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ASSOCIATIONS BETWEEN PSYCHOSOCIAL STRESSORS, GENES, AND CARDIOVASCULAR DISEASE IN AT-RISK ADULTS

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ASSOCIATIONS BETWEEN PSYCHOSOCIAL STRESSORS, GENES, AND CARDIOVASCULAR DISEASE IN AT-RISK ADULTS

DISSERTATION

A dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy in the
College of Nursing
at the University of Kentucky

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2020

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ABSTRACT OF DISSERTATION

ASSOCIATIONS BETWEEN PSYCHOSOCIAL STRESSORS, GENES, AND CARDIOVASCULAR DISEASE IN AT-RISK ADULTS

Psychosocial stressors have a significant adverse impact on cardiovascular health. While better medical treatments and increased emphasis on healthy lifestyle have improved cardiovascular health for many in the United States over the past 50 years, there are persistent inequities in cardiovascular disease (CVD) rates, with the highest rates among populations burdened by chronic exposure to psychosocial stressors such as discrimination and anxiety, among others. Genetic factors may interact with these stressors further influencing the rates of CVD in these populations. The purpose of this dissertation is to examine associations among psychosocial stressors and other CVD risk factors, and the influence of genetic variations on these associations in populations burdened with significant cardiovascular health inequities. Specific aims of this dissertation were to: (1) systematically review instruments measuring ethnic discrimination in Hispanic populations; (2) examine the association between psychosocial stressors and other CVD risk factors in adults with a high burden of health disparities who are at-risk for CVD; and (3) explore the moderation effect of genetic variants on the relationships between psychosocial stressors and CVD risk factors.

This dissertation includes three original manuscripts. The first specific aim was addressed by the first paper which is a systematic review of psychometrically tested instruments used to measure ethnic discrimination is Hispanic adults. Six instruments were reviewed. Of these, the “Experiences of Discrimination” instrument, available in both English and Spanish, was a valid and reliable instrument that provided the most concise measure of lifetime experiences of ethnic discrimination in Hispanic adults. Specific aims two and three were addressed by the second and third papers. The second paper was a secondary analysis of data examining the association between anxiety and inflammation, and the moderation of this relationship by single nucleotide polymorphisms (SNPs) associated with systemic inflammation, including rs1205, rs1800797, and rs4129267, located on the *CRP* gene, *IL-6* gene, and *IL-6R* gene, respectively. Findings revealed that only the *IL-6R* SNP, rs4129267, moderated the

association between anxiety and inflammation. In the final paper, results are reported from a study conducted to assess whether experiences of ethnic discrimination predict depressive symptoms in Hispanic adults, and to examine if a SNP on the *COMT* (catechol-o-methyltransferase) gene moderated this relationship. Results revealed that experiences of ethnic discrimination and *COMT* genotype are independently associated with increased depressive symptoms.

The findings from the studies conducted for this dissertation suggest that addressing psychosocial stressors such as experiences of discrimination and anxiety is critical to cardiovascular health promotion. Further, the results provide preliminary evidence of moderating effects of genetic variants on associations between psychosocial stressors and CVD risk. These findings fill an important gap in the body of knowledge related to CVD inequities in populations burdened by psychosocial stressors. Further research is needed to better understand the effects of such stressors as experiences of discrimination on CVD and genetic variants explore the unique experiences of these populations which may affect their CVD risk, as well as exploration into how best integrate genetics into CVD prevention efforts to maximize the impact of interventions.

KEYWORDS: cardiovascular disease, health inequity, stress, discrimination, inflammation, depressive symptoms

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ASSOCIATIONS BETWEEN PSYCHOSOCIAL STRESSORS, GENES, AND CARDIOVASCULAR DISEASE IN AT-RISK ADULTS

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DEDICATION

In loving memory of my grandfather, Donald Kaas. Throughout his life, he was an admirable, selfless, strong-willed, humorous, observant, loyal, and extraordinary person. I miss him dearly. He was there for all of the big moments in my life, and I only wish that he could have been here for this moment, too.

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CHAPTER 1. INTRODUCTION

1.1 Cardiovascular Disease Risk

Cardiovascular disease (CVD) is the leading cause of death globally.[1] In 2016, CVD accounted for over 17.6 million deaths worldwide, and is expected to account for over 23.6 million deaths by the year 2030.[1] In the United States (U.S.), CVD is present in 48% of adults aged 20 years or older, and causes 1 in 3 deaths nationally.[1] For many in the U.S., however, better medical treatments and increased emphasis on healthy lifestyle have significantly improved cardiovascular health over the past 50 years. Notably, though, these improvements do not uniformly impact all, resulting in disparately higher rates of CVD in certain U.S. populations.

Among those most adversely affected by high rates of CVD are rural-dwelling adults.[2,3] Across the nation, rural-dwelling adults experience higher CVD rates than urban-dwelling adults. A recent analysis of 2014 National Vital Statistics System mortality data showed that for adults less than 80 years of age, 42.6% of deaths due to heart disease in rural areas were in excess of what would have been expected compared with only 27.8% in non-rural areas.[4]

Notably, rural Kentucky adults have significantly higher rates of CVD than other U.S. communities, whether rural or urban. A report of 2014 cardiovascular death rates across U.S. counties showed the highest rates of CVD in the rural counties of Eastern Kentucky.[5] This is unsurprising given that among rural Kentucky adults, there are significantly higher rates of tobacco use,[6] physical inactivity,[7] obesity,[8] poor dietary patterns,[7] and hypertension[9] compared with the general population, placing this population at higher risk for CVD. Further adding to the rural Kentuckians' CVD risk is

the rural environment in which this population lives, and the unique stressors that may arise from this environment, such as lack of access to medical care, lack of access to healthy lifestyle resources, and high levels of poverty.[9,10] Downstream effects of these stressors may include increased anxiety levels or symptoms of depression, and in turn, further increased CVD risk.

Similar to rural populations, racial and ethnic minorities in the U.S. carry a heavier burden of CVD morbidity and mortality. For example, African Americans and Native Americans have among the highest rates of CVD risk factors, such as obesity, type 2 diabetes, and hypertension, leading to disproportionate rates of CVD and CVD-related deaths.[11,12] Hispanic adults are also at increased risk for CVD due to the high rates of CVD risk factors also seen in this population. Compared to non-Hispanic white adults in the U.S., Hispanic adults have higher levels of obesity, physical inactivity, hypercholesterolemia, and diabetes.[13] Hispanics also experience psychosocial stressors similar to those experienced by rural Kentucky adults, such as lack of access to medical services and higher levels of poverty, which increases CVD risk.[14] However, Hispanics also experience other psychosocial stressors, such as ethnic discrimination, which are more unique to their population and may also worsen their CVD risk.[15] The high prevalence of traditional behavioral CVD risk factors, coupled with the psychosocial stressors experienced by this population, could further exacerbate the already high CVD risk in Hispanic adults.

1.2 Psychosocial Stressors and Cardiovascular Disease Risk

Preventing CVD remains a major challenge especially in high-risk populations including in rural and Hispanic adults. While multiple factors are known to influence risk

in these populations, psychosocial stressors may be particularly significant contributors, although the stressors experienced in each community may be unique. In this dissertation, associations between the effects of psychosocial stressors unique to each population and CVD risk will be examined.

Ethnic discrimination is a notable psychosocial stressor experienced by U.S. Hispanic communities. This stressor results from negative transactions between ethnic minority individuals or groups and their environment that exceed coping resources or threaten the well-being of the individual or group.[16-18] Ethnic discrimination as defined by Clark and colleagues are the "beliefs, attitudes, institutional arrangements, and acts that denigrate individuals or groups because of phenotypic characteristics or ethnic group affiliation."[19] Considering this, some have hypothesized that stress responses such as hormonal dysregulation,[20] cellular aging,[21] and changes to allostatic load may influence health outcomes in the presence of discrimination-related stress.[16,22] Further, given the pervasiveness of ethnic discrimination across many areas of life, and given that experiences of discrimination accumulate over a person's lifetime, ethnic discrimination is a key contributor to chronic stress. Unlike the brief disruption to allostasis and rapid return to pre-stress equilibrium associated with acute stress, with chronic stress, the continuously activated stress response adversely impacts both physical and mental health.[23-25]

There are many types of racial and ethnic discrimination, ranging from insults and racial slurs to denial of equal treatment and acts of violence against those who are racial and ethnic minorities.[26] In the U.S., nearly 70% of Hispanic adults report personal experiences of ethnic discrimination during their lifetime.[27] These experiences occur in a

variety of situations including when seeking employment, in interactions with authorities, when seeking health care services, and in interpersonal interactions.[27,28] In Hispanic individuals, experienced ethnic discrimination has been consistently associated with negative health outcomes, including negative mental health outcomes.[15] Specifically, experiences of discrimination have been linked to increases in CVD risk factors such as hypertension, systemic inflammation, anxiety, and depression.[29-32]

Anxiety is another example of a stressor associated with increased CVD risk that may be experienced by the two populations of interest in this dissertation. Higher levels of anxiety have been demonstrated to be associated with increased risk for CVD in otherwise healthy individuals.[33] There are several proposed mechanisms through which anxiety is thought to be associated with increased CVD risk. Among the proposed mechanisms is a link between high anxiety and unhealthy lifestyle behaviors that, in turn, increase CVD risk.[34] Others have reported that CVD risk is increased in individuals with high anxiety due to over-activation of both the sympathetic nervous system and the hypothalamic-pituitary-adrenal axis, which can lead to increased plasma catecholamine levels, resulting in endothelial damage, atherosclerosis, and coronary artery disease.[34] A final proposed mechanism suggests that anxiety may increase CVD risk through inflammatory pathways.[35,36]

Similar to anxiety, depression is a mental health condition that is associated with increased CVD risk.[37] Although the etiology is not well understood, it is commonly accepted that depression is influenced by a combination of environmental, physiological, psychosocial, and genetic factors. For example, in addition to psychosocial influences, such as ethnic discrimination, prevailing physiological theories support that depression

may be due to monoamine neurotransmission system dysfunction that leads to decreased levels of monoamine neurotransmitters such as serotonin and dopamine.[38] Serotonin is currently thought to be associated with depressive symptoms through low serotonin function resulting from tryptophan depletion, the precursor amino acid to serotonin.[39] Dopamine, another monoamine neurotransmitter, has been found to have a key role in motivation and salience,[40] and abnormal dopamine levels have been associated with depressive symptoms such as anhedonia, disrupted sleep, and decreased motivation.[41]

1.3 The Influence of Genes on Cardiovascular Disease Risk Factors

Traditionally, CVD prevention efforts have focused primarily on behavioral, environmental, and psychosocial factors which place individuals at increased risk for CVD. However, more recently, there has been increasing evidence genetic influences associated with CVD risk, and research has been focused on identifying genetic variants that are associated with the development of CVD.[42] Genetic variants, including single nucleotide polymorphisms (SNPs), have been linked to increased CVD risk through the association with CVD risk factors, such as hypertension,[42] increased levels of inflammatory biomarkers,[43,44] and increased depressive symptoms.[45]

Depression is a known risk factor for CVD.[37,46,47] There is also growing evidence that there is a genetic component to depression. For example, in a twin study examining both monozygotic and dizygotic twins, results indicate that the heritability of depression may be as high as 38%.[48] Results from family studies suggest there is up to a threefold increase in lifetime risk of depression among first-degree relatives of persons with depression.[49] Considering that abnormal levels of monoamine neurotransmitters, such as serotonin and dopamine, are thought to be associated with the development of depression,

genes within the dopaminergic neurotransmission system, such as the catechol-o-methyltransferase (COMT) gene, have frequently been targeted in investigations of the genetic predisposition for depressive symptoms.

Similarly, increased levels of systemic inflammation have been associated with increased development of CVD.[50,51] Although behavioral factors,[52] such as physical activity and diet quality, and psychosocial stressors, such as anxiety,[36] are associated with levels of inflammation, inflammation is also known to have a strong genetic component.[43] Several common SNPs, located within or near inflammatory response genes, including several interleukin variants, have been shown to be associated with increased levels of circulating inflammatory biomarker proteins, particularly when a SNP resides within a key regulatory region of the gene.[44,53]

1.4 Interactions between Genes, Stress, and Cardiovascular Disease Risk

Although considering genetic variants associated with CVD risk factors may help further our understanding of CVD risk, genetic variants are not solely responsible for the development of CVD. Rather, genetic variants should be considered within the context of other known CVD risk factors. An example of this is examining interactions among CVD risk factors and genetic variants, as interactions among CVD risk factors have been recognized as an important part of CVD etiology.[54] In populations, such as Hispanics and rural Kentucky adults, with known cardiovascular health inequities, genes associated with CVD risk factors may further exacerbate existing inequities.

The identification of interactions among genetic variants and CVD risk factors, such as psychosocial stressors, should be of importance in the populations with disparate rates

of CVD. Better understanding and clarification of these relationships and interactions among CVD risk factors and genes would add to the knowledge of the etiology of CVD and provide evidence to support identification and implementation of early prevention strategies for those most vulnerable to the development of CVD.

1.5 Purpose of Dissertation

The purpose of this dissertation is to examine associations between psychosocial stressors and CVD risk factors, and the influence of genes on these associations in populations burdened with significant CVD health disparities. The specific aims of this dissertation were to: (1) systematically review instruments measuring ethnic discrimination in Hispanic populations, (2) examine the association between psychosocial stressors and CVD risk factors, and (3) explore the moderation effect of genetic variants on the relationships between psychosocial stressors and CVD risk factors.

1.6 Summary of Subsequent Chapters

Chapter 2 presents a systematic review of instruments measuring ethnic discrimination in Hispanic adults. An electronic search of PubMed, PsychInfo, and Sociological Abstracts was conducted. Keywords were “discrimination”, “Hispanic”, “Latino”, “measurement”, “racial discrimination”, and “ethnic discrimination.” After applying inclusion and exclusion criteria, 16 articles were included in this review, from which six measures of ethnic discrimination were identified. Instruments varied in number of items, constructs measured, and timing of discrimination experiences. For example, in contrast to the 9-item “Experiences of Discrimination” (EOD) scale that measures in how many situations and how often discrimination is experienced, the 70-

item “Perceived Ethnic Discrimination Questionnaire” includes 5 subscales and assesses lifetime and past-week discrimination experiences. The reliability and validity of all instruments was assessed. The conclusions drawn from this review guided the selection of the instrument used to measure experiences of ethnic discrimination in the study described in chapter four of this dissertation.

Chapter 3 presents a secondary analysis of data examining the relationship between anxiety and systemic inflammation in individuals at risk for CVD, and whether SNPs associated with inflammation moderate this relationship. A total of 398 adults residing in rural Kentucky participated in this study. Anxiety was measured using the Brief Symptom Inventory. Protein levels for C-reactive protein (CRP) and interleukin-6 (IL-6) were measured in serum, and genomic DNA was assayed for SNPs in the *CRP*, *IL-6*, and *IL6R* genes. Hierarchical multiple linear regressions were performed to examine if anxiety predicted inflammation (as measured by CRP and IL-6), and if SNPs moderated these associations.

