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INTEGRATING BIOMEDICAL AND PSYCHOSOCIAL APPROACHES TO STUDY PAIN IN PEDIATRIC SICKLE CELL DISEASE

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DEDICATION

I would like to dedicate this document to my family and friends and my partner, Joe Schacht, for their unwavering support of my education.

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ABSTRACT

Sickle cell disease (SCD) is a group of inherited blood disorders characterized by recurrent pain. A distinctive obstacle to managing pain in this condition is the substantial heterogeneity of outcomes observed in children, including the rate, intensity, duration, and extent of disability from pain. Previous studies have largely examined this heterogeneity by focusing on either biomedical or psychosocial approaches to this condition rather than pursuing an integrated approach that is consistent with modern conceptualizations of pain. In addition, few studies have examining genetic heterogeneity in pediatric SCD, which remains one of the only strategies for establishing a preventative approach to pain in this condition. In contrast to these approaches, the present project describes an integrated biomedical and psychosocial approach to studying pain in pediatric SCD that consisted of two studies: (a) a study that examined an integrated, biopsychosocial model of pain in SCD and (b) a study that examined a novel genetic marker of pain variability in SCD. The results from these studies are provided along with discussion, clinical implications, and future directions for this work.

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CHAPTER 1

INTRODUCTION

Sickle cell disease (SCD) is a group of inherited blood disorders that affect approximately 1 in 400 to 500 newborns each year in the United States. The majority of these children are of African descent, though children from the Mediterranean, Middle East, Central America, and Central India are also affected (Gustafson, Bonner, Hardy, & Thompson, 2006). Painful vaso-occlusive episodes or 'crises' are considered the hallmark symptom of SCD. These episodes occur when abnormal red blood cells polymerize (i.e., change structure) and occlude areas of the microcirculation, resulting in reduced blood flow, tissue damage, and a painful inflammatory response.

Pain episodes typically begin in childhood and can occur as early as the first year of life (Gill, et al., 1995); thus, pain is a salient issue for children with SCD. A distinct obstacle to effective management of pain in SCD is the substantial heterogeneity observed in children, including the rate, intensity, duration, and extent of disability from pain. Modern theoretical models suggest that this heterogeneity may be explained by a range of factors, including genetic and biological vulnerability and well as psychological and social processes (Embury, 2004); however, previous research has predominantly focused on either biomedical or psychosocial models of pain with little interdisciplinary integration of theory and methodology. Additionally, few studies have focused on identifying biomarkers of risk for pain, such as genetic markers, which may provide a method for identifying children at risk for pain and preventing adverse outcomes.

In contrast to these approaches, the current project describes an interdisciplinary approach to studying pain in children with SCD that was undertaken through two studies: (a) a study that examined an integrated, biopsychosocial model of pain in SCD and (b) a study that examined a novel genetic marker of pain variability in SCD. The current project focused specifically on children for two primary reasons. First, a focus on children allowed for a discussion of prevention and early intervention strategies. Second, research specific to children is especially needed given the unique circumstances of childhood, including the experience of a disease in the context of rapid developmental changes and the influence of important social environments, such as caregivers, on how children respond to pain.

In order to provide a detailed background for the project, the dissertation document begins with a review of research on pain in pediatric SCD, including the clinical significance of pain, extent of pain heterogeneity, and previous theoretical and methodological approaches and findings. The dissertation follows with details of the two studies, including research aims and hypotheses, methods, and results. The document concludes with a discussion of the results, clinical implications, and future research directions. In order to aid the reader with specific terminology, an appendix of key terms and definitions is provided in Table 1.1.

1.1 Clinical Significance of Pain in Pediatric SCD

In children with SCD, recurrent pain from vaso-occlusive episodes may exert an impact on virtually all aspects of quality of life. Pain episodes can occur as early as the first year of life and persist into adulthood (Gill, et al., 1995; Platt, et al., 1991). Pain episodes are the most frequent source of morbidity and health care utilization in patients,

with some children requiring emergency room care or hospitalization for severe episodes (Platt, et al., 1991). The unpredictable and debilitating nature of pain in SCD exerts a significant toll on the lives of children and their families. In addition to frequent medical visits, recurrent and severe episodes may lead to frequent school absences, academic attainment problems, reduced opportunity for physical recreation and social activities, and feelings of depression and anxiety (Anie, 2005; Edwards, et al., 2005; Fuggle, Shand, Gill, & Davies, 1996). Recurrent pain can also result in changes to areas of the peripheral and central nervous system responsible for processing pain, placing children at risk for the development of chronic pain over time (Smith & Scherer, 2010). Given the range of potential physical and psychosocial consequences for children with SCD, the identification of predictors of pain and the development of optimal prevention and intervention strategies in pediatric SCD are clearly important.

1.2 The Issue of Pain Heterogeneity

A distinct obstacle to effective management of pain in SCD is the substantial heterogeneity of outcomes in children, including the rate, intensity, duration, and location of pain and the extent of disability from pain (Gil, et al., 1993; Gil, Williams, Thompson, & Kinney, 1991; Platt, et al., 1991). This section provides descriptions of pain in children with SCD to illustrate this variability.

