Class	665	-12065	24274	24598	24780
6 Class	665	-12212	24600	24996	25245

LCA: Latent Class Analysis; N: Number of parameters in each model; AIC: Akaike Information Criterion; BIC: Bayesian Information Criterion; ICL-BIC: Integrated Completed Likelihood - Bayesian Information Criterion

Chapter 5: Genetic Determinants of Mental Stress Induced Myocardial Ischemia

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Abstract

Background: Mental stress-induced myocardial ischemia (MSIMI) is a transient myocardial ischemic response to a standardized mental stress challenge, common among patients with coronary artery disease (CAD). Several stress-related mechanisms have been postulated for MSIMI, such as autonomic nervous system imbalance and hyperactive immune/inflammatory responses to stress and endothelial and/or microvascular disease. However, the role of genetic predisposition in the occurrence of MSIMI, especially genes involved with stress-related pathways, has not been studied in detail.

Methods: 496 whites and 276 African-Americans with established CAD underwent two myocardial perfusion SPECT scans, one at rest and one with mental stress. MSIMI was calculated as difference between the perfusion defect scores during mental stress and at rest. Genome-wide single nucleotide polymorphisms (SNPs) were directly genotyped, and then used to impute to the 1000 Genome reference panel for association analysis of MSIMI. In addition to a genome-wide association study (GWAS), we examined the association between MSIMI and 286 pre-defined candidate genes involved in either stress-response physiology and/or those with an established association with CAD.

Results: The mean age of the study sample was 60 years (SD= 10 years), 253 (33%) were women, and 276 (36%) were African-American. Out of the 286 candidate-genes, *FGF5* (Fibroblast Growth Factor 5) gene on chromosome 4 was significantly associated with MSIMI ($P= 4.7\times10^{-5}$) at the Bonferroni-adjusted significant threshold. This gene was significantly associated in both whites (P= 0.0005) and African-Americans (P= 0.03). For the GWAS analysis, one SNP in SCD5 (Stearoyl-CoA desaturase 5) gene was significantly associated MSIMI ($\beta= 2.68$, $P= 2.0\times10^{-9}$) only in African-Americans.

Conclusion: The *FGF5* gene, which is strongly associated with systolic & diastolic blood pressure and part of the RET signaling pathway (important for neuronal cells survival), is associated with MSIMI. A SNP in SCD5 gene was associated with MSIMI in African-Americans only, and is warranted of further replication. These findings further delineate the pathophysiological importance of hemodynamic changes with mental stress and the neuro-hormonal origins for the occurrence of MSIMI.

Introduction:

Mental stress-induced myocardial ischemia (MSIMI) is a transient myocardial ischemic response to a standardized mental stress challenge.[9] MSIMI is analogous to conventional exercise or pharmacologically-induced myocardial ischemia during standard cardiac clinical testing, except that the stressor used is a standardized, laboratory based psychological stress challenge.[9] MSIMI is associated with a twofold increased risk of future cardiac events, which is similar to ischemia induced by conventional stress testing (exercise or pharmacological stress testing).[10] However, MSIMI appears to be a unique phenomenon, in that it occurs at lower levels of oxygen demand, and is usually not associated with severity of coronary artery disease (CAD).[9,46,57]

Several stress-related mechanisms have been postulated for MSIMI, including hyper-activation of cerebral regions responsible for emotions, memory and sympathetic activation,[58,59] imbalance in sympathetic-parasympathetic stimulation in response to stress,[60,61] hyperactive response of inflammatory systems to stress,[60-62] and endothelial dysfunction and/or microvascular disease.[57,61] However, the role of genetic predisposition in the occurrence of MSIMI has not been studied in detail. Identifying genetic polymorphisms which are associated with MSIMI can provide further insights into mechanistic pathways responsible for this phenomenon and can also potentially provide new targets for devising preventive strategies and/or therapies. Only one previous study has explored genetic determinants of MSIMI,[63] which was limited to five single nucleotide polymorphisms (SNPs) of β1-adrenergic receptors (*ADRB1*) and β2-adrenergic receptors (*ADRB2*). Hence, in our study of patients with stable CAD, we investigated the associations between genetic polymorphisms and MSIMI by performing an exploratory genome-wide association analysis (GWAS). We further investigated the association

between a-priori defined set of candidate genes (those related to stress-response physiology and/or those with an established association with CAD) and MSIMI.

Methods:

Study sample

Between June 2011 and March 2016, we enrolled 950 individuals (627 men, 323 women) with stable coronary artery disease in two parallel studies with similar protocols: The Mental Stress Ischemia: Prognosis and Genetic Influences Study (MIPS) (N=636) and the Myocardial Infarction and Mental Stress Study (MIMS) (N=314). Both studies shared testing and data collection protocols, as well as study staff, investigators, facilities and equipment, but there were some differences in the inclusion and exclusion criteria (**chapter 5, supplementary table 1**). A detailed protocol with inclusion and exclusion criteria has been previously described.[135] This research was approved by the Emory University Institutional Review Board, and all participants provided informed consent.

Phenotype measurement

Each study participant underwent single-photon emission tomography (SPECT) imaging studies; at rest, and with mental stress, with ^{99m}Tc-Sestamibi, at the dose of 10-14 mCi for rest imaging and 30-40 mCi for stress imaging, based on weight.[159] Mental stress was induced by a standardized social stressor using a public speaking task, as previously described.[134,135]

SPECT images were interpreted using accepted methodology by two experienced readers blind to patients' data using a 17-segment model. Disagreements between the two readers were resolved by consensus and a third reader if needed. Each myocardial segment was scored from 0 (no abnormality) to 4 (absent perfusion), and summed scores were calculated in a conventional

fashion, yielding a summed stress scores (SSS) for mental stress, and a summed rest score (SRS) for rest, each with a theoretical range of 0 to 68. A summed difference score (SDS) was calculated for mental stress by subtracting the SRS from the SSS. The SDS is a semi-quantitative measure of the number and severity of reversible (ischemic) myocardial perfusion defects.[136]

We used validated instruments to collect demographic, behavioral, social and health status data. Socio-economic status was assessed using income status (categorized as below or above family poverty-line income); smoking status was categorized into current smokers or non-current smokers. Hypertension, hyperlipidemia, and diabetes were ascertained by research staff during the clinic visit through a detailed medical history. Angiographic data were obtained from the most recent coronary angiogram documented in the patient's medical record. CAD severity was quantified using a cut-off of 70% blockage in any major arteries.

Genotype measurement

Genomic DNA (gDNA) was extracted from blood or saliva samples of study participants. Each gDNA sample was quantified using the PicoGreen assay, standardized to 50ng/mL, and processed following the standard Illumina protocol including hybridization, incubation and scanning. We used Illumina's multi-ethnic global array (MEGA) platform, which is optimized for genome-wide association studies in multi-ethnic populations.

Participants were excluded if they had an overall SNP call rate (ratio of measured SNPs per participant over the total number of SNPs in the dataset) < 95% or sex mismatch between genotypic and phenotypic measurements. In addition, individual SNPs were excluded from the analyses if they belonged to non-autosomal chromosome, missing rate greater than 5%, ethnicity-specific Hardy-Weinberg Equilibrium (HWE) p-value less than 0.0001 or a minor allele frequency (MAF) less than 0.05.[125] Using the measured SNPs, we performed genome-wide

imputation using 1000 Genomes (Phase 3) panel as the reference. This imputation was performed using the Michigan Imputation Server,[126] and after the quality control (MAF $\geq$ 0.05; removal of duplicate, non-SNP or monomorphic sites; imputation $R^2 \geq 0.50$), 5,504,202 SNPs were analyzed.

Candidate genes were selected based on following criteria:

I) Genes associated with stress response: We used a systems biology-pathway driven approach for selection of candidate genes. Body systems informed this selection are: Autonomic (sympathetic/parasympathetic) nervous system[127] (20 genes), Renin-Angiotensin-Aldosterone system[128] (7 genes), Inflammation and immunity[129,130] (10 genes), and Endothelial systems[131-133] (5 genes).

II) All genes found to be associated with cardiovascular disease (279 total genes from 161 loci significantly associated with CAD) in recently published genome-wide association studies.[65-69]

In total, we curated 321 (42 stress-related, 279 CAD-related) candidate genes for our analyses (details in **chapter 5, supplementary table 2**).