Chapter 4 presents findings from a genetics sub-study that was leveraged on the “Corazón de la Familia” study. The sample consisted of 124 community-dwelling Hispanic adults. This study was conducted to investigate the effects of ethnic discrimination-related stress and a SNP on the catechol-o-methyltransferase (COMT) gene on symptoms of depression in Hispanic adults at risk for CVD. Cross-sectional data were analyzed to examine the relationship between lifetime experiences of ethnic discrimination and depressive symptoms; between Val158Met SNP and depressive symptoms; and to explore the moderating effect of Val158Met SNP on the association between discrimination and depressive symptoms in Hispanic adults. Ethnic

discrimination was measured using the Experiences of Discrimination instrument, and the Patient Health Questionnaire-8 (PHQ-8) was used to assess depressive symptoms. Genomic DNA isolated from saliva samples was assayed for a SNP in the *COMT* gene. A hierarchical multiple linear regression was performed to examine if experiences of ethnic discrimination and the Val158Met polymorphism predicted depressive symptoms, as well as to examine if the Val158Met polymorphism moderated the relationship between experiences of discrimination and depressive symptoms.

Chapter 5 is an integrated discussion based on the findings of the three studies presented in this dissertation. This chapter synthesizes how psychosocial stress, and genes impact CVD risk in populations that suffer from significant health inequities, and how these findings address the current gaps in the literature. Lastly, implications for nursing practice and future directions for research in these populations are proposed.

CHAPTER 2. SYSTEMATIC REVIEW OF THE VALIDITY AND RELIABILITY OF INSTRUMENTS MEASURING ETHNIC DISCRIMINATION IN HISPANIC ADULTS

2.1 Introduction

In the United States (U.S.), racial and ethnic discrimination is a prevalent and pervasive problem for those belonging to minority groups. Discrimination, as defined by Clark and colleagues (1999), consists of the "beliefs, attitudes, institutional arrangements, and acts that denigrate individuals or groups because of phenotypic characteristics or ethnic group affiliation."[1] Different types of racial and ethnic discrimination have been described in the literature. Contrada et al. (2001) identified five types of racial and ethnic discrimination, which include verbal rejection (ethnic and racial slurs or insults), disvaluation (negative or low expectations of individuals based on their race or ethnicity), inequality-exclusion (denial of equal treatment), threat-aggression (threats or acts of physical harm), and avoidance (shunning, exclusion).[2] These forms of discrimination adversely affect multiple areas of individuals' lives, including when seeking employment, housing, education, voting, health care, and in dealings with authorities.[3]

Racial and ethnic discrimination are often considered to be a type of stressor, and are commonly examined within the context of stress and coping theories and frameworks.[4-7] As a stressor, discrimination has been theorized to affect an individual's physical, psychological, social, functional, and spiritual well-being.[4,8] Experiences of racial and ethnic discrimination have been associated with many negative physical health outcomes in minority populations, such as hypertension, low birth weight, and systemic inflammation, as well as negative mental health outcomes, including anxiety, early initiation of substance abuse, psychosis, and depression.[9-12]

Hispanics are the largest and fastest growing minority population in the U.S., currently representing over 18% of the nation's population.[13,14] Nearly 70% of U.S. Hispanics report experiencing ethnic discrimination at some point during their lifetime.[9,13] Considering the association between ethnic discrimination and negative health outcomes, this level of experienced ethnic discrimination is alarming, and poses a risk to the health of those in this population, as well as a burden to the U.S. health system.

To date, numerous instruments have been created to measure discrimination in racial and ethnic minorities. The majority of these instruments have been developed to measure the experience of discrimination in African American populations, and include survey specific to the experiences of this population, rather than for other ethnic and racial minority groups.[15] As racial and ethnic minority groups are not all the same, it is important to have instruments that are specific to the population in which they are being used. Additionally, many instruments measure one specific dimension of discrimination, such as workplace discrimination, or measure racial discrimination as one part of a larger scale measuring overarching constructs, such as stress, coping, or acculturation.[16] Discrimination is a pervasive and prevalent problem, and as such, it is vitally important to have valid and reliable instruments to be able to measure the construct of ethnic discrimination in this population. Thus, the purpose of this paper is to provide a systematic review of instruments measuring ethnic discrimination that have been psychometrically tested and used in Hispanic populations.

2.2 Methods

To identify relevant publications for review, a systematic search of the literature was conducted electronically using PubMed, Sociological Abstracts, and PsychInfo

databases. Keywords for the search were "discrimination", "racial discrimination", "ethnic discrimination", "Hispanic", 'Latino", and "measurement.” Additionally, reference lists for all articles selected for full-text review were examined for relevant citations.

Inclusion criteria for studies were: 1) studies that included a measurement of racial or ethnic discrimination; 2) use of the instrument in an adult Latino or Hispanic population; 3) reporting validity and reliability assessments; 4) written and published in English; and 5) published within the last 20 years, between 1997 and 2018. Exclusion criteria were: 1) studies in which discrimination was measured as a component of a larger scale or as a single question or 2) review articles.

2.3 Results

The initial database search yielded 909 articles. Nine records were removed because of record duplication. Titles and abstracts of the 900 articles were evaluated for inclusion, from which 32 articles were selected for full-text review. A total of 868 articles were excluded based on title and abstract review due to failure to meet inclusion criteria. After full-text review, 16 articles were excluded on the basis of including adolescent or child participants, lack of reported validity or reliability statistics for the discrimination instrument used in the study, or assessing experiences of racial discrimination without an instrument (e.g. one survey question created by investigators). The final review included 16 articles (Figure 2.1).

The 16 articles included in this review described 6 measures of racial/ethnic discrimination (Table 2.1). In the majority of the articles (n = 14), authors reported validity and/or reliability statistics of an instrument used to measure discrimination as one

of many aspects of a larger study whereas two articles written for the purposes of psychometrically validating the measurement of discrimination (Table 2.2). In ten of the 16 articles, investigators measured discrimination in Hispanic-only samples, while six articles measured discrimination in multi-ethnic samples which included Hispanic adults.

2.3.1 Detroit Area Study Discrimination Questionnaire

The Detroit Area Study Discrimination Questionnaire (DAS-DQ) was developed for the Detroit Area Study in 1995.[17] The DAS-DQ consists of 15 items and is comprised of 2 scales: The Everyday Discrimination Scale and the Major Experiences of Discrimination. The DAS-DQ is used to measure self-reported discrimination based on three categories: frequency of everyday mistreatment, recent experiences of discrimination, and lifetime history of experienced discrimination.[18] Although the DAS-DQ was originally developed with a sample of mostly African Americans and white participants, the DAS-DQ, and its subscales, have since been used in Hispanic populations.[19-21]

2.3.1.1 The Everyday Discrimination Scale

The Everyday Discrimination Scale is a 9-item scale that measures minor experiences of unfair treatment that are categorized as occurring chronically and routinely.[17] The nine items of this scale address being treated with less courtesy or respect than others, receiving poorer service in stores or restaurants than others, people treating you like you are not as smart as they are, others acting as if they are better than you, people being afraid of you, people thinking you are dishonest, being called names or being insulted, and being threatened or harassed. To each question, respondents answer

the frequency with which they have experienced each situation. Response options include never (1), rarely (2), sometimes (3), and often (4). Scores for the Everyday Discrimination Scale are calculated summing the nine items, with higher scores representing more frequent experiences of everyday discrimination.[17] Of the DAQ-DS scales, the Everyday Discrimination Scale is the most commonly used, particularly in Hispanic populations.[9,19-23] It has also been used to assess construct validity in new discrimination instruments, and has been translated from English to Spanish for this purpose.[24] Internal consistency reliability of the Everyday Discrimination Scale has been well-established. In studies with large Hispanic samples, Cronbach's alpha values ranged from 0.86-0.91.[9,19-22]

Taylor, Kamarck, and Shiffman (2004) were the first to validate the Everyday Discrimination Scale. This was in an African American sample and in the study, convergent validity of this scale was established by examining the relationship between the Everyday Discrimination Scale and Ecological Momentary Assessment.[18] Molina, Alegria, and Mahalingham (2013) later tested the validity of the Everyday Discrimination Scale in a sample of over 2,000 Hispanic adults using Confirmatory Factor Analysis (CFA).[23] Results of the CFA were consistent with factor analyses conducted in African American samples by Taylor and colleagues (2004), and revealed a single-factor solution.[23] The comparative fit index indicated a good fit to the data (.91), and the root mean square error of approximation was .06, which provides evidence for the validity of the Everyday Discrimination Scale in Hispanic samples.[23]

2.3.1.2 Major Experiences of Discrimination

The Major Experiences of Discrimination Scale measures self-reported major experiences of unfair treatment, such as violations of human rights.[18,25] An example item from this instrument is: "At any time in your life, have you been unfairly fired?" Scores are based on the number of items that the respondent has indicated experiencing. A follow up question, at the end of this instrument, asks the respondent to select what they believe to be the main reason for their experience. Response items to this question include race, gender, age, and religion, among other potential sources of discrimination. The Major Experiences of Discrimination originally consisted of 6 items,[17] however, a 9-item [26] version and a 19-item version [27] have since been developed for use in the Midlife in the United States (MIDAS) study, and the YES Health Study. The Major Experiences of Discrimination has been shown to be reliable and valid in a sample of African Americans, but reliability and validity testing has yet to be conducted in a Hispanic population.[18]

2.3.2 Perceived Ethnic Discrimination Questionnaire- Community Version

The Perceived Ethnic Discrimination Questionnaire-Community Version (PEDQ-CV) is a 70-item questionnaire that measures perceived experiences of ethnic discrimination, defined by authors as unfair treatment attributed to one's ethnicity.[15] The PEDQ-CV was created to measure ethnic discrimination in community-based samples of any racial or ethnic background.[15] The PEDQ-CV was developed based on the Perceived Ethnic Discrimination Questionnaire (PEDQ), which measures perceived ethnic discrimination in samples of students.[2,15] The PEDQ-CV was used in two studies in this

review, with samples that include Hispanic adults.[15,28] Validity and reliability testing of the PEDQ-CV was originally conducted with a multi-ethnic sample, of which, 27% were Hispanic.[15] In this study, validity and reliability was examined with each sub-group in the sample, including with Hispanics. The second study in which the PEDQ-CV was used measure discrimination also used a multi-ethnic and multi-racial sample, of which 48% were Hispanic.[28] However, the reliability statistics reported for the PEDQ-CV in this study were for the multi-ethnic sample as a whole, and were not reported for the Hispanic sub-sample.

The PEDQ-CV is comprised of several sub-scales, including: Lifetime Exposure, Discrimination in the Media, Discrimination against Family Members, Discrimination in Different Settings, and Past Week Discrimination. For all scales except the Past Week Discrimination Scale, respondents indicate how often they have had these experiences over the course of their lifetime on a 5-point Likert scale. This Likert scale ranges from 1 ("never happened") to 5 ("happened every day"). The last scale, the Past Week Discrimination Scale, consists of 10 items, which asks about experiences of discrimination in the previous week. Responses to these items are ranked on a 4-point Likert scale ranging from 0 ("never in the past week") to 3 ("3 or more times in the past week.").[15]

Psychometric testing of the PEDQ-CV was conducted with a sample of college students and community-dwelling adults, of whom 27% were Hispanic. Principal component factor analysis was conducted on the 34 items of the Lifetime Discrimination Scale, which resulted in 7 factors with eigenvalues greater than 1. These 7 factors were then rotated using the Varimax method, where 4 final factors were identified. Three of

these factors, Stigmatization/Disvaluation, Threat and Aggression, and Exclusion/Rejection closely aligned with the factors of the original PEDQ instrument. Three or more items items loaded onto each factor and together the three factors accounted for 52% of the variance.[15] The fourth factor was identified as discrimination at work.[15]

To address construct validity of the PEDQ-CV, known-group comparisons were conducted between African Americans and Hispanics. Prior research findings have indicated that public attitudes toward African Americans are more negative than towards Hispanics, and these findings were reflected in the group comparisons between African American and Hispanic participants in this study.[28] Based on PEDQ-CV scores, African Americans reported significantly more experiences of discrimination than Hispanics, providing evidence of construct validity of the PEDQ-CV.[28] Internal consistency for the PEDQ-CV was adequate, as evidenced by Cronbach's alpha values >.75 for all scales and subscales of the PEDQ-CV, and >.93 for Hispanics specifically on the Lifetime Exposure scale of the PEDQ-CV. While the PEDQ-CV has been frequently used by investigators, it has not been commonly used in Hispanic populations. In a study by Beatty Moody and colleagues (2016), the PEDQ-CV was shown to have acceptable reliability, with Cronbach's alpha values of .91 for the Lifetime Exposure scale and >.78 for the subscales, as well as a Cronbach's alpha of .92 for the Past Week scale. However, these values were based on results from a multi-ethnic sample, and not a solely Hispanic sample.[28]

2.3.3 Brief Perceived Ethnic Discrimination Questionnaire- Community Version

A brief version of the PEDQ-CV was created based on the full-length version of the PEDQ-CV.[15] The Brief PEDQ-CV consists of 17 items derived from the original 34 items included in the Lifetime Exposure scale of the PEDQ-CV. This brief version was created to be used when the PEDQ-CV would create too much participant burden or when time constraints necessitate a shorter instrument.[15]

The principal component factor analysis that was conducted during the psychometric testing of the PEDQ-CV guided the creation of the Brief PEDQ-CV. Sixteen of the 17 items included in the Brief PEDQ-CV were chosen to be included by selecting the four items which had the highest factor loadings on each of the four subscales of the full PEDQ-CV instrument (exclusion/rejection, stigmatization/discrimination, discrimination at work/school, threat/aggression).[15]

Reported internal consistency was acceptable for the Brief PEDQ-CV. In a Hispanic sample, the Cronbach's alpha value for the Lifetime Exposure Scale was .88, and Cronbach's alpha values for all subscales were reported to be >.69 in the psychometric testing conducted by Brondolo and colleagues.[15] In 3 other large studies with samples of U.S. Hispanics with various countries of origin, the PEDQ-CV maintained acceptable internal consistency with Cronbach's alpha values >.73 for all scales and subscales in each study.[29-31]

Construct validity of the Brief PEDQ-CV was evaluated using convergent, concurrent, and discriminant validity.[15] Convergent validity was tested with a sample of student participants, while concurrent and discriminant validity were tested with community samples. Convergent validity was examined by correlating Brief PEDQ-CV

scores with other measures of racial discrimination. Brief PEDQ-CV scores were highly correlated with the Latino Perceived Racism Scale ($r = .57$, $p < .001$).[15] Concurrent and discriminant validity were tested by comparing Brief PEDQ-CV scores with Primary Appraisals of Racist Interactions scores. Providing evidence for concurrent validity, Brief PEDQ-CV scores were correlated with appraisals of threat and harm included in the Primary Appraisals of Racist Interactions, while Brief PEDQ-CV scores were not associated with appraisals of challenge or perceptions of benefit, providing support for discriminant validity.[15] In no other studies were validity statistics reported for the PEDQ-CV.