According to the largest prospective epidemiological study of newborns and adults with SCD up to 66 years of age, approximately 30-40% of patients rarely to never have pain, 50% have several episodes per year, and 20% have frequent episodes per year (Platt, et al., 1991; Schechter, 1999). According to daily diary studies, pain is frequently managed at home, with approximately 40% of children having one episode per month

and 12% having more than two episodes per month (Dampier, et al., 2004; Shapiro, et al., 1995). In addition to the rate of pain in SCD, pain intensity, duration, and location also vary across patients. Many children experience low levels of pain that are managed at home without medication (Gil, et al., 2000). When medication and health care services are used, they tend to occur at high levels of pain, ranging from a six to seven on a ten-point scale. The duration of pain episodes has ranged widely, with some studies reporting a range of one to six days and others reporting durations that have lasted over two weeks (Dampier, et al., 2004; Gil, et al., 2000; Jacob, et al., 2003). For location, most pain is reported in the legs, though pain in the arm, knee, abdomen, and back are also common (Graumlich, et al., 2001). Pain that leads to health care use tends to occur predominantly in the chest, abdomen, or lower back (Jacob, et al., 2003).

Children with SCD may also experience a range of outcomes in response to pain. While some children cope relatively well with pain, children with recurrent and severe episodes and those with limited resources for managing pain may experience detrimental outcomes. As noted previously, functional consequences to pain can include activity disruption, school absences, anxiety and depression, and reduced physical and social recreation (Anie, 2005; Edwards, et al., 2005; Fuggle, et al., 1996; Gil, et al., 1991).

1.3 Theoretical Models of Pain in Pediatric SCD

The issue of pain heterogeneity in SCD is compounded by the myriad of factors that likely influence the pain experience. These include numerous biological systems, psychological factors, and social influences. Although theoretical models are not always explicitly noted in individual studies of pain in SCD, research can be broadly categorized into studies that focus on biomedical versus psychosocial factors. These models are

briefly described, along with common examples of each type of research in pediatric SCD.

The biomedical model conceptualizes disease in terms of measurable biological variables that indicate the presence or absence of disease or disease severity (Engel, 1977). With regard to pain, biomedical models have historically focused on the biological mechanisms of tissue damage that produce the experience of pain (Keefe & France, 1999; Melzack, 1996). The intent of this research is to identify potential routes for medical treatments that can prevent or repair tissue damage or that can alleviate pain (Keefe, Abernethy, & Campbell, 2005). The application of the biomedical model to sickle cell pain can be observed in large-scale epidemiological studies that have examined biological predictors of pain heterogeneity, studies of genetic variability in SCD, and basic science research that attempts to define the correlates and mechanisms of sickle cell vaso-occlusion (Embury, 2004; Higgs & Wood, 2008; Platt, et al., 1991; Sebastiani, Ramoni, Nolan, Baldwin, & Steinberg, 2005; Steinberg, 2005). Medical management of SCD pain is directed towards providing pain relief and preventing serious consequences, such as organ damage, through the use of opiate medication, non-steroidal anti-inflammatory medications (i.e., NSAIDS), and blood transfusion (AAP, 2002). Novel biomarkers that are linked to risk for pain (e.g., genetic markers) may provide a method of early identification and prevention of severe pain episodes, in addition to new treatment targets for SCD pain (Higgs & Woods, 2008).

Psychosocial models suggest that pain is best conceptualized by considering psychological and social variables, in addition to biological mechanisms. The goal of this research is to identify psychosocial factors that affect pain or adjustment to pain,

particularly those that may be modifiable by behavioral intervention. Psychological variables include thoughts, feelings, and behaviors related to the pain experience, while social variables include aspects of the social environment that influence the communication of pain or responses to pain. Psychosocial approaches to pain frequently pull from the theoretical underpinnings of the biopsychosocial model (described below); however, biological variables are not commonly assessed or integrated with the findings of psychosocial variables in these studies (Burlew, Telfair, Colangelo, & Wright, 2000). The application of a psychosocial approach to SCD can be observed in studies of child and caregiver psychological variables and familial influences in relation to pain and functional outcomes (Gil, Abrams, Phillips, & Keefe, 1989; Kliewer & Lewis, 1995; Lutz, Barakat, Smith-Whitley, & Ohene-Frempong, 2004). Psychosocial treatments in pediatric SCD have focused on education about SCD and the implementation of behavior changes (e.g., new coping strategies for pain) in the child and family (Chen, Cole, & Kato, 2004).

In contrast to these approaches, an integrated, biopsychosocial approach to pain incorporates biological and psychosocial variables as well as their interrelationships. This model proposes a dynamic view of pain, in which pain can be both the antecedent and consequence of biological, psychological, and social variables. This view of pain was derived from modern theories of pain, including aspects of gate control theory and social learning theory (Keefe & France, 1999). Gate control theory suggests that the experience of pain can be modulated at multiple levels of the central nervous system, including areas of the brain responsible for psychological constructs (e.g., attention and mood) (Melzack, 1996). This theory predicts that psychological factors will moderate the pain experience.

Social learning theory suggests that children learn to communicate and respond to pain through the social environment, such as by modeling adults or by reacting to the responses of caregivers (Craig, 1975; Craig, Lilley, & Gilbert, 1996). Few applications of integrated, biopsychosocial approaches exist; however, they have the potential to guide integrated biomedical and psychosocial approaches for pain in pediatric SCD.