Statistical analysis

We compared subject baseline characteristics according to the race (whites vs. African-American) and by study using either the t-test for continuous, normally distributed variables or the chi-square test for categorical variables. We also compared the baseline characteristics between subjects with and without genetic information in our final analytical sample. For GWAS analysis, we performed multivariable linear regression models with ischemia with mental stress (mental stress SDS) as outcome, and each SNP as the main predictor variable (using additive

model of genotyping), adjusting for resting perfusion defect (SRS), age, sex and indicators of population stratification. Indicators of population stratification were created by principal component analysis using R, and the first 10 principal components were adjusted for in the analysis. Since the SDS for mental stress had a skewed distribution, while the SSS was approximately normally distributed, we used the SSS score as dependent variables while adjusting for the rest score (SRS). Because of the mathematical relationship between these scores, the coefficient from a model with SSS as dependent variable, adjusted for SRS, is identical to that from a model where the dependent variable is the SDS. This strategy allowed us to obtain less biased standard errors and p values. GWAS analysis was conducted using RVTEST,[138] and R; and meta-analysis of individual study results was conducted using METAL.[139] For candidate gene analysis, summary results from individual, non-synonymous SNPs were aggregated to give gene-based test of significance using the web-based tool FUMA.[140] Gene-based analysis was performed using SNP-wise test, where the sum of –log of individual SNP P-value is used as summary statistic for the gene-level analysis.[141] Regional plots for the top associations were obtained using a web-based tool.[160] For GWAS analysis, we used a P-value threshold of 5×10^{-8}; for candidate-gene analysis, a Bonferroni adjusted P-value threshold of 1.75×10^{-4} (0.05/286) was used.

Results:

Sample Characteristics

One hundred and seventy-eight out of the total of 950 subjects had either missing genotype information (due to lack of sufficient serum or saliva sample) or missing information on the outcome, leaving a final analytical sample size of 772. When we compared the analytical sample to the subjects with missing information by study, subjects with missing information

were more likely to be women in MIPS study, whereas in they were less likely to be women in MIMS study. Among the cardiovascular risk factors, only heart failure prevalence was higher in missing subjects of MIMS study, while none of the cardiovascular risk factors were different among subjects with and without genetic information in MIPS study (**chapter 5, supplementary table 3**).

The mean age of the analytical sample was 60 years (SD= 10 years), 253 (33%) were women, 276 (36%) were African-Americans, and 139 (19%) had family income below the poverty line (**chapter 5, table 1**). As expected, the prevalence of cardiovascular risk factors was high in this population, including hypertension (77%), dyslipidemia (82%), and type 2 diabetes (32%). Furthermore, 52% had a previous myocardial infarction (MI), and 75% had a previous revascularization procedure. When patient characteristics were examined by race **(chapter 5, table 1)**, African-American subjects were younger (55 vs. 63 years), more often female (49% vs. 24%) and below poverty line (35% vs. 10%), as compared to whites. African-American subjects also were often current smokers, had a higher prevalence of hypertension, and diabetes, a higher BMI, and were more likely to have a previous MI, and a previous revascularization procedure. Comparison of baseline characteristics by study-type (**MIPS vs. MIMS, chapter 5, table 1)** mostly reflected study design differences.

Genome-Wide Association Analysis (GWAS) Results

After the quality-control and exclusion of low-frequency variants, 5,504,202 SNPs were tested for the association with MSIMI. The mean summed-difference score (SDS) for mental stress in the entire population was 0.77 units (SD = 1.93).

Using Quantile-Quantile plots (**chapter 5, figure 1**), we compared the observed P-value distribution with the expected P-value distribution (for the 5,504,202 tests of associations) in our

entire study cohort (N = 772), and did not observe any substantial inflation (inflation factor = 0.99). No SNP association reached the pre-defined genome-wide significance threshold of 5×10^{-8} in the entire study sample (**chapter 5, figure 2 shows the Manhattan Plot for GWAS, and table 2 describs the top 10 associations**). The most significant SNP (*rs17196120*) was negatively associated with mental stress ischemia (regression co-efficient = -0.9, P= 9.9×10^{-7}), each minor allele (allele = T) associated with 0.9 points lower summed difference score, as compared to having both major alleles (genotype = CC) (**chapter 5, table 2).** This SNP showed consistent results in terms of both directionality and effect sizes among both whites (regression co-efficient = -0.8, P= 8.3×10^{-5}) and African-Americans (regression co-efficient = -1.2, P= 0.002)**.** This SNP was mapped to chromosome 11, and is an intronic variant of a gene without known function (*LOC105369449*). Among the remaining top 10 associations, a cluster of SNPs in chromosome 11 (*rs2284301, rs592521, rs71526466, rs592532, rs506354*) were mapped to a region with multiple genes (**chapter 5, figure 3: Regional Plots for Top 10 Associations)**.

Race-specific GWAS analysis did not show any global inflation of P-values in either whites (inflation factor = 0.99) or African-Americans (inflation factor = 0.99) (**chapter 5, supplementary figure 1).** Although there was no genome-wide significant SNP observed in whites, two SNPs reached genome-wide significance in African-Americans (*rs36008702, rs12498940*) (**chapter 5, supplementary table 4, supplementary figure 2**). These two SNPs are located in an intronic region of the gene *SCD5* in chromosome 4.

Candidate Gene Analysis Results

Among 321 candidate genes from stress-related pathways and CAD GWAS (**chapter 5, supplementary table 2**), 286 genes were analyzed in the final sample. Out of all of these 286 genes, only the *FGF5* (Fibroblast Growth Factor 5) gene on chromosome 4 reached the

Bonferroni-adjusted significant threshold (P= 4.7×10^{-5}, **chapter 5, table 3**). The *FGF5* gene was significantly associated in both whites (P= 0.0005) and African-Americans (P= 0.03) (**chapter 5, table 3**), and the race-specific regional plots for the *FGF5* gene showed similar results from individual SNP association tests (**chapter 5, figure 4**).

Among the candidate genes curated through stress-related pathways, only the *COMT* (Catechol-O-Methyltransferase) gene showed significant results (at conventional p value cut-off of 0.05), and this association was only observed in whites (P= 0.003), and not in African-Americans (P= 0.70). Detailed results for each candidate gene are shown in **chapter 5, supplementary table 5.**

Discussion:

MSIMI, which is a transient myocardial ischemic response to a standardized mental stress challenge,[9] is hypothesized to be manifestation of stress-response pathophysiology[58,59] among subjects with pre-existing CAD. Understanding whether genetic mechanisms associated with stress-response can provide further insight into pathophysiology of MSIMI. In our study of individuals with pre-existing, stable CAD, candidate-gene specific analysis showed that the fibroblast growth factor 5 (*FGF5)* gene, which codes for the fibroblast growth factor protein, is associated with MSIMI. This association was present in both whites and African-Americans, and is independent of age, sex, and perfusion-defect burden at rest, an indicator of severity of CAD.

The *FGF5* gene (chr4:81,187,742 - 81,257,834) encodes a member of the fibroblast growth factor (FGF) family. FGF family members possess broad mitogenic and cell survival activities, and are involved in a variety of biological processes, including embryonic development, cell growth, morphogenesis, tissue repair, tumor growth and invasion. Additionally, this gene is involved in GDNF (Glial cell line-derived neurotrophic factor)/RET

(rearranged during transfection) signaling pathway, which is important for survival of neuronal cells including peripheral autonomic and sensory neurons and central motor and dopamine neurons.[161] *FGF5* gene has been consistently associated with systolic and diastolic blood pressure, and hypertension in multiple ethnicities,[162-165] and is also associated with CAD prevalence, with a SNP in the regulatory region of the gene (rs10857147) showing significant association with CAD (odds ratio for minor allele T vs. major allele A = 1.06, P= 3.4×10^{-8}).[68,69]

For the GWAS analysis of the entire study cohort, even though the results were non-significant at the pre-defined P-value threshold, we did find a cluster of SNP associations on chromosome 11 (64 – 65 MB position), which is dense in genes with relevant physiological functions. This gene cluster on chromosome 11 is associated mainly with the uricosurics pathway, the glycogen metabolism pathway, and GDNF/RET signaling pathway, the latter being also influenced by *FGF5* gene. Significant gene-expression levels are found in the urinary system, the GI tract, and the musculoskeletal, hematopoietic and endocrine systems;[166] and genetic diseases most commonly associated with this cluster are multiple endocrine neoplasia's, and autosomal recessive bleeding disorder,[166] signifying the importance of this gene cluster for overall endocrine function. For the race-specific GWAS analysis, we identified 2 significant associations among African-American participants; both SNPs are located in the Stearoyl-CoA Desaturase 5 gene (*SCD5*), which is suspected to be an important regulator of fat metabolism, with a potential role in obesity and dyslipidemia.[166] However, given the small sample of the African-Americans in our study, and the minor allele frequency ~ 0.05 for both the significant SNPs, this finding requires further replication.

To the best of our knowledge, only one study by Hassan et al[63] has investigated the association between genetic variants and MSIMI. These investigators examined five SNPs of β1-

adrenergic receptors (*ADRB1*) and β2-adrenergic receptors (*ADRB2*) in a small sample (N= 148) of patients with stable CAD. Polymorphisms in these genes alter the effects of epinephrine on cardiac and vasculature physiology and these polymorphisms have been associated with cardiovascular disease.[166] Hassan et al. found a significant association between a variant of the *ADRB1* gene (rs1801252: substitution of major allele adenine by guanine) and MSIMI at the significance level of 0.05, but no other genes involved in stress-response pathways were analyzed and there was no adjustment for multiple testing and population stratification. In our analyses, neither the five SNPs tested by Hassan et al, nor the genes (*ADRB1, ADRB2*) showed any relationship with MSIMI in whites or African-Americans.

