Quantitative Sensory Testing Reference Values for Healthy African American Adults

BY

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THESIS

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This thesis is dedicated to my parents Judy and Finley Powell, my husband, David Roach, and to my children, Myles Christian Powell Gibbs, and Crystal Michelle Roach, without whom it would not have been accomplished. To my mother who has always said, “keep your eye on the mark”, and encouraged me throughout this process. To my father who died May 2015, after I received my acceptance into the program. He instructed me to follow my dream, “and the rest will follow”. To my husband, who learned to walk this journey with me, and helped strengthen me through his support and unconditional love. To my children, who watch my every move. They inspire me to be the best example for them.

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Contribution of Authors

Chapter 2 is a literature review used to examine the context of my dissertation topic and explore for gaps in the literature as well as future approaches for advancing the science. This work resulted in a published manuscript (as Roach, K.L., Hershberger, P.E., Rutherford, J.N., Molokie, R.E., Wang, Z.J., Wilkie, D.J. (2018) The AVPR1A Gene and Its Single Nucleotide Polymorphism rs10877969: A Literature Review of Associations with Health Conditions and Pain. *Pain Management Nursing*. On-line March 2, 2018.) In this body of work, I am the primary author and initiator of the literature review. Dr. Hershberger help to guide me through the process of independently crafting a literature review. My mentor Dr. Wilkie and my advisor Dr. Rutherford offered insight and constructive critiques to the direction of my research at various stages of the review. Drs. Molokie and Wang are experts in the field who offered additional insight and interpretation for future research based on the findings from the review.

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LIST OF ABBREVIATIONS

ASD	Autism Spectrum Disorder
AVPR1A	Arginine Vasopressin Receptor 1A gene
AVP	Arginine Vasopressin
CD	Cool detection
COMT	Catechol-O-methyltransferase
DNA	Deoxyribonucleic acid
CPTh	Cold pain threshold
EFNS	European federation of neurological societies
GPCR	G protein-coupled receptor
HPTh	Heat pain threshold
IP3	Phosphatidylinositol
MD	Mechanical detection
MPTh	Mechanical pain threshold
OPR	Opioid Pain Reliever
OX	Oxytocin
PCR	polymerase chain reaction
PKC	Protein kinase C
PT	Pain threshold
QST	Quantitative Sensory Testing
RNA	ribonucleic acid
SCD	Sickle Cell Disease
SCT	Sickle cell trait
SNP	Single Nucleotide Polymorphism
WD	Warm detection

SUMMARY

This dissertation contains two manuscripts intended for publication. The first manuscript (chapter 2) is a literature review examining the gene AVPR1A and its pain related single nucleotide polymorphism rs10877969. The second manuscript (Chapter 3) is a databased paper exploring quantitative sensory testing reference values in a sample of 124 healthy African American adults. The abstracts for each manuscript is presented in this summary.

Manuscript 1:

Purpose and Objectives: Pain is the quintessential symptom for individuals suffering from sickle cell disease (SCD). Although the degree of suffering and the cost of treatment is staggering, SCD continues to be grossly understudied, including lack of data for pain related genes and prevalence of polymorphisms in this population. This lack of data adds to the inadequacy of pain therapy in this population. Pain genetics investigators have recently examined allele frequencies of single nucleotide polymorphisms from candidate genes in people who suffer with SCD. One of the genes identified was the arginine vasopressin receptor 1A gene (AVPR1A) and its associated single nucleotide polymorphism (SNP) rs10877969. Progress in explaining pain related polymorphisms associated with SCD can be facilitated by understanding the literature. The purpose of this literature review was to describe mechanisms of the polymorphic gene, *AVPR1A*, and the phenotypic variations associated with its SNPs relative to health conditions and pain.

Data Sources: A comprehensive search was conducted using PubMed, PubMed (OMIM), Cumulative Index to Nursing and Allied Health Literature (CINAHL), Web of Science, Embase, and GeneCards.

Data Synthesis: The search from the six different databases revealed 230 published articles.

Published studies were included if the research addressed AVPR1A and was a full manuscript in a peer reviewed journal, English language, a human or animal study, and published 2009 to present. Abstracts were included if they were in English and provided information not found in a full manuscript. After applying inclusion criteria, twenty-four studies remained for use in this manuscript.

Findings: The results of this review revealed that AVPR1A is associated with behavioral phenotypes, which include pair bonding, Autism Spectrum Disorder, musical aptitude, infidelity, altruism, monogamy, mating, substance abuse, and alcohol preference. In addition, there were associations with pain, stress pain by sex, and sickle cell pain. Summary of this literature could provide insights for future pain research of this SNP in people with SCD.

Manuscript 2:

Background: There are only a few studies in the literature reporting quantitative sensory testing (QST) reference values for healthy African Americans. Those that are reported have a small sample size and do not cover older adults.

Purpose: The purpose of this study is to describe the range of reference values for detection of warm and cool sensation, hot and cold pain threshold, and mechanical pressure (in grams of force) sensation at six body sites, three sites per participant (practice site, upper body and lower body), and to compare mean thermal (warm and cool detection, heat and cold pain threshold) values for differences by age, sex, and testing site location (upper body and lower body).

Method: This was a cross-sectional comparative study of a pain-free adult sample. One-hundred twenty-four participants were recruited for this study (Females 61 (49.2%), Age range 18-69

years: young adults 18-39 years and older adults 40-69 years). Quantitative sensory testing (QST) was used to obtain thermal and mechanical reference values at six body sites.

Results: In this study, women were more sensitive to thermal and mechanical pain modalities than men. Older adults were more sensitive to thermal and mechanical pain modalities than younger adults. When comparing the means of QST reference values at the upper and lower body, it was shown that the upper body was more sensitive to thermal and mechanical pain detection than the lower body. It was also shown here that the reference values for healthy African Americans were more sensitive than the reference values of healthy individuals from other populations.

Conclusion: This is the first study to obtain QST reference values in a large sample of healthy pain-free younger and older adults. These reference values for healthy adults will allow comparisons of QST results obtained in the clinical population, something that was heretofore missing. Of particularly significant importance, our study adds to an emerging body of literature confirming that, in contrast to conventional clinical wisdom, African American adults have lower pain thresholds than do White adults.

I. Introduction

A. Background

In the United States, over 100 million adults have some type of common chronic pain [1, 2], which results in an economic burden ranging between $560 to $635 billion annually [3]. As defined by the International Association for the Study of Pain, pain is: *"an unpleasant sensory and emotional experience associated with potential or actual tissue damage, or described in terms of such damage"* [4]. The sensation of pain follows a path of transduction, transmission, modulation and perception. The types of pain experienced are acute pain, chronic pain, and neuropathic pain. Acute pain has a biological purpose, which is self-limiting, and may last up to 6 months. Chronic pain, which is considered a disease state, may last well beyond normal healing time, does not serve a biological purpose [5], and the end point is usually unpredictable. Neuropathic pain, the result of nerve injury or disease, may be either acute or chronic, and affects the function of the somatosensory nervous system [5]. Sickle cell pain may be acute, chronic, or neuropathic and is influenced by genetic variability [6-10].

B. Disparities in the Pain Experience

Racial and ethnic disparities are pervasive in our health care system [3]; pain is no exception. Oligoanalgesia, ineffective treatment of pain, is found at a higher rate in racial and ethnic minorities [11]. Unrelieved pain results in unnecessary suffering, lost days from work and school, delayed healing, functional disability, and increased hospital length of stays [12]. Despite the high prevalence of painful conditions, African Americans are routinely under-treated for their pain [1, 11, 13-20]. Barriers to adequately address this problem are lack of sufficient information about genetic variability in sickle cell pain and reference values for measures of nervous system function in healthy African American adults.

1. How African Americans are viewed related to pain

The lack of patient- physician relationships especially in the emergency department may play a role in patient stereotypes, and the failure to treat pain [11]. Healthcare providers tend to believe that African Americans do not feel pain with the same intensity as other racial/ethnic groups [21]. Five studies were reviewed that reported disparities in pain. Overall, it was reported that regardless of setting, African Americans received inadequate analgesia compared to their White counterparts [2, 12, 13, 15, 22]. Furthermore, physicians and other health care professionals underestimated the severity of African Americans' pain [2, 13, 15], and their pain medication was less likely to be adequate, even if they had similar pain profiles [2].

2. Sickle cell and pain

Although this dissertation is not about sickle cell pain per se, it is a pain condition that is prevalent among African Americans and is therefore used as an example of a pain experience. Sickle cell disease is a global health problem, but in the United States, impacts predominantly African Americans [23]. It is a group of inherited blood-related disorders involving hemoglobin. It has protective properties in areas of the world that had malaria and was transported to the Americas during the slave trade. Sickle cell disease (SCD) was first described in the medical literature over 100 years ago [24]. It is a painful disease state that disproportionately impacts African Americans in the United States [11, 12, 16, 25]. The sickled shape of the cells causes pain from vaso-occlusion. Every organ system in the body is impacted from an early age. People with SCD are plagued with stroke, blindness, acute chest syndrome, heart attacks, renal failure, leg ulcers, avascular necrosis, liver damage, splenic sequestration, and chronic pain to name a few [23]. With the exception of the stem cell transplant, which is curative, the treatment for sickle cell crisis has not changed in many years [8, 26-28]. The

treatment consists of oxygen, intravenous fluids, hydroxyurea, antibiotics, and pain medications [29].

When patients with SCD have severe pain crisis that results in a visit to the emergency department, they typically have significant wait times for pain control compared to other racial/ethnic groups in the hospital setting [29]. Sickle cell pain can be further exacerbated by stress and perceived injustice [26]. In addition to this, patients with SCD have been heavily impacted by the opioid crisis. In many cases they are not able to get medications with which they had their pain well controlled for years, because physicians have been pressured to decrease the number of opioid prescriptions. Now physicians must follow stringent opioid prescription guidelines that may legitimize the biases that some healthcare providers already have [30, 31].

Patients with SCD are often stigmatized and viewed as drug-seeking, when in fact most do not become addicts [31]. According to the Centers for Disease Control, between 1999 and 2013, ninety-five (95) patients with sickle cell disease died from an opioid pain reliever (OPR) and 174,959 non-sickle cell patients died from OPR [32]. Despite this fact, patients with SCD are in increasing numbers being denied access to opioids, even in cases where they were well managed for years [30-32]. They are forced to manage pain crisis with visits to the emergency department where they are unfairly stigmatized by some physicians who have never previously treated a patient with SCD [30, 32], and fail to understand that higher tolerance to opioid drugs may be secondary to the improved SCD survival rates. The SCD group would benefit from research that provides for comparison reference values for thermal and mechanical detection and threshold in a healthy African American sample.

C. Approaches to understanding pain

The next two chapters of this dissertation include two manuscripts. Chapter two is a literature review for the gene AVPR1A and its pain related SNP rs10877969. Chapter three is a data base paper reporting study results of nerve function in healthy African American adults. Each chapter describes the problem, purpose, methods, results, discussion, and conclusion relevant to the manuscript's topic.

1. Genetics

Genetic variability is suspected to have a role in the perception of pain [33-38]. For example, some of these variations affect metabolism of medications which may result from alterations in pathways [39, 40]. Because of this potential variability, approaches to gain a better understanding of pain may be found by exploring genetic markers, such as single nucleotide polymorphisms (SNPs). A SNP is a single base pair substitution that may result in changes in the downstream sequence, protein expression, or phenotypic changes in appearance, disease risk, and response to drugs or the environment [41]. For example, a larger number of patients with SCD have a cytochrome P450 2D6 polymorphism that decreases the conversion of codeine to morphine resulting in decreased analgesia [39, 40].

Analysis of SNPs related to this gene were the result of the observation that some pediatric patients who were on hydroxyurea that had decreased pain relief from codeine therapy [39, 40]. There are many pain-related genes and SNPs. In one study, allele frequencies were examined using 115 SNPs from 49 pain related genes in a sample of 199 patients with SCD [42]. The AVPR1A gene and its SNP rs10877969, was among the 115 SNPs analyzed [42]. AVPR1A influences capsaicin induced pain levels in humans [43-45]. It was also shown to be significantly related to pain, stress, and sex [45]. This study examined the influence of this SNP on capsaicin

pain levels that appeared only in men who had stress at the time of experimental testing, and these findings were not repeated in women [46]. This SNP was selected for further analysis because of findings from previous studies in which there was a report of a relationship between sickle cell pain and stress.

Progress in explaining effects of pain related polymorphisms can be facilitated by understanding the literature. Pain genetics investigators have recently examined allele frequencies of single nucleotide polymorphisms from candidate genes in people who suffer with SCD. One of the genes identified was the arginine vasopressin receptor 1A gene (AVPR1A) and its associated single nucleotide polymorphism (SNP) rs10877969. The purpose of this literature review was to describe mechanisms of the polymorphic gene, *AVPR1A*, and the phenotypic variations associated with its SNPs relative to health conditions and pain. Published studies were included if the research addressed AVPR1A and was a full manuscript in a peer reviewed journal, English language, a human or animal study, and published 2009 to present. Abstracts were included if they were in English and provided information not found in a full manuscript.

To understand the research findings related to AVPR1A, 24 articles on this topic were identified and grouped by category: mechanisms and health problems. Mechanisms included articles exploring biochemical pathways, genomics, vasopressin, and oxytocin. The health problems were divided into three subgroups: (1) behavior/social, which included autism, bonding, stress, aggression, sex differences, and addiction; (2) pain, which included nociceptive and neuropathic; and (3) sickle cell disease.

The results of this review revealed that AVPR1A is associated with behavioral phenotypes, which include pair bonding, Autism Spectrum Disorder, musical aptitude, infidelity, altruism, monogamy, mating, substance abuse, and alcohol preference. In addition, there were

associations with pain, stress pain by sex, and sickle cell pain. Summary of this literature could provide insights for future pain research of this SNP in people with SCD.

Conclusions of the literature synthesis were that future pain-related SCD research should focus on rs10877969 to explore differences between genotypes in African Americans compared to individuals of African origin and to examine associations with acute or chronic pain of SCD. Another conclusion was that research is needed to determine if stress or sex are associated with AVPR1A and clinical SCD pain. Finally, information gained from this review will serve to increase understanding of the contributions that genetics and genomics play in health science to aid in health promotion and disease prevention, inform policymaking groups, and guide future research that further generates new knowledge for clinical practice and is focused on improving safe and effective pain management [47].

2. Quantitative sensory testing

Quantitative sensory testing (QST) is a widely-accepted method for examining the functional status of the somatosensory nervous system by using various modalities such as cool, warm and mechanical sensation detection and pain thresholds [48, 49]. QST is a standardized psychophysical method that includes a group of tests to evaluate the function of peripheral neuronal fibers (myelinated Aα, Aβ, Aδ and unmyelinated C-fibers) and their pathways to the brain [49]. Thermal testing examines the functionality of thinly myelinated A-delta fibers and unmyelinated C-fibers, and mechanical testing (von Frey filaments) examines the functionality of Aβ myelinated fibers [49]. In recent years, QST has been used with increasing frequency for assessment of the human nociceptive (the response by the nervous system to potentially harmful or harmful stimuli) system and to explore the functionality of the somatosensory system [49].

QST reference values are important because they provide a frame of reference for pathological nerve function conditions. These reference values exist for Asian, Latino, and White populations, but only to a limited degree in the African American population. Availability of QST reference values for healthy African American adults would allow healthcare professionals to develop and provide accurate treatment of individuals who have chronic debilitating pain in this population [49]. When searching for QST reference values for experimental pain in African American adults the reports of findings are limited. Some studies report findings from samples that include African American samples, but the sample sizes are typically small, and the age range generally limited to college-age students [17, 50, 51].

There are differences in QST responses by sex, age, and test site. Structural, functional, and biochemical changes are thought to occur as a part of the aging process, for example, a decrease in the density of unmyelinated C fibers have already begun to occur as early as 30 to 60 [52, 53]. Age related reduction of Substance P, a neurotransmitter of the nociceptive peripheral afferent nerves, in human skin is related to nerve function changes in the aging process. In the central nervous system, degenerative changes have been found in the spinal dorsal horn of older adults, which include loss of myelin and loss of serotonergic and noradrenergic neurons [52]. In addition, changes in the brain include neuronal death [52] and pain perception and pain reaction decrease (increase in threshold) have been shown in older adults [54].

Many studies have shown that men and women perceive and process pain differently. Investigators have explored gender pain differences using multiple modalities and test sites, where women on average are more sensitive than men [55-59]. In healthy individuals, age dependency has been observed in most investigated parameters. Pain perception and pain reaction decrease with age (increase in pain threshold) [54]. In healthy individuals, the location

of the test site may play a role in response time to the sensation. Upper body locations tend to have a shorter response than lower body testing sites. This may be due to the length of the neuron and action potential travel time to the dorsal horn of the spinal column and brain. Only a few studies have reported quantitative sensory testing (QST) reference values for healthy African Americans, and those studies are limited in sample size and age of participants. The purpose of this study was to describe the range of reference values for detection of warm, cool, and mechanical sensations and hot, cold, and mechanical pain thresholds in healthy African American adults and older adults. We also examined the reference values for differences by sex, age, and body test site. This was a cross-sectional comparative study of a pain-free sample of 124 African American adults and older adults (age 18 to 69 years, 49% female). The difference in the QST values between upper and lower body were significant across all thermal modalities, but not for the mechanical modality. These reference values for healthy African American adults and older adults indicate that they are more sensitive than indicated by the reference values of healthy individuals from other populations. The reference values for African American adults and older adults can be used to evaluate patients with acute and chronic pain syndromes.

D. Conclusion

Together, this work is important because it synthesizes the role of the AVPR1A gene and its pain related SNP rs10877969, which provides a lens through which the variation of QST reference values can be viewed. The reference values obtained from healthy African American adults in this study will allow health care providers to improve treatment of individuals from this population who have chronic debilitating pain. The misconception of African Americans not feeling pain like the rest of the population has proven to be detrimental to those suffering from

chronic pain syndromes like SCD, especially when health care professionals are biased in this belief.

The next approach will be to continue to compare the reference values from the healthy individuals to those who have chronic pain. In addition, further exploration of the SNP for the AVPR1A gene will be analyzed in the healthy adult population. The genetics information will be analyzed to explore variations in the QST reference values to understand the contributions of genetics to pain experiences and potential therapeutics. This work will lead to comparisons between QST values from healthy African American adults and patients with SCD to broaden the knowledge of the pain process in the SCD population.

II. The AVPR1A Gene and its Single Nucleotide Polymorphism rs10877969: A Literature Review of Associations with Health Conditions and Pain

Arginine vasopressin is a 7-transmembrane domain G-protein polypeptide that has been identified as being involved in many neurological functions, including aggression, bonding, sex behavior, autism, and schizophrenia [29]. The single nucleotide polymorphism (SNP) of the arginine vasopressin receptor 1A (AVPR1A) gene (rs10877969), is one of 115 polymorphisms of 49 genes that were previously identified as a candidate pain SNP [30]. A SNP is a common type of base pair substitution, which may cause changes in protein expression that lead to differences in response to drugs, appearances, and response to environment. SNP rs10877969 is found in the promoter region of the AVPR1A gene on chromosome 12. Although this SNP has been identified as a pain related SNP, association studies need to be performed. Some investigators have reported that rs10877969 plays a role in acute, neuropathic and stress related pain [31, 32]. In recent studies, investigators found that Catechol-O-methyltransferase (COMT), that metabolizes catecholamines, and AVPR1A are involved in pain modulation [33, 34]. Some of the studies contained in this review, include research that was completed using animal models. These models are important because, some mechanisms are more readily accessible in animal models. Important functions of a deoxyribonucleic acid (DNA) sequence tend to be highly conserved during evolution, the homolog (similar counterpart) will be recognizable in other species and in humans (ortholog) [35]. For genes not yet extensively studied in humans, the research in animals can be informative to guide human research.