1.4 Previous Research Findings

The following sections describe primary research findings from each theoretical approach of pain in SCD. While a detailed description of the methodology of these studies is beyond the scope of this proposal, additional information on methodology is provided in Table 1.2. Additionally, a summary of primary research findings is provided in Table 1.3. The sections below focus predominantly on established research that has been replicated in multiple studies with children. Research on adults is also presented if literature is absent in a particular area or in cases where studies included both children and adults with SCD. Seminal studies or newer, promising areas of research are also highlighted.

Biomedical Findings. Biological predictors of pain have been predominantly studied by large-scale epidemiological investigations through the Cooperative Study of Sickle Cell Disease (the CSSCD), a consortium of multiple sites that treat children and adults with SCD. Research on genetic modifiers of disease severity have increased in recent years, though only a handful of markers of pain in SCD exist to date. This section will briefly review the most common laboratory and genetic markers of pain in SCD. Where the term “pain rate” is used in this section, pain was assessed based on the rate of health care utilization for pain (e.g., number of emergency room visits, hospitalizations,

and health care contacts for pain) within a specified range of time (e.g., within the past year).

Laboratory Markers. The most prominent laboratory predictors of pain in SCD include hematocrit/hemoglobin and fetal hemoglobin. Hematocrit is the ratio of red blood cells to total blood volume. Hematocrit is also a derivative of hemoglobin, which is the amount of iron-containing protein in red blood cells. Although higher hematocrit is associated with a lower incidence of anemia, higher hematocrit is associated with a higher pain rate and a shorter duration between pain episodes (Dampier, Ely, Brodecki, & O'Neal, 2002; Platt, et al., 1991). Higher hematocrit levels may be an indicator of higher blood viscosity, a measure of resistance to blood flow, which is a contributing factor to vaso-occlusion (Platt, et al., 1991). Fetal hemoglobin is a form of hemoglobin expressed during the first six months of life. Persistently high levels of fetal hemoglobin are present in some children with SCD, exerting a protective effect on pain. Specifically, higher fetal hemoglobin levels are associated with a lower pain rate and longer duration between episodes (Dampier, et al., 2004). Fetal hemoglobin may exert this protective effect by inhibiting the polymerization of red blood cells. Fetal hemoglobin is almost exclusively measured in children with sickle cell anemia (versus other subtypes; see below) (Platt, et al., 1991).

Several additional biological correlates of pain variability have also been identified; however, hematocrit/hemoglobin and fetal hemoglobin are most consistently included in studies and have been found to be better predictors of pain rate, above and beyond several other laboratory markers (Platt, et al., 1991). Additional factors commonly assessed in clinical studies include coagulation factors (e.g., platelets) and

immune cells involved in the inflammatory response to pain (e.g., white blood cells). Vaso-regulatory factors that modify the constriction and dilation of red blood cells are also strongly implicated (Embury, 2004). (Genetic variability in a specific vaso-regulatory system, the endothelin system, will be the focus of the Study Two of the dissertation, discussed in great detail later).

Genetic Factors. As the result of the great phenotypic heterogeneity observed in patients, modern views of SCD suggest that it is a multifactorial disorder influenced by several genetic and environmental modifiers, including possible gene-gene and gene-environment interactions and epigenetic factors that modify gene expression (Higgs & Wood, 2008). Genetic variability can be examined as broadly as the level of SCD subtypes and at the level of the individual.

Sickle cell disease is comprised of several subtypes. The most common subtype is homozygous HbSS or sickle cell anemia, which is estimated to affect approximately 60% of those with SCD in the United States. Subtypes are distinguished by variations in hemoglobin. Additional forms of SCD include HbSC, comprised of hemoglobin S and C, and the $HbS\beta^0$ and $HbS\beta^+$ thalassemias, comprised of hemoglobin S and a mutant beta globin gene. HbSC is estimated to affect approximately 30% of those with SCD and the thalassemias together are estimated to affect approximately 10% of those with SCD in the United States (Hassell, 2010). Children with $HbS\beta^0$ have a beta globin gene that has been rendered inactive, while children with $HbS\beta^+$ have reduced levels of normal beta globin (Frennette & Atweh, 2007). Children with the HbSS and $HbS\beta^0$ subtypes tend to exhibit a more severe disease trajectory versus the HbSC and $HbS\beta^+$ subtypes, including a higher pain rate and longer duration of pain episodes (Dampier, et al., 2004; Platt, et al., 1991).

This finding may be explained by the "dosage" of the sickle cell trait and the tendency towards sickle cell polymerization in the HbSS and HbSβ^0 subtypes. Although variability exists at the level of SCD subtypes, there is much greater heterogeneity observed within subtypes, suggesting that additional biological and environmental factors are involved (Embury, 2004; Platt, et al., 1991).

The presence or absence of alpha thalassemia trait represents another aspect of genetic variability. Alpha thalassemia trait is present when one or more of the alpha globin genes composing hemoglobin have a genetic mutation (Platt, et al., 1991). The presence of this trait has been associated with a higher pain rate; however, the trait has ameliorative effects on other aspects of disease severity, such as anemia (Gill, et al., 1995). The effects of alpha thalassemia trait on pain rate have been explained by higher hematocrit levels observed in these patients, previously noted to increase blood viscosity and to contribute to vaso-occlusion (Embury, 2004; Platt, et al., 1991).