Our study has several strengths. To the best of our knowledge, this is the first study to comprehensively assess genetic variants related to MSIMI. Our study population was clinically well characterized. Myocardial ischemia was assessed using a state-of-the-art method for ischemia assessment and scans were read by experienced readers according to established methods. Also, genetic variants (i.e., SNPs) were measured and imputed with good genome-wide coverage across ethnicities.

However, some limitations should also be noted. Although larger than any previous studies on genetic correlates of MSIMI, our study was under-powered to assess genome-wide significant associations, especially among race-specific sub-groups. Due to sample-size limitations, we chose the candidate gene approach as the main analysis approach, but a major limitation this method over the GWAS analysis is that the choice of candidate genes is limited by current knowledge, and hence we might miss genetic determinants of MSIMI in novel genes and inter-genic regions, which could be discovered through GWAS analysis. However, we examined

many candidate genes involved in plausible biological systems, to make sure we covered the majority of genes which may have a role in the pathogenesis of MSIMI.

In conclusion, we found that among stable CAD subjects, FGF5 gene, which codes for the fibroblast growth factor protein, is associated with MSIMI in both whites and African-Americans. This gene is found to be associated with systolic & diastolic blood pressure, and further delineates the pathophysiological importance of hemodynamic changes with mental stress for the occurrence of MSIMI. Our novel analysis also uncovered RET signaling pathway as a suspected pathophysiological mechanism for MSIMI, and this pathways importance for survival of neuronal cells points towards neuro-hormonal origin of MSIMI.[51] Our findings need to be confirmed in a larger sample-size of subjects, and the clinical importance of these finding needs further exploration.

Chapter 5, Table 1: Descriptive Characteristics of the Study Population According to Race and Study Type

Variables	Whites (N= 496)	African-Americans (N= 276)	MIPS Study (N= 549)	MIMS Study (N= 223)	Total Population (N= 772)
Demographic Factors					
Age, Mean (SD)[1,2]	63 (10)	55 (10)	64 (9)	50 (7)	60 (10)
Women, N (%)[1,2]	119 (24%)	134 (49%)	133 (24%)	120 (54%)	253 (33%)
African-American, N (%)[2]	-	-	138 (25%)	138 (62%)	276 (36%)
Below poverty line, N (%)[1,2,3]	50 (10%)	89 (35%)	79 (14%)	60 (30%)	139 (19%)
Lifestyle Factors and Medical History					
Current smokers, N (%)[1,2]	54 (11%)	55 (20%)	63 (11%)	46 (22%)	109 (14%)
Hypertension, N (%)[1,2]	350 (70%)	243 (88%)	408 (74%)	185 (83%)	593 (77%)
Dyslipidemia, N (%)	408 (82%)	225 (82%)	454 (83%)	179 (80%)	633 (82%)
Diabetes, N (%)[1]	140 (28%)	109 (39%)	179 (32%)	70 (31%)	249 (32%)
BMI, Mean (SD)[1,2]	29 (5)	32 (7)	29 (5)	32 (8)	30 (6)
Previous MI, N (%)[1,2]	214 (43%)	184 (67%)	175 (32%)	223 (100%)	398 (52%)
History of heart failure, N (%)[1,2]	50 (10%)	48 (17%)	82 (15%)	16 (7%)	98 (13%)
Previous revascularization, N (%)	384 (77%)	197 (71%)	406 (74%)	175 (78%)	581 (75%)

CAD ≥ 70% stenosis, N (%)[4]	369 (84%)	196 (81%)	388 (82%)	177 (85%)	565 (83%)

SD: Standard Deviation; MI: Myocardial Infarction; BMI: Body Mass Index; CAD: Coronary Artery Disease

[1] P value < 0.05 for Whites vs. AA comparison

[2] P value < 0.05 for MIPS vs. MIMS Study comparison

[3] 28 observations missing

[4] CAD severity based on coronary angiography results prior to revascularization procedures (if any); 90 observations missing

Chapter 5, Table 2: Top 10 Associations of the Genome-Wide Association Analyses for Mental-Stress Induced Myocardial Ischemia in the Entire Study Cohort

SNP	Chr	Position (GRCh37/hg19)	Allele	MAF	Closest Gene	Functional Consequence	Effect Size (Meta-Analysis)	P value* (Meta-Analysis)	Whites P Value	AA P value
rs17196120	11	97109243	T/C	0.077	LOC105369449	Intron variant	-0.90	9.90E-07	8.35E-05	0.00213
rs8092282	18	76167327	A/G	0.071	-	Intergenic variant	-1.14	1.04E-06	0.000622	0.00049
rs59897174	11	97109738	C/G	0.077	LOC105369449	Intron variant	0.89	1.17E-06	9.63E-05	0.0022
rs1038475735	3	71009992	A/AT	0.165	FOXP1	Intron variant	0.89	1.43E-06	0.0003719	0.00095
rs28498422	18	76155008	A/T	0.075	-	Intergenic variant	-1.08	2.21E-06	5.18E-04	0.00129
rs2284301	11	64501991	T/C	0.132	RASGRP2	Intron variant	-0.70	2.47E-06	6.51E-05	0.0116
rs592521	11	64518525	A/G	0.117	PYGM	Intron variant	0.75	2.78E-06	7.91E-05	0.0114
rs71526466	11	64518530	A/ATG	0.117	PYGM	Intron variant	0.75	2.78E-06	7.92E-05	0.0114
rs592532	11	64518517	T/C	0.117	PYGM	Intron variant	0.75	2.79E-06	7.92E-05	0.0115
rs506354	11	64518504	T/C	0.117	PYGM	Intron variant	0.75	2.82E-06	7.92E-05	0.0114

SNP: Single Nucleotide Polymorphism; Chr: Chromosome, GRCh37/hg19: Genome Reference Consortium Human Build 37; MAF: Minor Allele Frequency

* All analyses were adjusted for age, sex and indicators of population stratification

Chapter 5, Table 3: Association between Mental-Stress Induced Myocardial Ischemia and the Pre-Defined Candidate Genes, for the Entire Study Cohort (Candidate Genes with Association P-values less than 0.05)

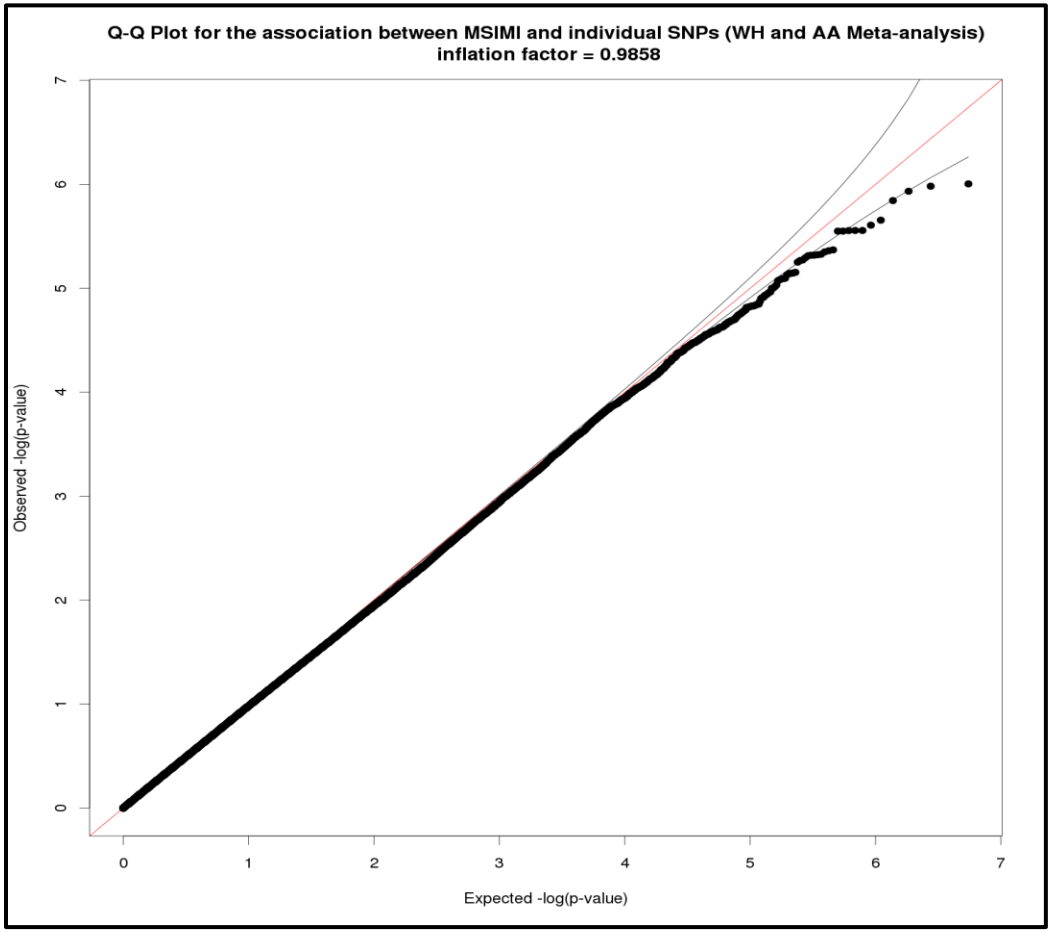
GENE	CHR	BP Position (GRCh37/hg 19)	Z-Statistics (Meta-Analysis)	P value (Meta-Analysis)	Whites P Value*	AA P value*	Gene Product	Gene Function
FGF5	4	81177753 - 81267834	4.069	4.71E-05	0.00049	0.032	Fibroblast growth factor family of proteins	Regulation of cell proliferation and cell differentiation
HGFAC	4	3433614 - 3461211	2.531	0.011	0.135	0.026	Peptidase S1 protein family	Activates hepatocyte growth factor (HGF)
RBPMS2	15	65022091 - 65077786	2.511	0.012	0.075	0.070	RNA recognition motif (RRM)-containing protein family	Development and dedifferentiation of digestive smooth muscle cells
UMPS	3	124439213 - 124474040	2.472	0.013	0.078	0.077	Uridine 5'-monophosphate synthase	Formation of uridine monophosphate (UMP), an energy-carrying molecule in many important biosynthetic pathways
ABCG5	2	44029611 - 44076004	2.464	0.013	0.055	0.121	ATP-binding cassette (ABC) transporters	Selective transport of dietary plant sterols & cholesterol in digestive system and for excretion
RAB11FIP4	17	29708642 - 29875236	2.404	0.016	0.044	0.185	RAB11 Family Interacting Protein 4	Regulator of endocytic traffic by participating in membrane delivery (cell endocytosis)
PRDM8	4	81095033 - 81135483	2.274	0.022	0.033	0.338	Histone methyltransferases family of proteins	Involved in the control of steroidogenesis