The state of the science relative to pain management is currently being expanded, in part because of animal models. For example, animal models were highly instrumental for the recent advances in the understanding of SCD pain mechanisms. The emergence of SCD mouse models demonstrated their suitability as stable models for the study of pain in SCD, which include

"knockout mouse" lines carrying exclusively human globulins after knocking out the mouse globulin gene in the Berkeley sickle cell mouse [36-43]. The use of animal models have led to a better understanding of the types of pain commonly experienced by individuals who have SCD pain. From findings of the animal studies, mechanisms have been revealed and interventions have been explored for the neuropathic pain that this population experiences more than commonly thought [44-47].

We have entered the era of personalized medicine. As a result, nurses are poised to assess, treat, and educate patients about genetically influenced components of disease [48]. Genomic discoveries are rapidly advancing the science of healthcare [49]. Using skilled assessments, nurses who are knowledgeable about genetics and genomics have the ability to help individuals understand and avert potential disorders and resulting morbidity and mortality [50].

The discovery that the SNP rs10877969 has a pain interaction associated to stress and sex, led our team to ask, "what additional findings about the function of this SNP could inform future research focused on the association of this SNP to variables important to pain of SCD?". Specifically, a review of the AVPR1A literature can provide insights for future pain research of this gene and its associated SNP (rs10877969) in people with sickle cell disease (SCD) and other pain conditions. Patients suffering with SCD frequently report chronic, debilitating pain, yet the degree of that pain varies from patient to patient, even among individuals with the same sickle cell genotype [51]. Identifying genetic polymorphisms and their influence on pain phenotypes may result in a better understanding of some of the variations seen in SCD pain [34]. Currently there is a lack of data for explaining pain related polymorphisms associated with SCD, and this significantly impedes the progress in this area of research. The purpose of this literature review was to examine the monoamine gene receptor AVPR1A and its SNP (rs10877969) to explore its

mechanisms, associations with health conditions, and contributions to pain, including sickle cell pain. Explanation of genetic terms are listed in table 1.

A. Method

A search of PubMed, PubMed (OMIM), Cumulative Index to Nursing and Allied Health Literature (CINAHL), Web of Science, Embase, and GeneCards was conducted using the following search terms: "rs10877969" and rs10877969 initially yielded three citations. Because AVPR1A, the gene of interest, is a subgroup of the vasopressin receptor family [52], the search was broadened using the terms "Vasopressin", "AVPR1A", "AVPR1A and polymorphism", "AVPR1A and pain", "AVPR1A and pain and polymorphism", and "AVPR1A and sickle cell anemia or sickle cell disease" as search terms. The search from the six different databases revealed 230 published articles.

As part of the selection process, a study was included if it was a full manuscript, published in a peer reviewed journal, written in English, and reported on human or animal studies. Abstracts were included if they were in English and provided information not found in a full manuscript. The search years were limited to 2009 to present, because most SNP research related to pain was conducted starting in 2011. Publications were excluded if they were commentaries, editorials, unpublished dissertations, primarily review articles, educational material or grey literature (scientific meetings, reports: preliminary, technical, government, or documents) [53, 54]. Retrieved articles were screened based upon title and abstract, and then a second review was performed. Two hundred-thirty articles were screened by title and abstract, of those and 147 were excluded. Ancestral search, and recommendations from experts in the fields, were reviewed for further articles germane to the review topic and were not limited by years, but were limited by all the other exclusion criteria. After reviewing studies from ancestral references, and expert recommendations, 10 additional articles were acquired for eligibility determination.

After further scrutiny, 50 articles were excluded for non-specificity; those articles mainly focused on AVPR1B or AVPR2A. The remaining 43 full text articles were reviewed, of which an additional 19 were excluded because they did not provide new information about AVPR1A. Twenty-four articles remained (Figure 1) for final synthesis.

B. Results

1. Sample Characteristics

The 24 retained articles were grouped into the following categories based on content: mechanisms and health problems. Mechanisms included articles exploring biochemical pathways, genomics, vasopressin, and oxytocin. The health problems were divided into three subgroups: (1) behavior/social, which included autism, bonding, stress, aggression, sex differences, and addiction; (2) pain, which included nociceptive and neuropathic; and (3) sickle cell disease (Table 2).

The AVPR1A studies included humans (adults and pediatrics) and animals (rodents) and focused on mechanisms and health problems. The range of the sample sizes for the human studies were from 33 to 1517. Ten (42%) studies included humans, nine (37%) studies included animals, three (13%) included both humans and animals, and two (8%) studies used cDNA from humans or animal. Eleven (38%) studies included mechanisms and eighteen (62%) included health problems: behavior/social (n = 11), pain (n = 6), and SCD (n = 1). Some of the articles included more than one subgroup. Only one citation addressed AVPR1A and SCD.

In only four human studies, investigators reported ethnicity. One research group reported studying Caucasians only whereas another group reported studying African Americans (adults) and African origin (pediatrics), a third group of researchers reported studying individuals from West Africa, and a fourth group reported studying individuals from Korea.

2. Mechanism

Although arginine vasopressin receptor 1A gene (AVPR1A) has three distinct receptors:AVP_{1a}, AVP_{1b}, and $AVP_{2;}$ [52]. For the purposes of this paper, our focus will be on the V_{1a} receptor. The protein name is Vasopressin V1a receptor, and the gene family is arginine vasopressin and oxytocin receptors [28].

The human AVPR1A gene is located in the long arm of chromosome 12 (12q14.2) [31, 52, 55-60]. The protein coded from this gene behaves as a receptor for arginine vasopressin and belongs to a family of G-protein coupled receptors that include oxytocin. This family of G-coupled receptors mediates cell proliferation and contraction, platelet aggregation, glycogenolysis, and coagulation factor release [28, 52]. AVP_{1a} acts by binding to AVPR1A where it stimulates Gq and activates phospholipase C-beta, leading to the release of diacylglycerol and phosphatidylinositol (IP3). The latter induces the release of calcium from the endoplasmic reticulum [59]. Protein kinase C is activated by diacylglycerol and calcium. The gene is conserved in the human, rhesus monkey, chimpanzee, dog, cow, chicken, rat, frog, zebrafish, and C. elegans [28, 55]. In some species, the 7 trans-membrane peptide hormone consisting of nine amino acids (nonapeptide) has lysine in the 8^{th} position, but in mammals it most commonly has arginine in the 8^{th} position, which is why it is referred to as arginine vasopressin [52]. The human sequence is: Cys-Tyr-Phe-Gly-Asn-Cys-Pro-Arg-Gly. The receptors for AVP_{1a} are located in the kidney, liver, peripheral vasculature, and the brain [61].

There are several aliases for the arginine vasopressin receptor 1A: Vascular/Hepatic-Type Arginine Vasopressin Receptor, AVPR V1a, AVPR1, V1aR, V1-Vascular Vasopressin Receptor AVPR1A, SCCL Vasopressin Subtype 1a Receptor, Antidiuretic Hormone Receptor 1A, Antidiuretic Hormone Receptor 1a, V1a Vasopressin Receptor, and Vasopressin V1a Receptor

[28]. For the purposes of this paper we will use AVPR1A to discuss this gene and AVP_{1a} to discuss the receptor.

3. History of AVPR1A Receptor Cloning

The cloning, sequencing, and functional expression of human cDNA for AVPR1A was described in 1994 by Thibonniert and his research team. They explored structure and functional expression of AVPR1A using cDNA isolated from the liver human subjects [56]. In 1996, the same research team used northern blotting to show the presence of mRNA transcript (5.5kb) expressed in the liver, kidneys, heart, and skeletal muscle [57]. In the same year, further exploration with human genomic libraries showed AVPR1A was included in its entirety along with two coding exons (6.4kb), and PCR analysis of AVPR1A using hybrid somatic cells demonstrated localization to chromosome 12 [57]. The gene was physically mapped to the 12q15-q15 region via in situ hybridization [57].

Results from the inositol phosphate production and calcium mobilization experiments confirm that the receptor function was preserved and coupled to phospholipase C [62]. Using a rat tissue of cloned rat liver cDNA that encoded the AVPR1A gene, it was observed that the amino acid identifies amongst members of the G protein-coupled superfamily and AVPR1A are congregated inside the transmembrane domains [63]. There is a 24-30% sequence identity with the following receptors: human 5HT1a, rat substance P, rat D2 dopamine, and rat endothelin A [63]. Binding to this receptor occurs in the AVP binding site at the N terminal end of the AVPR [61]. Researchers examined the N-terminus of $V_{1a}R$ and analyzed individual residues that contributed to the agonist specific interaction [61], Evaluating the mechanisms of action, researchers found that Arg46 has a critical function for receptor activation and high affinity agonist binding [61]. In addition, arginyl, a residue of arginine, is conserved in all

neuropophysial peptide hormone receptors, and may have a crucial role in the GPCR super family for agonist:receptor interaction [61].

Hasan et al. (2006) explored four novel SNPs in the promoter region of AVPR1A to evaluate if they could be used as a marker for divergent platelet aggregation response to AVP. There was a significant correlation ($r = 0.59$; $p<0.001$) between responses to AVP and those to adenosine diphosphate (ADP), but no differences in AVP – induced aggregation between subjects with and without variant alleles for the four SNPs. As a result, the four promoter region SNPs of the AVPR1A gene may not be useful as genetic markers for platelet aggregation heterogeneity [64].

In another study, investigators explored the role of the AVPR1A in cardiovascular homeostasis using gene targeting (Koshimizu et al., 2006). There was no significant difference between the two groups for heart rate or cardiac function or heart weight. AVPR1A plays a role in normal resting arterial blood pressure regulation mainly by its regulation of circulating blood volume and baroreflex sensitivity, and mice showed altered vasopressor responses to AVP stimulation [62].

4. AVPR1A and Oxytocin

The human AVPR1A receptor belongs to the prefamily of seven, transmembrane segment receptors with a significant sequence identity with the other members of the AVP-oxytocin family of receptors [62]. This receptor possesses high homology with other receptors found in the AVP and oxytocin family [62]. The peptides AVP and oxytocin (OT) are closely related. Vasopressin and oxytocin are nonapeptides located in the pituitary, consisting of nine amino acids arranged in a cyclic structure, and differs at only the 3 and 8 positions. Arginine and phenylanine in vasopressin are replaced by leucine and isoleucine in oxytocin [65-69]. The structure of AVP has a strong similarity to that of oxytocin, and oxytocin appears to have an

interaction with AVPR1A. Human AVP and oxytocin are located on the same chromosome, 12q14-q15, are separated by less than 15bp in most species, both neuropeptides are synthesized in the hypothalamus and secreted from the posterior pituitary gland into the bloodstream [52, 70, 71]. AVP's two primary functions are: water and salt retention and vasoconstriction, other functions include cell proliferation, platelet aggregation, release of factor VIII, von Willebrand factor, social recognition, and circadian tau [29, 52, 62, 68, 72], whereas the major role of oxytocin, is to regulate parturition, lactation, and bonding behavior [73].

5. Behavioral Phenotypes

AVP plays a role in social behavior, jet-lag, sexual motivation, and maternal bonding (close relationship to oxytocin) [58, 60, 74-79]. Researchers examined transgenic mice that had neuroanatomical patterns of AVP_{1a} receptors in the brain similar to prairie voles, a species used widely as an animal model in pair-bonding research, and reported that gene expression for receptor AVPR1A may have functional association to species-typical behaviors in males [60]. Young et al. (2003) later went on to report that prairie voles and montane voles had species-specific behavior patterns associated with differences in a microsatellite in the 5' regulatory region that encodes AVPR1A [74], where selective blocking of AVPR1A in the ventral pallidum, a reward circuit pathway, decreased pair bonding in prairie voles, therefore AVPR1A is critical for pair ponding [74]. While studying double knockout mice that lacked receptors for both AVP_{1a} and AVP_{1b}, it was found that these mice were resistant to jet-lag, suggesting that vasopressin signaling may also be a target for managing circadian misalignment [79].

Studies, in both humans and animals, on the roles of oxytocin and arginine vasopressin (AVPR1A) have shown they play a role in social processes in mammals for social deficits and

neurodevelopmental disorders including autism spectrum disorder, and ejaculatory function [65, 67, 75].

The 5'flanking region of AVPR1A contains three polymorphic microsatellite repeats ($[GT]_{25}$, RS1, and RS3): where activation stimulates phospholipase C. Differing lengths of these repeats have been described in various studies and are associated with behavioral traits, thereby suggesting that they are relevant for brain function related to emotional arousal and social behavior. Subjects with shorter RS3 (repeat sequence microsatellite) have been reported to show less altruistic behavior. Several AVPR1A promoter SNPs were reported to be associated with autism spectrum disorders. Longer AVPR1A RS3 alleles were reported to be associated with elevated levels of prepulse inhibition (the weaker prestimulus inhibits reaction to a subsequent stronger stimulus), particularly in healthy males [29]. This AVPR1A association with the well-known neurobiological phenomenon of prepulse inhibition suggests that a weak startling sound or non-noxious tactile stimuli in those with longer AVPR1A RS3 alleles could blunt future reactions to strong startling stimuli and this decreased startle effect may differ by sex [29].

Yang et.al investigated the association of Korean Autism Spectrum disorder (ASD) families with three AVPR1A SNPs. They demonstrated a possible association between the SNPs and the phenotype of ASD. The findings were statistically significant for association between autism and SNPs in in the promoter region of chromosome 12 [59].

6. Pain

Vasopressin has been described as playing a crucial role in the modulation of pain and several studies have shown that AVP can regulate the pain process in the brain through the mediation of central cholinergic and opioidergic systems [31, 78]. In a recent study, a three-way interaction between, genetics (AVPR1A, re10877969), sex, and acute stress in both mice and humans was demonstrated. In male volunteers reporting stress at the time of testing (capsaicin

pain) that differed based on genotype, and no effects of stress on genotype were found in women [78]. Allele frequencies of rs10877969 differed in White and Asian versus African American volunteers, and the lower pain rating of male volunteers was not observed in African Americans (Mogil et al., 2011). The involvement of AVPR1A in capsaicin pain indicates that vasopressin may have analgesic properties against this pain modality [78]. The mouse AVPR1A gene, encoding for AVP_{1a}, translates to humans in its role in pain and genotype interacts with sex and acute stress [78]. Further examination of the model revealed an endogenous pain relief pathway induced by stress and mediated by AVPR1A, which was replicated cross species (between mouse and human) in a three-way interaction among genotype, stress level, and sex [80].

Another genetic association model was used to observe physical impairment and acute pain. Parr, Fillingim, Wallace, et al (2012) examined AVPR1A and SNP rs10877969 along with SNPs of other genes and described it as having an impact on physical impairment and range of motion. Flexion and range of motion deficit was associated with AVPR1A and COMT, while internal rotation deficit and abduction deficit was associated with AVPR1A. Physical impairment in range of motion and strength had a stronger association with variation in inflammatory and pain modulating genes in comparison to psychological factors in which all predictors had *p*-values of less than .05 [81]. It was later reported that, after muscle injury, neuronal excitability and vasopressin-mediated endogenous analgesia may affect the pain-related influence of emotional or cognitive states [82]. The interaction between pain catastrophizing and depression revealed the phenotype for shoulder pain duration. SNPs from the gene AVPR1A were determined to be possible predictors for shoulder pain duration. Future investigations may explore similar interactions to determine the clinical applicability for the prediction of treatment outcomes.

Studies indicate that both OT and AVR play a role in the inhibition of nociception, but the identity of the main receptor remains obscure. Peng, Qu, Qiu, et al (2015), demonstrated the antinociceptive effect of formalin induced spontaneous nociception. In this study, they showed that spinal AVP reduced pain responses when mice were injected with 5% formalin, which induced spontaneous nociceptive behavior (licking and lifting of the injection site). Spinal dose reduced formalin-induced spontaneous nociception in wild-type mice and had no effect on mice that had been genetically modified (AVP_{1a} receptor knock-out, Peng, 2015).

Oxytocin has been shown to medicate pain by blocking the activity of blocking A delta and C fibers, and by activating various pathways in the neuronal system. Studies that explore analgesia at the periphery have indicated oxytocin receptor (OTR) involvement whereas others have shown AVPR1A involvement [70, 83].

Using Cytidine-5'-diphosphate-choline (CDP-Choline; citicoline) in the rat model, Bagdas et al. (2013), examined analgesic responses via CDP-choline. CDP-Choline enhances central and peripheral vasopressin levels in acute, inflammatory and chronic injury-induced neuropathic pain models in rats without any motor impairment [31]. Centrally administered CDP-choline elicited an analgesic effect through central cholinergic and opioidergic systems, and demonstrates evidence for the utility of centrally acting cholinergic and vasopressinergic agonists in the management of acute and neuropathic pain [31, 84].

7. Sickle Cell Disease

Recently published, [30] reported on patients with SCD from two institutions who participated in a pain study exploring pain phenotypes. The population consisted of African American adults and pediatrics patients of African origin. [30] explored 115 pain related SNPs in 49 candidate genes and compared them to current literature. The genes and related SNPs, are thought to be a part of the monoamine neurotransmitter system and have been previously

implicated in pain. The AVPR1A gene and a related SNP (rs10877969) were identified in this study as prospective candidate for pain genetics studies [30].

C. Discussion

The purpose of this literature review on the polymorphisms of AVPR1A was to examine its mechanisms and associated health conditions, including SCD. This review indicates that studies on pain-related SNPs are limited in number, and studies on pain related SNPs in the sickle cell population are even more limited. In a recent study, this SNP, rs10877969, was found to have a possible role for differences in the perception of pain.

In the mechanisms section, we explored current research on the function of this receptor, and current mechanisms for cloning and genotyping. AVPR1A was found to be highly linked with the oxytocin receptor [52, 65, 67, 70, 71, 85], which explains some of the overlap in functions.

AVPR1A binding has been shown to be highly linked to behaviors and behavioral health conditions. The most prevalent health condition associated with behavior was Autism Spectrum Disorder (ASD) [59, 65, 77]. Associated with features seen in ASD, AVPR1A was seen to linked to associated behaviors such as bonding, stress, and addiction (hyper fixation) [58, 59, 65, 74, 76, 85-87]. In a recent study exploring genetic factors and physical impairment, it was shown that AVPR1A has a role in pain modulation for acute shoulder pain [82]. More recently, a three-way interaction was demonstrated between sex, environment, and stress. This interaction was shown in both the animal and human models [78, 80].

Although the findings regarding AVPR1A and SCD are limited to only one publication, the findings from another study suggested a link to SCD pain for newly explored pain SNPs [30]. Jhun and colleagues [34] found that DRD3 Ser9Gly and COMT Val158Met may contribute to pain heterogeneity in SCD. They also found that patients were likely to have a higher acute care

utilization for acute pain if they had the COMT 158 Met allele or Met/Met genotype. Similar clinical SCD investigations are warranted for AVPR1A.