Additional studies of genetic variability through the CSSCD have examined associations between single nucleotide polymorphisms (SNPs), which are genetic mutations at the level of a single gene, and pain rate. A few promising studies have found that genetic variation underlying fetal hemoglobin levels is associated with pain rate. In Lettre, et al. (2008), authors found that five SNPs accounted for over 20% of the variance in fetal hemoglobin levels (see Table 1.3 for specific SNPs). Their statistical model also demonstrated that the pain rate of patients with SCD could be predicted by genotype (Higgs & Wood, 2008; Uda, et al., 2008). Although this research appears promising for developing markers of risk for pain, few other examples of studies examining genetic risk for pain in SCD exist.

Brief Summary of Findings. Biomedical approaches to SCD have identified consistent predictors of pain rate; however, there is still a large amount of unexplained variability in pain that is not accounted for by existing biomarkers. For example, hematocrit and fetal hemoglobin collectively account for about five percent of the variance in pain rate (Platt, et al., 1991; Lettre, et al., 2008). Additionally, little is known about biomarkers of pain features aside from rate (e.g., intensity, duration), and pain rate has been almost exclusively defined by using health care utilization as a proxy. Rather than being a pure measure of pain rate, health care utilization measures can also capture aspects of the health care environment and experience (e.g., relationships with health care personnel) (Smith, et al., 2005). Additionally, this method does not capture pain that is managed at home without health care contact (Shapiro, et al., 2005).

Establishing laboratory and genetic markers of pain variability appears to be a promising approach to identifying children at risk for severe pain, and it remains the only approach with the potential to lead to preventative approaches to pain in SCD (Frennette & Atweh, 2007). Additionally, identifying novel genetic markers may provide new routes for treatment (Higgs & Woods, 2008). However, existing markers cannot identify children at risk for pain reliably, and current treatment approaches depend on children demonstrating morbidity (e.g., severe pain episodes) prior to treatment. Additional research is clearly needed in this area, particularly in terms of understanding genetic variability. Study Two of this proposal will target this particular limitation of research by studying a novel genetic marker of pain variability, discussed in greater detail later.

Psychosocial Findings. Psychosocial approaches to SCD pain have established several important correlates of pain variability. These include both child- (e.g., child coping) and family-level factors (e.g., caregiver coping).

***Child Factors*.** At the child level, several studies have established the importance of coping strategies in relation to pain variability. Using the stress and coping framework of Lazarus and Folkman (1984), coping with regard to pain can be defined as the thoughts or behaviors children use to manage pain or pain-related stressors. In SCD, two primary types of coping constructs have been studied: coping attempts and negative thinking. Coping attempts are active behaviors or ways of thinking about pain that a child uses to reduce pain, such as distraction, calming statements, or increasing activity level. Negative thinking is focused on rumination or magnification of pain, such as thinking the pain will never end, that no one can help with the pain, or thinking about the negative impact of pain on one's life (Gil, et al., 1991; Rosenstiel & Keefe, 1983).

Through the use of multiple methods, including correlational, experimental, and intervention designs, several studies have established that active coping attempts tend to be related to better pain outcomes versus negative thinking. Specifically, coping attempts are related to lower pain sensitivity, lower health care utilization, and higher activity levels. In contrast, negative thinking is associated with higher pain sensitivity, higher health care utilization, lower activity levels, higher ratings of distress, and poorer psychological adjustment (Anie & Green, 2012; Gil, et al., 1989; Gil, et al., 1991; Thompson, Gil, Burbach, Keith, & Kinney, 1993). Intervention research has demonstrated that active coping strategies can be learned by children with SCD and can modify pain sensitivity. Gil and colleagues (1997, 2001) demonstrated that a brief

training in active coping strategies, including deep breathing, relaxation, and guided imagery, reduced the use of negative thinking and lowered pain sensitivity during a standardized laboratory task in children who practiced the coping strategies. Similar findings have been demonstrated in adults with SCD (Gil, et al., 1996, 2000).

An additional psychological factor that may be important for pain in SCD is mood. Mood can be defined as an emotional state that varies on two dimensions: positive and negative. Positive mood is the degree to which individuals feel joyful and energetic. In contrast, negative mood is the degree to which individuals feel sad, nervous, angry, or distressed (Laurent, et al., 1999; Watson, Clark, & Tellegen, 1988). Gil and colleagues (2003) examined mood in 37 adolescents with SCD using a daily diary approach. Diaries were collected for up to six months. Mood was assessed by asking adolescents the degree to which they felt different positive and negative emotions. Adolescents reported on pain intensity, duration, health care utilization, distress, and activity levels as primary outcomes. The authors found that negative mood was related to greater pain intensity, greater health care utilization, and lower activity levels and that positive mood was related to lower pain intensity, lower health care utilization, and higher activity levels. These authors have also demonstrated similar findings with adults (Gil, et al., 2004). In addition to these findings, mood is widely viewed to be an important psychological correlate of pain in both SCD and other pain conditions (Keefe, et al., 2005; Porter, Gil, Carson, Anthony, & Ready, 2000; Porter, et al., 1998; Serjeant, et al., 1994).

***Social Factors*.** Although social factors may not directly impact the pain experience, they are relevant for two primary reasons: (a) social factors can influence important psychological factors that directly impact pain (e.g., the development of coping

skills) and are therefore integral to interventions for pain and (b) social factors can influence the treatment of pain in children through caregiver and provider decision-making (Kenneth, Lilley, & Gilbert, 1996). For children with SCD, the most widely studied social factor is the family environment.