2.3.4 Experiences of Discrimination

The Experiences of Discrimination (EOD) Scale is a 9-item scale that measures self-reported experiences of discrimination in 9 specified situations, such as discrimination experienced in restaurants or when seeking employment.[24,32] The EOD was developed with a multi-ethnic sample, and has since been used primarily with African American and Hispanic samples. [24,33-36] The EOD was developed in English and Spanish and can be administered and scored using 2 different methods. One method is to use the "situation" version of the EOD. For the situation version, individuals report in which of nine situations they have experienced discrimination. To score, the sum is taken of the number of situations in which a participant has reported experiencing discrimination, with higher scores indicating more experiences of discrimination. For the second method, individuals are asked to report how often they have experienced discrimination in each of the nine situations. For this frequency version, values are assigned based on the frequency of occurrences of discrimination the respondent has

reported for each situation. Responses of "never" are given a value of 0, "once" a value of 1, "2 to 3 times" a value of 2.5, and "4 or more times" a value of 5.[24] To score, these values are then summed across the 9 items.

Reliability of the EOD was tested using Cronbach's alpha and test-retest reliability. In the Hispanic subset of study participants from the original article describing this instrument, Cronbach's alpha values for both frequency-scored and situation-scored versions of the EOD were reported to be >.71.[24] These values were similar to the Cronbach's alpha values obtained in a study with a sample of pregnant Latina women.[36] Test-retest reliability for the EOD was examined and determined to be acceptable, with all correlations greater than 0.69 for EOD scores.[24]

Construct validity for the EOD was tested by examining correlations between EOD and the Major Experiences of Discrimination and Everyday Discrimination Scales from the Detroit Area Study- Discrimination Questionnaire.[17,24] Statistically significant correlations were found between these three discrimination scales. Structural Equation Modeling was also used to determine if the discrimination scales were measuring the concept of "self-reported racial discrimination." In the resulting model, controlling for social desirability, the EOD instrument had the highest correlation with the "self-reported racial discrimination" construct.

2.3.5 General Ethnic Discrimination Scale

The General Ethnic Discrimination Scale (GED) is an 18-item instrument that measures perceived ethnic discrimination, and was created to measure perceived ethnic discrimination in any ethnic group.[37] The GED is a modified version of the Schedule of Racist Events, which is an instrument that was developed for use specifically in African

American populations.[38] To create the GED, the stem of the Schedule of Racist Events items were changed from "because you are Black" to "because of your race/ethnic group."[37] The language of the scale was also simplified so that it could be used with respondents who use English as a second language. It has been used in multi-ethnic studies, with participants of varying race and ethnicity.[37,39,40]

Each item of the GED assesses racial/ethnic discrimination in a different area of life, such as at work, when seeking healthcare, and while in public places. Respondents answer each item three times, considering how many times they have had these discrimination experiences in the past year, in their lifetime, and their appraisal of the stressfulness of the experience. These constitute the three subscales of the GED. Scores are calculated by summing the scores of each of the three subscales.

Cronbach's alpha values were used to establish the internal consistency reliability of the GED. For the Hispanic subset of the original study sample, Cronbach's alpha values ranged from 0.93-0.94 for all subscales of the GED.[37] In another study, with over 200 Hispanic college students, Cronbach's alpha for the GED Lifetime Discrimination subscale was 0.91 and 0.92 for the two administrations of the GED.[39]

Confirmatory Factor Analysis was used to examine if the three subscales of the GED measured the construct of perceived ethnic discrimination similarly to the three subscales of the Schedule of Racist Events. This testing was completed in a multi-ethnic sample, and all subscales were significant at $p < .05$ for all ethnic groups. Structural Equation Modeling also provided evidence that the GED models perceived ethnic discrimination in the same manner as the Schedule of Racist Events.[37]

2.4 Discussion

Through this literature review, six instruments that are used to measure ethnic discrimination in Hispanics were identified. There were several commonalities among the instruments. All were multi-item scales that relied on self-report to measure ethnic discrimination. Most scales aimed to measure discrimination across multiple settings (e.g. at work, in dealings with authorities, at school, etc.), and across different timeframes (past week, past year, lifetime).[15,17,24,37] There is evidence to support the internal consistency reliability of each instrument included in this review (all Cronbach's alpha values were > .69) among Hispanic-only or multi-ethnic samples. Additionally, there is evidence to support the validity in Hispanic-only and/or multi-ethnic samples in each of the instruments.

Factor analyses were conducted to assess instrument validity for several of the instruments in this review. Confirmatory factor analysis was conducted with the Everyday Discrimination Scale and the GED; principal factor analysis was conducted for the PEDQ-CV. Factor analyses revealed several factors, including where individuals experience discrimination and frequency of the discriminatory experiences. For the Everyday Discrimination scale, results of both exploratory factor analyses conducted with a large sample of African Americans [18] and confirmatory analyses later conducted with Hispanics [23] revealed a single factor for measurement of discrimination defined as experiences of unfair treatment. This contrasts with 3 factors identified through confirmatory factor analysis for the GED after it was modified for use with Hispanic populations. Two of the 3 factors identified (Recent Discrimination, Lifetime Discrimination) are time-dependent whereas the third factor (stress appraisal) considers an individual's perception of the discriminatory experience.[37] This is in contrast to the

single factor of the Everyday Discrimination scale, the purpose of which is to measure experiences of daily, routine, and minor discrimination. Factor analyses of both the PEDQ-CV and the Brief PEDQ-CV revealed 4 factors, more than any other of the instruments. These factors were found to identify types of discrimination experienced rather than amount of discrimination experienced within specific time periods.[15]

Other validity testing conducted with these instruments included convergent, divergent, concurrent, and discriminatory validity testing. The authors of the PEDQ-CV demonstrated construct validity by using known-group comparisons, where the PEDQ-CV scores among racial/ethnic groups aligned with rates of experienced discrimination reported in the literature.[15] The brief version of the PEDQ-CV had evidence of convergent, concurrent, and discriminant validity. Brief PEDQ-CV scores were highly correlated with other measures of racial discrimination, as well as appraisals of threat and harm.[15] Conversely, Brief PEDQ-CV scores were not strongly correlated with appraisals or perceptions of benefit or appraisals of challenge, which are included in the Appraisals of Racist Interaction scales.[15] Finally, the EOD scale was highly correlated with the Major Experiences of Discrimination Scale and the Everyday Discrimination Scale, providing evidence for concurrent validity of this scale.[24]

Racial/ethnic discrimination is considered to be a type of stress and is examined as part of stress frameworks.[2,15,37] Thus, the instruments used to assess experiences of racial/ethnic discrimination should be modeled similarly to instruments measuring stress, and include measurements of stress appraisal, as is included in the GED.[37] The GED is the only instrument that includes a measurement of stress appraisal in addition to frequency of discriminatory experiences. However, in order to comprehensively measure

stress, frequency of experiences should also be included in the measurement. Each instrument in this review included a measurement of frequency of discriminatory experiences.

These instruments were not initially created for sole use with Hispanic samples, but each instrument in this review has been used with Hispanic samples. Of note, not all instruments in this review have had psychometric testing completed in entirely Hispanic samples, although some studies reported psychometric testing results in multi-ethnic samples, in which Hispanics were included. For all instruments, internal consistency reliability was tested using Cronbach's alpha values. For the instruments that were tested in multi-ethnic samples, Cronbach's alpha values were reported for the Hispanic sub-samples for most instruments. However, not all instruments had reported validity testing results for Hispanic-only samples or Hispanic sub-samples. For the EOD scale and the GED scale, the researchers conducted and reported validity testing results for the full sample despite the fact that testing was conducted in multi-ethnic samples.[24,37] To confirm the validity of these instruments in Hispanic populations, future validity testing needs to be conducted with Hispanic-only samples or, for multi-ethnic samples, with Hispanic-only subsamples.

When using instruments in Hispanic samples, another consideration needs to be the language in which the instrument is written. In the U.S., approximately 62% of adult Hispanics are bilingual, speaking both Spanish and English, with 38% of those primarily using Spanish.[41] Considering this, when conducting research in Hispanic populations, Spanish and English language versions should be available for participants to select from to increase accuracy of the measurements and to ensure participant comprehension. The

EOD scale and the Everyday Discrimination Scale are the only instruments in this review to have a version of the instrument that has been translated and psychometrically tested in the Spanish language. Of note, the psychometric testing for both instruments were conducted on data collected using both the Spanish and English versions thus providing weaker evidence for the validity of the Spanish version than if psychometric testing had been conducted separately on the English language and Spanish language versions.[24]

There was also a large range in the lengths of the instruments in this review. The shortest instrument was the Major Discrimination Scale, which consisted of 6 items. Most other instruments were similar in length, ranging from 9 to 18 items. The longest instrument was the PEDQ-CV, consisting of 70 items. The abbreviated version of this instrument, the Brief PEDQ-CV, comprised of 17 items, allows the use of an instrument similar to the PEDQ-CV without the burden of 70 items full length PEDQ-CV. The brevity of the Everyday Discrimination Scale, the Major Discrimination Scale, and the EOD may make them more appealing instrument choices when considering participant burden and available time.

In summary, reliability and validity has been demonstrated in all instruments. However, availability of Spanish and English language versions of the instrument, length, and the populations in which the instruments have been tested should be considered. The Everyday Discrimination Scale was found to be the most commonly used instrument; however, it only captures everyday discrimination, and not lifetime exposures or stress appraisals. Comparatively, the 9-item EOD scale has been translated and preliminarily validated in Spanish. Further, it has also shown good reliability in Hispanic populations

and shows promising construct validity in a multi-ethnic sample with a large proportion (43%) of Hispanic participants.[24]

2.5 Conclusion

Racial/ethnic discrimination is of critical concern in the U.S. High levels of experienced discrimination have been associated with negative physical and mental health outcomes. To accurately explore the effect of ethnic discrimination on health outcomes, measures of racial and ethnic discrimination must be reliable and valid. Additionally, the instruments must be available in the appropriate language for the population in which it is being used. The EOD, in Spanish and English, shows promising validity and reliability in Hispanic populations. Further research is needed to analyze the validity of this measurement in Hispanic-only populations, as well as the validity of the Spanish version of the EOD. However, the EOD is a succinct instrument and should be considered for use when measuring ethnic discrimination in Hispanic populations.

Table 2.1 Description of 6 Instruments Measuring Ethnic Discrimination in Hispanic Samples				
Instrument (year)	Number of Items	Response Options	Scoring and Range	Sample Item
Everyday Discrimination Scale (1997)	9	Almost every day (5) At least once a week (4) A few times a month (3) A few times a year (2) Less than once a year (1) Never (0)	Scores are calculated by summing items. Scores range from 0 to 45.	You are treated with less courtesy than other people.
Major Discrimination Scale (1997)	6	At least once in my lifetime but not in the past 12 months (2) At least once in the past 12 months (1) Never (0)	Scores are calculated by adding all responses in each category. Past 12-month experiences are summed for "recent exposures." Responses from the past 12 months or lifetime are summed for "lifetime exposures"	Have you ever been unfairly stopped, searched, questioned, physically threatened, or abused by the police?
Perceived Ethnic Discrimination Questionnaire-Community Version (2005)	70	Scale ranging from 1 (never happened) to 5 (happened very often)	Subscale scores are computed as mean of item responses. Total score may be computed as the mean of subscale scores. Scores range from 0 to 5.	Have others threatened to hurt you (ex: said they would hit you)?

Table 2.1 continued				
Brief Perceived Ethnic Discrimination Questionnaire-Community Version (2005)	17	Scale ranging from 1 (never happened) to 5 (happened very often)	Scores are computed as the mean of item responses. Scores range from 0 to 5.	Have others made you feel like an outsider who doesn't fit in because of your dress, speech, or other characteristics related to your ethnicity?
Experiences of Discrimination (2005)	9	For situation version, questions are answered yes (1) or no (0). For frequency version, responses include once (1), two or three times (2), four or more times (3).	Answers are summed across items. For situation version, scores range from 0 to 9. For the frequency version, scores range from 0 to 45.	Have you ever experienced discrimination been prevented from doing something, or been hassled or made to feel inferior in getting credit, bank loans, or a mortgage because of your race ethnicity, or color?
General Ethnic Discrimination Scale (2006)	18	Items are scored on Likert-type scales from 1 (never) to 6 (Almost all the time). Each question is answered three times: past year experiences, lifetime experiences, and stress appraisal	Items are summed within subscales. Recent discrimination scores range from 18-108; Lifetime scores range form 18-108. Appraisal scores range from 17-102.	How often have you been treated unfairly by institutions because of your race/ethnic group?