This review explored the AVPR1A SNP and associated health problems (social/behavioral, pain, and sickle cell disease). Limitations of this review include the recognition that SCD is understudied and additional studies need to be done to explore polymorphisms related to pain that could result in more desirable patient outcomes with respect to pain. Other studies, such as those reported in languages other than English may provide additional insight into the function of AVPR1A.

In summary, future pain related SCD research should include exploring rs10877969 using sufficiently sized samples, and further exploring differences between genotypes in African Americans compared to individuals of African origin. In addition, studies are warranted to explore AVPR1A for associations with acute or chronic pain of SCD and if stress or sex are associated with AVPR1A and clinical SCD pain. Information gained from this review will serve to increase understanding of the contributions that genetics and genomics play in health science to aid in health promotion and disease prevention, inform policymaking groups, and guide future research that further generates new knowledge for clinical practice and is focused on improving safe and effective pain management [50].

Figure 1

Search strategy

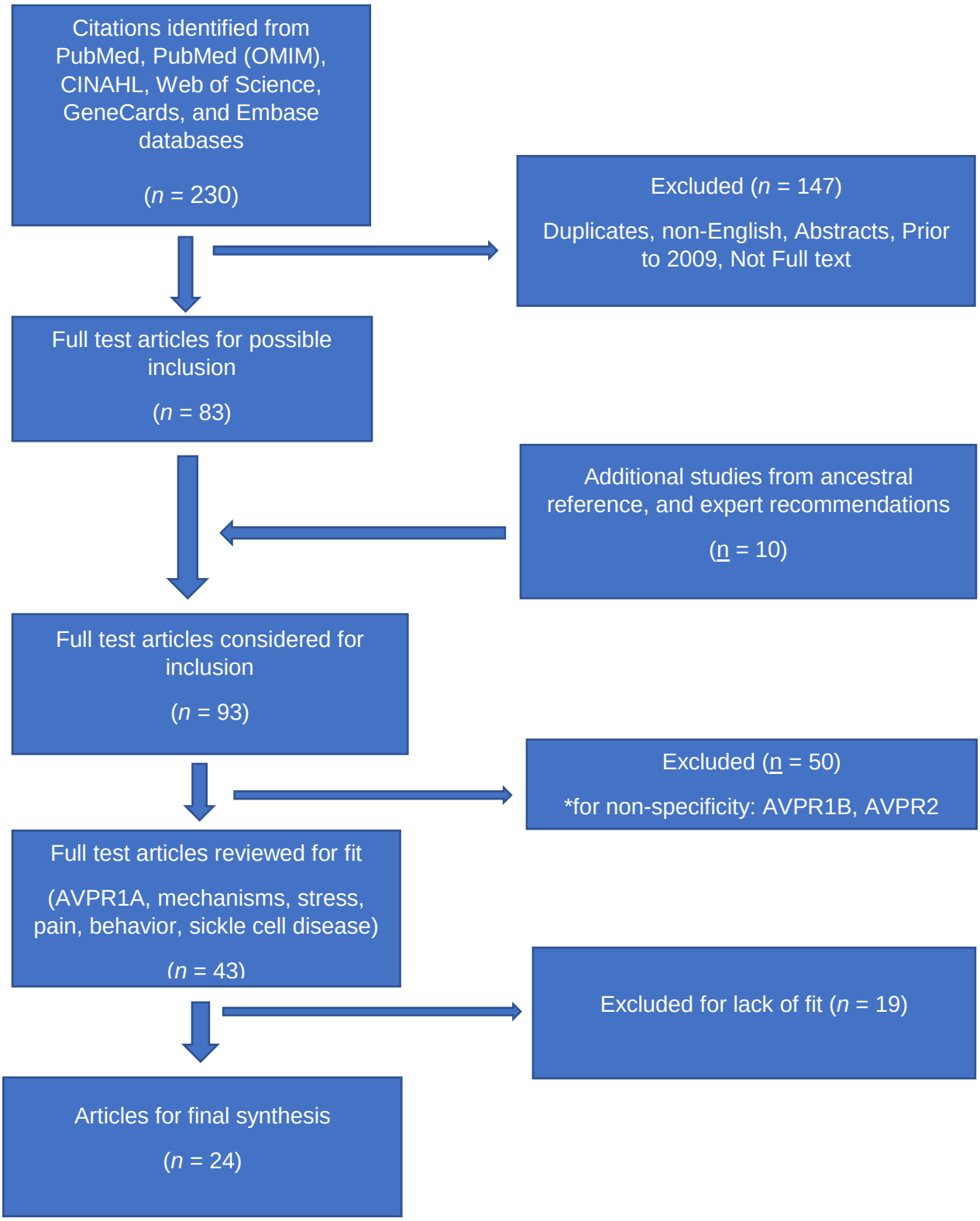

Table 1

Glossary of genetic terms

Term	Definition
5’ flanking region	The end of the DNA strand that is linked by a 3’carbon sugar (Strachan, Goodship, & Chinnery, 2014).
Allele	“Any one of two or more alternate forms of a gene located at the same locus” (Lashley, Kasper, & Schneidereith, 2016), pg. 489, e.g., one of G, A, T, C.
Conserve sequence	DNA sequence that is highly similar or identical across organisms; suggests an important function (Strachan et al., 2014).
G Protein	Guanine nucleotide-binding proteins, or G proteins, act as molecular switches inside cells to transmit signals from a variety of stimuli outside a cell to its interior and play important roles in pain modulation (Ahmad & Dray, 2004).
Gene	A functional DNA unit of heredity that is used to make a product, such as a protein (Strachan et al., 2014).
Homolog	Sequences of two or more genes that are highly similar due to a strong evolutionary relationship (Strachan et al., 2014).
Knockout gene	The targeted creation of a null allele within a predetermined gene (Strachan et al., 2014).

Ortholog	Equivalent genes present in two or more species that evolved from a common evolutionary ancestor (Strachan et al., 2014).
Phenotype	"A physical appearance resulting from the interaction between genes and the environment" (Lashley et al., 2016), pg. 498.
Polymorphism	DNA variants at a significant frequency in the population (Strachan et al., 2014).
Precision Medicine	An approach to the treatment and prevention of disease that considers the individual variability of environment, genes, and lifestyle of each individual (Filipski, 2016, June 28).
Promoter region	A region of DNA that initiates transcription of a gene (Strachan et al., 2014).
Sequence Identity	The number of nucleotides that are matched exactly between two different sequences (Rameez, 2017, April 24).
Single Nucleotide Polymorphism (SNP)	A type of variation in a single base pair of the DNA at specific location in the genome (Lashley et al., 2016).
Transgenic mice	Artificially introduced DNA becomes incorporated into the germ line (Strachan et al., 2014).

Table 2

Summary of the Findings

Author Year	Study Purpose	Sample Description	Key Concept(s) & Major Findings	Conclusions Study Limitations
Morel et al., 1992	Examine cloned cDNA encoding hepatic AVPRIA.	Animals (Rats)	*Mechanisms*. Cloned cDNA encodes hepatic AVPR1A. The hepatic cDNA encodes the seven transmembrane domains and binds AVPR1A. The binding affinities of the cloned cDNA is similar to those of the native rat AVPR1A DNA. The corresponding mRNA is dispersed in the rate tissues where AVPR1A are known to be located.	Amino acid identities amongst members of the G protein-coupled superfamily and AVPR1A are congregated inside the transmembrane domains. There is a 24-30% sequence identity with the following receptors: human 5HT1a, rat substance P, rat D2 dopamine, and rat endothelin A.
Thibonnier, M., 1994	Describe the cloning, sequencing and functional	Human Animal	*Mechanisms*. The structure and functional expression of human	Human AVPR1A receptor belongs to the prefamily of seven-transmembrane segment receptors

	expression of cDNA AVPR1A.		AVPR1A cDNA isolated from the liver.	with a significant sequence identity with the other members of the AVP-oxytocin family of receptors.
Thibonnier, M., 1996	Describe genomic characteristics, tissue expression, chromosomal localization, and regional mapping of the human AVPR1A gene.	Human	*Mechanisms*. Exploration of northern blotting showed presence of mRNA transcript (5.5kb), expressed in the liver, kidneys, heart, and skeletal muscle. Exploration with human genomic libraries showed AVPR1a is included in its entirety and includes two coding exons (6.4kb). When analyzing AVPR1A by PCR using hybrid somatic cells, localization to chromosome 12 was revealed, and use of in situ hybridization	AVPR1A was physically mapped to chromosome 12, region12q14-q15.

			demonstrated that the gene was physically mapped to the 12q15-q15 region.	
Young, L., 1999	Behavior response differences in the prairie vole verses the montane vole are related to AVPR1A receptor binding pattern differences between the species.	Animal (mice)	*Behavior*. Transgenic mice that have neuroanatomical patters of AVPR1A receptors in the brain similar to prairie voles. Gene expression for receptor AVPR1A located it the brain, may have a functional association to special typical behaviors in males.	Arginine vasopressin administered centrally, increases affiliative behavior in the male monogamous prairie vole and not the promiscuous male montane vole.
Birnbaumer, M. 2000	Explore the biological effects of AVPR1A.	cDNA isolation (tissue variability)	*Mechanisms*. Comparison of the structure of the three receptor subsites of AVP: structure, signaling, and testing. Comparison of AVR to OTR.	Maintenance of water homeostasis is the most important physiological role of vasopressin

Hawtin, S. R., 2002	Explore the $V_{1a}R$ N-terminus and analyze individual residues that contribute to the agonist specific interaction, evaluate the required side-chain properties and suggest a mechanism of action.	Cell culture (HEK 293T cells)	*Mechanisms*. Arg^{46} had a decreased affinity for AVP leading to a 1300-fold increase with K_d, to the wild-type, but not with any class of antagonist. N-terminus agonist recognition site was restricted to six residues (Leu^{42}, $Gly4^{3}$, Asp^{44}, Val^{45}, Arg^{46}, Asn^{47}). Constructs without Arg^{46} had impaired intracellular signaling.	Arg46 has a critical function for receptor activation and high affinity agonist binding. Arginly is conserved in all neurohypophysial peptide hormone receptors, and may have a crucial role in the super family of GPCRs for interaction of agonist:receptor. May play a role in substance P binding.
Lim, M. 2004	Demonstrate the role of AVR1A expression in social bonding.	Animals (voles)	*Behavior*. AVR1A is expressed in a higher concentration in monogamous, prairie voles than found in promiscuous, meadow voles.	Similar polymorphisms in the AVPR1A promoter region humans has been linked to autism. Human AVR1A polymorphisms may

			Partner preference on meadow voles was increased when exposed to AVR1A gene (via gene transfer) in the ventral forebrain.	contribute to human variability in social behavior.
Hammock, E. 2005	To determine whether or not intraspecific variation in the microsatellite is sufficient to change gene expression.	Animals (prairie voles)	*Behavior*. There are significant genotype differences in the frequency of pup licking and grooming. In partner preference, long and short allele spent equal amounts of time in total social contact and long allele males spent more time with their partner compared with the stranger in long allele males, and in short allele males. Long allele-males displayed	Species specific patterns of AVPR1A expression appear to be regulated by differences in a microsatellite in the 5' regulatory region of the gene encoding AVPR1A. Vasopressin systems contribute to male, not female, species-typical behaviors.

			partner preference, whereas short-allele animals did not.	
Koshimizu, T. 2006	Explore the role of the AVPR1A in cardiovascular homeostasis using gene targeting.	Animals (mice)	*Mechanisms*. No significant difference between the two groups for HR or cardiac function. No difference in heart weight. Mice showed altered pressor responses to AVP stimulation.	AVPR1a plays a role in in normal resting arterial BP regulation mainly by its regulation of circulating blood volume and baroreflex sensitivity.
Hasan, K 2007	Evaluate four SNPs in the promoter region of AVPR1A to evaluate if they could be used as a marker for divergent platelet aggregation response to AVP.	n = 33 Human (Adult)	*Mechanisms*. Significant correlation ($r = 0.59$; $P<0.001$) between responses to AVP and those to ADP. No differences in AVP – induced aggregation between subjects with and without variant alleles for the four SNPs.	Four promoter region SNPs of the AVPR1A gene may not be useful as genetic markers for platelet aggregation heterogeneity. Platelet aggregation varies among individuals.

Walum, H., 2008	Investigate if the variability in the 5' flanking regions of AVPR1A affects p [air-bonding behavior in prairie voles.	Human model n = 552 Adults Twin pairs Spouses and offspring; Animals (Prairie voles)	*Behavior*. Men that were homozygous for the 334 allele were more likely to be unmarried than the other men.	Results suggest an association between single gene and pair-bonding behaviors in humans, and indicate a well characterized influence of AVP on pair-bonding in voles may be of relevance to humans.
Meyer-Lindenberg, A. 2009	Explore differences in amygdala	Humans (Adults)	*Behavior*. Significant association in AVPR1A with human personality traits. Alleles for polymorphic	Human amygdala function is strongly associated with genetic variation in AVPR1A.

	activation related to specific alleles.	n = 258	microsatellite repeats linked to autism are associated with differential amygdala activation and personality traits in humans.	
Yang, S. 2010	Determine the association between arginine vasopressin receptor 1A gene (AVPR1A) and autism spectrum disorder (ASD).	Humans n = 151 trios (triplet sets)	*Behavior*. Significant association between autism and SNPs (rs7294536, rs10877969, and between autism and haplotype model. ADR-I scores for failure to develop peer relationships were higher in individuals with the AA genotype than n subjects with the AG genotype.	Possible association between the two SNPs and ASDs.
Maher, B.S., 2011	Explore association between AVPR1A	Human (Adults)	*Behavior*. Associations to DUD were detected in three SNPs in AVPR1A.	AVPR1A may influence the risk of dysregulated behavioral

	and drug use disorders (DUD)	n = 757		outcomes through social/affiliative behaviors.
Mogil, J. 2011	Explore the effects of Avpr1a pain and analgesic responses in humans and rodents.	Humans (Adults) n = 104 Animals (Mice)	*Pain*. No genetic association with either SNP was seen in overall mean pain ratings.	Role of AVPR1A in pain is specific to the chemical/inflammation modality, most chronic pain states feature inflammation, and the capsaicin test is thought to be an excellent model of human clinical pain.
Ukkola-Vuoti, L., 2011	Investigate the role of AVPR1A, serotonin, and dopamine systems in active and passive listening to music in	Human (Adults and Pediatrics) n = 437	*Behavior*. Genetic association between amounts of active and passive current and lifelong listening to music. Association with the AVPR1A receptor gene that mediates the effects of highly conserved AVP	Willingness to listen to music is related to neurobiological pathways affecting social affiliation and communication. Similarities between human and animal song have been detected.

	families tested for music aptitude.	Age = 8-93 years	suggests that listening to music is related to the pathways affecting attachment behavior and social communication.	
Jern, P. 2012	Investigate associations between SNPs linked to OT and AVP receptor genes and ejaculatory function.	Human (Adult) n = 1517 twin males	*Behavior*. Heterozygote effect on one SNP in the OTR gene (rs75775), individuals had a significantly elevated risk for symptoms of premature ejaculation.	Rare variants in AVP genes may have significant effects on premature ejaculation.
Bagdas, D. 2013	Assess possible role of AVP receptors in the analgesic effect of CDP-choline on acute and	Animals (rats)	*Pain*. Intracerebroventricular administration of CDP-choline increased plasma vasopressin levels in a dose-dependent and time-dependent manner. Intracellular administration of CSP-choline	*Intracerebroventricular administration of CDP-choline elicits an analgesic effect in rat models of acute and neuropathic pain. Central AVP_1 and AVP_2 are involved in the analgesic effect of

	neuropathic pain in rats.		increases Ach levels in the caudate nucleus and periaqueductal gray.	CDP-choline. Histological studies suggest that AVP-containing fibers may play a role in the pain modulating nuclei of the brain.
Juif, P. 2013	Characterize the effects of physiological blood concentrations of OT and AVP on spinal nociception and on pain responses.	Animals (Rats)	*Pain*. Action potentials mediated by C-type nociceptive fibers was strongly reduced (antinociception) after IV injections of low doses of OT (<5 μg) or AVP (<500 pg). An increase (pronociception) was observed at higher doses.	Blood levels of OT and AVP modulate nociception, windup plasticity and pain responses. These effects remain to be identified but are likely to be C-type nociceptors
Yamaguchi, Y., 2103	Identify candidate signaling molecules that might contribute to jet-lag.	Animal model (mice)	*Behavior*. V1a and V1b receptor double knockout mice in a light controlled environment.	Mice without arginine vasopressin 1a and 1b receptors were resistant to jet-lag. Results suggest that vasopressin signaling may be a

				target for managing circadian misalignment.
George, S., 2014	Identify genetic and psychological interactions predictive of exercise-induced shoulder pain phenotypes.	Human	*Pain*. After muscle injury, neuronal excitability and vasopressin-mediated endogenous analgesia may affect the pain-related influence of emotional or cognitive states. The interaction between pain catastrophizing and depression revealed the phenotype for shoulder pain duration.	SNPS from the gene AVPR1A were determined to be possible predictors for shoulder pain duration. Future investigations may explore similar interactions to determine the clinical applicability for the prediction of treatment outcomes.
Moons, W., 2014	Assess the baseline and post stressor levels of plasma OT, plasma AVP and its	Human (Adults) n = 166	*Behavior*. Women with high levels of post stressor OT and the GG genotype felt the most positive affect after the stressor.	Oxytocin and vasopressin receptor genes interact with levels of OT and AVP to predict sex-specific emotional response to stress.

	effect on positive affect, and anger.	(68 men/98 women)	Men with high levels of post stressor AVP polymorphism reposted more post stressor anger than non-carriers.	Biological sex, receptor polymorphisms, and endogenous neuropeptide levels jointly predict emotional outcomes in humans.
Jhun, E. H., et.al.; 2015	Identify genetic polymorphisms and their influence on pain phenotypes to explain pain variances seen in SCD.	Humans (Adult and pediatric) African origin African American n = 199	*Pain*. Genotype and allele frequencies of SNPs were found to be different between AA cohort and West A cohort; or adult and pediatric cohort. Candidate pain genetic studies may aid in designing precision pain medication. Sickle Cell Disease	Differences were found between the two cohorts, but it is unclear as to whether the difference was attributed to being African vs African American, or pediatric vs adult. Relation to monoamine neurotransmitter and TNP (Transient Receptor Potential Channels) and its implication to pain for targeted therapies.
Peng, F, 2015	Explore the role of spinal vasopressin	Animals (Mice)	*Pain*. Spinal AVP dose dependent reduced formalin-induced	Spinal AVP can exert an antinociceptive effect on the

	and its impact on $GABA_A$ receptor function in mice.	spontaneous nociception in wild-type mice. AVP had no effect on formalin-induced spontaneous nociception in AVPR1A receptor in knock-out (V_{1A} -/-) mice. Antinociceptive effect of AVP was reversed by co-treated $GABA_A$ receptor against. AVP potentiated GABA-activated currents in wild-type littermates, but not V_{1A} -/- mice.	second phase of formalin-induced spontaneous nociception. A novel mechanism of AVP analgesia by enhancing $GABA_A$ receptor function in primary sensory neurons via AVPR1A receptors.

Articles are listed chronologically because some of the articles include information relevant to more than one category.