FNDC3B	3	(171747418 - 172129455)	2.19	0.028	0.030	0.445	Fibronectin Type III Domain Containing 3B	Positive regulator of adipogenesis
COMT	22	(19919130 - 19967498)	2.136	0.032	0.003	0.697	Catechol-O-methyltransferase	Inactivation of catecholamine neurotransmitters, including the neurotransmitters dopamine, epinephrine, and norepinephrine, and catechol hormones
PCIF1	20	(44553267 - 44586662)	2.117	0.034	0.236	0.052	PDX1 C-Terminal Inhibiting Factor 1	Transcription elongation or in coupling transcription to pre-mRNA processing
KDELR2	7	(6475584 - 6533873)	2.109	0.034	0.103	0.179	KDEL Endoplasmic Reticulum Protein Retention Receptor 2	Retention of luminal endoplasmic reticulum proteins by transport to the cell Golgi apparatus and subsequent modification
TFPI	2	(188318957 - 188440487)	1.994	0.046	0.392	0.029	Member of serine protease inhibitor	Regulates the tissue factor (TF)-dependent pathway of blood coagulation
OAZ2	15	(64969772 - 65005480)	1.982	0.047	0.123	0.211	Ornithine decarboxylase antizyme family	Inhibits ornithine decarboxylase (ODC), the key enzyme in polyamine biosynthesis in the cell

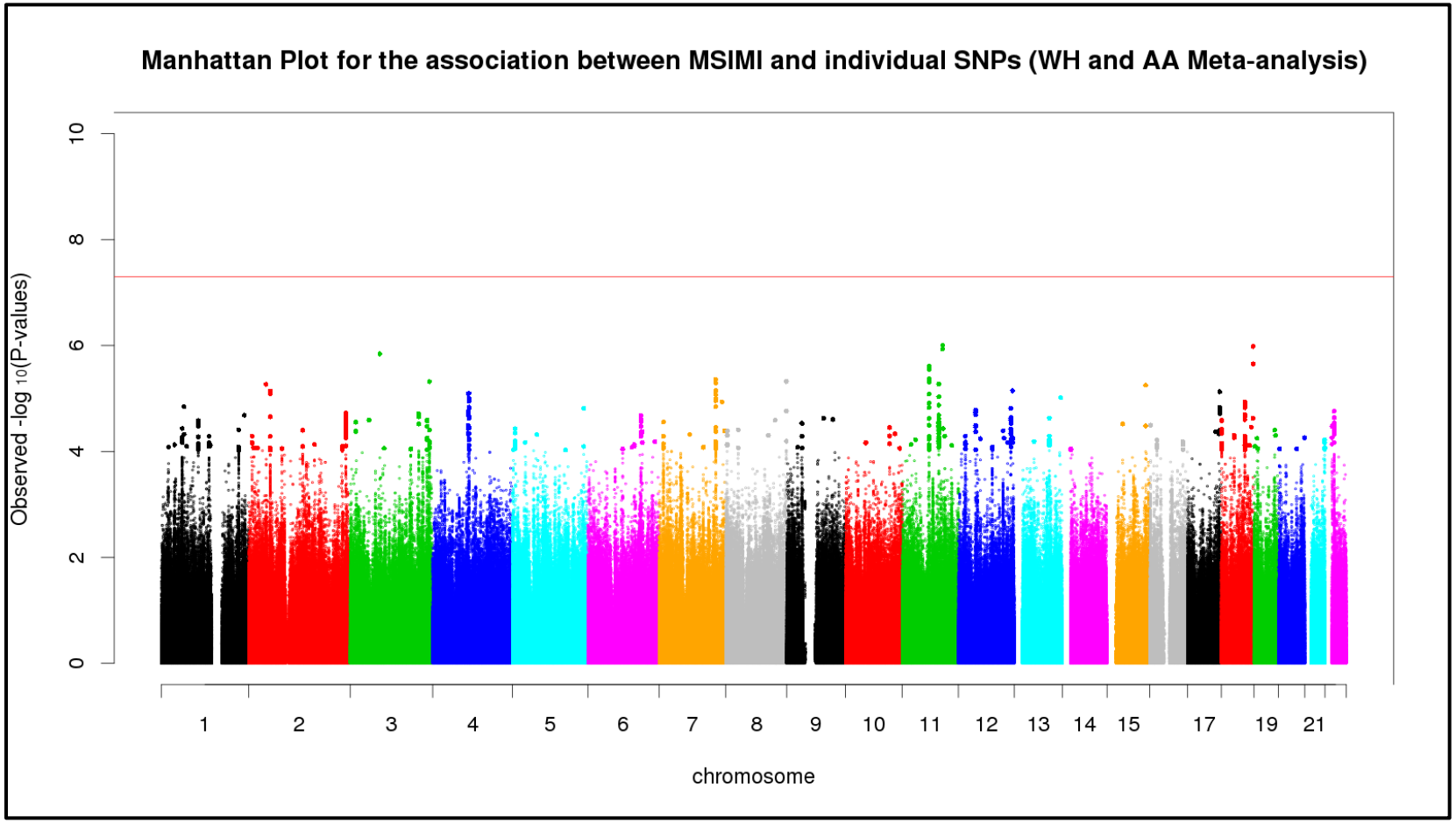
Chr: Chromosome, GRCh37/hg19: Genome Reference Consortium Human Build 37

* Individual SNP-level analysis was adjusted for age, sex and indicators of population stratification; Results of non-synonymous SNPs were combined together to derive gene-level associations using MAGMA for each race-specific cohort

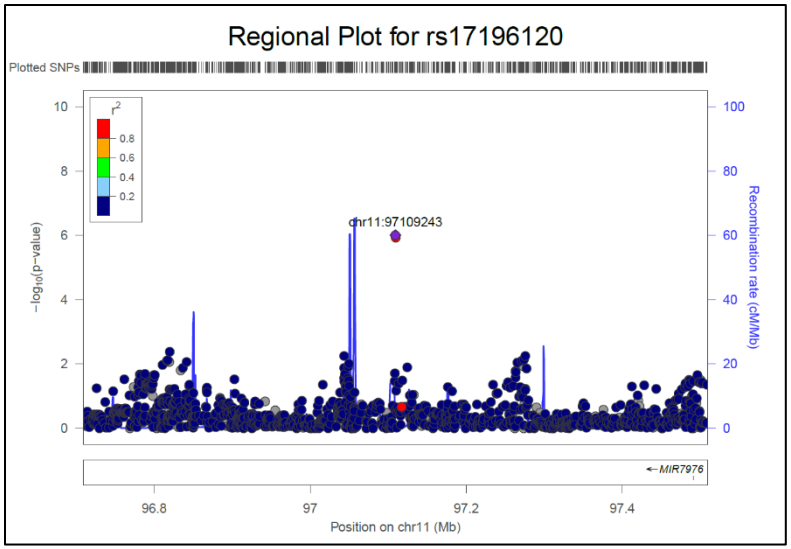
Chapter 5, Figure 1: Quantile-Quantile (QQ) Plot for the Genome-Wide Association Analyses for Mental-Stress Induced Myocardial Ischemia in the Entire Study Cohort, with Inflation Factor (Individual SNP tests observed P-values on Y-axis were plotted against expected P-value distribution for the 5.5 million SNP-tests on the X-axis; data-points around the line at 45 degrees represents lack of undue artificial inflation in the observed P-values)