Table 2.2 Psychometric Properties of 5 Instruments Measuring Ethnic Discrimination in Hispanic Samples				
First Author (year)	Design	Sample	Reliability	Validity
Everyday Discrimination Scale				
Perez (2008)	Cross-sectional	2,554 Hispanic adults in the U.S.	Cronbach's alpha 0.91	Not reported
Perez (2009)	Cross-sectional	3,899 Hispanic adults in the U.S.	Cronbach's alpha 0.87	Not reported
Chou (2012)	Cross-sectional	4,539 Asian, Hispanic, and African American adults in the U.S. 951 Hispanic participants.	Cronbach's alpha 0.91 in Hispanic participants	Not reported
Molina (2013)	Cross-sectional	2,554 Hispanic adults in the U.S.	Not reported	Confirmatory factor analysis supported the original single-factor solution. The single factor solution had a good fit to the data (Comparative Fit Index = .91)
Almeida (2016)	Cross-sectional	719 Hispanic adults in the U.S.	Cronbach's alpha 0.86	Not reported
Cobb (2017)	Cross-sectional	122 undocumented Hispanic adults in the U.S.	Cronbach's alpha 0.93	Not reported

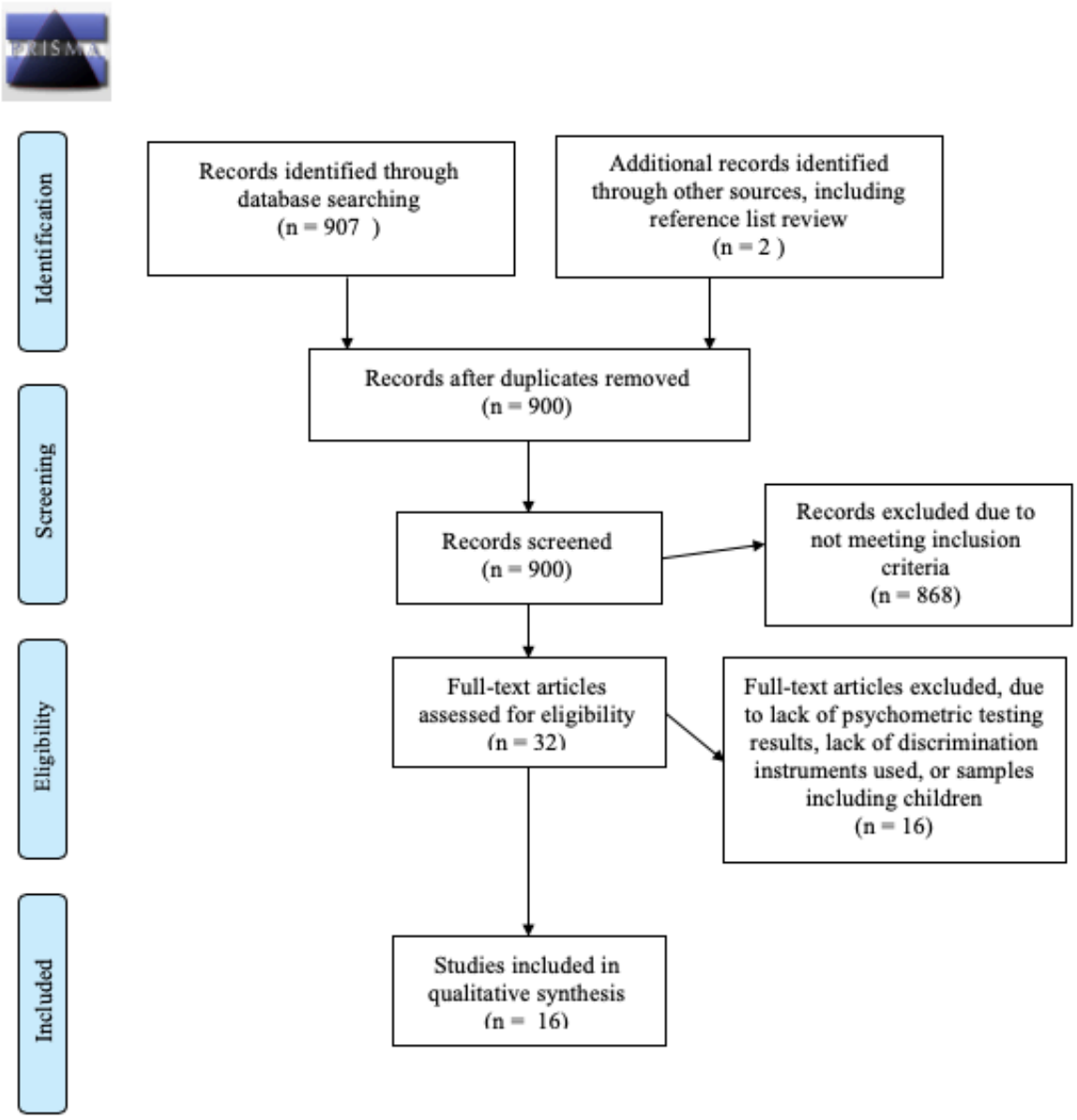
Table 2.2 continued				
Perceived Ethnic Discrimination Questionnaire- Community Version				
Brondolo (2005)	Cross-sectional	301 African American, Hispanic, white, Asian, Native American adults. 27% of the sample identified as Hispanic.	Cronbach's alpha >.75 for all scales and subscales of the PEDQ-CV. Cronbach's alpha >.93 for Hispanics on the Lifetime Exposure scale of the PEDQ-CV.	Principal factor analysis using the Lifetime Discrimination scale. Four final factors aligned with the original PEDQ scale and accounted for 52% of variance. Support for construct validity through known-group comparisons.
Beatty-Moody (2016)	Longitudinal	607 African American and Hispanic adult participants in the U.S. Hispanics made up 48% of the sample (n = 289).	Cronbach's alpha for the Lifetime Discrimination Scale was .91. All Lifetime Discrimination subscales had Cronbach's alpha values >.78. The Past Week scale had a Cronbach's alpha value of 0.92. Values are for the entire sample, not only the Hispanic participants.	Not reported

Table 2.2 continued				
Brief Perceived Ethnic Discrimination Questionnaire- Community Version				
Brondolo (2005)	Cross-sectional	340 college students and community-dwelling adults. 135 participants in the sample were Hispanic.	In the Hispanic sample, the Lifetime Exposure Scale had a Cronbach's alpha value of 0.88. All subscales had Cronbach's alpha values >.69.	Evidence to support convergent, concurrent, and discriminant validity. Brief PEDQ-CV was highly correlated with Latino Perceived Racism Scale and appraisals of threat and harm. The Brief PEDQ-CV was not associated with perceptions of benefit or appraisals of challenge in the Primary Appraisals of Racist Interactions scales.
Arellano-Morales (2015)	Cross-sectional	5,291 adult Hispanics in the U.S.	Cronbach's alpha 0.88 for the entire scale. All subscales had a reported Cronbach's alpha of >.74	Not reported
Ornelas (2016)	Cross-sectional	5,313 adult Hispanics in the U.S.	Cronbach's alpha for all subscales were >.73	Not reported
Corona (2017)	Cross-sectional	198 Hispanic college students in the U.S.	Cronbach's alpha 0.90	Not reported

Table 2.2 continued				
Experiences of Discrimination				
Krieger (2005)	Cross-sectional	The main study consisted of 616 multi-ethnic adult participants, 249 of which identified as Hispanic. In the validation study, the sample consisted of 208 participants; 110 were Hispanic.	Cronbach's alpha values for the frequency and situation versions of the EOD were >.71 for Hispanic participants. Test-retest reliability for the EOD was acceptable, with correlations >0.69 for EOD scores	Construct validity was assessed using correlations between EOD and the Major Experiences of Discrimination and Everyday Discrimination Scales, with significant correlations found between the scales. Structural Equation Modeling provided evidence that the EOD was measuring self-reported racial discrimination well.
Walker (2012)	Prospective observational	515 pregnant Latina women in the U.S.	Reported Cronbach's alpha values between 0.75-0.90	Not reported
Nguyen (2012)	Prospective observational	677 pregnant urban Black and Hispanic women.	Reported Cronbach's alpha value was 0.74.	Not reported.
General Ethnic Discrimination Scale				
Landrine (2006)	Cross-sectional	1569 multi-ethnic adults in the U.S. Hispanics made up 11% (n =174) of this sample.	Cronbach's alpha ranged from 0.93-0.94 for all subscales in the Hispanic segment of this study sample.	Confirmatory factor analysis was used to analyze if GED measured discrimination similarly to the Schedule of Racist Events. All subscales were significant at $p < .05$ for all ethnic groups.

Table 2.2. continued				
Cheng (2015)	Longitudinal	203 Hispanic college students in the U.S.	Cronbach's alpha ranged from 0.91-0.92 for the two administrations of the GED.	Not reported

Figure 2.1 PRISMA 2009 Flow Diagram



CHAPTER 3. INFLAMMATORY GENOTYPE MODERATES THE ASSOCIATION BETWEEN ANXIETY AND SYSTEMIC INFLAMMATION IN ADULTS AT RISK FOR CARDIOVASCULAR DISEASE

3.1 Introduction

Cardiovascular disease (CVD) is the leading cause of death globally.[1] In 2016, CVD accounted for over 17.6 million deaths, and by 2030, is expected to contribute to over 23.6 million deaths annually.[1] Preventing CVD remains a major challenge largely due to the multifactorial nature of CVD risk factors that range from genetic, to psychological and behavioral, to environmental factors, and interactions among these factors. While behavioral and environmental factors have been well studied, less is known about associations among psychological factors and CVD risk. For example, while anxiety is associated with increased risk for CVD,[2] the exact mechanism(s) underlying this relationship remain unclear.

Among the proposed mechanisms is a link between high anxiety and unhealthy lifestyle behaviors that, in turn, increase CVD risk.[3] In contrast, others have reported that CVD risk is increased in individuals with high anxiety due to overactivation of both the sympathetic nervous system and the hypothalamic-pituitary-adrenal axis, which can lead to increased plasma catecholamine release, resulting in endothelial damage, atherosclerosis, and coronary artery disease.[3] Yet another proposed mechanism suggests that anxiety may increase CVD risk through inflammatory pathways, however, evidence supporting this mechanism has been conflicting. While outcomes from some studies have suggested that anxiety is positively associated with levels of systemic inflammatory biomarkers including C-reactive protein (CRP) and interleukin-6 (IL-6),[4-6] results from other studies have not supported these findings.[7,8] Further investigation into associations

between anxiety and inflammation is warranted, as a clearer understanding of systemic effects of anxiety can improve CVD risk reduction strategies.

Inflammation is known to have a strong genetic component.[9] Several common genetic variants, or single nucleotide polymorphisms (SNPs), located within or near inflammatory response genes, have been shown to be associated with increased levels of the circulating inflammatory biomarker proteins, particularly when a SNP resides within a key regulatory region of the gene, such as a promotor region or an untranslated region (UTR) (e.g. the 5'- and 3'-UTRs). Among these are variants of the *CRP* gene (rs1205),[10] *IL-6* gene (rs1800797),[11] and *IL-6R* gene (rs4129267).[12] For example, rs1205 is located within the 3'-UTR region of the *CRP* gene, and inheritance of the minor T allele at this location has been associated with decreased levels of serum CRP protein levels.[13,14] Similarly, rs1800797 is located within the *IL-6* gene promotor at position (-597), and inheritance of the minor A allele has been associated with increased systemic levels of IL-6, and decreased systemic levels of CRP when compared to individuals who inherited the GG genotype.[11,15] Finally, rs4129267 is located in the intron of the *IL-6R* gene, and inheritance of the T allele has been reported to be associated with increased IL-6 levels.[16-18]

While studies have been conducted to better understand how inflammatory genotypes interact with CVD risk factors to influence systemic inflammation levels,[9,19] few studies have been conducted to examine whether inflammatory gene variants moderate associations between anxiety and systemic inflammation. Gaining a better understanding of the relationship between anxiety and systemic inflammation, as well as genes that may affect this relationship, would enlarge our current knowledge base of

health outcomes associated with anxiety, and ultimately aid in the identification of the mechanism(s) by which anxiety can influence CVD risk. The purpose of this study was to (1) examine the relationship between anxiety and systemic inflammation; and (2) determine if SNPs associated with inflammation moderate this relationship. We hypothesized that anxiety would be a predictor of systemic inflammation. Further, we hypothesized that inflammatory genotypes would moderate this relationship, with those with an rs1205 CC genotype, an rs1800797 A allele, or an rs4129267 T allele having higher levels of systemic inflammation in comparison to participants without these characteristics.

3.2 Methods

3.2.1 Study Design

This was a secondary analysis of data collected at baseline in a primary research study entitled "Gene Environment Interactions Regulating CVD Inflammation and Success of Behavioral Therapies." The data for the primary research study were leveraged on the HeartHealth in rural Kentucky study that was conducted from 2009 to 2012 to test the efficacy of a 12-week CVD risk reduction intervention among rural Kentucky adults with two or more CVD risk factors. The HeartHealth study protocol has been previously published.[20] The "Gene Environment Interactions Regulating CVD Inflammation and Success of Behavioral Therapies" study was conducted to investigate the impact of the HeartHealth CVD risk reduction intervention on systemic levels of inflammation and the influence of inflammatory genotypes on the response. Extensive baseline and post-intervention data were collected including sociodemographic data,

clinical history, blood pressure, height, weight, lipid profiles, and smoking status. For the purposes of the leveraged study, serum for measuring systemic levels of inflammatory biomarkers and saliva for DNA extraction were collected from participants who completed baseline and post-intervention data collection.

3.2.2 Sample and Setting

Participants in the HeartHealth study were community-dwelling adults in rural Kentucky. Participants were 18 years of age or older with two or more CVD risk factors, including: age > 44 years for men and >55 years for women, a positive family history of CVD, history of hypertension, abnormal lipid levels or diabetes; current smoker or tobacco user, body mass index >25 kg/m^2, a sedentary lifestyle, or a diet high in saturated fat. Individuals were excluded if they were non-English speaking, had a history of chronic drug abuse, end-stage renal, liver, or pulmonary disease; were diagnosed with active cancer, were diagnosed with gastrointestinal disease requiring special diets, had cognitive impairment, had physical activity limitations, or were taking protease inhibitors or other medications that interfere with lipid metabolism. Approval from the University of Kentucky Institutional Review Board was obtained prior to all study activities, and all participants provided written informed consent prior to commencement of study protocols.

3.2.3 Measures

3.2.3.1 Anxiety

Anxiety was measured using the Brief Symptom Inventory (BSI) Anxiety subscale.[21] The scale consists of six items that are used to quantitate the degree of anxiety

experienced by an individual. Each item is rated on a scale from 0 (not at all) to 4 (extremely) based on the distress caused by the symptom[21]; the items were summed and then the average was obtained, with higher total scores indicative of greater anxiety.[22] The instrument has demonstrated excellent reliability and validity in non-cardiac and cardiac samples.[21,23] The Cronbach's alpha value for the current study was .77.

3.2.3.2 Systemic inflammation

CRP and IL-6 are key inflammatory biomarkers.[24,25] C-reactive protein and IL-6 are two of the most commonly measured biomarkers to determine levels of systemic inflammation.[24,25] High sensitivity CRP (hsCRP) was measured using a Cholestech LDX® analyzer (Cholestech LDX Diagnostics, Hayward, CA), a point-of-care methodology. The Cholestech LDX® system uses reflectance photometry and has well-established accuracy and reproducibility for the detection of serum hsCRP.[26]

To measure serum IL-6 protein levels, 10 ml of whole blood was collected using anti-coagulant free red top vacutainers. Samples were maintained at room temperature for at least 30 minutes and no longer than 60 minutes to allow for clotting. Samples were then centrifuged at 1100 rpm for 15 minutes, after which serum was removed, aliquoted, and stored in cryovials at −80°C until analyzed. Serum IL-6 was analyzed using a 6-plex Millipore kit (EMD Millipore, Billerica, MA), and results were read using a Luminex IS100 (Luminex, Austin, TX), following manufacturers' protocols.

3.2.3.3 SNP genotyping

Whole expectorated saliva was collected from participants using Oragene® DNA Collection Kits. From these samples, genomic DNA was isolated according to the

manufacturer's instructions.[27] Purified DNA was suspended in 10mM Tris-HCl, 1 mM EDTA pH 8.0 (Thermo Fisher, Wilmington, DE), and DNA concentrations were measured using the NanoDrop-1000 spectrophotometer (Fisher Scientific, Fair Lawn, NJ). SNP genotyping of purified genomic DNA was performed utilizing Taqman® Genotyping Assay Kits and reagents (Applied Biosystems/Thermo Fisher Scientific. Carlsbad, CA) in the Roche LightCycler 480® Instrument (Roche Applied Science. Indianapolis, IN). For the purposes of this study, we examined a single SNP in each of the *CRP* (rs1205), *IL-6* (rs1800797), and *IL6R* (rs4129267) genes. All SNPs tests maintained Hardy-Weinberg equilibrium ($p > 0.05$). Genotypes were coded as follows: (1) for rs1205, participants homozygous for major C alleles were compared to those who were CT heterozygous and TT homozygous; (2) for rs1800797, participants who were major G allele homozygotes were compared to GA heterozygotes and minor allele A homozygotes; and (3) for rs4129267, participants homozygous for the major C allele were compared to those who were CT heterozygotes or were homozygous for the minor T alleles.