III. Quantitative Sensory Testing Reference Values for Healthy African American Adults

(Previously published as Roach, K.L., Hershberger, P.E., Rutherford, J.N., Molokie, R.E., Wang, Z.J., Wilkie, D.J. (2018) The AVPR1A Gene and Its Single Nucleotide Polymorphism rs10877969: A Literature Review of Associations with Health Conditions and Pain. *Pain Management Nursing*. On-line March 2, 2018.)

African Americans are consistently undertreated for post-operative pain, chest pain, and acute pain presenting to the emergency department [1, 2]. Unrelieved pain is a major health problem among African Americans, leading to unnecessary suffering, delayed healing, functional disability, increased length of hospital stays, and lost school and work days [3, 4]. Quantitative Sensory Testing (QST) is an objective method to identify sensitization in small nerves (A-delta and C fiber) [5-9]. Findings from several studies using QST among sickle cell disease (SCD) patients reveal that adequate reference values are unavailable for interpretation of QST results from African American adults [10-14]. Previous QST studies of healthy African American adults were based on relatively small samples [15]. Those studies primarily included young adults such as college-aged undergraduate students [11, 14, 16] or siblings of pediatric patients with SCD [10, 17, 18]. None of these studies included older adults or sampling plans balanced by age and gender. The lack of sufficient QST reference values for healthy African American adults, especially older adults, is a barrier to research that is needed to guide healthcare professionals in accurately treating pain in this population. The purpose of our study was to rectify this gap by characterizing thermal and mechanical QST reference values in a healthy sample of African American adults.

Racial and ethnic disparities are pervasive in our health care system [19]. These disparities occur in the context of numerous illnesses and syndromes [20]. Sickle cell disease and cancer are just two painful disease states that disproportionately impact African Americans. One in 365 African American newborns is diagnosed with sickle cell disease every year, and there are

190,000 new cancer cases in African Americans per year across age categories [21]. There are racial and ethnic differences in individual responses to pain [13, 15, 22-26]. These differences are typically misunderstood by health care providers because it is not known how healthy African American adults and older adults respond to painful stimuli [2, 3, 19, 27]. QST studies can help provide reference values in younger and older adults.

QST is a non-invasive method used to detect nerve damage at small and large sensory nerve endings that detect change in cold and warm temperatures and pressure and then communicate the information to the central nervous system. QST reference values for healthy adults have been shown to differ among race/ethnicities by age, sex, and body location [12, 13, 28]. Latin-Americans have lower thermal induced pain thresholds than those reported in the White population [24]. In a sample that was ≥ 85% White, age was a significant factor in thermal and mechanical thresholds with sensitivity decreasing with age [29]. Sex differences have been demonstrated in pain processing with women having a lower pain threshold to heat and mechanical stimuli [30-32]. Whereas age and sex differences have been explored with QST in Chinese, Hispanic/Latino, and White adults, there are no reports of those findings within the African American population.

QST can be conducted at various body sites. The most common test sites are the forearm, dorsum of the foot/hand, ventral forearm, and thenar eminence, with the most common sites tested being thenar eminence and the dorsum of the foot. [5, 7, 10, 17, 28, 33-38]. QST values differ between the upper and lower body sites, with the upper body sites detecting thermal stimuli earlier than lower body sites [5, 24, 39, 40].

The aim of this study was to describe the range of reference values for detection of cool, warm, and mechanical sensations and cold, hot, and mechanical pain thresholds, at six body sites

and to compare these reference values for differences by age, sex, and testing site location (upper body and lower body). Availability of reference values in a sample of healthy African American adults will help to broaden the understanding of pain and help improve the pain treatment of individuals in the African American's who suffer from altered pain conditions, including SCD.

A. Materials and Methods

1. Design

This was a cross-sectional comparative study of a pain-free adult sample. The Institutional Review Board at the University of Illinois at Chicago approved the study.

2. Participants

Participants in this study were pain-free African American adults. Inclusion criteria included: (a) African American, (b) health history negative for diabetes mellitus, polyneuropathy, hypertension, SCD, SCT, cancer, and current or chronic pain, (c) English fluency in speaking and reading, and (d) age ≥ 18 years. Exclusion criteria included: (a) being legally blind, (b) inability to physically or mentally to complete study measures, (c) use of prescription pain medications or recreational drugs, and (d) report of acute pain within the past 48 hours.

Volunteers were recruited from the UIC campus, surrounding communities, churches, local sororities, fraternities, community organizations, by word of mouth, flyers, and social media. The study was conducted at the University of Illinois at Chicago, College of Nursing (UIC CON). One hundred twenty-five individuals were recruited, gave informed consent and completed the measures (Table 1). One participant, who passed the verbal eligibility criteria, was removed from the study because she electronically reported race as Asian and parents as being born in India.

3. Quantitative Sensory Testing

We used well-validated measures for the thermal and mechanical QST [12]. The testing protocol was consistent with the FENS (European Federation of Neurological Societies) recommendations for testing Aβ, A-delta, and C fiber function [5]. QST measures response in 6 modalities: cool detection (CD), warm detection (WD), mechanical sensation, cold pain threshold (CPTh), heat pain threshold (HPTh), and mechanical pain threshold (MPTh).

a. Thermal.

The TSA-II NeuroSensory Analyzer (Medoc) was used for the measurement of the participants' thermal response values**.** The TSA-II is a precise, computer-controlled device capable of generating and documenting responses to highly repeatable thermal stimuli, such as cool detection, warm detection, cold-induced pain, and heat-induced pain. The TSA-II delivers quantitative assessment of small caliber sensory nerve function and was used to identify thermal pain thresholds. For participant safety, the TSA has software that halts the heating or cooling if any thermode problems are detected, and the TSA-II has a hardware mechanism that overrides the software and disconnects the thermode power if the temperature reaches 53º C to 54º C, the temperature at which tissue damage occurs.

In a private temperature-controlled research room, the TSA-II thermode with a Peltier element (30 x 30 mm) and a cooling water system was placed on the skin to deliver standardized stimuli for determination of cold detection, cold pain threshold, warm detection, and heat pain thresholds [7]. To avoid tissue damage, the limits protocol was used. This protocol has a predetermined cutoff temperature for all trials was 50º C for heat and 0º C for cold. Using this protocol, the probe was placed on bare skin, and the temperature increased from a baseline of 32ºC (adaptation temperature) with a 1º or 1.5ºC/second rate of rise for warm detection and heat

pain threshold, respectively. Cool detection and cold pain threshold were evaluated from the baseline of 32ºC with a 1º or 1.5ºC/second rate of decline. The participant responded to the temperature stimuli by pushing a button and the stimulus returned to the 32º C adaptation temperature. A threshold value was determined in accordance with increasing or decreasing temperature of the thermode contact surface [7]. There was a 30-second inter-stimulus interval between test repetitions. The warm and cool sensation detection was presented in three repetitions per site (averaged by site) and the heat and cold pain detection was presented in the three repetitions per site (averaged by site) [12]. The actual pain threshold was calculated from three consecutive individual values within an arithmetic average.

Detection of warm is usually 1-2ºC above 32ºC (the adaptation temperature) and is mediated by C fibers. Cold sensation usually occurs at a similar range below adaptation and mediated by A-delta fibers. Heat pain threshold is usually about 45ºC and is mostly mediated by C fibers with some A-delta fiber involvement. Cold pain threshold is the most variable and difficult to assess of all these modalities, but it is usually sensed at about 10ºC as mediated by a combination of both C and A-delta fibers. In a recent study, cold pain threshold was shown to be consistent in a 10-week test re-test study [41].

b. <u>Mechanical</u>

To capture the mechanical detection thresholds (Aβ nerve fibers), QST was conducted for mechanical detection/pain threshold using standardized, calibrated von Frey monofilaments**.** Von Frey filaments are measuring devices calibrated to bend at a set amount of force depending on the thickness of the filament. To ensure accurate testing of the detection threshold and pain threshold, the filament was placed in the same perpendicular manner to the area being tested until the filament showed an “s-shaped” bending pattern [7]. The contact time to the surface of the skin during testing was about one second. When a calibrated filament is

applied to the skin, a non-painful or a painful response to mechanical transient indentation of the tissue is produced by the filament. Seven filaments were used in sequence, from lightest to heaviest, starting with 3.84 (0.6g) and ending with 5.88 (60.0g). These filaments were selected based on previous studies that provided both clear non-painful sensations in all patients and a painful sensation in some patients as per the EFNS protocol [34].

4. Procedure

Data collection was schedule at times convenient to participants, including evening and weekends, to be sensitive to the needs of our target sample. Multiple contact information was collected (e-mail, mobile phone, and home phone) to maximize contact of the participants on the day before and the day after their appointments. The room where research data collection occurred was temperature controlled and adequate for the QST protocol. The testing occurred with the participant seated on a comfortable leather recliner and positioned at approximately 45ºC for adequate implementation of the QST protocol. After written consent was obtained, participants provided demographic information, and then QST reference values were collected.

Previous work by our team identified six test sites based on frequent references in the literature, and ease of access [5, 6, 9-11, 39, 40, 42-44]. QST reference values were obtained across the entire sample from a combination of six sites total: three upper extremities (forearm anterior, forearm posterior, and upper arm lateral) and three lower extremities (leg lateral, leg medial, and leg posterior). Each participant, however, contributed only three test sites: the practice site (anterior forearm), one upper site and one lower site. Test sites were randomly selected until a 20-subject quota was reached for each site. One site per limb was tested (Table 2). Over recruitment was required to obtain the appropriate number of older males.

The participants received instructions for QST testing procedures. For cool and warm detection, the participant was instructed to press the button when a cool/warm feeling was first detected. This was repeated for three to five trials depending on the detection points. For heat pain threshold and cold pain threshold, the participant was asked to push the button at the moment when the heat/cold sensation first felt painful.

Using the von Frey filament, starting at 0.6g of force, the participant was asked to report when they first felt a sensation and the strength of the filament was recorded. The filaments were tested in increasing order of force and testing stopped when the participant reported a force as being painful. As with the EFNS protocol, each site was tested with three repetitions for each filament size and a report of pain for any one of the repetitions was documented and testing stopped.

An incentive of $50 was provided to cover the cost of transportation, travel, and subjects' time to complete the study measures. Payment was rendered once all testing was completed.

5. Statistical Analysis

The data for the 6 modalities were examined by age group 18-39 years (i.e., younger adult) and ≥40 years (i.e., older adult), sex, each test sites, and location category of sites (i.e., upper vs. lower). The initial analysis described the modalities at the six different body sites using mean (*M*), standard deviation (reported as ±) and range. Additional analyses used independent t-test to examine the means for each modality by age, sex, and paired t-tests to examine the means of upper compared to lower body values. Analysis was performed with SPSS version 24 for windows and Stata version 14.2.

B. Results

Table 1 shows the sample demographic information. All participants were healthy pain-free adults, ranging in age from 18 to 69 years and were sensate to thermal and mechanical

detection (MD) of stimuli. The mean age was 38.6 ± 12.5, 64 (51.6%) were 18-39 years and 60 (48.4%) were ≥40 years of age, and 61 (49.2%) were female. The 124 participants identified as African American, including those who also reported being Hispanic/Latino (n=5) or multi-ethnic (n=4). Fifty participants (40.3%) had an associates degree or greater and 74 (59.6%) had some college or less.

Descriptive statistics for QST reference values are reported in Table 2. There were 124 participants providing QST data for the anterior forearm, and 48 to 53 participants providing QST data for the other five site subgroups (Table 2). Reference values across each modality generally did not fluctuate by site.

1. <u>Sex</u>

At the anterior forearm, differences in CD values were not statistically significant by sex, but WD values were significantly lower for females (M = 34.3 ± 1.1) than males (M = 34.8 ±1.2; t_{122} = -2.66, p = .009). On average females detected CPTh and HPTh (1.8 and 1.3 °C) earlier than males. CPTh values for females (M = 27.2 ± 3.6) were significantly higher than males (M=25.4 ± 5.9; t_{122} = 2.05, p = .043), and HPTh values were significantly lower for females (M=37.1 ± 3.4) than males (M=38.5 ± 3.7t_{122} = -2.09, p = .039). Differences in MPTh values were not statistically significantly by sex.

At the posterior forearm, across all modalities, none of the differences in the values were statistically significant by sex. For the lateral upper arm, detection differences were greater than 1°C across all thermal modalities and MPTh at 11.5g between females and males. Differences in CD, CPTh, HPTh, and MPTh values, however, were not statistically significant by sex. On the other hand, there was a statistically significant difference in WD values for females (M = 34.1 ± 1.0) than males (M = 35.8 ± 3.7; $t_{28.3}$ =-2.19, p = .037).

At the lateral leg, differences in CD, WD, CPTh, and MPTh values were not statistically significant by sex. At the medial leg, differences in CD, CPTh, HPTh, and MPTh values were not statistically significant by sex. Females responded to WD and HPTh on average 1.1 and 1.6 °C sooner than. These differences in WD values were statistically significantly lower for females (M = 34.8 ± 1.4) than males (M = 35.9 ± 2.5; t_{48} = -2.03, p = .048).

At the posterior leg site, differences in CD, WD, and CPTh values were not statistically significant by sex. Females detected CPTh, and HPTh on average earlier than males (1.2 and 2.2 °C respectively) and detected MPTh on average 13.4 g earlier than males. There was a statistically significant difference in HPTh values with female values being statistically significantly lower (M = 37.5 ± 3.2) than males (M = 39.7 ± 3.3; t_{47} = -2.42, p = .019). Similarly, mechanical threshold values were statistically significantly lower for females (M = 11.7 ± 16.50) than males (M = 25.1 ± 24.7; t_{47} = -2.2, p = .030) (Table 2).

2. <u>Age</u>

As shown in Table 2, at the anterior forearm, older adults detected HPTh on average 1°C earlier and mechanical threshold 6.7g less than younger adults, a statistically significant difference (younger adults M = 38.5 ± 3.4; older adults M = 37.5 ± 3.7; t_{122} = 2.34, p = .021). Differences in WD, CPTh, MPTh values were not statistically significant by age. There was a nearly statistically significant difference for CD scores for younger adults (M = 29 ± 1.9) by age.

At the forearm posterior site, differences in CD, WD, CPTh, HPTh, and MPTh values were not statistically significant by age. In the lateral upper arm, differences in CD, WD, and MPTh values were not statistically significant by age. Older adults experienced CPTh and HPTh on average 4.2 and 3.4 °C earlier and mechanical 7.3g less than younger adults, and these

differences were statistically significant for CPTh values (younger adults M=23.4 ± 7.6; older adults M = 27.6 ± 2.5; $t_{36.9}$ = -2.8, p = .008) and HPTh values (younger adults M = 40.6 ± 4; older adults M = 37.2 ± 3.3; t_{51} = 3.22, p = .002).

At the lateral leg site, differences in CD, WD, CPTh, HPTh, and MPTh values were not statistically significant by age. At the medial and posterior leg sites, for each of the modalities, none of the differences of the values were statistically significant by age (Table 2).

3. Upper and Lower body sites

Paired t-tests were used to examine the differences in the means of QST values between upper and lower body sites for individuals. Difference in the QST values between upper and lower body were significant across all thermal modalities but was not significant for the mechanical modality (Table 3). Thermal detection (CD, WD) and pain threshold (CPTh, HPTh) occurred earlier in the upper body sites compared to the lower body sites.

C. Discussion

This is the first study to provide extensive QST reference values for healthy African American adults, including older adults, with six modalities (CD, WD, CPTh, HPTh, MD, MPTh). We also examined differences in these reference values by sex, age, and body sites. As we expected, all participants were sensate to low thermal and mechanical stimuli which supports the validity of the rest of our test results. Our findings provide reference values for healthy African Americans. The results of this study are relevant, regardless of sex and age, QST reference values for African Americans in the mid-western United States using the limits protocol. Although, there were some statistically significant findings of differences by age and gender there were no clear patterns that would have been significant had we used the family confidence interval adjustments for the multiple t-tests that were used in this exploratory study.

However, the findings that upper sites were more sensitive than lower sites are robust, valid, and consistent with what other researchers have found.

1. Sex

Men and women differed significantly in their response to stimuli, with women being more sensitive to thermal stimuli than men. Mechanisms contributing to sex differences are not clear, although hormonal, cortical brain influence, social, environmental, and genetic factors have been found to influence pain perception [45, 46]. Previous investigators have explored sex-based pain differences using multiple modalities and test sites [46-50]. Fillingim et al., reviewed sixteen studies that explored gender differences in heat pain threshold [46]. Of those, findings from eleven studies revealed women had a lower heat pain threshold than men, whereas five studies showed no difference between women and men in this modality [46]. All of the studies used contact heat as the source and the stimulation sites varied between the face, hand, and arm (upper body). Of nine studies evaluated for cold pain response, six studies revealed women were more sensitive to cold pain threshold than men, whereas three studies revealed no difference in cold pain threshold [46]. Our lateral forearm site findings from adults and older adults, therefore, are consistent with the prior findings that women were more sensitive to cold and heat pain than men.

Men and women differ in their experience of mechanical sensation as well, with women exhibiting lower thresholds [29, 32, 44, 47, 51] [38]. Eight studies reported findings for mechanical pain threshold, and of those, six studies revealed women were more sensitive to mechanical pain than men, and the results from two studies revealed no difference between women and men [46]. Neziri found that sex, age, and body site were related to heat pain thresholds [9]. Wang et al., reported similar results in a sample of Chinese adults. For cold pain

threshold, men had lower thresholds (11°C) (lower sensitivity) than women (20.4°C) [44]. For heat pain threshold, men had higher thresholds (42.7°C) (lower sensitivity) than women (38.3°C) [44]. Overall, compared to men, women have a significantly lower pain threshold to heat and mechanical stimuli [7] and have a more pronounced sensitivity to cool detection and cold pain threshold [6, 40, 46, 47, 52-54]. On average, males tend to detect warm sensation at the anterior forearm, leg medial, and upper arm lateral at higher temperatures than females. The mean cold threshold is higher for females, and heat threshold is higher for men at the forearm anterior and leg posterior. Overall, our results that women detect thermal and mechanical changes earlier than men concur with findings in the literature [9, 46, 49, 55, 56].

2. Age

In healthy individuals, age dependency has been observed in most investigated parameters. Structural, functional, and biochemical neuronal changes are thought to occur as a part of the neuronal aging process [26, 57]. For example, a decrease in the density of unmyelinated C fibers, those fibers implicated in thermal sensation, has already begun to occur as early as age 30 to 60 [26, 57]. As age increases, thresholds for cold pain and heat pain also increase [6]**.** Age related reduction of Substance P, a neurotransmitter of the nociceptive peripheral afferent nerves, in human skin is thought to play a role in decreased sensitivity with the advancement of age. In the central nervous system, degenerative changes have been found in the spinal dorsal horn of aged adults, including loss of myelin, loss of serotonergic and noradrenergic neurons [57]. In addition, age-related changes in the brain include neuronal death [57]. Pain perception and pain reaction decrease with age (increase in threshold) [22]. Per recent studies, the changes result in less sensitivity to warm and painful stimuli and occurs at a greater frequency in the lower extremities, mostly tested at the knee [26].