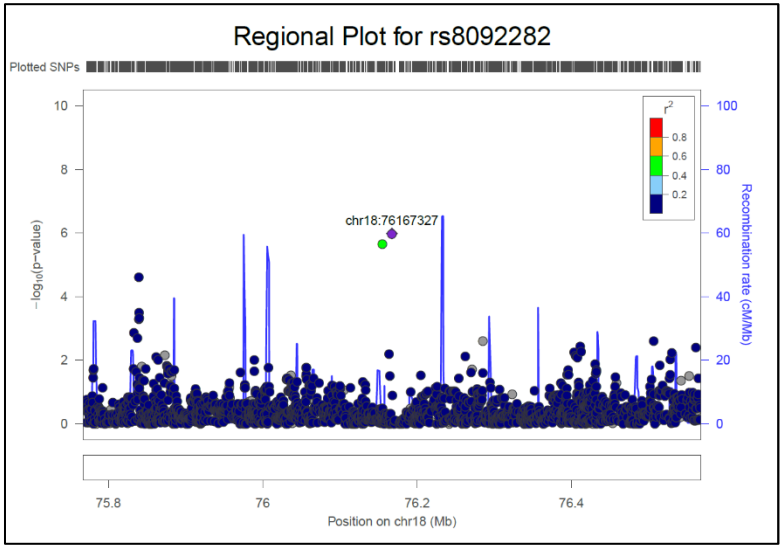


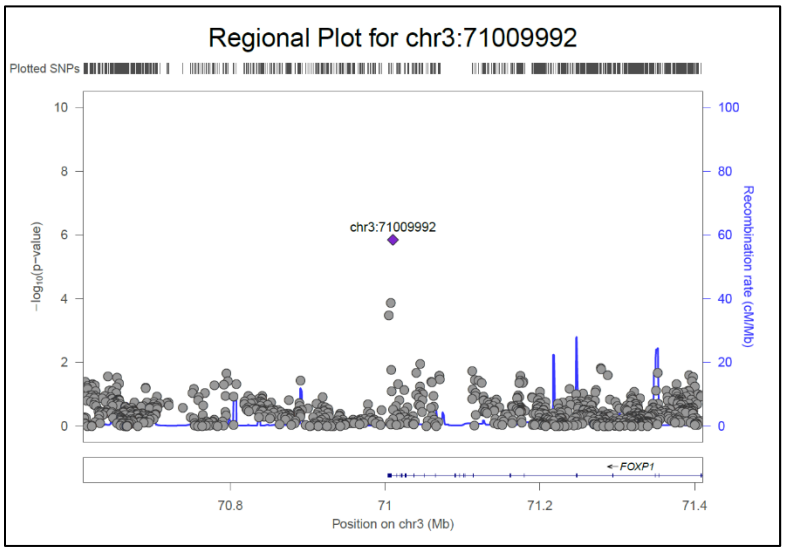
Chapter 5, Figure 2: Manhattan Plot for the Genome-Wide Association Analyses for Mental-Stress Induced Myocardial Ischemia in the Entire Study Cohort (P-values for each SNP are plotted according to their chromosome location on Y-axis, with X-axis indicating the magnitude of association as $-\log_{10}$ of P-values (i.e. 2 = 0.01, 3 = 0.001, 8 = $1*10^{-8}$); the red-line indicated the significant threshold of $5*10^{-8}$)

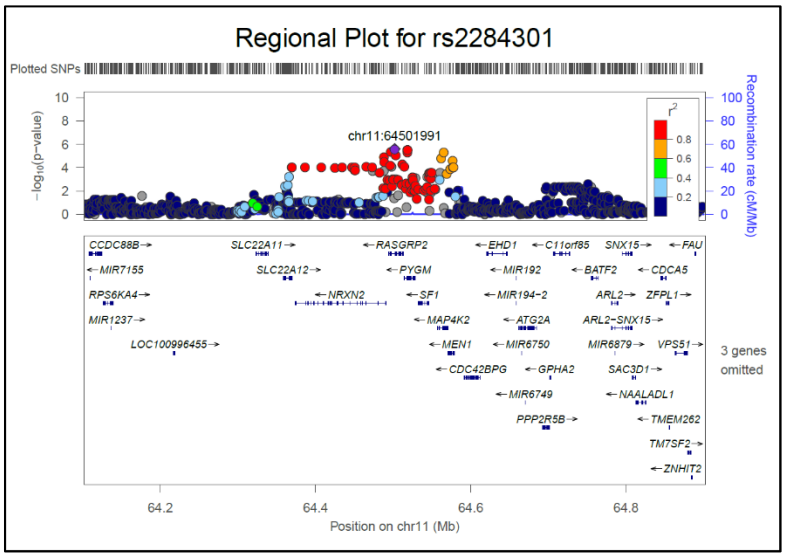


Chapter 5, Figure 3: Regional Plots for the Top 10 Significant SNPs in the Genome-Wide Association Analyses for Mental-Stress Induced Myocardial Ischemia, in the Entire Study Cohort

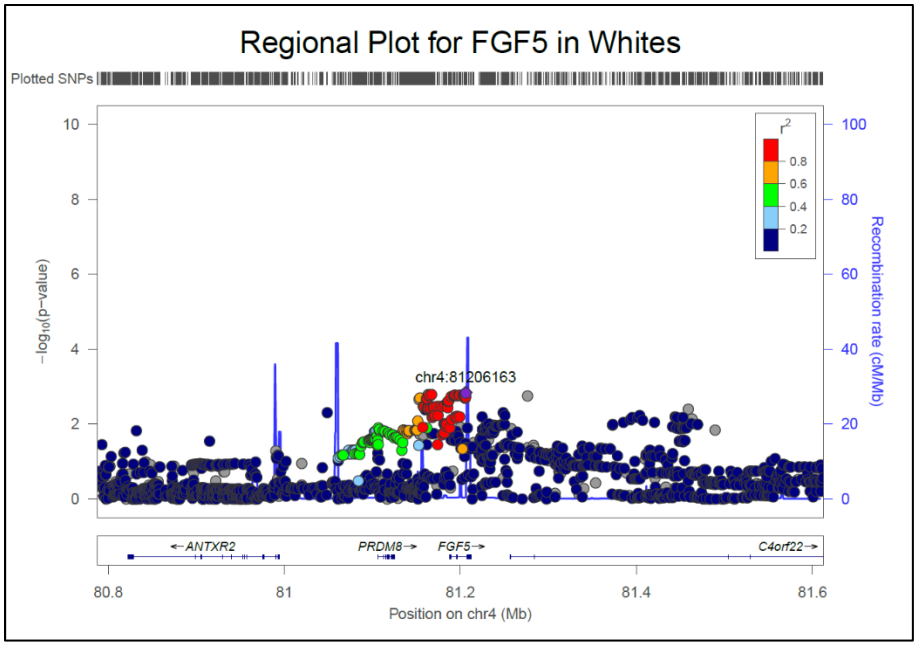


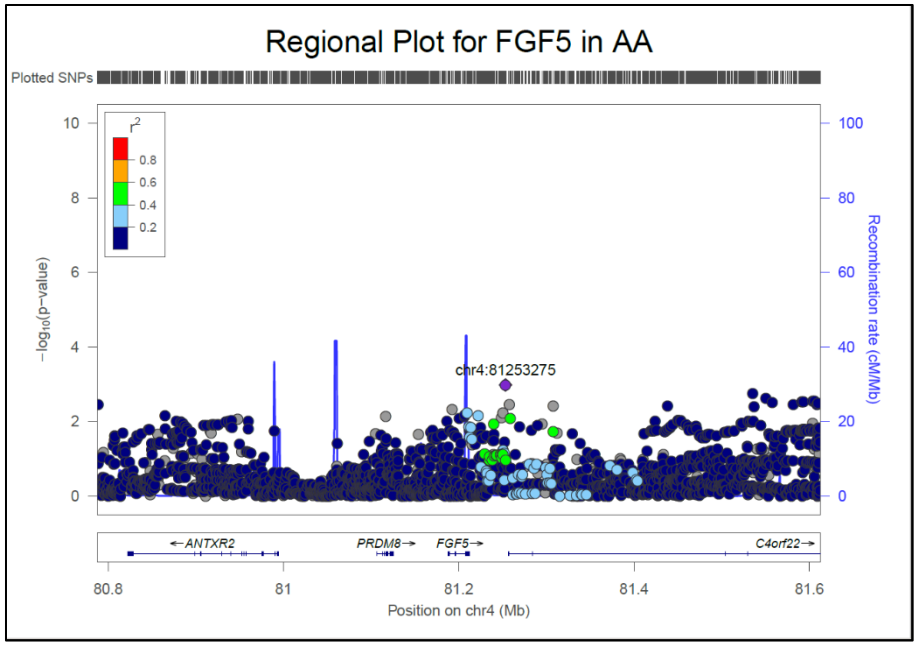






Chapter 5, Figure 4: Regional Plots for FGF5 (Fibroblast Growth Factor 5) Gene, Presented Separately in Whites and African-Americans