Several studies in SCD have focused on the contribution of family-level factors to how children respond to pain, with the strongest findings supporting the influence of caregiver coping and family functioning on children's responses to pain. Children's coping strategies tend to coincide with caregiver coping strategies for pain in SCD, suggesting that social modeling may be important for understanding the development and maintenance of coping strategies in children (Gil, et al., 1991; Kliewer & Lewis, 1995; Lutz, et al., 2004). Family functioning, defined by how a family cooperates together as a unit, has been consistently related to child and caregiver coping; however, the exact manner in which family processes influence child and caregiver coping is likely to be complex (Kliewer & Lewis, 1995; Lutz, et al., 2004). For example, greater family cohesion, a component of family functioning, has predicted active coping in children and caregivers in some studies and maladaptive coping in others (Brown, et al., 1993); thus, qualities of family functioning, such as the extent to which cohesion promotes active coping in children versus dependence on caregivers, may be important (Kliewer & Lewis, 1995).

Additional family-level variables that may be relevant include caregiver psychological functioning and caregiver-child communication around pain; however, research in this area is sparse and requires replication. Caregiver psychological functioning has been linked to child adjustment and rates of health care utilization in

SCD (Logan, Radcliffe, & Smith-Whitley, 2002; Thompson, et al., 1993). Child adjustment, which can include aspects of mood, may be an important correlate of adverse pain outcomes (Edwards, et al., 2005); thus, the extent to which caregivers influence child adjustment may be relevant for understanding pain in SCD. Although research is sparse for understanding caregiver characteristics that impact pain, this research may suggest opportunities for family-level intervention for pain.

***Brief Summary of Findings*.** Research has demonstrated that psychosocial factors are integral to understanding pain variability in children with SCD. The most consistent evidence exists in the area of coping, with child coping consistently associated with pain outcomes. Additionally, specific family-level factors, such as caregiver coping and family functioning, appear to be relevant for understanding the development and use of coping strategies in children. Psychosocial studies have also focused on a range of pain outcomes (e.g., pain features and functional outcomes). Although these studies tend to use small to moderate sample sizes, the effect sizes for child psychological variables, in particular, have been moderate to large in size, ranging from R^2 of .06 to .17. Nonetheless, this approach is limited by the absence of research on how family-level factors impact pain outcomes, in addition to their influence on child coping and adjustment.

Integrated Biopsychosocial Findings. Modern theoretical models of pain and chronic illness support an integrated approach to research that combines biomedical and psychosocial approaches (Engel, 1977; Keefe & France, 1999). There are currently no examples of this type of research focused on pain in children with SCD; however, two

examples of integrated research in SCD provide useful templates: the PiSCES project and biopsychosocial studies of adjustment in pediatric SCD.

The PiSCES Project. The stated aim of the Pain in Sickle Cell Epidemiology Study (PiSCES) project is to "validate a biopsychosocial model of SCD pain, pain response, and health care utilization in a large, multisite adult cohort" (Smith, et al., 2005). As this statement illustrates, the PiSCES project is designed to examine an integrated model of pain. The study is a large, multisite endeavor conducted as part of the CSSCD. Common biomarkers of pain variability (e.g., hematocrit), psychological factors (e.g., coping), and social factors (e.g., social support, health care issues) are examined. Pain outcomes, including rate, intensity, duration, location, and health care utilization, and medication usage are assessed via daily diaries. These characteristics demonstrate a true integration of biopsychosocial theory and methodology. Although several studies have been published from this project, the examination of interrelationships among biological and psychosocial predictors has not been published. Nonetheless, the methods of this study provide a useful template for how integrated research across multiple disciplines can be accomplished in SCD.

Biopsychosocial Models of Adjustment. Another potentially useful line of integrated research in SCD comes from studies that have applied a biopsychosocial model of adjustment to SCD in children (Burlew, et al., 2000; Thompson, et al., 1993). The strength of this research is that it has focused on integration of biopsychosocial factors in children; thus, these studies considered issues that are pertinent to a pediatric population (e.g., use of measures and constructs appropriate for children, examination of family-level factors). These studies have also focused on the assessment of moderators

and mediators of psychological adjustment, an approach that may be used to examine pain outcomes in SCD (Burlew, et al., 2000; Lutz, et al., 2004). Biopsychosocial studies of adjustment have generally focused on how the impact of disease parameters (e.g., biomarkers and clinical events) on child and caregiver adjustment (e.g., ratings of depression) is moderated or mediated by psychosocial factors (e.g., child and caregiver coping). This line of research has been successful in demonstrating the utility of using a biopsychosocial model in predicting variability in adjustment outcomes. For example, this research has consistently demonstrated that psychosocial variables may play a larger role in predicting adjustment in SCD than biological factors, a finding that is important to consider for intervention planning (Barakat, Schwartz, Simon, & Radcliffe, 2007; Burlew, et al., 2000; Lutz, et al., 2004).

Brief Summary of Findings. Currently, no published findings exist on the relative contributions of biological and psychosocial factors to pain in SCD, and an integrated model has yet to be used in children with SCD to predict pain. Examples of integrated approaches exist for adults with SCD and for children to predict adjustment outcomes; however, there are several unanswered questions with regard to how biopsychosocial factors impact pain in SCD.