A recent meta-analysis of 10 studies reported pain threshold (PT) using various stimuli. Five studies reported age-related increase in PT, and an additional study demonstrated no age-related increase in PT [26]. Age-related decline in sensitivity occurs at the forearm, thigh, and calf in addition, and differences between upper and lower body are more pronounced [57]. Among healthy study participants, age was related to all investigated QST parameters such as thermal testing (cool detection, warm detection, heat pain tolerance, cold pain threshold) and mechanical testing (vibration detection, von Frey filament) [7]. The largest age-related effects were detected for cold pain thresholds followed by heat pain thresholds [7]. In the current study, young adults tended to have a higher HPTh than the older adults (less sensitive) at the forearm anterior, upper arm lateral locations and a higher CPTh than older adults at the lateral upper arm. It has been reported in the literature that sensitivity to thermal detection varies with age [58] and others have reported decrease in sensitivity with the aging especially in the extremities [29, 59] with the greatest increase is at the HPTh [26]. HPTh at the posterior leg is consistent with the finding that sensitivity degrades at greater increments in the lower extremities [26]. Overall, in this sample older adults detected thermal and mechanical changes earlier than young adults.

Previous studies exploring thermal and mechanical pain threshold using college aged students reporting values for HPTh, CPTh, and MPTH, while our sample mean CPTh for younger and older adults were 26.1°C and 26.5°C, respectively, previously reported results were lower (9.8°C) [12]; HPTh was 38.5 °C in the younger adult and 37.8°C in the older adult and published results indicated 40.7°C – 45.2°C) [10-12, 14, 29, 33], and MPTh pressure comparisons were reported as 17g [17], and our adults young and old were 19.6g and 13.3g respectively. Most of the studies were from samples of African Americans, one study included pediatric participants, and another study did not report ethnicity.

3. Upper/Lower body site

Although many body sites have been used for QST the upper verses lower body sites have been consistently utilized in QST studies. Reported findings from upper verses lower have been the most consistent over time. Using quantitative sensory testing, Hagander (2002), recruited 46 healthy volunteers and used QST to explore cool and warm detection thresholds and pain thresholds on four different body test sites; the thenar eminence, volar surface of the wrist, dorsum of the hand at the first metacarpal space, and the dorsum of the foot on the non-dominant side [60]. The goal of their study was to determine if there was a significant difference in thresholds at the tested sites and to identify a preferred site for testing for each thermal modality [60]. The results revealed statistically significant thermal threshold differences between sites. When exposed to cool detection thresholds the hand had greater sensitivity than the foot. When evaluating warm detection threshold at the thenar eminence, the site had greater sensitivity than the wrist. There were no differences for sensitivity between the wrist and hand [60].When exploring heat pain, sites at the hand and foot did not differ in sensitivity [60]. Campbell et.al. used test sites at the thumb, trapezius, forearm, and quadriceps. Neziri et.al., used the lower back, suprascapular region and second toe or lateral leg to obtain reference values in healthy individuals [8, 9].

In the current study, we found that upper body sites were more sensitive to thermal and mechanical sensations. Though the sites chosen did not include glabrous tissue as seen at the thenar eminence, the results were similar. The sites at the upper body had greater sensitivity than the lower body, and thermal differences at the upper body sites were less than 1C°. We found that sensitivity to CD and CPTh were greater in the upper body sites while the lower body sites were more sensitive to WD and HPTh. QST reference values for this sample grossly varies from

those of other populations in the literature [9, 24, 26, 37-39, 60]. Some of the references values for our sample varied as much as 9 C° from reference values reported in the literature, see Table 4. Differences in thermal and mechanical responses between the upper and lower body may be related to the distance the impulse travels to trigger the response. Neuron length may contribute to this difference.

Unlike what was found by previous investigators, the results of this study indicate that African American adults and specifically females and older adults respond with greater sensitivity to thermal and mechanical stimuli than other racial/ethnic groups. In a 1943 study of 120 participants, African Americans perceived pain at a lower level than a Northern European sample of the same age and sex [22]. Similarly, pain thresholds (the stimulus when pain is first perceived) were lower in Mexican adults when compared to non-Hispanic whites without neuropathy (problems with nerve sensation) [24]. Results from other past studies showed, when exposed to experimental pain, African American adults demonstrated greater sensitivity. This was evidenced by lower tolerances for heat pain compared to White volunteers, heat pain threshold for African Americans [14], while in other studies, no differences were found between groups for most modalities[12]. In the current study, sensitivity of healthy African Americans adults was close to that of Hispanic/Latino adults in some parameters. In sum, African Americans are more sensitive to thermal and mechanical modalities across six sites than are Whites [24]. Our findings are consistent with this literature, but in our overall sample, African Americans are more sensitive to CD, WD, CPTh, HPTh, and MPTh than other racial/ethinic groups. This is important because it helps to guide practitioners in the requirement for earlier treatment of pain than what is required for other populations.

4. Clinical Implications and future research

The normative reference values obtained in this study using QST may be used to guide recommendations for treatment in patients with acute and chronic pain syndromes. Currently, the general public, policy makers, and practitioners tend to believe that African Americans have a high pain tolerance and do not require as much medication as other groups [2, 27, 61-64]. When patients from this population arrive in the emergency department with pain from sickle cell disease and other chronic pain conditions, they are often viewed as drug seeking [65]. Thus, our findings that African Americans actually have increased pain sensitives, hold significant potential to inform policy and evidence-based practice. Skin anatomy and physiology is an important avenue of research to pursue. Additional research is needed to explore QST at glabrous and hairy tissue types in African American adults since we tested only hairy tissue sites [66], with the thought being that hair may impede the transmission of the thermal sensation. In addition, it is important to explore the effect of melanin density, instead of social constructs of race, in the processing of thermal stimuli. Groups that are racially defined are not suitable for explaining differences in health care [19, 67, 68]. It is also important to consider the density of nerve fibers at the skin surface when obtaining QST data. It may be useful to consider this approach in a healthy population and could serve as another way to examine pain sensitivity instead of ethnicity.

The study is not without limitations. Although all 124 participants were tested at the anterior forearm site, the number of participants tested at the remaining five sites (subgroup) when examining additional subgroups of sex and age, the sample size ranged from 22 to 64 individuals. However, even these sub-categories are larger than what has been examined previously in the literature, signifying an important development in this area of research.

Random selection of the sample was not used in this study. All participants were from the same region of the country and were obtained via advertisement and word of mouth. It is unknown if daily climate, genetic, and epigenetic factors impacts responses to QST measures. All testing sites used hairy tissue. Every effort was made to maintain the accuracy of the protocol. In the statistical analysis, the false discovery rate or the Bonferroni family confidence interval for multiple comparisons were not used, which mean that statistically significant findings in this study could have occurred by chance.

D. Conclusions

Ours is the first study to report normative reference values for pain-free African American adults. These reference values for healthy adults will allow comparisons of QST results obtained in the clinical population, something that was heretofore missing. Secondly, our findings are based on a larger sample size than previously studied, with a nearly equal number of both younger and older adults, as well as nearly equal numbers of female and male participants. Further, our findings provide an important platform for future elucidation of mechanisms underlying differences between men and women, and younger and older adults. Of particularly significant importance, our study adds to an emerging body of literature confirming that, in contrast to conventional clinical wisdom, African American adults have lower pain thresholds than do White adults.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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Table 1 indicates demographic information of the sample.

Table 1

Demographic information

Sample Size	Frequency	Percent
Sex		
Female	61	49.2
Male	63	50.8
Age group (18 to 69 years)		
18-39 years	64	51.6
≥40 years	60	48.4
Education		
≤ 8th grade	3	2.4
Vocational	7	5.6
≤ High School	53	42.7
Associates	19	15.3
Bachelor	22	17.7
Masters	8	6.5
Doctoral	1	0.8
Other	11	8.9
Marital Status		
Single	88	71
Married/Partner	26	21
Widowed	2	1.6
Other	6	4.8
Not Reported	2	1.6
Income		
≤ 10K	35	28.2
11-20K	21	16.9
21-30K	17	13.7
31-40K	14	11.3
41-50K	10	8.1
>50K	22	17.7
Not reported	5	4

Table 2 Reference Values

Table 2

Indicates the range of reference values for cool detection (CD), warm detection (WD), cold pain threshold (CPTh), heat pain threshold- (HPTh), and Mechanical detection at six test sites for All, sex, and age group, and results of independent t-tests for sex and age groups. Thermal is measured in degrees Celsius (C), and mechanical is measured in grams of force.

	All			Female			Male						18-39 years			≥40 years				
	Obs	Mean (SD)	Min-Max	Obs	Mean (SD)	Min-Max	Obs	Mean (SD)	Min-Max	t	df	p	Obs	Mean (SD)	Min-Max	Obs	Mean (SD)	Min-Max	t	df	p
Anterior Forearm	124			61			63						64			60					
CD		29.2 (1.6)	18.4-31.5		29.4 (1.2)	24.9-31.4		29.1 (1.8)	18.4-31.5	.932	122	.352		29 (1.9)	18.4-31.3		29.5 (1.1)	26.3-31.5	-1.95	122	.053
WD		34.5 (1.2)	32.3-38.9		34.3 (1.1)	32.5-37.3		34.8 (1.2)	32.3-38.9	-2.66	122	.009		34.7 (1.3)	32.3-23.9		34.4 (1.1)	32.6-36.6	1.14	122	.258
CPTh		26.3 (5)	0-31		27.2 (3.6)	10.1-31		25.4 (5.9)	0-30.9	2.05	122	.043		26.1 (4.5)	0-30.9		26.5 (5.5)	0-31	-.463	122	.644
HPTh		37.8 (3.6)	33.1-48.5		37.1 (3.4)	33.1-45.3		38.5 (3.7)	33.5-48.5	-2.09	122	.039		38.5 (3.4)	33.1-48.5		37.5 (3.7)	33.1-46.8	2.34	122	.021
Mechanical		16.7 (22.2)	.6-60		14.1 (21.2)	.6-60		19.2 (23.2)	.6-60	-1.26	122	.210		19.9 (23.6)	.6-60		13.2 (20.4)	06-60	1.69	121.2	.094
Posterior Forearm	48			22			26						23			25					
CD		29.6 (1.1)	26.8-31.2		29.6 (1.2)	26.8-31.2		29.6 (.91)	28.2-31.5	.000	46	1.00		29.5 (1.1)	26.8-30.9		29.6 (1)	27.7-31.5	-.312	46	.757
WD		34.5 (1.5)	32.5-41.7		34.3 (1.1)	32.5-37.2		34.6 (1.8)	32.6-41.7	-.859	46	.395		34.2 (1)	32.5-36.6		34.7 (1.9)	32.6-41.7	-1.12	46	.267
CPTh		25.8 (6.6)	0-31		26.5 (6.4)	1.2-31		25.6 (6.8)	0-30.4	.654	46	.517		27.4 (2.1)	22.9-30.7		24.2 (8.7)	0-31	1.78	27.2	.087
HPTh		37.8 (4.1)	33-47.9		36.9 (3.6)	33-45.5		38.6 (4.4)	33.8-47.9	-1.44	46	.157		37.2 (3.6)	33-45.7		38.3 (4.5)	33.7-47.9	-.909	46	.368
Mechanical		17.4 (22.8)	.6-60		14.9 (21.9)	1.4-60		19.5 (23.8)	.6-60	-.700	46	.487		19.8 (1.6)	1.4-60		15.2 (21)	.6-60	.686	46	.496
Lateral Upper Arm	53			27			26						30			23					
CD		28.9 (1.3)	26-3.9		29 (1.1)	26.5-30.9		28.7 (1.4)	26-46.9	.648	51	.520		28.6 (1.2)	26-30.5		29.2 (1.2)	26.1-30.9	-1.77	51	.083
WD		34.9 (2.7)	32.7-46.9		34.1 (1)	32.7-36.4		35.8 (3.7)	32.7-46.9	-2.19	28.3	.037		35.5 (3.4)	32.7-46.9		34.2 (1.2)	32.7-37.5	1.65	51	.106
CPTh		25.2 (6.3)	0-30.7		26.6 (2.8)	16.6-30.6		23.7 (8.3)	0-30.7	1.67	30.5	.105		23.4 (7.6)	0-28.7		27.6 (2.5)	19.1-30.7	-2.80	36.9	.008

HPTh		39.1 (4)	33.2-49.6		38.1 (3.3)	33.7-46.3		40.2 (4.5)	33.2-49.6	-1.93	51	.060		40.6 (4)	35.4-49.6		37.2 (3.3)	33.2-46.3	3.22	51	.002
Mechanical		23.0 (26.2)	.6-60		17.4 (25.7)	.6-60		28.9 (26)	.6-60	-1.62	51	.112		26.2 (26.8)	.6-60		18.9 (25.5)	.6-60	1.00	51	.320
Lateral Leg	48			25			23						25			23					
CD		29.0 (1.4)	26-31.8		29.3 (1.6)	26.2-31.8		28.7 (1.3)	26-31.5	1.38	46	.174		28.7 (1.3)	26.2-31.8		29.3 (1.6)	26-31.5	-1.41	46	.164
WD		35.1 (1.5)	32.9-39.9		35.2 (1.6)	33-39.9		35.1 (1.3)	32.9-38.1	.295	46	.769		35 (1.2)	33-38.1		35.2 (1.8)	32.9-39.9	-.188	46	.852
CPTh		25.3 (5.7)	5.2-31.3		26.1 (4.7)	11.8-31.3		24.4 (6.6)	5.2-30.7	1.05	46	.298		24.8 (5.5)	5.8-31.3		25.7 (6)	5.2-31.1	-.565	46	.575
HPTh		39.1 (4.2)	33.2-48.2		38 (3.9)	33.2-46.2		40.3 (4.2)	33.4-48.2	-1.95	46	.058		39.6 (3.7)	34.5-45.2		38.5 (4.6)	33.2-48.2	.986	46	.329
Mechanical		23.9 (22.7)	.6-60		19.9 (21.8)	.6-60		28.2 (23.3)	.6-60	-1.27	46	.211		26.9 (21)	4-60		20.7 (24.4)	.6-60	.947	46	.348
Medial Leg	50			25			25						25			25					
CD		28.2 (1.5)	25.2-31.3		28.2 (1.5)	25.7-31		28.3 (1.6)	25.2-31.3	-.235	48	.815		28 (1.4)	25.2-31		28.4 (1.7)	25.9-31.3	-.894	48	.376
WD		35.4 (2.1)	32.9-46.5		34.8 (1.4)	33.2-39.4		35.9 (2.5)	32.9-46.5	-2.03	48	.048		35.6 (2.6)	33.4-46.5		35 (1.6)	32.9-39-4	.951	48	.346
CPTh		24.4 (7.5)	0-30.1		24.9 (7)	2.1-29		23.8 (8.2)	0-30.1	.535	48	.595		23.5 (7.6)	0-28.6		25.2 (7.5)	.2-30.1	-.814	48	.420
HPTh		39.0 (4.2)	33.6-48.4		38.2 (3.4)	33.9-46.7		39.8 (4.8)	33.6-48.8	-1.38	43.1	.176		39.8 (4.2)	34.5-48.4		38.2 (4)	33.6-46.9	1.40	48	.168
Mechanical		17.9 (23.1)	.6-60		17.6 (22.5)	1.4-60		18.2 (24.1)	.6-60	-.099	48	.921		20.7 (23.3)	1.4-60		15.2 (23)	.6-60	.842	48	.404
Posterior Leg	49			23			26						25			24					
CD		28.7 (1.9)	20.8-31.4		29.1 (1.2)	26-31.4		28.3 (2.3)	20.8-31.2	1.61	47	.113		28.8 (1.3)	26-30.7		28.6 (2.4)	20.8-31.4	.378	47	.707
WD		35.3 (1.8)	32.7-43.5		34.8 (1.1)	32.7-37.9		35.7 (2.2)	33.7-43.5	-1.76	47	.085		35.4 (1.5)	33.5-39.8		35.2 (2.1)	32.7-43.5	.341	47	.735
CPTh		25.0 (4.7)	6.7-30.6		25.7 (4.8)	7.2-30.6		24.5 (4.7)	6.7-29.3	.901	47	.372		25.7 (2.5)	19.3-29.9		24.3 (6.3)	6.7-30.6	1.02	30.1	.316
HPTh		38.7 (3.4)	33.2-45.7		37.5 (3.2)	33.2-44.1		39.7 (3.3)	34.8-45.7	-2.42	47	.019		38.6 (3)	33.8-44.1		38.8 (3.8)	33.2-45.7	-.261	47	.795
Mechanical		18.8 (22.1)	.6-60		11.7 (16.5)	.6-60		25.1 (24.7)	.6-60	-2.2	47	.030		19.3 (22.2)	.6-60		18.2 (22.4)	.6-60	.179	47	.858

Table 3 Indicates the results of the paired t-test for modality detection/threshold to upper body verses lower body

Table 3
Paired t-test

Modality		M (SD)	$t_{(123)}$	p
CD	Upper Lower	29.2 (1.3) 28.6 (1.7)	5.37	<0.001
WD	Upper Lower	34.6 (1.5) 35.2 (1.7)	-4.63	<0.001
CPTh	Upper Lower	25.9 (5.4) 24.8 (6.1)	8.6	<0.001
HPTh	Upper Lower	38.1 (3.7) 39 (3.8)	-4.19	<0.001
MPTH	Upper Lower	18.4 (22.4) 20.3 (22.5)	-1.56	.121

cool detection (CD); warm detection (WD); cold pain threshold (CPTh); heat pain threshold (HPTh), mechanical pain threshold (MPTh) (grams of force)

IV. Cited Literature

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C. Chapter 3

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APPENDICES

Appendix A

Research Proposal

2. Specific Aims. The goal of this research is to understand thermal/mechanical pain thresholds, and genetic contributions to pain perception in a healthy African American (AA) adult population. Over 100 million adults have some type of common chronic pain [1], which results in a socioeconomic burden that ranges between **$560 to $635 billion annually [2]**. Sickle cell disease and cancer are just two painful disease states that disproportionately impact the African American population. One in 365 AA newborns are diagnosed with sickle cell disease every year, and there are 190,000 new cancer cases per year in the population across age categories [101]. The Institute of Medicine (IOM) of the National Academy of Sciences reported racial and ethnic disparities are pervasive in our health care system [2]. Despite the high prevalence of painful conditions, African Americans are routinely under-treated for their pain [1, 3, 5-12]. These individuals may suffer from neuropathic pain, central sensitization, peripheral sensitization, or mixed sensitization. Unrelieved pain results in unnecessary suffering, delayed healing, functional disability, and increased hospital length of stays [4]. QST is a widely-accepted method for assessing cool and warm detection, pain thresholds and detection of pressure (in grams of force), and measures the functional status of the somatosensory system [16, 17]. QST reference values exist for the Asian, Latino, and White populations, and are useful for evaluating pathologic pain in these populations as a guide for treatment. Despite this, quantitative sensory testing (QST) normal reference values have not been established in this population [26, 90, 103, 113]. QST reference values for healthy AA adults would allow healthcare professionals to develop and provide accurate treatment of individuals who have chronic debilitating pain in this population.