Chapter 5, Supplementary Table 1: Study Design Similarities and Differences between MIPS and MIMS Study

Characteristics	**MIPS Study**	**MIMS Study**
Sample size	636	314
Age range	30 to 80 years	18 to 60 years
Sex ratio (M/F)	70:30	50:50
Inclusion criteria	Significant history of CAD during their lifetime: 6) Angiographically proven disease including at least 1 major vessel with evidence of disease 7) Prior myocardial infarction (>3 months) 8) Abnormal coronary intravascular ultrasound for at least 1 vessel 9) Previous bypass surgery or post percutaneous intervention (> 1 year) 10) Positive nuclear scan or exercise stress test)	Documented history of myocardial infarction within the previous 8 months
Common exclusion criteria	6) History of unstable angina or acute MI within the past week 7) Severe comorbid medical or psychiatric disorder that could interfere with study results, such as cancer, renal failure, current alcohol or substance abuse or schizophrenia 8) Uncontrolled hypertension and/or deemed to be unfit to withhold anti-ischemic medications by study cardiologist 9) Weight over 400 lbs 10) Pregnancy or breastfeeding	
Exclusion criteria differences	Individuals with inflammatory diseases (like rheumatoid arthritis, lupus), on dialysis, or having any organ transplant excluded	No such exclusion criteria

Chapter 5, Supplementary Table 2: Candidate Gene Information

Aim 3
Supplementary table

Chapter 5, Supplementary Table 3: Descriptive Characteristics of the Study Population According to Presence or Absence of Data in Final Sample

Variables	MIPS Study			MIMS Study		
	Analytical Sample (N = 549)	**Missing Information (N = 87)**	**Total Population (N = 636)**	**Analytical Sample (N = 223)**	**Missing Information (N = 91)**	**Total Population (N = 314)**
Demographic Factors						
Age, Mean (SD)	64 (9)	63 (8)	64 (9)	50 (7)	52 (7)	51 (7)
Women, N (%)[1,2]	133 (24%)	36 (41%)	169 (27%)	120 (54%)	35 (38%)	155 (49%)
African-American, N (%)	148 (27%)	32 (37%)	180 (28%)	140 (63%)	66 (73%)	206 (66%)
Below poverty line, N (%)[3]	79 (14%)	18 (21%)	97 (15%)	60 (30%)	31 (38%)	91 (33%)
Lifestyle Factors and Medical History						
Current smokers, N (%)	63 (12%)	13 (15%)	76 (12%)	46 (22%)	25 (29%)	71 (24%)
Hypertension, N (%)	408 (74%)	71 (82%)	479 (75%)	185 (83%)	69 (76%)	254 (81%)
Dyslipidemia, N (%)	454 (83%)	73 (84%)	527 (83%)	179 (80%)	72 (79%)	251 (80%)
Diabetes, N (%)	179 (33%)	29 (33%)	208 (33%)	70 (31%)	29 (32%)	99 (32%)
BMI, Mean (SD)	30 (5)	29 (6)	30 (5)	32 (8)	31 (8)	31 (8)
Previous MI, N (%)	175 (32%)	30 (34%)	205 (32%)	223 (100%)	91 (100%)	314 (100%)

History of heart failure, N (%)[2]	82 (15%)	10 (11%)	92 (14%)	16 (7%)	15 (16%)	31 (10%)
Previous revascularization, N (%)	406 (74%)	75 (86%)	481 (76%)	175 (78%)	76 (84%)	251 (80%)
CAD ≥ 70% stenosis, N (%)[4]	388 (82%)	65 (91%)	453 (83%)	177 (85%)	70 (84%)	247 (85%)

SD: Standard Deviation; MI: Myocardial Infarction; BMI: Body Mass Index; CAD: Coronary Artery Disease

[1] P value < 0.05 for analytical sample vs. missing information comparison in MIPS study

[2] P value < 0.05 for analytical sample vs. missing information comparison in MIMS study

[3] 38 total observations missing

[4] CAD severity based on coronary angiography results prior to revascularization procedures (if any); 114 total observations missing

Chapter 5, Supplementary Table 4: Top 5 Associations of the Genome-Wide Association Analyses for Mental-Stress Induced Myocardial Ischemia in the Whites and African-Americans Separately

SNP	Chr	Position (GRCh37/ hg19)	Allele	MAF	Closest Gene	Functional Consequence	Effect Size (Meta-Analysis)	P value (Meta-Analysis)	AF (Opposite Race)	Effect Size (Opposite Race)	P value (Opposite Race)
Top 5 Associations in Whites											
rs73145513	20	62454075	T/C	0.05	ZBTB46	Intron variant	-1.66	1.16E-07	-	-	-
rs710551	3	189698744	G/A	0.19	P3H2	Intron variant	-0.75	2.89E-07	0.27	0.20	0.35
rs710560	3	189703267	C/T	0.2	P3H2	Intron variant	-0.73	3.62E-07	0.40	0.11	0.60
rs837767	3	189697935	G/A	0.19	P3H2	Intron variant	-0.75	3.88E-07	0.38	0.19	0.33
rs710556	3	189701673	T/A	0.19	P3H2	Intron variant	-0.74	4.14E-07	0.25	0.17	0.45
Top 5 Associations in African-Americans											
rs36008702	4	83636618	C/G	0.06	SCD5	Intron variant	2.68	2.02E-09	0.12	0.02	0.90
rs12498940	4	83636723	A/G	0.06	SCD5	Intron variant	-2.68	2.06E-09	0.12	-0.02	0.90
rs4798138	18	3863668	T/C	0.11	DLGAP1	Intron variant	1.64	1.47E-07	0.55	0.03	0.75
rs139397294	11	1531279	C/G	0.22	-	Intergenic variant	-1.26	2.33E-07	0.22	-0.04	0.76
chr16:63687807	16	63687807	T/TTATTA TGTC	0.1	-	Intergenic variant	1.85	4.21E-07	-	-	-

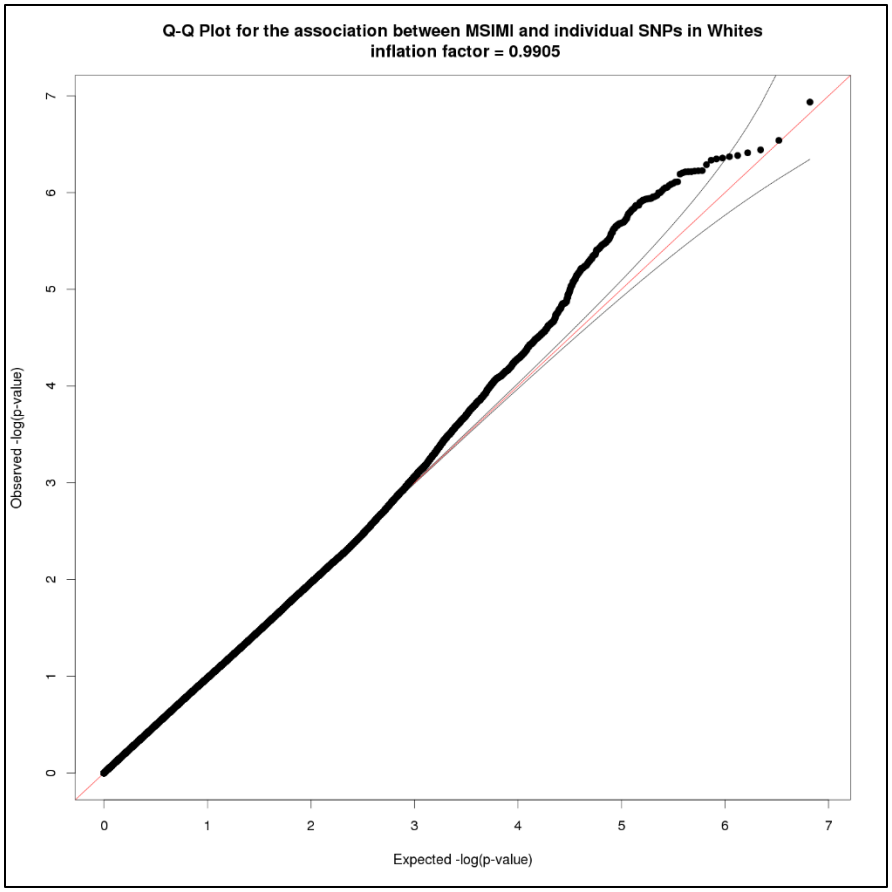
SNP: Single Nucleotide Polymorphism; Chr: Chromosome, GRCh37/hg19: Genome Reference Consortium Human Build 37; MAF: Minor Allele Frequency