First, we do not know the relative contributions (i.e., relative effect sizes) of biomedical versus psychosocial variables on pain variability, which is important when considering the allocation of resources to treat pain. Second, we do not know the contributions of biomedical and psychosocial to specific pain outcomes, which may be useful for tailoring treatment approaches to patients. For example, children may vary in the extent to which certain features impact quality of life (e.g., pain intensity versus

frequency). Third, no studies have examined how biopsychosocial variables interact to produce pain. For example, psychological factors may moderate the impact of biology on pain, as predicted by the gate control theory, or these variables may act independently of one another. The focus of Study One of this proposal is to examine an integrated model of pain in children with SCD, in order to address these limitations.

Table 1.1.
Glossary of Terms and Definitions

Term	Commonly Used Definitions
Biomedical Variables	
Hematocrit	The ratio of red blood cells to total blood volume
Hemoglobin	The iron-containing protein of red blood cells that transports oxygen from the lungs to the body
Fetal Hemoglobin	Form of hemoglobin expressed during the first six months of life; persistently high levels beyond the first six months of life are found in some individuals
Single nucleotide polymorphism	A mutation of a single nucleotide in a gene; also called a SNP
Psychological Variables	
Coping	Constantly changing efforts to combat internal or external events that are appraised by an individual as challenging
Child Coping	
Coping Attempts	Engaging in active thinking or behavioral strategies to reduce pain
Negative Thinking	Engaging in thoughts about pain that are characterized by rumination or magnification of pain
Caregiver Coping	
Active Coping	Engaging in active thinking or behavioral strategies to reduce pain (similar to child coping attempts)
Passive Coping	Engaging in passive strategies, such as rumination about pain (similar to child negative thinking)
Mood	A state of positive (e.g., happy, excited) or negative (e.g., sad, fearful) affect
Social Variables	
Social modeling	Display of behaviors to children that are then attended to, learned, and exhibited by children
Family functioning	The degree to which a family generally functions together as a unit
Pain Outcomes	
Pain rate/frequency	The number of pain episodes occurring in a specified period of time
Pain intensity	The subjective amount of pain experienced by a patient; can be a particular episode or average pain intensity
Pain duration	The length of time that a pain episode occurs; can be a particular episode or average pain duration
Pain location	The part or parts of the body in which the patient interprets the pain as occurring
Health care utilization	The degree to which a patient uses health care services for pain; in SCD, services may range from routine visits or calls to emergency room care or hospitalization
Functional outcomes	The degree to which pain interferes with a patient's emotional, social, and occupational well-being

Table 1.2.
Biomedical, Psychosocial, and Integrated Approaches to Studying Pain in Sickle Cell Disease

Theoretical Model	Methods	Key Variables	Key Outcomes	Strengths	Limitations
Biomedical Model	Large sample size, multi-site CSSCD	Biomarkers: Hematocrit Fetal hemoglobin Genetic variability (SCD subtypes, alpha thalassemia, variation in fetal hemoglobin)	Pain rate (often assessed by health care utilization)	Guide for medical management, including prevention and intervention Generalizability of findings is high	Ignores patient- and social-level factors Pain features aside from rate not widely studied
Psychosocial Model	Small-to-moderate, single site samples Systems approach (i.e., patient- and social-level factors) Multi-modal assessment of pain	Patient-level: Child coping Child mood Social-level: Parental coping Parental psychological functioning Family functioning	Multiple pain features: Rate Intensity Duration Location Health care utilization Functional outcomes	Guide for behavioral interventions Incorporates patient- and social-level factors Multimodal assessment of pain	Generalizability of findings only to one site No guide for prevention of pain No integration of biomedical and psychosocial variables
Biopsychosocial/ Integrated Model	Large sample size, multisite for CSSCD (could be variable) Interdisciplinary collaboration Systems approach Multi-modal assessment of pain	Biomarkers Patient- and social-level variables Interrelationships among variables: establishing key mediators and moderators of pain	Multiple pain features and outcomes Psychological adjustment	Guide for integrated approach to pain management Incorporates multiple levels of analysis and tests modern models of pain Resource sharing and collaboration	Different research approaches (e.g., measures, statistical methods) must be integrated No research focusing on pain in children with SCD

Note. This table provides descriptive information on biomedical, psychosocial, and integrated approaches to studying pain in sickle cell disease (SCD) based on common characteristics of existing studies. CSSCD = Cooperative Study of Sickle Cell Disease

Table 1.3.
Summary of Biopsychosocial Variables in Relation to Pain in Pediatric Sickle Cell Disease