3.2.3.4 Body Mass Index

Height and weight were measured with a professional grade stadiometer and a professional grade digital body weight scale at baseline for all participants. Body mass index (BMI) was calculated using the formula body weight (kilograms) divided by height (meters) squared. Body mass index was entered into analyses as a continuous variable.

3.2.3.5 Smoking status

Smoking status was determined from self-report. Participants were asked to identify if they were a never smoker, quit smoking 1 or more years ago, quit smoking less than one year ago, or were a current smoker. Responses were collapsed into 2 categories: participants who had never smoked or had quit 1 or more years prior were categorized as non-smokers; participants who had quit less than 1 year ago or were current smokers were categorized as current smokers.

3.2.3.6 Demographic characteristics

Demographic information was collected via self-report questionnaire. Data included age in years, race/ethnicity, and gender. For race/ethnicity, participants were asked to self-identify as being non-Hispanic white or Caucasian, non-Hispanic black or African American, Asian, Hispanic or Latino, American Indian or Alaskan Native, Native Hawaiian or other Pacific Islander, or other. Reflective of the rural Kentucky population, 89% of participants in the HeartHealth study self-identified as being non-Hispanic white or Caucasian. To control for population stratification, analyses in this study were limited to participants who self-identified as being non-Hispanic white.

3.2.4 Data Analysis

Descriptive analyses included frequency distributions or means and standard deviations, as appropriate for each variable of interest. Chi square analyses and independent t-tests were used to assess sociodemographic differences between genotypes.

Multiple linear regression modeling was conducted to examine whether anxiety was associated with serum CRP and IL-6, adjusting for age, gender, BMI, and smoking

status. To test moderation effects, hierarchical multiple linear regression modeling was conducted to examine if rs1205, rs1800797, or rs4129267 genotypes moderated associations between anxiety and serum hsCRP or IL-6. In each of the models, block one included age, gender, BMI, smoking status, and anxiety score, and genotype for either rs1205, rs1800797, or rs4129267, and the outcome variable of either serum hsCRP levels or serum IL-6 protein levels. An interaction term for anxiety and each genotype (anxiety * genotype) was entered in the second block of the regression model to test whether the relationship between anxiety and systemic inflammation varied according to genotype. For each significant interaction term, a subsequent simple slope analysis was conducted to determine the nature of the moderation effect.

To limit the overall Type I error rate, serum CRP and IL-6 protein levels were selected as the only outcome variables (i.e., indicators of the systemic inflammatory status) based on the reviewed literature.[2,4-6] For each outcome variable (CRP and IL-6 protein levels), three models were considered (one for each genotype). Given the purposeful limit imposed on number of cytokines and number of genotypes, we maintained an *a priori* alpha level of .05 throughout. Consistent with prior analyses and as a correction for skewed distributions, both hsCRP and IL-6 variables were log-10 transformed prior to analysis.

All data analyses were performed using SPSS Statistics for Windows version 26 (IBM Corp., Armonk, N.Y., USA) except for simple slope analysis, for which ModGraph Internet Version, version 3.0 (Victoria University of Wellington, Wellington, New Zealand) was used.[28,29]

3.3 Results

3.3.1 Sample Characteristics

Characteristics of the sample (N = 398) are summarized in Table 1. The majority of participants were female (73.4%) with a mean age of 51.4 ± 13.3 years. Most of the sample were non-smokers (88.7%). The mean BMI for this sample was 32.8 ± 7.4 kg/m^2. There were no significant differences in sociodemographic characteristics found when comparing genotypes for all three SNPs examined (data not shown).

3.3.2 Associations between anxiety and levels of inflammatory biomarkers

Table 2 shows results of the regression analysis conducted to examine if anxiety predicted serum hsCRP protein levels controlling for age, gender, BMI, and smoking status. While the overall model was significant ($F(5, 392) = 24.45$, $p < 0.001$), this was due to the significant associations of gender, smoking status, and BMI with serum hsCRP protein levels; anxiety was not a significant predictor of hsCRP protein levels. Additionally, the regression model examining whether anxiety predicted serum IL-6 protein levels was not significant (Table 3).

3.3.3 Moderating effects of inflammatory genotypes on associations between anxiety and levels of inflammatory biomarkers

Three models were analyzed to examine if the rs1205, rs1800797, or rs4129267 SNPs moderated the association between anxiety and serum IL-6 protein level. Of the three models, the two hierarchical linear regression models examining whether the rs1205 or rs1800797 SNPs moderated the association between anxiety and serum IL-6 protein level were not found to be significant ($F(7, 390) = 1.607$, $p = .132$ and $F(7, 390) = 1.56$, p

= 0.146, respectively). The third model which examined the moderation effect of the *IL-6R* gene SNP, rs4129267, was found to be significant ($R^2 = 0.042$, $F(7, 390) = 2.42$, $p = 0.019$). In this final model, the interaction term between anxiety and rs4129267 genotype was significant ($\beta = -0.235$, $p = 0.010$), indicating a significant moderation effect. The subsequent simple slope analysis revealed that anxiety was only significantly associated with IL-6 protein levels for those with an rs4129267 CC genotype ($b = 0.243$, $SE = 0.04$, $p < 0.001$) and not for those with a CT or TT genotype ($p = 0.770$) (Figure 1).

Three additional models examined whether rs1205, rs1800797, or rs4129267 SNPs moderated the association between anxiety and serum CRP level. In these models, there was no evidence that rs1205, rs1800797, or rs4129267 genotypes moderated the association between anxiety and serum CRP level.

3.4 Discussion

In previous studies, anxiety has been reported to be associated with increased levels of systemic inflammation.[2-4,30,31] However, this relationship has not been consistently supported in the literature,[7,8] and was not supported in this study (Tables 2 and 3). In accord with the results of our study, anxiety was not significantly associated with either serum hsCRP or IL-6 levels. The lack of consistency in the association between anxiety and systemic levels of inflammation suggests that other variables may influence this relationship.

Genetic variants previously associated with increased systemic levels of inflammatory biomarkers were examined as potential moderators of the association between anxiety and inflammation.[13,19,32] Of the three SNPs (rs1205, rs1800797, and rs4129267) examined in this study, the rs4129267 SNP, located on the *IL-6R* gene, was

found to have a moderating effect on the association between anxiety symptoms and serum IL-6 levels. Results of the simple slope analysis indicated that the association between anxiety and inflammation was only significant for individuals with the rs4129267 CC genotype. The *IL-6R* SNP, rs4129267, examined in this study has been reported to be in complete linkage disequilibrium with a second *IL-6R* SNP, rs2228145, with the minor C allele of rs2228145 co-inherited with the minor T allele of rs4129267.[16] The rs2228145 SNP is responsible for an amino acid change, Asp(358)Ala, that results in a modification to the structure of the IL-6 receptor.[16] This amino acid change increases the cleavage of the IL-6 receptor from the cell surface into the extracellular space, where the IL-6 receptor can then associate with free IL-6.[17,18,33] The resulting complex is then recognized by glycoprotein (gp) 130 structures on the membrane of most cells in the body, providing an alternate IL-6 signaling pathway, known as IL-6 trans-signaling.[17,18,33] As compared to classic IL-6 signaling, where IL-6 associates with membrane-bound IL-6 receptors that are expressed by only a few types of cells in the body, IL-6 trans-signaling can occur in many types of cells throughout the body, and is reported to be a pro-inflammatory process, and may explain findings from this study.[17]

The pro-inflammatory nature of the T allele of rs4129267 is reflected in our findings. There was no significant association between anxiety and IL-6 levels for those with the rs4129267 CT or TT genotype, but rather, as demonstrated in the simple slope analysis results, those with the CT or TT genotype had higher IL-6 levels, with no regard to level of anxiety. This suggests that individuals with CT or TT rs4129267 genotypes may experience persistent elevated systemic inflammation regardless of anxiety levels. Conversely, our findings suggest that those with an rs4129267 CC genotype, which has

not been reported to be associated with increased systemic inflammation, may experience an increased inflammatory response to stressors, such as anxiety.

Other SNPs examined did not have a moderating effect on associations between anxiety and levels of serum hsCRP or IL-6. Among these was rs1205. Located within the *CRP* gene, the minor T allele of rs1205 has been associated with lower circulating levels of CRP.[13,14,34] In the two models which examined rs1205 genotype, genotype was not a significant predictor of CRP or IL-6 levels, nor was there evidence that genotype moderated the relationship between anxiety and systemic inflammation. The second SNP examined in this study was rs1800797, located on the *IL-6* gene. The minor A allele of the rs1800797 SNP has been associated with increased IL-6 levels and decreased CRP levels when compared to GG genotypes.[11,15] Like rs1205, rs1800797 was not found to be associated with IL-6 or CRP levels, nor did it have a moderating effect on the relationship between anxiety and inflammation.

Of note, in the two models examining the moderation of rs1205 and rs1800797 on the association between anxiety and CRP, gender, BMI, and smoking status were found to be significant predictors of CRP. These are expected findings, as female gender, increased BMI, and smoking are well-established predictors of systemic inflammation, and are commonly adjusted for in analyses examining novel associations with systemic inflammation.[35]

This study adds to the current literature that reports an association between anxiety and systemic inflammation by suggesting that certain inflammatory genotypes may be more susceptible to the negative effects of anxiety, resulting in increased inflammation. Few studies have been conducted to examine associations between anxiety

and inflammation, and fewer still to test moderating effects of inflammatory genotypes on these associations. The findings from our study add to the knowledge of how specific genotypes might place some individuals at risk for increased systemic inflammation that can, in turn, increase risk for CVD. Additional research is needed to examine these relationships further, and to replicate and validate the findings of this preliminary study.

3.4.1 Strengths and Limitations

To control for population stratification, the analyses were limited to participants who self-identified as non-Hispanic white or Caucasian. It will be of interest in future studies to examine these relationships in more diverse samples. Second, while we had a large sample representative of rural populations at high risk for CVD, additional studies with larger samples will be critical both to verify our preliminary findings, and to support conducting a larger number of analyses with correction for multiple comparisons.

3.5 Conclusion

Anxiety is associated with increased risk for CVD. The mechanism that underlies this relationship is not known, although inflammatory pathways have been hypothesized to be a potential mechanism. In this study, anxiety was positively associated with serum IL-6 protein levels, but a moderation analysis indicated that this association was significant only for individuals with the rs4129267 CC genotype. These findings suggest that there may be genotypic differences in individuals' responses to anxiety, which may place certain individuals at higher risk than others for inflammation, and subsequently CVD. Further studies are needed to provide more robust evidence of these relationships,

from which interventions can be tailored based on genotypic differences to improve CVD prevention efforts.

Table 3.1 Participant Characteristics	
	Total Sample (N=398)
Age (years)	51.4 ± 13.3
Gender (male)	106 (26.6%)
Never or former smoker	353 (88.6%)
Body mass index (kg/m^2)	32.8 ± 7.4
Anxiety score	0.5 ± 0.5
hsCRP[a] (mg/L)	3.3 ± 3.7
IL-6[b] (pg/L)	16.4 ± 34.3
Values reported as either mean ± SD or n (%). [a] high sensitivity C-reactive protein [b] Interleukin-6	

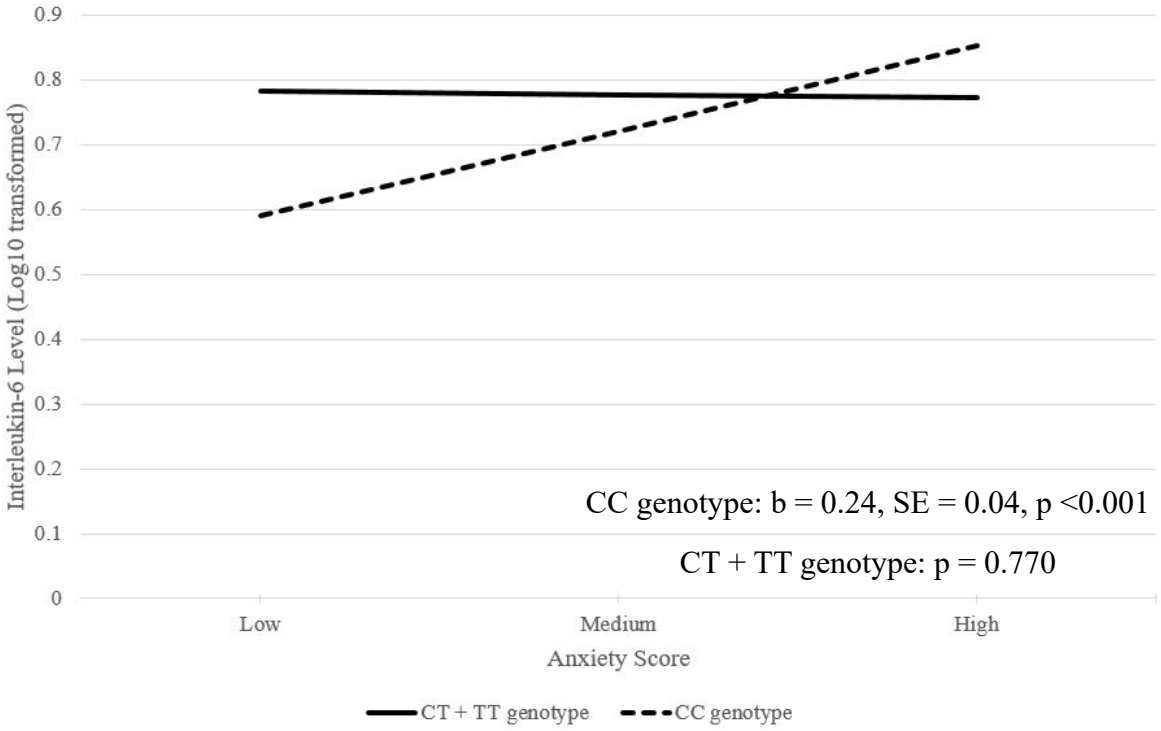
Table 3.2 Summary of Multiple Linear Regression Predicting Serum High Sensitivity C-reactive Protein Level (N= 398)				
Variable	B	**β**	95% CI for B	*p* value
Age	0.002	0.057	(-0.001, 0.006)	0.209
Gender (male)	0.218	0.191	(0.116, 0.319)	<0.001
Body mass index	0.029	0.425	(0.023, 0.035)	<0.001
Smoking status (never or former)	0.163	0.102	(0.022, 0.304)	0.023
Anxiety score	0.064	0.069	(-0.019, 0.148)	0.128
Overall: $R^2 = 0.238$, $p < 0.001$				