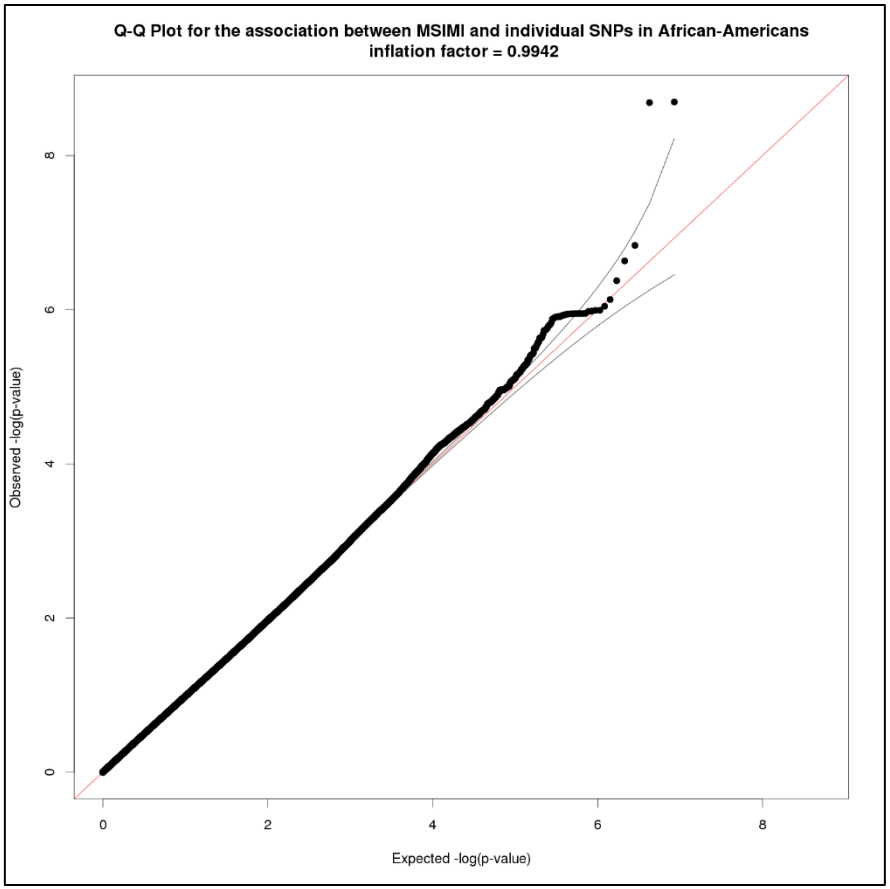
* All analyses were adjusted for age, sex and indicators of population stratification

Chapter 5, Supplementary Table 5: All Candidate Genes Results for the Association with Mental-Stress Induced Myocardial Ischemia in the Entire Study Cohort, and by Race

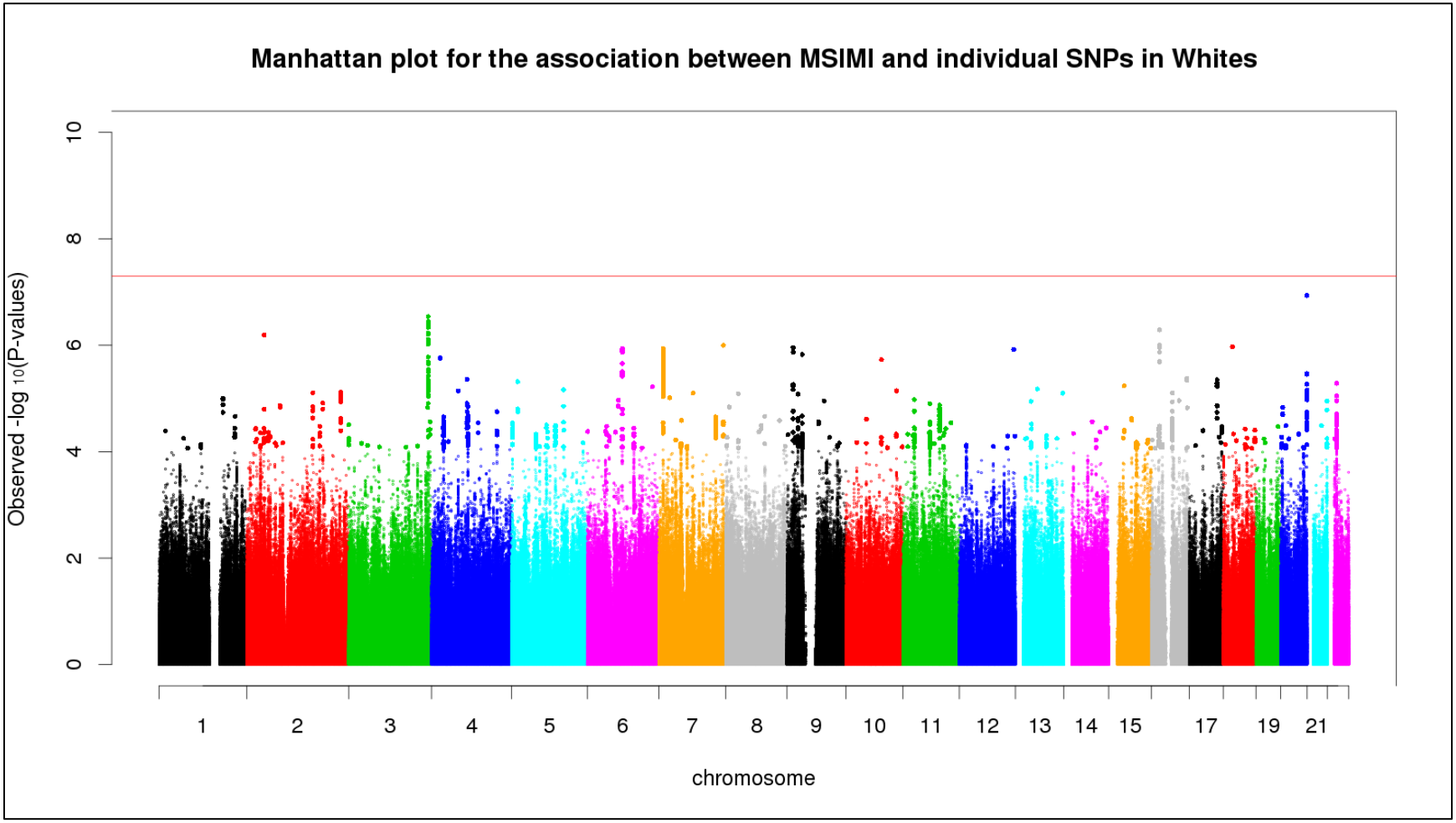
Aim 3
Supplementary table

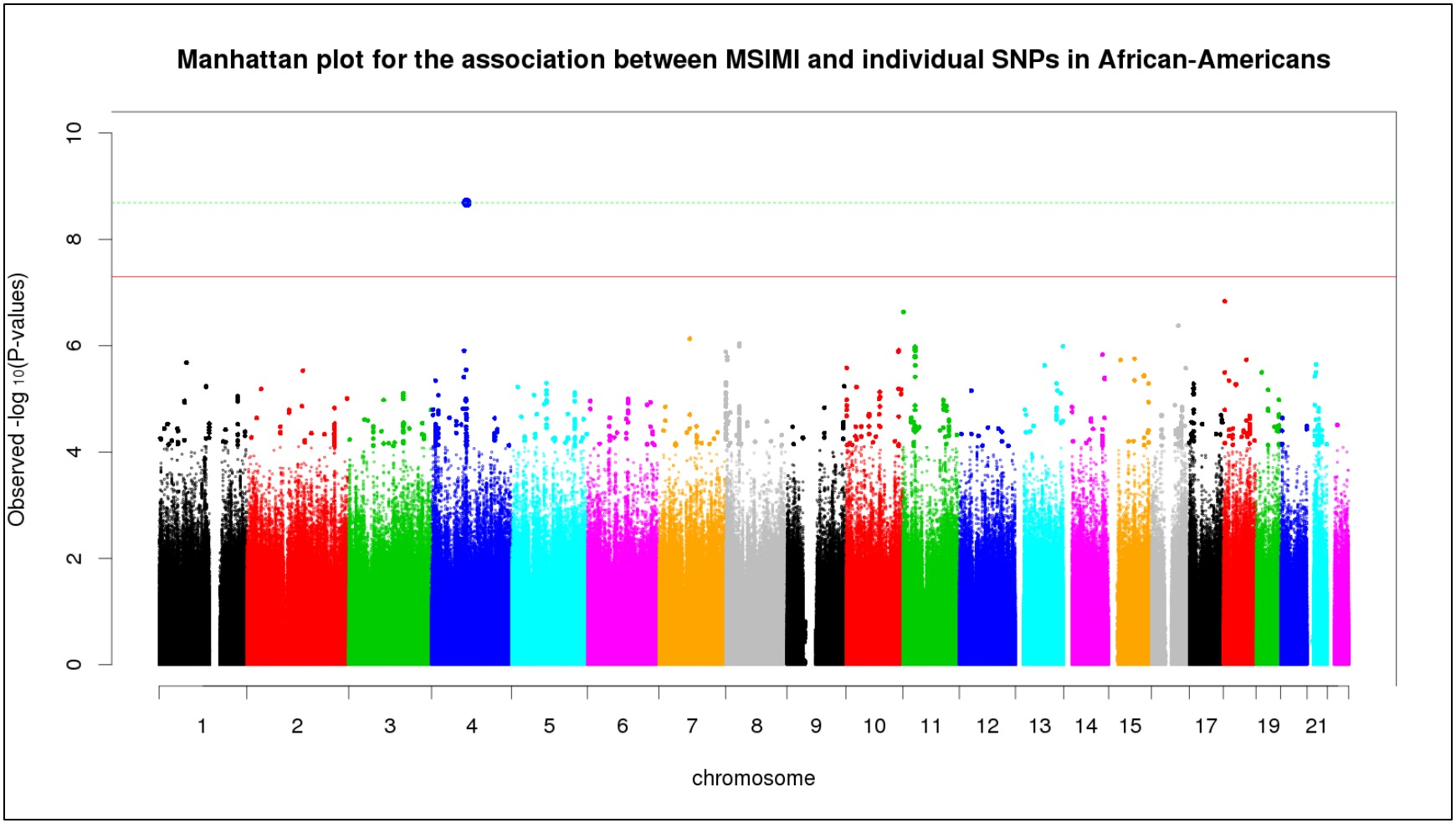
Chapter 5, Supplementary Figure 1: Quantile-Quantile (QQ) Plot for the Genome-Wide Association Analyses for Mental-Stress Induced Myocardial Ischemia in Whites (Left) and African-Americans (Right)