QST is an objective method to identify sensitization in small nerves (A-delta and C fiber), and to appraise the effectiveness of pain interventions and altered pain mechanisms [17, 26, 90-92]. Relatively few studies have examined normative pain data for the healthy African American adult population [97]. Recent studies have primarily used college aged undergraduate students [16, 95], while another study matched healthy volunteers to patients with sickle cell disease in a pediatric population [30, 93, 99]. QST reference values among ethnicities for healthy adults have varied [16, 95, 103]. Studies in the Latin-American population have had lower temperature induced pain thresholds than those reported in the White population [25]. Age was a significant factor in thermal and mechanical thresholds, and showed that sensitivity decreases with age [104]. Sex differences have been demonstrated in pain processing. It has been reported that women have a lower pain threshold to heat and mechanical stimuli [105-107]. QST can be sufficiently conducted at various body sites, the most common sites are the forearm, dorsum of the foot/hand, ventral forearm, and thenar eminence [17, 90, 93, 99, 103, 108-113]. Differences have been demonstrated between the upper and lower body [17, 24, 25, 90, 114]. There have been many studies using QST in the sickle cell population. The understanding of QST normative reference values in healthy AA adults is crucial for application to clinical practice [17]. In this study, sites will be chosen bases on commonly reported and easily accessible pain sites found in the SCD population. Equally important to QST is the role of genetics in pain perception. It has been demonstrated in recent studies that variations in the arginine vasopressin receptor gene AVPR1A promoter region, SNP (rs10877969), modulate pain sensitivity in both humans and mice, and have demonstrated a three-way interaction between genotype, stress, and sex [32]. The use of QST along with genetic analysis to obtain reference values in the healthy AA adult

population will contribute to better assessment and treatment of the of AA adults who suffer from altered pain conditions.

We will obtain normative QST values from **125 healthy African American adult volunteers**, for the purposes of characterizing thermal detection, thermal pain thresholds, and detection to pressure (in grams of force) using QST measures. All volunteers will provide demographic information and blood samples for genetic analysis. Measures include mechanical QST using von Frey filaments (pressure sensation and pain detection), thermal QST (cool/warm sensation detection, heat/cold pain detection thresholds), genotyping (rs10877969 SNP), and demographic comparisons.

Impact: Results from this study will provide normative reference values for warm and cool detection, hot and cold pain thresholds, detection of pressure and the sensation of pain from pressure. In addition, results from this study will aid in the examination of the genetic contribution to pain. The normative data obtained from this study will be used for comparison with the segments of the AA adult population who suffer from chronic pain. By establishing normative QST reference values and expanding the understanding of the role of the AVPR1A gene pain related SNP rs10877969, in healthy African American adult, the results of this study will help broaden the understanding of pain and allow for improved treatment of pain in this population. In this study of 125 healthy AA adults, the specific aims are:

Aim 1. To describe the range of reference values for detection of warm and cool sensation, hot and cold pain threshold, and pressure (in grams of force) sensation at six body sites, three sites per participant (practice site, upper body and lower body). The information generated by this aim is descriptive, there is no hypothesis.

Aim 2. To compare mean thermal (warm and cool detection, heat and cold pain threshold) values for differences by age, sex, and testing site location (upper body and lower body). HOs: Mean warm detection and hot pain threshold values will increase as age increases, will be lower in women than in men, and will be greater in the lower than upper extremities.

Aim 3. To compare mean hot pain threshold values by genotype for the SNP AVRP1A (rs10877969). (HO): Individuals who have the TT genotype will report less pain than those who have the CT AND CC genotype.

3. Research Strategy

A. <u>Significance</u>. A 2002 report indicated that over 100 million adults have some type of common chronic pain [1]. **The socioeconomic burden of chronic pain ranges from $560 to $635 billion annually** [8]. The Institute of Medicine (IOM) of the National Academy of Sciences reported that racial and ethnic disparities are pervasive in our health care system [2]. These disparities occur in the context of numerous illnesses and syndromes [100]. **Among African Americans, unrelieved pain is a major health problem that leads to unnecessary suffering, delayed healing, functional disability, increased length of hospital stays, and lost school and work days** [4, 89]. There are racial and ethnic differences in individual responses to pain. These pain behaviors are typically misunderstood by health care providers [1, 2, 4, 88]. **AA are consistently undertreated for cancer pain, post-op pain, chest pain, and acute pain presenting to the emergency department** [7, 88].

Quantitative sensory testing is a standardized psychophysical method that uses a group of tests to evaluate the function of neuronal fibers (myelinated Aα, Aβ, Aδ and unmyelinated C-fibers) and their pathways to the brain [17]. Thermal testing examines the functionality of thinly myelinated A-delta fibers and unmyelinated C-fibers, and mechanical testing (von Frey

filaments) examines the functionality of Aβ myelinated fibers [17]. In recent years, quantitative sensory testing (QST) has been used with increasing frequency for assessment of the human nociceptive (the response by the nervous system to potentially harmful or harmful stimuli) system, and to explore the functionality of the somatosensory system [17].

QST is a standardized, calibrated tool, used to measure individual pain processing differences by applying a consistent noxious stimulus [94]. In order to understand the meaning and implications of QST clinical values, it is necessary to have normative references values in which to make comparisons for populations with pain [26]. Although there has been an increase in the use of QST in clinical research, the usefulness remains limited due to the lack of normative data sets in large populations of healthy individuals [26]. QST reference values of healthy persons serve as the basis for the evaluation of neuronal pathological changes [17]. These reference values have been extensively examined in Asian and White subjects, most thoroughly in the German Network Study (DFNS), and to a lesser extent in Latino population [23, 25, 26, 90, 94-96, 108, 112, 114, 137-140]. Although QST studies have included healthy African American adults, the sample sizes have been small and the age range is typically college-aged young adults. Most importantly, **normative QST reference values for the adult AA population have not yet been established**. This lack of normative data is a barrier that must be addressed.

Pain differences among race/ethnicities. According to the National Institutes of Health (NIH), there are five racial categories: Black or African American, American Indian or Alaskan Native, Asian, Native Hawaiian or Other Pacific Islander, and White. They further recommended that racial categories should not be considered as biological constructs, and rather sociopolitical ones [141]. The Office of Management and Budget (OMB), adopted the recommendation that the

term ethnicity defines groups of people based on specific characteristics: shared culture, beliefs, history, language and experience. As a result two ethnic groups are included with race: Hispanic or Latino and not Hispanic or Latino [142].

The biopsychosocial model of pain perception and response suggests that the pain experience is influenced by a complex interaction of biological, behavioral, and cultural variables [1]. Investigators have explored pain differences using both clinical and experimental settings. In the clinical setting, **AA were more likely to experience more pain, even in cases where healthy young adults were the primary participants** [16, 96], and were more like to have lower tolerance for ischemic arm pain compared to non-Hispanic White patients. In addition, in the multidisciplinary pain setting, African Americans reported higher levels of pain and disability compared to white patients [16]. **African American and Latino patients reported greater post-operative pain compared to non-Hispanic White patients having the same surgical procedure** [143]. In the experimental setting, the history of pain sensation disparities research across U.S. populations reaches back over seventy years. In a 1943 study of 120 participants, African Americans perceived pain at a lower level than the Northern European of the same age and sex [23]. Similarly, pain thresholds (the stimulus when pain is first perceived) were lower in the Latino (Mexican) population when compared to the non-Hispanic whites without neuropathy (problems with nerve sensation) [25]. In a later study, **African Americans and Hispanics had lower cold and heat pain tolerances (the highest level of exposure to painful stimulation that the participant is willing to accept) when compared to white Americans** [11] (Table 1). When exposed to experimental pain, African American volunteers demonstrated greater sensitivity. Evidenced by lower tolerances for heat pain compared to White volunteers, heat pain

threshold for AA 42.3 (4.2) and for Whites 43.1 (4.8), with a magnitude of difference 0.8 [96]. In other studies, no differences were found between groups for most modalities, but AA volunteers rated their pain experience as more unpleasant at temperatures 46-47 °C [95]. In 1999, Edwards and Fillingim, reported that a sample of healthy African American college students had lower thermal pain tolerances than other ethnic groups [96]. The same group of students had a marginally greater number of pain sites and a significantly higher average pain severity over the previous month [96] when compared to the other groups. Campbell et.al, also reported lower heat pain tolerances among healthy young adult African Americans compared to similarly aged subjects from other groups [95]. Sheffield et al., found that AA participants rated pain stimuli as more unpleasant and rate it as more intense than Whites [22]. Rahim et al., (2007), observed strong group differences when comparing pain threshold to pain tolerance [11].

	Warm detection threshold (°C) (Upper body)	Warm detection threshold (°C) (Lower body)	Heat pain threshold (°C) (Upper body)	Heat pain threshold (°C) (Lower body)
NHB (n = 53)	35.9 (2.2)	37.0 (2.2)	41.8 (2.8)	41.9 (3.2)
NHW (n = 138)	35.2 (2.4)	37.1 (2.7)	43 (2.6)	47.2 (2.7)

Table 1. Heat Pain response for Non–Hispanic Blacks (NHB) and Non-Hispanic Whites (NHW) adapted from Riley 2015

In a QST study for heat pain and pressure pain comparing 27 healthy individuals to 27 age, sex, race, BMI, and education matched patients with sickle cell disease, **the SCD group reported significant differences between the groups at several QST measures, including heat pain tolerance and pressure pain threshold** [94]. Pressure pain threshold and heat pain tolerance were lower ($p<0.05$ and $p<0.001$, respectively) [94, 96]. This illustrates the value of using multiple pain measures and stimulus intensities when comparing thermal detection and thermal pain thresholds [94, 96]. Several investigators discuss behavioral components that are more frequent within ethnicities that may lead to increased pain, for example, catastrophizing, the belief that pain is inevitable, prayer, stoicism, fear of addiction, intolerable side effects, lack

of pain relief, warm showers, belief that a patient's race and gender guides care, reliance on complementary and alternative pain treatments, and the waiting until pain is severe before taking analgesics [1, 3, 5-9, 11, 16, 22, 88, 96, 144-148]. Psychological characteristics that go unmeasured are more likely to result in larger pain rating differences of pain unpleasantness and pain intensity [22].

Many studies have shown that health care providers believe that African Americans have less pain, and as a result are less likely to receive appropriate medications for pain relief, resulting in greater suffering, longer recovery and debilitation, and depression [1-3, 5-8, 11, 12, 22, 97, 102, 143, 147-151].

Pain differences related to age**.** In healthy individuals, age dependency has been observed in most investigated parameters. Structural, functional, and biochemical changes are thought to occur as a part of the aging process, for example, a decrease in the density of unmyelinated C fibers has already begin to occur as early as 30 to 60 [27, 119]. **As age increases, thresholds for cold pain and heat pain also increase [91].** This may be due to age related reduction of Substance P, a neurotransmitter of the nociceptive peripheral afferent nerves, in human skin. In the central nervous system, degenerative changes have been found in the spinal dorsal horn of aged adults, loss of myelin, loss of serotonergic and noradrenergic neurons [119]. In addition, age-related changes in the brain include neuronal death [119]. Pain perception and pain reaction decrease with age (increase in threshold) [23]. These findings were reported by Chapman in 1944: 10 to 22, 0.289 g cal; 23 to 44, 0.324 g cal; 45-85, 0.347 g cal. Per recent studies, the changes result in less sensitivity to warm and painful stimuli, and occurs at a greater frequency in the lower extremities, mostly at the knee [27]. Mixed findings have been reported for pain threshold (PT) using various stimulus mechanisms. Five studies were related to reports of age-

related increase in PT and the results of 5 additional studies demonstrated no age-related increase in PT [27]. The greatest decline in sensitivity occurs at the forearm, thigh, and calf in addition, and comparisons between upper and lower body are more pronounced [119]. Among healthy study participants, age was related to all investigated QST parameters such as thermal testing (cool detection, warm detection, heat pain tolerance, cold pain threshold) and mechanical testing (vibration detection, von Frey filament)

[17]. The largest age-related effects were detected for cold pain thresholds followed by heat pain thresholds [17] (table 2). The magnitude of difference between older and younger adults were as follows: Upper Body: cold pain 0.0, heat pain 0.6; Lower body: cold pain 2.8, heat pain 2.0. This leads to the expectation that normative QST values among healthy African American adults should be related in similar ways to increasing age.

Test Site	Age group (years)	Cold Detection Threshold (°C)	Warm Detection Threshold (°C)	Cool Pain (°C)	Heat Pain (°C)
Upper body	18 to 39	**29.65**	**34.2**	14.2 [1.3, 27.6]; **19.85**	42.3 ± 4.2; **42.75**
Upper body	≥40	**28.67**	**35.0**	14.2 [1.3, 27.6]; **18.6**	42.9[37, 47.4]; **42.8**
Lower body	18 to 39	**28.45**	**37.3**	10.8 ± 9.5; **20.4**	45 ± 2.7; **43.7**
Lower body	≥40	**28.37**	**37.47**	8 ± 8.8; **18.03**	47 ± 2.1; **43.72**
Upper body		**28.6 ± (1.8) [22.2, 31.1]**	**35.2 ± (2.2 [32.5, 42.3]**	**24.6 ± 6 [0.0, 29.4]**	**40.1 ± 3.9 [35.3, 48.4]**

Table 2. Normative values of QST per group of age African American, Latino, Current literature Adapted from Ezenwa 2014 and Gonzalez 2016

Pain differences related to sex**.** Many studies have shown that **men and women perceive and process pain differently**. Investigators have explored gender pain differences using multiple modalities and test sites [18-22]. Fillingim et al., reviewed sixteen studies that explored gender differences in heat pain threshold. Of those, findings from eleven studies revealed women had a lower heat pain threshold than men, while five studies showed no difference between in women and men for

heat pain threshold [18]. All of the studies used contact heat as the source and the stimulation sites varied between the face, hand, and arm (Upper body). Nine studies were used to explore results for cold pain [18]. Of those, results from six studies revealed women were more sensitive to cold pain threshold than men, while results from three studies revealed no difference in cold pain threshold [18]. The cold water test was used in all of the studies and the hand was used for the stimulation site (upper body). Pressure pain has been reported as being stronger in women [19, 104, 107, 118, 123]. Eight studies reported findings for pressure pain threshold, and of those, six studies revealed women were more sensitive to pain than men, and the results from two studies revealed no difference between women and men [18]. In all except one study, the method of testing was via pressure algometry, and for one the computer controlled pressure stimulator was used. The stimulation sites varied between finger, hand, face, trapezius muscle, ulna, masseter muscle, (all upper body sites), one study used the leg as a stimulation site (lower body). While exploring reference values of mechanical and thermal pain in a pain free population, Neziri found that sex, age, and body site were significantly related to heat pain thresholds [26]. Wang et. al., reported similar results in the Chinese population. For cold pain threshold, men had lower thresholds (11 °C) (lower sensitivity) than women (20.4 °C) with a magnitude of difference 9.4 ($F=16.296$, $p = 0.001$) [118]. For heat pain threshold, men had higher thresholds (42.7 °C) (lower sensitivity) than women (38.3 °C), with a magnitude of difference 4.4 ($F = 16.962$, $p = 0.001$) [118]. Additionally, hormonal, psychological, and musculature factors have been found to influence pain perception [104]. Overall, women had a significantly lower pain threshold to heat and mechanical stimuli [17] and have a more pronounced sensitivity to cold pain [18, 19, 91, 114, 124-126].

Pain differences related to sites. Using quantitative sensory testing, Hagander et.al (2002), recruited 46 healthy volunteers to explore cool and warm detection thresholds and pain thresholds on four different body test sites; the thenar eminence, volar surface of the wrist, dorsum of the hand at the first metacarpal space, and the dorsum of the foot on the non-dominant side [129]. The goal of their study was to determine if there was a significant difference in thresholds at the tested sites and to identify a preferred site for testing for each thermal modality [129]. The results revealed, statistically significant thermal threshold differences between sites. When exposed to cool detection thresholds the hand had greater sensitivity than the foot ($p < 0.001$). When evaluating warm detection threshold at the thenar eminence, the site had greater sensitivity than the wrist ($p < 0.001$). There were not differences between sites for sensitivity at the wrist and hand ($p = 0.0035$) [129]. Heat pain sites at the hand and foot did not differ in sensitivity [129]. Campbell et.al. used test sites at the thumb, trapezius, forearm, and quadricep. Comparisons between healthy volunteers and participants with SCD revealed differences in pressure pain threshold and heat pain tolerance were lower ($p<0.05$, $p< 0.001$; respectively) [94]. Neziri et.al., used the lower back, suprascapular region and the reference values in healthy individuals. QST references values for 300 healthy participants were obtained to be used to compare QST results of individuals who have central hyposensitivity and hypersensitivity [26].

In the proposed study, we will explore several test sites using QST. While there is overlap in some of the testing sites, the method of stimulation varies between the studies examined in the literature and the sites and stimulation modalities chosen for the current study. In the current study, test sites were chosen based on common pain sites for individuals who have SCD, ease of access,

Upper arm dorsal (n=20)
Forearm ventral (n=20)
Forearm dorsal (n=20)
Calf lateral (n=20)
Calf medial (n=20)
Calf posterior (n=20)

Table 3. Testing Sites

sufficient skin surface that allows full contact of the probe, and the ability to make slight changes in the position of the probe between stimuli [152] (Tables 3).

Genetic influence on pain. Genetics plays an important but still not fully-understood role in the variation in the perception of pain. Some of these variations affect metabolism of medications which may result from alterations in pathways. Genetic variability is suspected to have a role in the perception of pain across racial and ethnic groups. **Variants in genes have been suggested in pain associated with cancer and differences among minority groups** [1]. In a genetic association study, the single nucleotide polymorphism (SNP) rs10877969, located in the promoter region of the AVPR1A gene on chromosome 12 was found to influence capsaicin pain levels in humans (C/T allele) [32]. A SNP is a single base pair substitution that may result in changes in the downstream sequence, or protein expression, and may result in phenotypic changes in appearance, disease risk, and response to drugs or the environment [28]. Using a sample of 190 healthy volunteers, Parr et.al, explored the effect that genetic and psychological factors have on range of motion (ROM). They showed that pain related variables differ by SNP. In this study, physical impairment in ROM and strength had a stronger association with variation in inflammatory and pain modulation genes, specifically ADBR2 and AVPR1A (rs10877969) [81]. The number of AA participants enrolled in the study was not reported. It also appears that emotional state, genetic background, and sex are modifiers of the analgesic efficacy of a drug [80]. AVPR1A was found to be responsible for strain-dependent pain sensitivity to formalin and capsaicin pain levels. These findings suggested that AVPR1A modulates pain sensitivity in stress in men but not women. A single nucleotide polymorphism (rs10877969) within AVPR1A influenced capsaicin pain levels. Depending on genotype, reported capsaicin pain levels were lower, but only in males who reported stress at the time of testing [78]. **Recent evidence**

Figure 1

suggests that AVPR1A and variability in pain sensitivity is associated with ethnicity [78, 81, 153]. Allele frequencies for rs10877969 differed between AA verses Asian and White participants [78]. The lower pain rating was observed in Asian and White males, but not AA males [32]. However, in unpublished research examining AA volunteers who had SCD, this same SNP was found to be significantly related to citing stress as a pain aggravator for individuals who had the CC genotype (p=0.002) [154] (figure 1).