Table 3.3 Summary of Multiple Linear Regression Predicting Serum Interleukin-6 Protein Level (N = 398)				
Variable	B	**β**	95% CI for B	*p* value
Age	0.001	0.016	(-0.003, 0.005)	0.753
Gender (male)	-0.072	-0.061	(-0.190, 0.046)	0.232
BMI	0.004	0.062	(-0.003, 0.011)	0.216
Smoking status (never or former)	-0.133	-0.081	(-0.297, 0.031)	0.111
Anxiety score	0.100	0.103	(0.003, 0.197)	0.043
Overall: $R^2 = 0.022$, p = 0.116				

Table 3.4 Summary of hierarchical multiple linear regression to examine moderating effects of rs4129267 genotype on associations between anxiety and serum IL-6 protein level							
Variable	B	**β**	95% CI for B	*p* value	R	R^2	R^2 change
Step 1					0.159	0.025	0.003
Age	0.001	0.015	(-0.003, 0.004)	0.772			
Gender (male)	-0.073	-0.062	(-0.191, 0.045)	0.227			
BMI	0.004	0.062	(-0.003, 0.011)	0.222			
Smoking status (never, former)	-0.133	-0.081	(-0.296, 0.031)	0.112			
Anxiety score	0.100	0.103	(0.003, 0.196)	0.044			
rs4129267 (CC)	0.057	0.054	(-0.047, 0.160)	0.281			
Step 2					0.204	0.042	0.017
Age	0.000	0.008	(-0.004, 0.004)	0.867			
Gender (male)	-0.075	-0.064	(-0.192, 0.042)	0.207			
BMI	0.005	0.065	(-0.002, 0.011)	0.197			

Table 3.4 continued							
Smoking status (never or former)	-0.143	-0.087	(-0.306, 0.020)	0.085			
Anxiety score	0.243	0.251	(0.098, 0.388)	0.001			
rs4129267 (CC)	0.191	0.182	(0.047, 0.336)	0.010			
Anxiety * rs4129267 genotype	-0.252	-0.235	(-0.443, -0.061)	0.010			

Figure 3.1 Association between anxiety and IL-6 by rs4129267 genotype



CHAPTER 4. EXPERIENCES OF ETHNIC DISCRIMINATION AND VAL158MET POLYMORPHISM ARE ASSOCIATED WITH DEPRESSIVE SYMPTOMS IN HISPANIC ADULTS AT RISK FOR CARDIOVASCULAR DISEASE

4.1 Introduction

Hispanics are the largest and fastest growing minority population in the U.S., currently representing 18.3% of the nation's population.[1] Nearly 70% of U.S. Hispanic adults report experiencing ethnic discrimination during their lifetime.[2] Discrimination, as defined by Clark and colleagues, consists of the "beliefs, attitudes, institutional arrangements, and acts that denigrate individuals or groups because of phenotypic characteristics or ethnic group affiliation."[3] Racial and ethnic discrimination adversely affects multiple areas of individuals' lives, including employment, housing, education, voting, health care, and in interactions with authorities.[4]

Discrimination is a chronic stressor that is associated with negative physical and mental health outcomes in racial and ethnic minority populations.[5-7] This includes a higher propensity for the development of cardiovascular disease (CVD). In a multi-ethnic sample from a national longitudinal study, Everson-Rose and colleagues (2015) reported that a one standard deviation increase in experiences of lifetime discrimination was associated with an 11% increase in CVD risk. Further, experiences of racial/ethnic discrimination have also been associated with known CVD risk factors, including elevated levels of systematic inflammation, elevated blood pressure, and feelings of anxiety.[2,8-10] Although associations between discrimination and depression in Hispanic adults have not been well-studied, strong associations between experiences of discrimination and depressive symptoms have been demonstrated in research with African American populations.[11-13]

The underlying pathway of this relationship is not well understood. Chronic stressors, such as experiences of discrimination, have been proposed to result in dopaminergic pathway dysregulation in the brain, beginning with increased levels of dopamine in response to the stressor.[14,15] In response to this overstimulation of the dopaminergic system, an adaptive downregulation of the dopaminergic system may occur, which includes decreased transmission of dopamine throughout the brain.[16,17] Subsequently, the decreased transmission of dopamine is associated with common depressive symptoms including anhedonia, decreased motivation, sleep disruption, and decreased energy.[18,19]

Given the role of dopamine in depressive symptoms, genetic variants associated with dopamine activity may affect the relationship between chronic stress, such as discrimination, and depressive symptoms. One such variant is the Val158Met polymorphism in the catechol-o-methyltransferase (*COMT*) gene, located on chromosome 22. This gene encodes the COMT enzyme that is responsible for dopamine degradation in the prefrontal cortex.[20] COMT with the common valine (Val) allele is more stable than COMT with the less abundant methionine (Met) allele.[21] COMT enzymatic activity in Val-Val homozygous individuals may be increased by as much as 50%, relative to Met-Met individuals, while COMT activity in heterozygous individuals may be increased by as much as 12% relative to Met-Met individuals.[22] Combined with elevated levels of dopamine resulting from prolonged stress, such as experiences of discrimination, the decreased dopamine degradation seen with the Met allele may increase the body's compensatory response and ultimately decrease the body's responsiveness to dopamine. This could result in higher risk for depressive symptoms in those heterozygous or homozygous for the Met allele.[23]

Better understanding of the relationship between ethnic discrimination and depressive symptoms, and identification of genes that influence this relationship, could support early identification of people at greatest risk for depressive symptoms who are appropriate for early intervention. The purpose of this study was to examine the relationship between lifetime experiences of ethnic discrimination and depressive symptoms; between Val158Met polymorphism and depressive symptoms; and to explore the moderating effect of Val158Met polymorphism on the association between discrimination and depressive symptoms in Hispanic adults.

4.2 Methods

4.2.1 Study Design

This was an analysis of data from a genetic sub-study that was leveraged on the "*Corazón de la Familia* (Heart of the Family)" study. The *Corazón de la Familia* study is a randomized controlled trial conducted to examine the effects of a family dyad intervention to reduce CVD and type 2 diabetes (T2D) risk in Hispanics with two or more risk factors. Extensive baseline data were collected as part of the *Corazón de la Familia* study, including sociodemographic data, clinical history, and depressive symptoms from both members of the participating family dyad. The sub-study was conducted to investigate the effects of experiences of ethnic discrimination and genetic variations on symptoms of depression in Hispanic adults at risk for CVD. For the purposes of the leveraged study, saliva for genomic DNA extraction and subsequent genotyping was collected from participants who completed baseline data collection.

Prior to commencement of the study, all study protocols and materials were approved by the University of Kentucky Institutional Review Board. Prior to participation in any study procedures, written informed consent was provided by all participants. Following informed consent, data collection questionnaires were completed by each member of the participating dyad separately. Saliva samples were collected from participants after completion of the data collection questionnaires.

4.2.2 Sample and Setting

Participants were community-dwelling Hispanic adults who resided in central Kentucky. Family dyads were recruited including (1) a primary participant who had two or more risk factors for CVD or T2D, but did not have a diagnosis of either condition, and (2) a co-participating family member with or at risk for CVD or T2D. Other inclusion criteria were that participants were primary Spanish speakers 18 years of age and older. Individuals were excluded from this study if they had cognitive impairment that precluded either member of the dyad from understanding the consent process or participating in the intervention as evaluated using the Spanish-language version of the Mini-Cog; had a major psychiatric (e.g., schizophrenia) condition; or had medical contraindications to participating in a lifestyle intervention. Either member of the parent study's family dyad was eligible to participate in the genetic sub-study, and the sample in this study consists of both principal members of the dyad, as well as family dyad members.

4.2.3 Measures

4.2.3.1 Sociodemographic and clinical characteristics

Data were collected via self-report at baseline of the parent study. These data included participant age and sex. Socioeconomic status was assessed using a measure of financial comfort in which participants were self-categorized into 2 groups: having enough or more than enough money to make ends meet or having not enough money to make ends meet. Educational level was collected, and participants were categorized as having a high school education or less or as having more than a high school education. Marital status data were collected, and participants were categorized as being single, divorced, or widowed as compared to married or cohabitating. Additionally, clinical information was collected which included a list of currently prescribed medications.

4.2.3.2 Acculturation

Marin's Acculturation Scale was used to measure acculturation. This 12-item scale measures acculturation to U.S. culture among Hispanic individuals. The 12 items of this instrument address three domains of acculturation: "language use", "media", and "ethnic social relations".[24] Participants respond to each item on a 5-point Likert scale that ranges from 1 (only Spanish) to 5 (only English). The score is calculated by summing across the 12 items, with higher scores indicative of more acculturation to the U.S. culture. In this sample, the reliability of this instrument was found to be acceptable, with a Cronbach's alpha of 0.80.

4.2.3.3 Experiences of discrimination

Ethnic discrimination is the unfair treatment of individuals or groups based on phenotypic characteristics or ethnic group affiliation.[3] Experiences of ethnic discrimination were assessed using the Experiences of Discrimination (EOD) instrument. This is a 9-item instrument that asks the respondent to identify situations in which they have ever experienced discrimination as a result of their ethnicity, such as at school or work or in accessing medical care or seeking housing. For any situation in which discrimination has been experienced, the participant identified how often; response options consisted of "Never" (score = 0), "once" (= 1), "2-3 times" (= 2.5), and "4 or more times" (= 5). The score is summed across the 9 items and ranges from 0 to 45; higher scores indicate more frequent experiences of discrimination. Reliability and validity for English and Spanish versions of the EOD have been demonstrated in Hispanic populations, with Cronbach's alpha values ranging from 0.71 to 0.90 for both English and Spanish language versions.[25-27] In this sample, the Cronbach's alpha for the Spanish language version of the EOD instrument was 0.77.

4.2.3.4 Val158Met Polymorphism

Two milliliters of whole expectorated saliva were collected from participants using Oragene® DNA OG-500 Collection Kits. Genomic DNA was isolated from these samples according to the manufacturer's instructions.[28] Purified DNA was suspended in 100 μL of elution buffer (New England Biolabs, Inc, Ipswich, MA), and purified DNA was quantified by UV absorbance with a NanoDrop 2000/2000c Spectrophotometer (Thermo Scientific, Waltham, Massachusetts). Samples were then genotyped for the

Val158Met SNP in the *COMT* gene. Genotyping of purified genomic DNA was performed utilizing Taqman® Genotyping Assay Kits and reagents, according to the manufacturer's protocol (Applied Biosystems/Thermo Fisher Scientific. Waltham, Massachusetts). Genotypes were determined using a CFX Real Time PCR system (BioRad). The Val158Met SNP maintained Hardy-Weinberg equilibrium ($p > 0.05$). For the purposes of the analysis, participants were categorized as having a Val/Val genotype or Val/Met + Met/Met genotype.

4.2.3.5 Depressive symptoms

The Patient Health Questionnaire-8 (PHQ-8) consists of 8 items used to assess depressive symptom frequency over the previous two weeks.[29] The 8 items are based on Diagnostic and Statistical Manual of Mental Disorders (DSM) IV diagnostic criteria for depressive disorders.[29] Scores range from 0 to 27, with each item scored as 0 (not at all), 1 (several days), 2 (more than half the days), and 3 (nearly every day). Higher PHQ-8 scores indicate higher levels of depressive symptoms. Spanish and English versions have been shown to have high specificity and sensitivity when compared to structured psychiatric interviews conducted by mental health professionals,[29,30] as well as appropriate validity and reliability.[30,31] In this sample, internal reliability was acceptable, with a Cronbach's alpha value of 0.83.

4.2.4 Data Analysis

Descriptive analyses to characterize the sample-included frequency distributions or means and standard deviations, as appropriate for each variable of interest.

Hierarchical multiple linear regression modeling was conducted to examine (1) the relationship between discrimination experiences and depressive symptoms (block 1 of the hierarchical regression model); (2) the relationship between Val158Met polymorphism and depressive symptoms (block 2 of the model); and (3) whether the Val158Met polymorphism moderated the association between experiences of discrimination and depressive symptoms (block 3 of the model). The model was adjusted for age, gender, acculturation, marital status, educational level, and financial status. Experiences of discrimination was included in block 1 of the model, in addition to the control variables listed. The Val158Met polymorphism was entered into the second block of the regression model. An interaction term for discrimination and Val158Met (discrimination * Val158Met) was entered in the third block of the regression model.

All statistical analyses were performed using SPSS version 26 (IBM, Armonk, NY), and an *a priori* alpha level of 0.05 was used to determine significance in all analyses.[32] All variables were examined to ensure they met the statistical assumptions for the analyses performed.

4.3 Results

4.3.1 Sample Characteristics

Characteristics of the sample are summarized in Table 1. The majority of participants were female (74.2%) with a mean age of 40.2 ± 9.3 years. The mean acculturation score for this sample was 20.9 ± 5.2, showing that the sample was less acculturated to the U.S. culture. In this sample, 79% reported their household income to

be enough or more than enough to make ends meet. Participants reported low levels of experienced ethnic discrimination (2.9 ± 5.1) and depressive symptoms (2.7 ± 3.3).

4.3.2 Associations between experiences of discrimination, Val158Met polymorphism, and depressive symptoms

Results of the hierarchical multiple linear regression are presented in Table 2. Block 1 examined the association between experiences of discrimination and depressive symptoms ($F(7, 116) = 2.151$, $p = 0.044$). Findings from this block showed experiences of discrimination to be a significant predictor of depressive symptoms ($p = 0.034$), with more experiences of discrimination associated with higher depressive symptoms. In block 2, the Val158Met polymorphism was assessed as a predictor of depressive symptoms ($F(8,115) = 2.471$, $p = 0.016$). Regarding the Val158Met polymorphism, carriers of the Met allele had higher levels of depressive symptoms compared to those homozygous for the Val allele. Block three of the regression examined if the Val158Met polymorphism moderated the association between experiences of discrimination and depressive symptoms. The final block of the model was significant ($F(9, 114) = 2.200$, $p = 0.027$), and revealed significant main effects of age ($p = 0.017$), experiences of discrimination ($p = 0.041$), and the Val158Met polymorphism ($p = 0.049$) on depressive symptoms. However, the interaction term between experiences of discrimination and the Val158Met polymorphism was not significant ($p = 0.682$), indicating that the Val158Met polymorphism does not moderate the relationship between experiences of discrimination and depressive symptoms. In all three blocks of the regression model, of the control variables, only age was associated with depressive symptoms, with depressive symptoms decreasing with age.