Biomedical Variables	Relationship to Pain Outcomes	References
Hematocrit (Hct)	↑Hct ↑pain rate (health care utilization)	Dampier, et al., 2004; Platt, et al., 1991
Fetal hemoglobin (HgF)	↑HgF ↓pain rate (health care utilization)	Dampier, et al., 2004; Platt, et al., 1991
SCD Subtype	HbSS, HbSβ^o > HbSC, HbSβ^+ for pain rate and duration	Dampier, et al., 2004; Gil, et al., 1991; Platt, et al., 1991
Alpha thalassemia trait	Presence of trait ↑pain rate (health care utilization)	Gill, et al., 1995; Platt, et al., 1991
Single nucleotide polymorphisms (SNPs): *BCL11A* rs4671393 *HBS1L-MYB* rs28384513 rs9399137 rs4895441 *β-globin gene cluster XmnI* rs7482144	Collectively, all five SNPs significantly predicted ↓pain rate (health care utilization) in Lettre, et al., 2008 No statistically significant effect for individual SNPs	Lettre, et al., 2008; Uda, et al., 2008
Psychological Variables	**Relationship to Pain Outcomes**	**References**
Child coping	↑ Coping attempts ↓pain sensitivity, ↓health care utilization, ↑activity levels ↑ Negative thinking ↑pain sensitivity, ↑health care utilization, ↓activity levels	Anie, et al., 2002; Gil, et al., 1989, 1991, 1993, 1997, 2001 Adults: Gil, et al., 1996, 2000
Mood	Negative mood ↑pain intensity, ↓activity levels ↑ Positive mood ↓pain intensity, ↑activity levels, ↑school attendance	Gil, et al., 2003 Adults: Gil, et al., 2004
Social Variables	**Relationship to Child Outcomes**	**References**
Parental coping	Child coping for pain mirrors caregiver coping	Gil, et al., 1991; Kliewer & Lewis, 1995; Lutz, et al., 2004
Family functioning	Complex, depends on extent to which family promotes adaptive coping	Brown, et al., 1993; Kliewer & Lewis, 1995; Lutz, et al., 2004

CHAPTER 2

THE CURRENT PROJECT

In reviewing biomedical and psychosocial approaches to SCD pain, it is clear that both approaches have contributed to the current understanding of pain and treatment approaches for pain in SCD; however, there are few examples of studies that integrate biomedical and psychosocial approaches to pain as well as minimal research on genetic markers of pain heterogeneity. The current project aimed to build from previous research through two studies described below: (a) a study that examined an integrated, biopsychosocial model of pain in pediatric SCD and (b) a study that examined a novel genetic marker of pain in SCD.

2.1 Study One: Examine an Integrated, Biopsychosocial Model of Pain in Pediatric SCD

Modern theoretical models of pain, including gate control theory and the biopsychosocial model of pain, suggest that a combination of biological, psychological, and social factors contribute to pain. Specifically, these models suggest that psychological factors, such as coping and mood, modulate the relationships between biological factors and pain (Melzack, 1999). The biopsychosocial model also proposes that individual reactions to pain are often established and reinforced by the social environment (Keefe & France, 1999). Previous cross-sectional, experimental, and daily diary studies by Gil and colleagues (1989, 1991, 1997, 2001, 2003) have indicated that psychological factors, such as coping and mood, may play a central role in understanding

pain variability, including pain sensitivity, pain intensity, and health care utilization for pain. Additional research on the social environment of children with SCD indicates that caregiver and familial factors, such as caregiver coping and family functioning, may influence children's development of strategies to manage pain (Kliewer & Lewis, 1995; Lutz, et al., 2004).

Despite these findings, there is little information on how these variables collectively influence pain. The following aims and hypotheses focused on developing an integrated, biopsychosocial model of pain in pediatric SCD.

Study One: Aims and Hypotheses.

Aim I: Compare the effect sizes of biological and psychological factors in relation to multiple pain outcomes in pediatric SCD. For Aim I, effect sizes were examined to better understand the role of specific biological (sickle cell subtype; hematocrit and fetal hemoglobin) and psychological (child coping and mood) factors previously demonstrated to be predictive of pain in SCD. This information may be useful for understanding the relative contributions of biomedical and psychological factors to multiple pain features for the purposes of allocating resources to treat pain and to tailor treatment approaches to specific pain features. To assess pain, multiple pain outcomes were assessed, including pain rate, intensity, duration, and health care utilization. For Aim 1, the following hypotheses were made:

Hypothesis 1: Biological variables (sickle cell subtype; fetal hemoglobin and hematocrit) will be associated with pain rate and health care utilization for pain, with an approximate, cumulative R^2 of .05 or higher. This effect size estimate was derived from two large, epidemiological investigations that consistently supported an R^2 of .05 for

these biological variables when pain rate was assessed based on health care utilization (Lettre, et al., 2008; Platt, et al., 1991). Hypothesis 2: Child psychological variables (child coping and mood) will be associated with pain intensity and health care utilization for pain, with approximate R^2 values of .12 for pain intensity and .08 for health care utilization, respectively. These effect size estimates were based on previous research estimating the effects of coping and mood on pain outcomes in SCD, with effect sizes ranging from .06 to .17 for pain intensity and .06 to .10 for health care utilization (Gil, et al., 1989; Gil, Abrams, Phillips, & Williams, 1992; Gil, et al., 2003; Gil, et al., 1991). These estimates were averaged to develop specific hypotheses. No hypotheses were provided for pain duration due to an absence of published effect sizes; analyses with pain duration were considered exploratory.