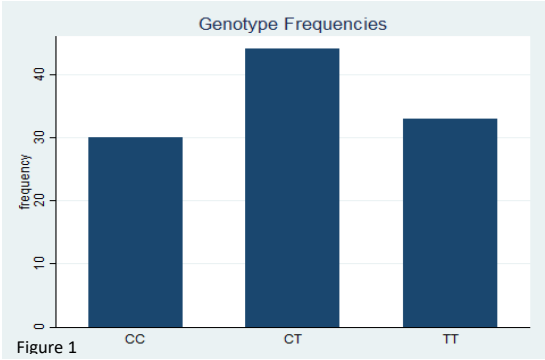

Figure 1

Finally, **AVPR1A (rs10877969) is also significantly related to acute sickle cell disease (SCD) pain** (p=0.026), a condition also associated with stress and inconsistent findings related to sex [155] (Figure 2). In the current study, it is hypothesized, when comparing genotype with mean hot pain threshold values, participants who have the TT genotype will report less pain than those who have the CT AND CC genotype.

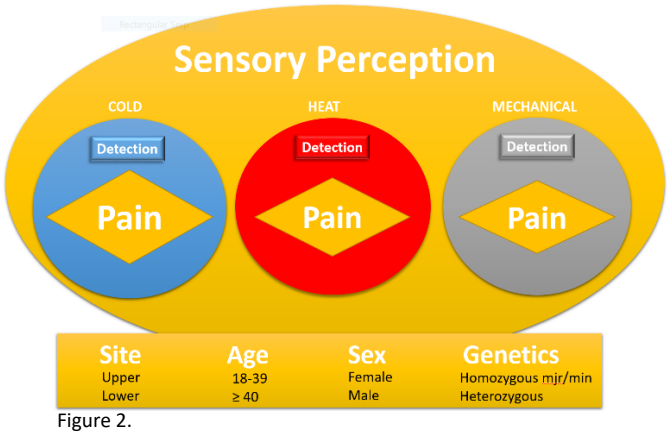

Figure 2.

<u>QST tool for determining pain.</u> Quantitative Sensory Testing (QST) is a standardized valid and reliable tool used to assess the function of afferent nerve fibers (A-delta and C), by examining responses to warm and cool sensation, heat and cold thermal pain thresholds, and pressure (in grams of force). Recently, QST was used to determine the reference values for pain thresholds for mechanical and thermal stimuli in pain-free adults: Europe n=300 (racial and ethnic demographics were not reported), Latino n=83, AA n=23, AA n=30 and Asian, n=20 [92, 104,

111, 118, 156]. There are few QST studies for the healthy AA adult population. Four pain studies exploring ethnic differences in healthy adults were found. Only one study was found using QST to explore thermal pain thresholds in African Americans (N=120 [62 African Americans and 58 Whites]) healthy young college-aged students [95]. **QST norms that are specific for African American adults are not available for most parameters.** Investigators of a recent study, reported QST findings in the AA adult population, and reported that the QST values for cold pain differed from those found in the literature [156] (Table 4). QST studies in the adult AA population are limited. They include college-age students, a limited age range, and small sample sizes. It is important to explore experimental pain responses in the African American population amongst healthy individuals who are pain free. Extensive amounts of QST data have been generated in the AA adult population for individuals who have cancer, sickle cell disease, arthritis, and perioperative pain to name a few, however reference values for healthy AA adults with a wide age range (18 to 74 years) does not exist. **These normal reference values are necessary to guide treatment in this population** [16]. The proposed study is designed to explore the nature of normal thermal and mechanical pain thresholds in healthy African Americans, in order to gain an appropriate base for

Test Site	Cool Detection Threshold (°C)	Warm detection thereshold (°C)	Cold pain (°C)	Heat pain (°C)
Upper body (AA)	28.6 ± 1.8 [22.2, 31.1]	35.2 ± 2.2 [32.5, 42.3]	24.6 ± 6.0 [0.00, 29.4]	40.1 ± 3.9 [33.9, 49.1]
Upperbody (Current Literature)			14.2 [1.3, 27.6]	42.3 ± 4.2
Lower body (AA)	28.2 ± 1.2 [25.2, 30.3]	35.8 ± 2.4 [33.2, 46.5]	23.3 ± 6.4 0.0, 28.7]	4.06 ± 3.8 [35.3, 48.4]
Lower body (Current Literature)			10.8 ± 9.5	45 ± 2.7

Table 4. Normative values of QST
African American and Current literature
Adapted from Ezenwa 2016

comparison and accurate interpretation using QST reference values in the AA adult population with pain syndromes. There must be an understanding of the unique variance that each group contributes to the overall pain experience [16].

B. Innovation. This study is innovative because we will describe QST reference values in the healthy African American adult population (Figure 2). There are currently three studies that have used QST to explore multidimensional pain in the African American population. One of the studies includes college-aged students and the other two include matched pediatric, adolescent, and adult participants without differentiating between the groups in the results. The data gained from this research, will allow further studies to proceed that require comparison values of normative quantitative sensory testing reference values in the healthy African American population. Having access to normative reference values from healthy volunteers will allow for comparison of values, accurate interpretation of the values, and appropriate pain treatment. It will also serve as a baseline for future studies examining the same population living in different regions of the country with varying temperatures (mild, moderate, extreme heat, and extreme cold), exploration of nerve fiber density on pain, and the role of melanin concentration on pain perception. In addition, the research will provide information about differences in QST based on age, sex, and body site. Furthermore, this innovative study will explore the genetic component of QST in the healthy African American adult population as it relates to arginine vasopressin SNP AVPR1A (rs 10877969) and its role in the variability of responses. Overall, the findings from this study have the potential to change the way we interpret pain and guide treatment for individuals with chronic pain as we broaden our understanding of the degree in which age, sex, race, and genotype impacts pain. In addition, it will begin to fill the gap of normative QST reference values for the African American adult population.

C. Approach

C1. **Preliminary studies.** Sickle cell pain is a multidimensional problem that has no satisfactory therapy and requires further research. Patients suffering from sickle cell disease (SCD) commonly have varying degrees of chronic debilitating pain and episodic acute pain. Genetic factors have been shown to influence how individuals experience pain. The purpose of this study was to explore pain related genetic factors in patients with sickle cell disease. The investigators for this study used a quasi-experimental, descriptive, and comparative design.

Patients with sickle cell disease, sample of 115 individuals (mean age 34.1 ± 12.3 [range 15-70 years], 68% female) was 97% African American with SCD (81% SS, 10% SC, 9% other). Genotyping was performed on the SNP by the MassARRAY iPLEX Platform. Negative binomial regression of patient utilization found that, controlling for age, sex, and SCD type, subjects with either CC or CT genotype had significantly higher utilization than those with TT genotype **(p=0.026).** This SNP was not a significant predictor for CPI in this study. The same population was further analyzed, **N=107** (a few samples were loss during processing), mean age 35.2 ± 12 years [ranged from 19-70 years], female (74(68.5%) and male 34 (32.7%), were asked to characterize their pain experience (pain now, least pain in 24 hours, worst pain in 24 hours, and stress) and demographic sex and age. A polymorphism of vasopressin (rs10877969) was analyzed along with the other variables. The frequency of rs10877969 homozygous CC genotype was 30 (28%), heterozygous (CT) was 44 (41%), and homozygous TT genotype was 33 (31%). The mean pain intensity scores were 3.8±3.1 for pain now, 3.1±2.7 for least pain, and 5.4±3.5 for worst pain. Thirty-five (33%) patients reported stress aggravated their pain and 72 (67 %) did not. Age, sex, and pain intensity scores were not associated with genotype at a statistically significant level. Genotype and citing stress as a pain aggravator were significantly related

(p=0.002); 10% of patients with CC genotype cited stress, compared with 48% of patients with CT and 33% of patients with TT. The frequency of rs10877969 homozygous CC genotype was 30 (28%), heterozygous (CT) was 44 (41%), and homozygous TT genotype was 33 (31%). When examining stress and genotype, individuals with sickle cell disease with the **CC** allele are more likely to have no stress, **CT** are more likely to have stress, and **TT** are equally likely to have stress or no stress. The covariates pain least and sex were nearly significant, p>0.056 [154].

Design. A cross-sectional comparative study of **125 healthy Adult African American adult volunteers** will provide demographic information, normative QST values, and blood samples for DNA analysis. Normative QST values were obtained using QST, testing a combination of six sites total, three on the upper and three lower extremities (Table 3).

Setting. The study will be conducted at the University of Illinois at Chicago, College of Nursing (UIC CON). The CON includes a lobby area where the subjects were initially met. Research data collection will be conducted in a temperature controlled and adequate for the QST protocol. During testing, the participants will be seated in a comfortable leather chair reclined at approximately 45 degrees for comfort and relaxation during the study.

Sample. Based on 2010 census data, 32.9% of 2.7 million people within Chicago city limits are African American. For those who meet the eligibility criteria, we anticipate an attrition rate of 10% and an 80% enrollment for rate for volunteers. We based these number on accruals, withdrawals, and deaths in previous studies conducted at UIC, including a 13-year longitudinal study of patients with sickle cell disease (SCD) and sickle cell trait (SCT) carriers. For another local study, 99 healthy African Americans were successfully recruited using community engagement strategies that will be replicated in the proposed study; attrition at 24 months was only 9%. We obtained a sample of 125 healthy volunteers with complete data, from the UIC

campus, surrounding communities, churches, local sororities, fraternities, community organizations, and word of mouth.

Eligibility criteria-healthy volunteers. Inclusion criteria: (a) African American, (b) health history negative for diabetes, hypertension, SCD, SCT, cancer, current or chronic pain, (c) speaks and reads English; and (d) age ≥ 18 years. Exclusion criteria: (a) legally blind, (b) unable physically to complete study measures, or (c) report using prescription pain medications or recreational drugs.

Retention Strategies. Data collection will be schedule at times convenient to participants, including evening and weekend appointments. Multiple contact information will be collected (e-mail, mobile phone, and home phone) to maximize my ability to contact the volunteer on the day before and the day after their appointments. Participants will receive an incentive of $50 to cover the cost of transportation, travel, and their time to complete the study measures.

Sample Power. Aim 1 Using the mean, standard deviation, and range. We will describe the reference values for the healthy African American adult population for detection of warm and cool sensation, hot and cold pain threshold, and pressure (in grams of force) sensation at six sites, and describe the average

variability within the sample for each of the six sites being tested. **Aim 2** To compare mean thermal (warm and cool detection, heat and cold pain threshold) values for differences by age, sex, and testing site location (upper body and lower body). Using t-test and paired t-test, the proposed sample of 125 healthy subjects will give us power >.99, alpha 0.05. **Aim 3** To compare mean hot pain threshold values by genotype for the SNP AVRP1A

(rs10877969). Using ANOVA, the proposed sample of 125 healthy subjects will give us power >.99, alpha 0.05.

Procedures. Research assistants (RAs) will be trained in all study procedures prior to subject recruitment. Calibration of the QST protocol will be performed to assure results similar to an internationally recognized QST team. Once the calibration will be completed, a role-play check demonstration protocol will be performed to check adherence, by the test administrators. The procedure for performing the test is standardized and always follows the same test sequences and the use of the same calibrated thermal and mechanical test stimuli [17]. As described in table 5, at the time of a scheduled session, the research assistant will state to the participants, that the investigator is testing a new set of pain measures to better understand pain. After written informed consent is received, the participants will be orientated to QST testing procedures and a blood sample will be collected for DNA analysis. The QST measure will be conducted to determine the thermal (cool detection/cold pain, warm detection/heat pain) and mechanical (pressure detection/pain threshold) responses. Three sites will be tested starting first with cool detection, warm detection, cold pain, heat pain in all sites and conclude with the progressive intense mechanical stimuli in each of the three test sites. Using a 0 to 10 Likert scale, subjects will be asked to report the intensity of the pain they experienced from the mechanical pressure, if any, and the intensity of the cold pain and heat pain experienced. The participants will receive a 24-hour follow-up call and will be asked to rate their worst pain, least pain in the last 24 hours, and current pain.

Screening and Consent
Study visit
Blood Sample
Thermal Quantitative Sensory Testing Conducted (TSAII) **3 randomly selected sites**
Mechanical Quantitative Sensory Testing Conducted (von Frey filaments) **3 randomly selected sites**
Follow up call 24 hour
Adapted from Wilkie et.al.

Table 5. Flow Chart: Study progression through

Measures. We will use well-validated measures for the quantitative sensory testing (QST) study with the 125 health AA adult participants [95]. This protocol is consistent with the FENS (European Federation of Neurological Societies) recommendations for testing Aβ, A-delta, and C fiber function [90].

Heat and Cold Threshold	Gender differences	Ethnic Differences
Arm, Forehead, forearm, suprascapular region, low back, lateral leg, Leg L5, leg L4, foot dorsal surface, foot sole, hand thenar area, hand dorsum, face, upper extremity, lower, wrist, extremity, thigh, Volar arm, upper back T2-8, lower back, thumb, trapezius, quadricep, T10-L3, deltoid, periumbilical	Arm, Forearm, C7 spinous process, cheek, L1 spinous process, lateral leg, thenar eminence, foot, hand dorsum, upper back T2-8, lower back T10-L3,	Forehead, Forearm

Table 7. Test Sites: Thermal, gender, ethnicity

QST: Thermal. The TSA-II NeuroSensory Analyzer (Medoc) will be used for the measurement of thermal and sensory response values**.** The TSA-II is a precise, computer controlled device capable of generating and documenting responses to highly repeatable thermal stimuli, such as cool detection, warm detection, cold-induced pain, and heat-induced pain. The TSA-II delivers quantitative assessment of small caliber (A-Delta and C fiber) sensory nerve function and is used to identify thermal pain thresholds. Test sites will be randomly selected until a 20-subject quota is reached for each site. One site per limb will be tested (Table 6). Over testing may be required to obtain the appropriate number of older males.

Body Site and Age		Cold Pain	Heat Pain
Upper body	**18-39 yr**	14.2 [1.3,27.6][129]	42.3±4.2[95]
	>= 40 yr	14.2 [1.3,27.6][129]	42.9 [37,47.4][129]
Lower body	**18-39 yr**	10.8±9.5[157]	45±2.7[157]
	>= 40 yr	8±8.8[157]	47±2.1[157]

Table 6. Published Normal scores for thermal pain detection (threshold) (modified from Wilkie et.al)

In the literature, multiple sites were used (table 7). In a private temperature controlled research room, the TSA-II thermode with a Peltier element and a cooling water system will be placed on the skin, cold and warm detection thresholds will be determined, followed by cold pain and heat pain thresholds [17]. The temperature will increase from a baseline of 32ºC with a 1º or 1.5ºC/second rate of rise (warm/heat pain) from 32ºC with a 1º or decrease from a baseline of 32ºC with 1.5ºC/second rate of decline (cool/cold pain), until the volunteer responds by pressing a stop button, which is connected to the computer unit. A threshold value will be determined in accordance with increasing or decreasing temperature of the thermode contact surface [17]. There is a 30-second inter-stimulus interval between the three testing sites. To avoid tissue damage, the cutoff temperature for all trials was 50º C for heat and 0ºC for cold. The participant will respond to the temperature stimulus by pushing a button and the stimulus returned to the adaptation temperature. We will use the limits protocol, the warm and cool sensation detection will be presented in three repetitions per site (averaged by site) and the heat and cold pain detection was presented in the three repetitions per site (averaged by site) [95]. The actual pain threshold will be calculated from three consecutive individual values as an arithmetic average, the TSA device automatically records the average sensory threshold. Detection of warm is usually 1-2º C above 32º C (the adaptation temperature) and is mediated by C fibers. Cold sensation usually occurs at a similar range below adaptation and mediated by A-delta fibers. Heat pain threshold is usually about 45º C and is mostly mediated by C fibers with some A-delta fiber involvement. Cold pain threshold is the most variable and difficult to assess of all these modalities, but it is usually sensed at about 10º C as mediated by a combination of both C and A-delta fibers.

For participant safety, the TSA has software that halts the heating or cooling if any thermode problems are detected, and the TSA-II has a hardware mechanism that overrides the software and disconnects the thermode power if the temperature reaches 53º C to 54º C, the temperature at which tissue damage occurs. The TSA-II has been used to compare sensory responses of White and African American healthy young adults. Results from these studies have varied results, some of the studies indicate no statistically significant ethnic differences for thermal pain thresholds, while other indicate African American participants having responses that indicate greater sensitivity when compared to White participants [10, 11, 96, 108, 111]. To determine the average value for each stimulus, which the TSA-II system automatically calculates and includes the variance within the repetitions for the stimuli, these values are compared to the norms for body are location age and gender. The sites are categorized as a normal response or response for each pain stimuli, each of which represents different fiber functions. When participant report that a stimulus is painful, they will be asked to rate the intensity of pain for that stimulus.

QST: Mechanical. To capture the mechanical detection thresholds, QST will be conducted for pressure detection/pain threshold using standardized, calibrated von Frey filaments**.** von Frey filaments are measuring devices calibrated to bend at a set amount of force depending on the thickness of the filament. To ensure accurate testing of the detection threshold and pain threshold, the filaments will be placed in the same perpendicular manner to the area being tested until the filament shows an "s-shaped" bending pattern [17]. The contact time to the surface of the skin during testing, should be about two seconds. When a calibrated filament is applied to the skin, a non-painful or a painful response to mechanical transient indentation of the tissue is produced by the filament. Seven filaments will be used starting with 3.84 (=0.6 g) and ending with 5.88 (=60.0 g). These filaments were selected based on previous studies that provided both

clear non-painful sensations in all patients and a painful sensation in some patients as per the European Federation of Neurological Societies (EFNS) protocol [109]. The participant will be instructed to close his/her eyes, and then will be asked to report when they feel a sensation at the designated site. Is a sensation is detected, the participant will be asked to report whether or not the sensation is painful. The filaments will be tested in increasing order of force and testing at that site will be stopped when the participant reports a force as being painful. The participant will then be asked to report the pain intensity of the sensation on a 0 to 10 pain intensity scale. The von Frey filaments will be calibrated monthly using a microbalance to ensure they remain undamaged and accurate. As with the EFNS protocol, each site will be tested with three repetitions for each filament size and a report of pain for any one of the repetitions will be scored.

Pain measures. The participants will be given self-report pain measures to validate that they were pain free. The tools will be administered, and responses will be captured using a pen tablet computer for data capture.

PAIN*Report*It©, is the first electronic version of the McGill Pain Questionnaire (MPQ 1970 version), and is a valid and reliable way to measure pain in the cancer [158] and sickle cell population. PAINReportIt was self-administered and required minimal previous exposure to computers.

Demographic variables. The variables will include: age, gender, ethnicity, education, substance use history, concurrent illnesses, and medical history checklist. PAINReportIt, which will be completed by the participant at the beginning of the study to verify that he/she is pain free.

Genetic Studies. DNA from peripheral blood samples and finger sticks will be extracted using QuickGene DNA extraction method (QuickGene-mini80 isolation device, Fujifilm). DNA

samples will then be aliquoted into Eppendorf tubes and stored in a research freezer at -80º C. Major polymorphisms and single nucleotide polymorphisms (SNPs) will be analyzed. Genotyping will be performed using the Sequenom® MassARRY iPLEX Platform. (Sequenom, San Diego, CA, USA). Genetic data will be analyzed for Hardy-Weinberg equilibrium, followed by statistical analysis chi-squared, Fisher's exact test, and other methods to determine the contribution of both SNPs and effects of covariates (sex, age, and test site). The contribution of the SNP (AVPR1A) will be analyzed at the system network level using an open source software (Cytoscap) that is designed to intergrate and visualize functional effects of the SNP in a complex network.