4.4 Discussion

The demonstration of a significant association between experiences of ethnic discrimination and depressive symptoms in this study adds to a growing body of research in which similar outcomes have been reported in studies conducted with other populations.[12,33,34] For example, in a study conducted to investigate the association between experiences of racial discrimination and depressive symptoms in older African American adults, Nadimpalli and colleagues (2015) found for every increase in a measure of discriminatory experiences, participants were 1.2 times more likely to report higher levels of depressive symptoms.[11] Similar findings have been reported in studies conducted with multi-ethnic samples.[35] The few studies conducted with Hispanics of Mexican origin,[36] Hispanic adolescents[37,38] and emerging Hispanic adults[39] have also shown a positive relationship between experiences of discrimination and depressive symptoms. Although few studies have been conducted to investigate the association between experiences of discrimination and depressive symptoms in a heterogeneous sample of U.S. Hispanic adults, as this study was, Torres and Ong (2010) examined these associations in a sample of 91 Hispanic adults that, similar to our sample, were from various countries of origin.[40] Similar to the findings from this study, Torres and Ong also found that greater experiences of discrimination were positively associated with depressive symptoms.[40] Findings from this study add to a limited, but growing, body of research showing these associations in Hispanic adults in the U.S.

Findings from other studies have indicated there are associations between the Val158Met polymorphism and depressive symptoms, with the Met allele associated with higher levels of depressive symptoms.[41-44] For example, in a sample of 405 people, Aberg and colleagues found that depressed participants were nearly 1.5 times more likely to

have a Val/Met or Met/Met genotype.[41] Similarly, we found the Val158Met polymorphism was found to be an independent predictor of depressive symptoms in our sample, with the Met allele associated with increased depressive symptoms.

The Val158Met polymorphism is located in the gene that encodes catechol-*O*-methyltransferase, or COMT, an enzyme that catabolizes catecholamines, such as dopamine, to physiologically inactive metabolic products.[20,44] The Val158Met polymorphism has been associated with a three to four fold variation in COMT activity, with those homozygous for the Val allele having higher COMT enzymatic activity than those homozygous for the Met allele.[21,22] Our findings show increased depressive symptoms in participants with at least one Met allele. This coincides with the outcomes of several well-designed studies, where the Met allele was also reported to be positively associated with depressive symptoms.[41-43] The increased risk for depressive symptoms seen with the Met allele may be due to lower COMT enzyme activity associated with the Met allele.[21,22] The slower COMT enzyme activity results in consistently higher levels of dopamine. The overstimulation caused by the high levels of dopamine may, in turn, decrease the body's responsiveness to dopamine.[16] This decreased responsiveness to dopamine could result in the presence of depressive symptoms.[45]

Conversely, there have been studies which report the Val allele of the Val158Met polymorphism to be associated with increased depressive symptoms.[46-48] Of note, the sample populations in these studies are primarily of European/Caucasian or Asian descent. Further, a recent meta-analysis reported differences in the Val158Met allele associated with depression risk in two different ethnic populations.[48] In the East Asian sample in the meta-analysis, the Met allele was found to be the allele associated with

depression, while in the European sample, the Val was the allele associated with depression.[48] To our knowledge, the association between the Val158Met polymorphism and depressive symptoms has not been investigated in Hispanic individuals, and may explain differences in our findings than in those who report the Val allele to be associated with increased depressive symptoms.

While investigators have examined biological mechanisms that could potentially underlie the association between ethnic discrimination and health outcomes, this association is still not well understood.[5,7,49,50] Due to the stressful nature of ethnic discrimination, and effect of stress on dopamine levels in the brain,[51] the Val158Met polymorphism was investigated as a potential moderator of this relationship. It was hypothesized that the Val158Met polymorphism would moderate the relationship between experiences of discrimination and depressive symptoms, given the genotype's association with dopamine activity,[20] and the role of dopamine in depression.[19] However, findings from this study indicated no moderation effect of Val158Met polymorphism on this relationship, suggesting that experiences of discrimination may not impact depressive symptoms through dopaminergic pathways, and instead are associated through alternative pathways. Our results differ from findings in previous studies in which the Val158Met polymorphism was reported to moderate associations between psychosocial stressors and depressive symptoms.[41,52,53] However, these studies differ from the present study as they were conducted in mainly samples with European ancestry, and examined different psychosocial stressors, such as adverse life events and experiences of childhood trauma.[41,52,53]

This study further strengthens the knowledge base about the impact of experiences of ethnic discrimination on mental health, specifically on symptoms of depression. This sample of Hispanic adults reported relatively few experiences of ethnic discrimination, and relatively low levels of depressive symptoms. Despite this, experiences of discrimination were predictive of increased depressive symptoms, suggesting that this is an important risk factor for depressive symptoms that should be considered in this population. Additionally, this study adds to the knowledge of how the Val158Met polymorphism may influence depressive symptoms in Hispanic populations, and presents preliminary findings identifying individuals who may be at higher risk for depressive symptoms.

4.4.1 Strengths and Limitations

This study has several strengths and limitations that should be noted. First, the sample size in this study was relatively small (N = 124) and reported experiences of discrimination and depressive symptoms were low in this sample. To better understand this phenomenon, and to verify our preliminary findings, larger studies with more variance in discrimination experiences and depressive symptoms are needed. Additionally, in the present study, the use of anti-depressant medication was unable to be controlled for in the analysis due to lack of medication data for all participants. For the participants who did have complete medication data (n = 102), no participants reported anti-depressant medication use. Future research should aim to have complete medication records for all participants as to be able to adjust analyses for the use of anti-depressant medications.

4.5 Conclusion

Experiences of ethnic discrimination are associated with increased depressive symptoms in Hispanic adults, and findings from this study provide further evidence of this relationship. The mechanisms underpinning this relationship remain unclear, as the Val158Met polymorphism was not found to moderate the association between experiences of discrimination and depressive symptoms. However, findings from this study did reveal the Val158Met polymorphism to be an independent predictor of depressive symptoms, with the Met allele associated with increased depressive symptoms. These findings suggest that both experiences of discrimination and Val158Met genotype may influence depression in Hispanic adults, which may place certain individuals at higher risk than others for depression, and subsequently CVD.

Table 4.1 Participant Characteristics (N = 124)	
Age (years)	40.2 ± 9.3
Gender (male)	32 (25.8%)
Region of Origin Mexico Caribbean Central America South America	 100 (80.7%) 3 (2.4%) 4 (3.2%) 17 (13.7%)
Marital status Single, divorced, or widowed Married or cohabitating	 37 (29.8%) 87 (70.2%)
Financial status Not enough to make ends meet Enough or more than enough to make ends meet	 26 (21.0%) 98 (79.0%)
Education level High school or less More than high school	 86 (69.4%) 38 (30.6%)
Acculturation	20.9 ± 5.2
Experiences of discrimination	2.9 ± 5.1
Val158Met Polymorphism Val/Val Val/Met + Met/Met	 48 (38.7%) 76 (61.3%)
Depressive Symptoms	2.7 ± 3.3
Values reported as either mean ± SD or n (%).	

Table 4.2 Summary of hierarchical multiple linear regression to examine moderating effects of Val158Met genotype on associations between experiences of discrimination and depressive symptoms

Variable	B	**β**	95% CI for B	*p* value	R	R^2	R^2 change
Block 1					0.339	0.115	0.115
Age	-0.071	-0.201	(-0.135, -0.008)	0.028			
Gender (male)	0.959	0.127	(-0.415, 2.333)	0.170			
Marital status (single, divorced, widowed)	0.378	0.052	(-0.900, 1.655)	0.559			
Financial status (enough or more than enough to make ends meet)	0.674	0.083	(-0.772, 2.121)	0.358			
Education level (high school or less)	-0.609	-0.085	(-1.943, 0.725)	0.368			
Acculturation	-0.010	-0.016	(-0.131, 0.111)	0.871			
Experiences of discrimination	0.124	0.192	(0.010, 0.238)	0.034			
Block 2					0.383	0.147	0.032
Age	-0.077	-0.217	(-0.140, -0.014)	0.017			

Table 4.2 continued							
Gender	0.828	0.687	(-0.533, 2.189)	0.231			
Marital status	0.187	0.026	(-1.086, 1.460)	0.772			
Financial status	0.443	0.055	(-1.001, 1.887)	0.544			
Education level	-0.602	-0.084	(-1.918, 0.714)	0.367			
Acculturation	-0.023	-0.036	(-0.143, 0.097)	0.708			
Experiences of discrimination	0.146	0.224	(0.031, 0.260)	0.013			
Val158Met (Val/Val)	1.260	0.186	(0.055, 2.465)	0.041			
Block 3					0.385	0.148	0.001
Age	-0.077	-0.217	(-0.140, -0.014)	0.017			
Gender	0.824	0.109	(-0.543, 2.190)	0.235			
Marital status	0.200	0.028	(-1.079, 1.479)	0.757			
Financial status	0.451	0.056	(-0.998, 1.901)	0.538			
Education level	-0.590	-0.082	(-1.912, 0.732)	0.379			
Acculturation	-0.019	-0.030	(-0.141, 0.104)	0.764			

Table 4.2 continued							
Experiences of discrimination	0.170	0.260	(0.007, 0.332)	0.041			
Val158Met	1.407	0.208	(0.005, 2.810)	0.049			
Discrimination * Val158Met	-0.048	-0.054	(-0.277, 0.182)	0.682			

CHAPTER 5. CONCLUSION

5.1 Background and Purpose

The overall purpose of this dissertation was to examine associations between psychosocial stressors and CVD risk factors, and the influence of genes on these associations in populations burdened with significant cardiovascular health disparities. This dissertation includes three original manuscripts. The first manuscript is a systematic review of instruments that have been psychometrically tested and used to measure ethnic discrimination is Hispanic adults. In the second paper, findings from a secondary analysis of data conducted with a rural Kentucky population are reported. The purpose of the second paper was to examine associations between anxiety and serum levels of CRP and IL6, and the moderation of this relationship by genetic variants located on the *CRP* gene, *IL-6* gene, and *IL-6R* gene. In the final paper, results from a study conducted to examine whether experiences of ethnic discrimination predicted depressive symptoms in Hispanic adults, and to explore if a genetic variant on the *COMT* (catechol-o-methyltransferase) gene moderated this relationship are reported.

Considering the high prevalence of CVD, and the high rates of mortality associated with CVD,[1] particularly in the rural Kentucky[2] and Hispanic[3] communities, prevention of CVD remains a critical priority. However, in high-risk populations, CVD prevention remains a major challenge. Although CVD risk is multifactorial and involves genetic, behavioral, environmental, and psychosocial factors, CVD prevention efforts to date have focused primarily on behavioral and environmental risk factors. Far less is understood about how psychosocial factors, including psychosocial stressors, and genetic variants affect CVD risk.[4]

Psychosocial stress is a type of stress that occurs when an individual's environmental demands tax or exceed the individual's adaptive capabilities.[5] Multiple factors are known to influence CVD risk in rural and Hispanic populations, however, psychosocial stressors may be particularly significant contributors to CVD risk. Examples of relevant environmental stressors in rural and Hispanic adult populations include experiences of discrimination, low socioeconomic status, and limited access to healthcare. Experiences of these stressors lead to psychosocial stress, which has been associated with increased physiological stress responses and psychological symptoms, which in turn, may further increase CVD risk. It is of particular importance to examine psychosocial factors that impact CVD risk in U.S. rural and Hispanic populations who experience unique psychosocial risk factors that may further exacerbate CVD risk.[2,3]

This dissertation was guided by a conceptual framework that was based on the "Pathways between Racism and Health" conceptual framework created by Paradies and colleagues in 2013.[6] The original model was based on the findings of a systematic review and meta-analysis, and described multiple pathways through which racial discrimination is associated with physical and mental health outcomes, including through psychological stress processes.[6] For the purposes of this dissertation, the framework was modified based on existing literature which describe pathways between general psychosocial stressors and physical and mental health outcomes (Figure 5.1). The modified framework presents a pathway linking psychosocial stress to psychological symptoms and mental and physical health outcomes and includes genetic variants as a potential moderator of the association between psychosocial stressors and psychological symptoms.

The purpose of this chapter is to summarize, synthesize, and discuss the findings of the three studies in this dissertation. In this chapter is a discussion of how the outcomes of these studies further the knowledge and the state of the science regarding stress, genes, and CVD risk in populations suffering from cardiovascular health disparities. Lastly, recommendations are made for practice and future research directions.

5.2 Summary of Findings

Chapter 2 of this dissertation is a systematic review of instruments measuring ethnic discrimination that have been psychometrically tested and used with Hispanic adult populations. The review study included 16 articles in which six measures of racial and/or ethnic discrimination in Hispanic adults were described. In the majority of the articles (n = 14) psychometric testing of the instrument was reported as part of the findings from a larger study. Two articles were written specifically to report results of validity and reliability testing of the instrument. In 10 of the articles, the instruments were used in Hispanic-only samples, and in the six remaining articles, the instruments were used in multi-ethnic samples which included portions of Hispanic adults. The instruments varied in length, ranging from 6 to 70 items, and all instruments relied on self-report to measure ethnic discrimination. Most instruments aimed to measure discrimination across multiple settings (e.g., at work, at school, in public locations), but the instruments varied in the timeframes in which they ask respondents to report experiences of ethnic discrimination (e.g. past week, past year, lifetime). While all instruments were found to be valid and reliable measures of ethnic discrimination, only two instruments had Spanish language versions that were psychometrically tested. The availability of Spanish and English language versions of the instrument, length, and the populations in which the

instruments have been tested were considered. While evidence supports the reliability and validity of each instrument, the Experiences of Discrimination (EOD) instrument has been shown to be valid and reliable in both Spanish and English. Further, the EOD is a concise instrument, limiting participant burden, while reliably and validly measuring lifetime experiences of ethnic discrimination in Hispanic adults across 9 different settings.[7] These findings provide important guidance to the scientific community as they can serve as a guide when selecting appropriate instruments to measure lifetime experiences of ethnic discrimination.

Chapter 3 was a secondary analysis of data examining whether anxiety predicted levels of systemic inflammation, and if three genetic variants associated with inflammation moderated this relationship in a sample of rural Kentucky adults with two or more CVD risk factors (N = 398). These variants assessed for moderation effect included single nucleotide polymorphisms (SNPs) rs1205, located on the C-reactive protein *(CRP)* gene,[8] rs1800797 (Interleukin-6 (*IL-6)* gene),[9] and rs4129267 (Interleukin-6 Receptor (*IL-6R)* gene).[10] Findings from this study indicated that anxiety was positively associated with IL-6 protein levels. Based on results of the moderation analysis, this association was significant only for individuals with the rs4129267 CC genotype, and not for those with CT or TT genotypes, nor for the rs1205 or rs1800797 SNPs. These findings suggest that there may be genotypic differences in individuals' responses to anxiety, which may place certain individuals at higher risk than others for inflammation, and subsequently CVD. Although further research is needed to verify these findings, results from this study add to the body of knowledge describing potential mechanism(s) through which anxiety is associated with inflammation. Further, these findings provide

preliminary insight into factors that may underlie the relationship between psychosocial stress and CVD risk.