Chapter 5, Supplementary Figure 2: Manhattan Plot for the Genome-Wide Association Analyses for Mental-Stress Induced Myocardial Ischemia in Whites (Upper) and African-Americans (Lower)




Manhattan plot for the association between MSIMI and individual SNPs in African-Americans
Observed -log 10(P-values)
0
2
4
6
8
10
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
17
19
21
chromosome

Chapter 5, Supplementary Analyses Table: Association between CAD Genetic Risk Score and Mental Stress Induced Myocardial Ischemia, and the Interaction Effect of Psychosocial Distress

Exposure	Full Sample: Adjusted[1] β (95% CI)	Low Symptoms: Adjusted[1] β (95% CI)	Mild Symptoms: Adjusted[1] β (95% CI)	Moderate Symptoms: Adjusted[1] β (95% CI)	High Symptoms: Adjusted[1] β (95% CI)	P-value for Interaction
Unweighted Genetic Risk Score	-0.08 (-0.22 to 0.05)	-0.13 (-0.36 to 0.10)	0.07 (-0.29 to 0.44)	-0.13 (-0.40 to 0.14)	-0.21 (-0.62 to 0.19)	0.77
Weighted Genetic Risk Score	-0.13 (-0.27 to 0.01)	-0.14 (-0.37 to 0.09)	0.02 (-0.34 to 0.37)	-0.20 (-0.46 to 0.06)	-0.24 (-0.68 to 0.20)	0.63

[1] β indicated the adjusted change in mental stress summed rest score, with each standard deviation increase in respective genetic risk score; Results adjusted for age, sex, indicators of population stratification, and summed rest score

Chapter 6: Implications

In this chapter, we will first enumerate the key findings of the dissertation, discuss in detail strengths and limitations of this work, and then conclude with future directions.

Key Findings:

In aim 1, we found that among individuals with stable coronary artery disease (CAD), women with higher psychosocial distress, defined as a composite measure of psychosocial symptom scales (depression, PTSD, anxiety, anger, hostility, and perceived-stress) using latent class analysis, showed significantly higher incidence of future cardiovascular events, while there was no such association in men. These findings were independent of traditional CAD risk factors.

In aim 2, we did not find any association between this composite measure of psychosocial symptom scales and mental stress-induced myocardial ischemia (MSIMI) in the overall population. However, similar to the results in aim 1, women with higher psychosocial distress showed significantly higher resting perfusion defects, while there was no such association in men.

In aim 3, a candidate-gene specific analysis showed that the *FGF5* gene, which codes for the fibroblast growth factor protein, is associated with MSIMI. This protein, which is a member of the fibroblast growth factor (FGF) family, has been consistently and significantly associated with systolic and diastolic blood pressure as well as hypertension[162-165] in multiple ethnicities, and is also associated with CAD prevalence.[68,69] In an exploratory GWAS analysis, none of the individual SNPs reached the significance threshold for their association with MSIMI. We did not find any significant interaction between the genetic risk score (based on the genes associated

with CAD) and psychosocial distress indicator, for the association with MSIMI. Also, no effect modification of sex was observed on either the association between genetic risk score and MSIMI or the interaction between psychosocial distress and genetic risk score (i.e. three-way interaction between sex, psychosocial distress and genetic risk score was non-significant).

Strengths & Limitations

This dissertation is bolstered by detailed evaluations of physical, psychosocial and genetic profiles of study participants. To the best of our knowledge, this is the first analysis to investigate the association between a comprehensive measure of psychosocial distress and different cardiovascular outcomes (CVD events in aim 1, MSIMI in aim 2). Furthermore, in addition to psychosocial and physical measures, we comprehensively assessed the genetic variants related to the occurrence of MSIMI, using both hypothesis testing (candidate gene analysis) and hypothesis generating (GWAS) analyses (aim 3). Our study population was well characterized clinically with thorough exposure assessment of psychosocial factors across multiple domains. Genetic variants (i.e., SNPs) were measured using the most updated chips on the market and extensive quality control was performed. Also, cardiovascular events and MSIMI were independently adjudicated by experienced cardiologists using established protocols.

As with any studies, ours are not without limitations. Measurement bias for exposure (observed psychosocial distress) is an important issue for both aims 1 and 2, as all these factors are self-reported. For example, it is possible that the lack of association between distress and cardiovascular outcomes in men is due to measurement bias, as men might under-report depressive symptoms, as compared to women.[148] The main limitation of the latent class analysis is that because the LCA variable is not directly observed, there is a possibility that our psychosocial distress construct was not a proper representation of true underlying distress.

However, we believe that our latent class variable was a valid measure, since there was a clear separation of symptomatology levels across classes. Furthermore, we followed sound statistical methodology, and results for each observed psychosocial phenotype showed similar trends when analyzed separately.

Another potential limitation in our analyses for aims 1 and 2 is that results might have been affected by a collider bias,[149] as we recruited individuals with established CAD. Traditional confounding adjustment may not have been sufficient to correct for this problem. Furthermore, even though this is the largest study of MSIMI using myocardial perfusion imaging, our sample size was inadequate to investigate the association between psychosocial distress and specific CVD outcomes in addition to a composite endpoint. Also, as the study design for aim 2 is cross-sectional, we cannot infer causality based on these data, and it is not possible to ascertain whether some of the risk factors adjusted for in the analysis are mediators or confounders of the association between psychosocial status and myocardial perfusion.

For aim 3 our study was under-powered to assess genome-wide significant associations, especially among race-specific subgroups. Due to this limitation, we chose a candidate gene approach as our main analytical strategy. A major limitation of the candidate gene approach over the GWAS analysis is that the choice of candidate genes is limited by current knowledge, and hence we might have missed genetic determinants of MSIMI involving novel genes and inter-genic regions, which could have been discovered through a GWAS analysis. However, we examined many candidate genes involved in plausible biological systems to make sure we covered the majority of genes which may have a role in the pathogenesis of MSIMI.

Conclusion & Future Directions

In conclusion, we found that, among CAD patients, a higher level of psychosocial distress, measured as a composite measure of variety of symptom scales, is associated with higher cardiovascular events and higher resting perfusion abnormalities in women, but not in men. Although the clinical translation of our findings requires further evaluation, these data suggest the central role of psychosocial stress in defining pathways of CVD risk among women, and the potential importance of incorporating regular assessment of psychosocial measures in cardiovascular practices. Equally important will be to explore treatment modalities for decreasing the effects of psychosocial distress on CVD, including holistic approaches like meditation or relaxation techniques in addition to pharmacological treatment.[150]

From our novel genetic analysis, we uncovered the GDNF (Glial cell line-derived neurotrophic factor)/RET (rearranged during transfection) signaling pathway as a possible pathophysiological mechanism for MSIMI. This pathways is implicated in the survival of neuronal cells, and thus it points to the neuro-hormonal origins of MSIMI.[51] This finding may be of value in future pharmacogenomics efforts involving MSIMI, although it will need to be confirmed in larger studies and its therapeutic implications need further exploration.

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