Protocol adherence check. Formal training will be provided to research assistants and investigators. Periodic reliability assessments will be performed on the RAs implementation of the protocol on a random sample of 20% of the QST measures. The RAs will be re-assessed in their technique. Although this assessment cannot be blinded, the RA will not be informed of the reliability check until after the data collection procedure. If there is deviation from the initial training. The RA will be re-trained to the protocol.

Statistical Analysis. Data management and preliminary data analysis procedures will be supervised by Drs. Wilkie and Yao. Data will be analyzed using the statistical analysis programs, SPSS version 24 and Stata version14©. Descriptive statistics, ANOVA and Fishers exact test will be used to analyze the data. In the case of missing data, multiple imputations will be used to generate multiple complete datasets on which statistical inference will be performed. Missing at random assumption will be assessed and if necessary sensitivity analysis will be performed using pattern mixture methods. A *p* value equal to or less than .05 will be considered statistically significant.

Aim 1. Using the mean, standard deviation, and range, describe the reference values for detection of warm and cool sensation, hot and cold pain threshold, and pressure (in grams of force) sensation at six sites. Describe the average variability within the sample for each of the six sites being tested and report the normative data.

Aim 2. To compare mean thermal (warm and cool detection, heat and cold pain threshold) values for differences by age, sex, and testing site location (upper body and lower body). **(HO):** Mean warm detection and hot pain threshold values will increase as age increases, will be lower in women than in men, and will be greater in the lower than upper extremities.

Aim 3: To compare mean hot pain threshold values by genotype for the SNP AVRP1A (rs10877969). (HO): Individuals who have the TT genotype will report less pain than those who have the CT AND CC genotype.

Timeline. I will prepare study materials, collaborate with the team to be trained and calibrate, and calibrate equipment. I will recruit volunteers and complete their data collection. I will write manuscripts as data are available and the final report in the last 6 months (Table 8).

Table 8.	2017									2018			
Dissertation Timeline	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sept**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**
Submit grant proposal													
Preliminary Examination													
Assuming the Preliminary Examination is passed:													
Clean data													
Data analysis DNA analysis													
Prepare grant proposal for dissertation													
Submit manuscript 1 for publication													
Submit manuscript 2 for publication													
Dissertation writing/finalization													
Submit final dissertation to Committee													

Potential Problems and Alternate Plans. The protocol was pilot tested with 25 adults with SCD. In the past using the same recruitment strategy and recruitment area. Loss of DNA samples are potential problems. In a previous study using the same recruitment strategy and recruitment area, twice as many volunteers were recruited for a study. One hundred twenty-five healthy AA adult participants were recruited for this study. Calibration of the QST measures can be another problem. QST is a "subjective method" and cooperation of the participant is crucial for it to work properly. This protocol required a monthly calibration of the TSA-II device and von Frey filaments. Inability to obtain a DNA sample can be another problem. If the participant's blood collection does not yield enough blood for DNA analysis, then there will be missing data for that sample.

Summary. At the conclusion of this study, I will have descriptive QST information for a healthy African American adult sample. Combined with the demographics to be collected and the SNP data and its role in sensory and pain perception, I will have a rich foundation of information to use as a comparison for adults of African American descent who have chronic pain. The **scientific premise** of this study is strong because there is a clear need for establishing normative QST values in the healthy African American adult population. There are no studies that focus on the adult population that spans beyond college aged students, with a large sample size. Results of this study will allow for the comparison of normative values from healthy adults with those who suffer from chronic pain experiences, like SCD and cancer. Limitations for this study are that the data generated will be specific for this region of the country and will not account for the regional temperature variations. Future studies, could replicate the approach used in this research in different regions of the country to evaluate if there are regional differences and then make appropriate comparisons which individuals who have chronic pain in those regions. A

further limitation is that the emphasis on the six sites means that the relationship between QST values and the SNP data cannot be reported for the full sample of 125 participants.

4. Human Subjects

Characteristics of Human Subjects

A sample of 125 healthy (pain free) volunteers completed the study. The participants were recruited from the University of Illinois at Chicago (UIC) campus, surrounding communities, and churches. Sickle cell organizations and social media will be used.

Inclusion criteria for the **healthy volunteers**: (a) African American, (b) health history negative for diabetes, hypertension, cancer, sickle cell disease, sickle cell trait, and current acute or chronic pain, (c) speaks English; and (d) age 18 years or older. **Volunteers will be excluded** if they are: (a) legally blind, (b) physically unable to complete study measures, or (c) report using pain medication or recreational drugs. **If the person was not eligible, I thanked the participant using the following statement:** Thank you for your interest in participating in this study. Based on the answers to our eligibility questions, you are not eligible to participate in this study. You might be eligible to participate in our future studies. Again, thank you for speaking with me.

Sources of Research Material

For the aim 1 procedure, healthy participants completed PAIN*Report*It © and were tested using TSA-II NeuroSensory Analyzer (Medoc) and standardized and calibrated von Frey filaments, which provided the sources of data. Data collection procedures required 2 -3 hours at one data collection point for the healthy participants. They provided DNA data via blood sample from venipuncture or finger stick. For all participants, all subject identifiers will be removed when data are analyzed.

Recruitment and Consent Plans

Healthy volunteers were recruited in person after referral or direct community outreach at churches, college student center, grocery, drug stores, and community health screening events. Online listservs and social networks (Facebook, Craigslist, Google+, Twitter, and others), church and community organizations newsletters, text messages, and e-mail, helped to achieve sufficient outreach to the African American population. Subjects who reply to announcements for the study will be evaluated for inclusion and selected until the following all categories reach quota: body site (upper and lower) and the age: 18-39 and ≥40 years. The male:female ratio within each category will be 1:1 (±1). Over sampling may be required to obtain the number of male participants needed for the study design. This design will provide valuable normative QST data, genetic information, and demographics in the healthy (pain free) adult African American population.

These processes were previously used in this community with great success. At a time, just prior to the data collection session, informed consent procedures was performed. Written consent forms were obtained, where the study was described in specific detail, time required of the participants, and the assurance of confidentiality. All materials will be approved by the IRB at the University of Illinois at Chicago IRB and will be HIPAA compliant.

Potential Risks

The risks of this study are primarily those regarding generation of pain from testing with the TSA-II NeuroSensory Analyzer (Medoc) and standardized and calibrated von Frey filaments,

fatigue, bruising, from the blood draw, and loss of privacy. The likelihood of these risks are very small.

Procedures for Protecting Against or Minimizing any Potential Risks

If urgent and unmet health needs of any patient was discovered while the study is being conducted, rapid appropriate referrals were made. The volunteers were informed that they have the right to refuse any or all the study procedures at any time without consequences. I have received training in the ethical treatment of research subjects and biohazards associated with handling biological specimens. Prior to the commencement of the study procedure, I executed confidentiality statements prior to contact with human subjects.

Confidentiality has been maintained through the use of participation codes and investigator sensitivity to privacy thresholds of the participants. For all data analysis procedures, the patient's name will be removed from the data forms and only code numbers will identify the subject data. In the database, all patient identifiers will be encoded with 56 key encryption to protect patient privacy.

Benefits to Subjects to Knowledge Gain

There may be no direct benefit to individual subjects. This study has tremendous potential to inform future studies on QST by providing reference values for adults and older adults. These reference values can be used for comparison to those from adults or older adults who have nociceptive and central or peripheral sensitization-related pain, which could considerably improve management of pain. This study has tremendous potential to inform about the age and sex determinants for QST data in the adult African American population. This study has the potential to inform about the pain related genetic determinants of single nucleotide polymorphism AVPR1A (rs10877969).

Data Safety and Monitoring Plan

Although this study was not an intervention study or a clinical trial, to ensure confidentiality, each subject was assigned a code number. All data are coded with the code number and linked to the patient's name. The patient's name will be removed from the database when the statistical analyses are conducted. Demographic information and chart review information will be pass protected with strong passwords and located on a reliable and secure nursing research server. Access to the drive is password restricted and can only be accessed via an application designed for data download. Only the programmer and statistician will have authority for direct access to the database server and files. The team in the Nursing Health Informatics Core is skilled in developing and deploying user-friendly, secure, and reliable programs for data collection and tracking. Firewall protection, antivirus software, and spy tracking software will be loaded onto each computer for additional safety. The staff members in the nursing information systems office are well-trained and responsible for maintaining the network security and adhering to UIC policy and procedure regarding security for the research data and protected health information. The College of Nursing research servers have firewall protection and access through the firewall is restricted and closely monitored. Data on server will be backed up each night; backups are stored nightly in water- and fireproof cabinets. Completing the testing with the QST protocol are low risk. Appropriate format for monitoring with prompt reporting of adverse effects to the IRB and NIH Program Officer**. Known potential adverse effects were monitored, such as pain from testing with the TSA-II NeuroSensory Analyzer (Medoc) and standardized and calibrated von Frey filaments**. Any adverse events were reported to the IRBs and NIH program officer. We will evaluate the progress of the study, including periodic assessments of data quality and timelines, participant recruitment, accrual and

retention, participant risk verses benefit, and other factors that could impact the outcomes of the study. We will monitor for scientific or therapeutic developments that may have an impact on the safety of the participants or the ethics of the study.

Inclusion of Woman and Minorities

We will strived for a sample that is at least 50% female. All of the participants will be of African American descent. I am sensitive to the cultural issues in research and data, and will use strategies for recruitment which are culturally sensitive, targeted study brochures, community-based advertising, direct discussion in person or via telephone, discussions about the study with community resources (churches, community newsletters and information boards), and other outreach strategies aimed at aiding and maintaining trust between the population of interest and the researchers.

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Appendix B

IRB Approval Letter

Notice of Determination of Human Subject Research

October 24, 2017

20171136-107994-1

Keesha Roach, MS

Biobehavioral Health Science

845 S. Damen Ave

Phone: (312) 952-1317 / Fax: (312) 996-1819

RE: **Protocol # 2017-1136**
Quantitative Sensory Testing Reference Values for Healthy African American Adults

Sponsor(s): None

Dear Keesha Roach:

The UIC Office for the Protection of Research Subjects received your "Determination of Whether an Activity Represents Human Subjects Research" application, and has determined that this activity **DOES NOT meet the definition of human subject research** as defined by 45 CFR 46.102(f).

Specifically, this research will involve a secondary analysis of de-identified data, where the information for this study will be obtained from a previously performed study. The research will not involve interactions or interventions with living individuals for research purposes. The research will also not involve private identifiable information about living individuals. This research will therefore NOT involve human subjects.

You may conduct your activity without further submission to the IRB.

If this activity is used in conjunction with any other research involving human subjects or if it is modified in any way, it must be re-reviewed by OPRS staff.

cc: Diana J. Wilkie, Biobehavioral Health Science. M/C 802

Appendix C

Permission to Reprint Copyright Material

15 March 2018

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A copy of this letter is included for your records.

Thank you for your kind consideration of this request.

Sincerely,

Keesha L. Roach
3120 NW 31st Blvd
Gainesville, FL. 32605

Accept your approved request

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The AVPR1A Gene and Its Single Nucleotide Polymorphism rs10877969: A Literature Review of Associations with Health Conditions and Pain

Author: Keesha L. Roach, Patricia E. Hershberger, Julienne N. Rutherford, Robert E. Molokie, Zaijie Jim Wang, Diana J. Wilkie

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Education

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1999 – 2001	**Bachelor of Science in Nursing** Loyola University Chicago, School of Nursing, Chicago, IL
2004 - 2007	**Masters of Science in Nursing** DePaul University, School of Nursing, Chicago, IL
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PROFESSIONAL NURSING PRACTICE

2001 – 2003	Registered Nurse, Medical and cardiac intensive care units University of Chicago Hospitals, Chicago, IL
2006 – 2013	Registered Nurse, Adult Critical Care Nurse (PACU, ER, ICU) Professional Nursing Incorporated, Chicago, IL
2008-2010	Safety Review Specialist (contract to Abbott and Baxter pharmaceuticals) Delta Pharma, Deerfield, IL
2010 – Present	Patient Care Leader, Cardiovascular Intensive Care, Home Infusion, and Home Health, Advocate Healthcare, Oak Brook, IL

TEACHING EXPERIENCE

2009-2010	Clinical Nursing Instructor Oakton College, Skokie IL
2015 – 2016	Teaching Assistant (Pathophysiology) University of Illinois at Chicago, College of Nursing, Chicago, IL

RESEARCH EXPERIENCE

1987 – 1989	Research Assistant National Institute of Alcohol Abuse and Alcoholism National Institute of Mental Health National Institutes of Health, Bethesda, MD

1991-1994	Research Assistant, Howard Hughes Medical Institute University of Chicago, Chicago, IL
1994 – 1996	Research Assistant The Pasteur Institute, Lille and Paris, France
1996-1997	Scientist I, Sequana Therapeutics, Inc, San Diego, CA
2002 – 2002	Research Assistant, Department of Anesthesia and Critical Care University of Chicago Hospitals, Chicago, IL
2016 – Present	Graduate Research Assistant (R01HL124945) Phenotypic Characterization of Chronic Pain in Adults with Sickle Cell Disease University of Illinois at Chicago, College of Nursing, Chicago, IL
2017 – Present	Research Program Director/Coordinator Center for Palliative Care Research and Education University of Florida, Gainesville FL

Honors and Awards

2015 Dean's PhD Student Award
2015 Robert Wood Johnson Foundation Future of Nursing Scholar
2016 George H. Miller Fund Health Science Research (HSSR) Award
2016 American Pain Society Young Scientist Travel Award
2017 Dean's PhD Student Award
2018 PRICE Travel Award

Grants

2015 Diversity Supplement, National Heart, Lung, and Blood Institute 1R01HL124945S1

Training Programs

2016 Summer Genetics Institute Fellow, National Institute of Nursing Research (NINR)

Peer Reviewed Scientific Publications

Stoffel, M., Bell, K.L., Blackburn, C.L., Powell, K. L., Seo, T. S., Takeda, J., Vionnet, N., Xiang, K. S., Gidh-Jain, M., Pilkis S., J., Ober, D. & Bell, G. I. (1993). Identification of Glucokinase Mutations in Subjects with Gestational Diabetes Mellitus. *Diabetes, 42*, 937-940. PMID: 8495817

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Manuscripts in Preparation

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Roach, K.L., Jhun, E.H., Yao, Y., Ezenwa, M.O., Suarez, M.L., Molokie, R.E., Wang, Z.J., & Wilkie, D.J. (In preparation, February 2017). Pain Factors and Acute Care Utilization Associated with the SNP AVPR1A in Patients with Sickle Cell Disease. *Biological Research for Nursing*.

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LePrete, F., Vionnet, N., **Powell, K. L.**, Froguel, P., Passa, P. (1996, March). Prevelence de Sept Mutations Implique Dans le Diabete Non-Insulin Dependent Dans Une Population d'Indiens de Pondichery. Poster presented at the meeting of ALFEDIAM, Paris

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Roach, K.L., Jhun, E.H., Yao, Y., Ezenwa, M.O., Suarez, M.L., Molokie, R.E., Wang, Z.J., Wilkie, D.J., (2016, September). Pain Factors Associated with Arginine Vasopressin Receptor 1A SNP (rs10877969) in Patients with Sickle Cell Disease. Poster presented at the 16th Congress on Pain for the International Association for the Study of Pain (IASP), Yokohama, Japan.

Ezenwa, M.O., Yao, Y., Wang, Z.J; Suarez, M.L., Zhao, Z., Carrasco, J., Angulo, V., Shuey, D., **Roach, K.**, Mandernach, M.W., Molokie, R.E., & Wilkie, D.J. (2016, September). Perceived Discrimination is Related to Emotional Impact Scores of the Patient-Reported Outcome Measure, the Adult Sickle Cell Quality-of-Life Measurement (ASCQ-Me). Poster presented at the 16th Congress on Pain for the International Association for the Study of Pain (IASP), Yokohama, Japan.

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Schlaeger, J.M., Suarez, M.L., Pauls, H., Steffen, A. D., **Roach, K**., Hughes, T.L., Wilkie, D.J. (Submitted August 24, 2017 American College of Nurse Midwives). Vulvodynia Pain Characteristics: An On-line Survey Pilot Study.

Schlaeger, J.M., Patil, C.L., Suarez, M.L., Pauls, H., Steffen, A.D., **Roach, K.,** Hughes, T.L., Wilkie, D.J. (Accepted for Paper Presentation December 15, 2017, Midwest Nurses Research Society). Vulvodynia Pain Intensity and High-Risk Pain Relief Strategies: A Pilot Study. April 13, 2018 (Poster presentation).

Roach, K.L., Molokie, R.E., Wang, Z.J., Ezenwa, M.O., Shuey, D.; Angulo, V.; Suarez, M.L.; Schlaeger, J.E., Yao, Y.; Wilkie, D.L. (2018, March) PAIN*Report*It Number of Neuropathic pain descriptors Pain Scale Validation in Adults with Sickle Cell Disease. Southern Nurse Research Society (Podium presentation).

Roach, K., Yao, Y., Suarez, ML, Angulo, V., Shuey, D., Ezenwa, MO, Rutherford JN, Schlaeger, JM, Patil, C, Fillingim, RB, Wang,ZJ, Molokie, RE, Wilkie, DJ., (2018, March) QST normative values in African Americans reveal sensitivity to thermal and mechanical stimuli. American Pain Society, Pain Summit. (Poster presentation).

Schlaeger, J.M., Patil, C.L., Suarez, M.L., Pauls, H., Steffen, A.D., **Roach, K.L**., Hughes, T.L., Wilkie, D.J. Vulvodynia, "A Really Great Torturer": A Pilot Study to Examine Pain Intensity and High-Risk Pain Relief Strategies. *Journal of Pain*. 19(3): S107. doi: 1016/j.jpain.2017.12.254 (Poster presentation).

Roach, K.L., Yao, Y., Suarez, M.L., Angulo, V., Shuey, D., Ezenwa, M. O., Rutherford, J. N., Schlaeger, J.M., Patil, C.L., Fillingim, R.B., Wang, Z.J., Molokie, R.E., Wilkie, D.J. Age Differences in Normative Values of African American Adults in Quantitative Sensory Testing (QST). International Society for the Study of Pain 17th World Congress (Poster Presentation).

Schlaeger, J.M., Patil, C.L., Steffen, A.D., Pauls, H., Suarez, M. L., **Roach, K.L**., Hughes, T.L., Wilkie, D.J. Non-drug Pain Relief Strategies Used by Women with Vulvodynia: A Pilot Study (Submitted 3/19/18). International Association for the Study of Pain 17th World Congress (Poster Presentation).

Research Experiences

Project manager for internet-based vulvodynia pain study.

Media

Hostettler, S. (2015, October 28). UIC selected for 'Future of Nursing' grant, names two scholars. UIC
News. Available at https://news.uic.edu/uic-selected-for-future-of-nursing-grant-names-two-scholars.

Professional Memberships

2015 – Present	Midwest Nursing Research Society (MNRS)
2015 – Present	American Pain Society (APS)
2015 – Present	International Association for the Study of Pain (IASP)
2017-- Present	Southern Nurses Research Society (SNRS)

Professional License and Certification

2001 -- Present	Registered Nurse, Professional License

Board Member
2017-Present Have a Heart for Sickle Cell Anemia Foundation