In Chapter 4, results of a study conducted to examine relationships between lifetime experiences of ethnic discrimination, depressive symptoms, and genetic variants associated with depressive symptoms are reported. This was a cross-sectional analysis of data from 124 Hispanic adults at-risk for or with diagnosed CVD. Analyses were conducted to determine whether there were significant associations between lifetime experiences of ethnic discrimination and depressive symptoms or between Val158Met polymorphism and depressive symptoms, and to explore the moderating effect of Val158Met polymorphism on the association between discrimination and depressive symptoms. The results of the analyses revealed that experiences of ethnic discrimination and allelic variants of the Val158Met polymorphism were independent predictors of depressive symptoms, with more experiences of ethnic discrimination and the Met allele independently associated with increased depressive symptoms. Variants of the Val158Met polymorphism did not moderate the association between experiences of ethnic discrimination and depressive symptoms in this sample. This study is among the first conducted to examine associations between ethnic discrimination, the Val158Met polymorphism, and depressive symptoms in a Hispanic sample. Similar to the previous study in Chapter 3, replication of this study is needed to verify the findings. However, these preliminary findings provide a unique contribution to the growing body of scientific knowledge regarding influences of genes on factors associated with CVD risk, such as depressive symptoms. Further, the study provides a much-needed focus on genetic influences on health outcomes in Hispanics, a population seldom included in genetic

studies. Finally, findings from this study provide further evidence of the association between experiences of discrimination and negative health outcomes.

5.3 Impact of Dissertation on the State of the Science

Cardiovascular disease remains the leading cause of death in the U.S., despite ongoing prevention efforts.[1] Nationally, it is estimated that nearly half of all adults aged 20 years and older have CVD.[1] However, certain populations within the U.S. suffer disproportionately from CVD, and have a higher CVD risk burden than other populations. Both adults residing in rural Kentucky and Hispanic adults suffer higher rates of CVD and have higher prevalence of CVD risk factors than the general population.[2,3,11-14] The increased risk for CVD seen in these two populations is further compounded by the presence of unique psychosocial stressors which may increase CVD risk in these populations. The findings from this dissertation further expand our understanding of psychosocial stressors, including anxiety and experiences of discrimination, and genetics as CVD risk factors in populations at increased risk for CVD. Specifically, the outcomes of the studies 1) provide evidence of valid and reliable instruments to measure ethnic discrimination experiences in Hispanic adults; 2) suggest that the relationship between anxiety and systemic inflammation in rural Kentucky adults is moderated by a genetic variant in an inflammatory pathway; and 3) indicate that experiences of ethnic discrimination and a genetic variant associated with dopamine degradation predict depressive symptoms in Hispanic adults.

In Chapter 2, I conducted a systematic review of the literature in which 16 articles describing six measures of ethnic discrimination appropriate for use in Hispanic adult

populations were identified. In Chapter 2, the validity and reliability of each instrument, as well as considerations for their use, were discussed. Findings from this study highlight the inconsistencies in the validity and reliability testing of these instruments with Hispanic populations; some instruments completed psychometric testing with multi-ethnic and multi-racial samples and did not differentiate results by ethnic or racial sub-groups, while others did conduct psychometric testing with entirely Hispanic samples. Further, there is a lack of psychometric testing conducted to validate Spanish versions of these instruments, which are necessary when used with Hispanic samples in the U.S, as 62% of Hispanics in the U.S. are bilingual, and nearly 40% primarily use Spanish.[15] To confirm the validity of these instruments for use in Hispanic populations, future validity testing needs to be conducted with Hispanic-only samples, and both English and Spanish versions need to undergo validation prior to use with Hispanic samples.

In Chapter 3, the relationship between anxiety and systemic inflammation, as well as the possible moderation of this relationship by genetic variants associated with inflammation were explored. Literature about the association between anxiety and inflammation has been contradictory, with findings from some studies supporting this relationship and others disputing it. Our findings did not support a relationship between anxiety and systemic inflammation. The lack of consistent findings in regard to this relationship suggests that additional factors may influence this relationship. Thus, three SNPs associated with inflammation were examined as potential moderators. Of the three SNPs, rs4129267 was the only SNP found to have a moderating effect, with the association between anxiety and IL-6 significant only for those with a rs4129267 CC genotype, and not those with a CT or TT genotype. This is consistent with the current

knowledge about the pro-inflammatory properties associated with the T allele of the rs419267 SNP.[16-18] Our findings suggest that individuals with the CT or TT rs4129267 genotype may experience persistent elevated systemic inflammation regardless of anxiety levels. Conversely, those with an rs4129267 CC genotype, which has not previously been associated with increased systemic inflammation, may experience an increased inflammatory response to stressors, such as anxiety. These findings add to the knowledge about possible mechanism(s) through which anxiety is associated with systemic inflammation. Additionally, these findings can guide future work aimed at identifying individuals who are at higher risk than others for inflammation, and subsequently CVD, based on genotypic differences identified through this study.

The findings described in Chapter 4 support the body of knowledge outlining the negative health effects associated with experiences of discrimination. Although the association between experiences of ethnic discrimination and depressive symptoms has been previously illustrated, these findings add to the literature by providing evidence of this relationship in an entirely Hispanic sample with diverse countries of origin. Further, the findings from this study attempted to describe how experiences of discrimination influence depressive symptoms through the examination of the Val158Met polymorphism as a potential moderator of this relationship. While the Val158Met polymorphism was not found to moderate the relationship between experiences of discrimination and depressive symptoms, the Val158Met polymorphism was found to be an independent predictor of depressive symptoms in this sample. These findings provide a foundation for future work surrounding the biological mechanism(s) which may underpin the association between discriminatory experiences and depressive symptoms in

Hispanic adults. Additionally, our findings provide evidence supporting the association between the Met allele and increased depressive symptoms, a relationship that has been previously described in the literature. These findings suggest that there may be genotypic differences in risk for depressive symptoms, which may place certain individuals at higher risk than others for depressive symptoms, and subsequently CVD, than others. Confirmation of these findings in future work could support interventions tailored based on genotypic differences to ultimately improve CVD prevention efforts.

The findings from this dissertation support the need to identify and address psychosocial stressors, which may influence CVD risk in populations that already bear a high CVD risk burden. By beginning to identify specific stressors, such as experiences of ethnic discrimination, and anxiety, as well as their interactions with genetic variants, such as the rs419267 SNP, we can see that there are certain novel predictors that are associated with increases in known CVD risk factors, including depressive symptoms and inflammation. These findings may help to explain why certain populations are at higher risk for CVD than other populations. Further, identification of these risk factors can help to inform future CVD prevention efforts, and to ensure that interventions are tailored to the populations of interest in order to increase efficacy of the interventions.

5.4 Recommendations for Practice and Research

This dissertation provides groundwork for identifying relevant psychosocial stressors that influence CVD risk in at-risk populations. It is well documented that certain populations suffer disproportionately from CVD. As such, the identification of novel CVD risk factors, including psychosocial stressors specific to these populations, are key

to better understanding cardiovascular health disparities, such as those seen in rural Kentucky and Hispanic adults. Further research is needed to provide more robust evidence of the associations between anxiety and inflammation and experiences of discrimination and depressive symptoms. This future work should be conducted in large samples with more variance in the levels of anxiety and experiences of discrimination and should be able to adjust analyses for all relevant confounding factors. Additionally, further research should aim to identify additional psychosocial stressors that are relevant to the populations of interest.

It is also important that future research be conducted to investigate potential interactions among and genetic influences on psychosocial and other CVD risk factors. Expanding our knowledge about biological and genetic pathways that may affect CVD risk will help to identify individuals and groups at the highest risk for CVD. Future research examining genetic variants and CVD risk should be conducted in large samples, in which comparisons could be made across homogenous sub-samples. It would also be beneficial to use genetic ancestry markers to control for confounding. Further, future research should examine other types of genetic variants and changes, such as DNA methylation, and how these changes may alter CVD risk. Increasing our knowledge about how genetic variants affect CVD risk can help to inform and guide tailoring of CVD interventions so that those at highest risk for CVD receive more intensive intervention.

Upon identification of psychosocial stressors and genetic variants that may influence CVD risk, targeted interventions should be created and tested for effectiveness. Such interventions tailored to specific populations or to individuals within populations,

can be developed to address the most relevant risk factors in order to increase effectiveness of CVD prevention efforts.

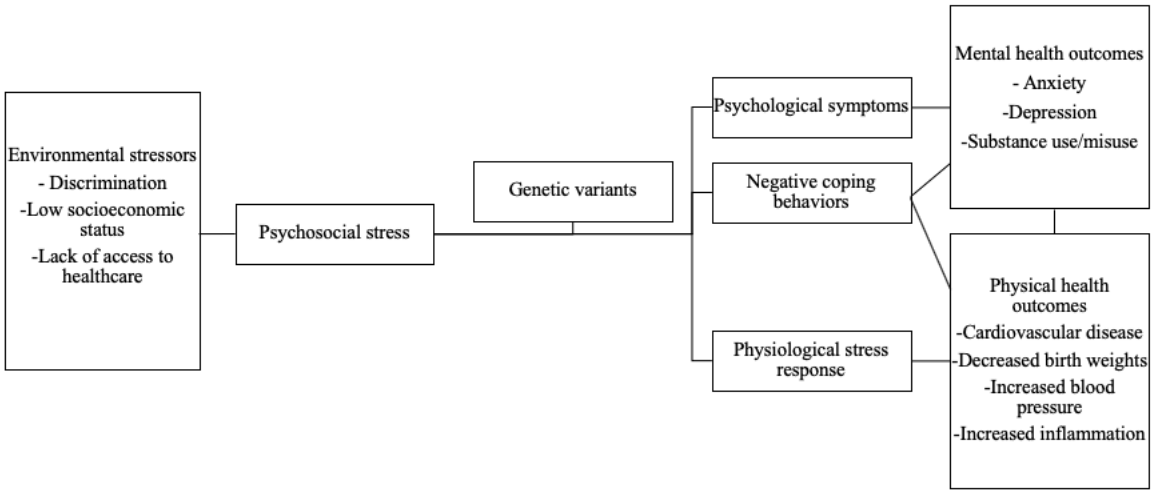
5.5 Limitations

Although this dissertation has filled several important gaps in our knowledge of how psychosocial stressors, genes, and the interaction between these influence CVD risk in at-risk populations, some limitations of this work should be acknowledged. Primary among these, Chapters 3 and 4 describe preliminary findings of associations between CVD risk factors and genetic variants as well as moderating effects of these variants on CVD risk. Study replication is needed to confirm the findings. Specific to the study in which associations between depressive symptoms, experiences of discrimination, and genotypic variations in Hispanics were examined, a larger study in which associations could be examined in more homogenous groups such as populations representative of specific countries-of-origin or genetic ancestry, will be more informative and is recommended for future research.

5.6 Conclusions

Findings from this dissertation suggest that unique psychosocial stressors influence CVD risk in populations known to suffer from cardiovascular health inequities. Psychosocial stressors, in combination with genes and behavioral CVD risk factors, may further increase the prevalence of CVD in rural Kentucky and Hispanic adults. Further studies are needed to provide more robust evidence of these relationships, from which interventions can be tailored to improve CVD prevention efforts in both of these populations.

Figure 5.1 Modified conceptual framework



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Professional Experience

Dates	Institution and Location	Academic Position
2018-present	University of Kentucky, College of Nursing, Lexington, KY	Program Coordinator, *Corazón de la Familia*
2018- 2019	University of Louisville School of Nursing, Louisville, KY	Lecturer
2016-2018	University of Kentucky College of Nursing, Lexington, KY	Graduate Research Assistant

Dates	Institution and Location	Clinical Position
2016-2019	University of Kentucky Chandler Medical Center, Medicine Intensive Care Unit, Lexington, KY	Staff Nurse
2014- 2016	University of Kentucky Chandler Medical Center, Lexington, KY	Nursing Care Technician

Awards and Honors

2019	College of Nursing Dissertation Award, University of Kentucky
2018-2020	Fellowship, DREAM Scholars Program, College of Nursing, Center for Clinical and Translational Science, and Center for Health Equity Transformation, University of Kentucky
2018	Fellowship, Summer Genetics Institute, National Institute of Nursing Research at the National Institutes of Health
2018	Top abstract, American Society of Preventative Oncology Annual Meeting

2016	Inducted, Sigma Theta Tau International Nursing Honor Society
2015	UKHealthCare Commitment to Nursing Excellence Award
2015	Cum Laude, University of Kentucky
2015	Inducted, American Association of Colleges of Nursing Student Policy Summit Academy
2014	Dean's Interprofessional Honors Colloquium
2013-2015	University of Kentucky College of Nursing Undergraduate Research Certificate

Publications

Articles

Key, K.V., Adegboyega, A., Bush, H., Aleshire, M., Contreras, O., Hatcher, J. (2020) #CRCFREE: Using Social Media to Reduce Colorectal Cancer Risk in Rural Adults. *American Journal of Health Behavior.* 44(3):353-363. doi:10.5993/AJHB.44.3.8

Adegboyega, A., Aroh, A., **Voigts, K.**, Hatcher, J. (2018). Regular mammography screening among African American (AA) women: Qualitative application of the PEN-3 framework. *Journal of Transcultural Nursing.* doi: 10.1177/1043659618803146

Hatcher, J., **Voigts, K.,** Culp-Roche, A., Adegboyega, A., Scott, T. (2018). Rural Grandparent Headed Households: A Qualitative Description. *Online Journal of Rural Nursing and Health Care*. 18(1). doi: http://dx.doi.org/10.14574/ojrnhc.v18i1.486

Book chapters, Reports, Monographs, Protocols

Breslin, J.D., & **Voigts, K.** (2014). Incorporating Critical Constituents: Integrating Students into Assessment Planning and Analysis. *2014 Briefs on Academic Support in Higher Education. ACPA* Academic Support Monograph Series.

Manuscripts Submitted for Publication

Key, K.V., Mudd-Martin, G., Moser, D.K., Rayens, M.K., Morford, L.A. Inflammatory genotype moderates the association between anxiety and systemic inflammation in adults at risk for cardiovascular disease. *Journal of Cardiovascular Nursing.* 2020

Published Abstracts

Voigts, K. & Mudd-Martin, G. (2019). Ethnic discrimination is associated with depressive symptoms in Hispanic adults at risk for cardiovascular disease. *Circulation*, 140(1), Suppl 1, Abstract 10895.

Alreshidi, S., **Voigts, K**., Lennie, T.A., Mudd-Martin, G. (2019). Family encouragement for a healthy diet predicts diet quality in Hispanic adults at risk for cardiovascular disease. *Circulation*, 140(1), Suppl 1, Abstract 11671.

Voigts, K., Adegboyega, A., Bush, H., Hatcher, J. (2018). Using Social Media to Reduce Multiple Risk Factors for CRC in Rural Appalachians: #CRCFREE. *Cancer Epidemiology, Biomarkers & Prevention,* 27(3):354. doi:10.1158/1055-9965.epi-18-0049