Aim II: Explore interrelationships among biological and psychological factors. Modern theoretical models of pain suggest that a combination of biological, psychological, and social factors contribute to pain. Specifically, theoretical models of pain suggest that psychological factors are involved in pain perception and may moderate the impact of biological factors on pain (Keefe & France, 1999; Melzack, 1999). Few studies have examined how biological and psychological factors work together to impact pain in pediatric SCD, which was the goal of Aim II. Two competing hypotheses about the relationships between biological and psychological factors and pain were made:

Hypothesis 3: Biological and psychological factors will operate independently of each other, such that child coping and mood have an additive relationship with biological factors (sickle cell subtype; fetal hemoglobin and hematocrit) in predicting pain outcomes. Hypothesis 4: Consistent with modern theoretical models of pain (Keefe &

France, 1999; Melzack, 1999), child coping and mood will moderate the relationship between biological factors and pain outcomes. Specifically, greater use of maladaptive coping strategies or negative mood will have a synergistic effect with biological risk; greater use of adaptive coping strategies or positive mood will buffer the effects of biological risk. Given the possibility of small effect sizes for biological predictors, these analyses were considered exploratory, with a focus on both effect sizes and statistical significance.

Aim III: Examine relationships between child psychological variables, caregiver psychological variables, family functioning, and pain outcomes. This aim focused on the relationships between child and caregiver psychological variables and family functioning as well as their relationship to pain outcomes. Results from this aim were anticipated to provide information that could inform mediation analyses to understand relationships between caregiver and family factors and child pain outcomes in SCD as well as potential mechanisms for these effects (e.g., child coping or mood). The following hypotheses were made:

Hypothesis 5: Child coping and mood will mirror caregiver coping and mood (e.g., higher adaptive coping in caregivers will be associated with higher adaptive coping in children). Hypothesis 6: Better ratings of family functioning will be correlated with higher coping attempts and lower ratings of negative thinking in children. Hypothesis 7: Caregiver coping, mood, and family functioning will be correlated with health care utilization. Specifically, higher active coping, higher positive mood, and higher family functioning will be associated with lower health care utilization, while higher passive coping, higher negative mood, and lower family functioning will be associated with

higher health care utilization. Exploratory analyses were conducted for pain rate, intensity, and duration.

2.2 Study Two: Examine a Novel Genetic Marker of Pain in Pediatric SCD

The great heterogeneity of pain observed in SCD has led to modern views of SCD as a multifactorial disorder influenced by several underlying biological and environmental factors (Higgs & Wood, 2008). The identification of biomarkers of pain risk, including genetic markers, has been proposed as a promising method for identifying children early in life and preventing adverse consequences from severe pain (Lettre, et al., 2008). Additionally, novel genetic markers may suggest new routes for treatment of pain (Higgs & Woods, 2008).

Previous epidemiological studies have found that variations in hematocrit and fetal hemoglobin levels and genetic variants hypothesized to be involved in the regulation of fetal hemoglobin are predictive of pain rate in SCD. Although these markers have explained statistically significant variability in pain rate, there is substantial variability in pain that remains unexplained. Additionally, aside from genetic markers of fetal hemoglobin, there are likely to be several other genetic and epigenetic factors that are predictive of pain severity (Higgs & Wood, 2008; Lettre, et al., 2008; Platt, et al., 1991).

Of the candidate genes that have been described in relation to pain, genes involved in vaso-regulatory systems have been strongly implicated. Vaso-regulatory factors are thought to contribute to both the initiation and duration of pain due to their influence on blood velocity and the transit time of sickled cells in the microcirculation. Levels of endothelin-1 (ET-1), a potent vaso-active peptide, increase in response to conditions of low oxygen and the sickling of cells in the endothelium that occur during

pain episodes. Due to its vaso-constrictive properties, ET-1 may delay the transit time of sickled cells out of the endothelium, thereby contributing to vaso-occlusion (Hebbel, Osarogiagbon, & Kaul, 2004). The biological actions of ET-1 are mediated by two receptors (ET-A, ET-B). The ET-A receptor is expressed on vascular smooth muscle cells and mediates vasoconstriction. In contrast, the ET-B receptor is expressed on endothelial cells and mediates vasodilation (Khodorova, Montmayeur, & Strichartz, 2009). Over the past several years, single nucleotide polymorphisms (SNPs) in coding regions for these receptors have been associated with several aspects of vessel regulation, including alterations in cardiovascular function and exercise-induced arterial adaptation (Dong, Wang, Zhu, Treiber, & Snieder, 2004; Iemitsu, et al., 2006).

A preliminary study of two specific SNPs in the endothelin system was undertaken to assist in the development of Study Two and to inform specific hypotheses. The following is a description of the findings from the preliminary study.

Preliminary Study of Endothelin Receptor SNPs and Pain Rate. A preliminary study of two SNPs in the ET-A and ET-B receptors (rs5333 and rs5351, respectively) was conducted with 20 children with SCD (all subtypes) ages 2 to 17 years and their families. The data was collected as part of a larger study of 61 children with SCD that examined plasma endothelin levels during procedural pain. Participants were recruited at the Center for Children's Cancer and Blood Disorders (the primary recruitment site for the current project). Genotyping for the ET-A and ET-B SNPs was an exploratory component of this study and samples were not available from all participants. Genotyping was conducted on 12 of 20 plasma samples for the ET-A SNP and 14 of 20 samples for the ET-B SNP (not all plasma samples were successfully amplified). The

breakdown of SCD subtypes within each SNP was as follows: ET-A A/A (HbSS *n* = 3, HbSβ^+ *n* = 1), A/G (HbSS *n* = 4, HbSC *n* = 1), and G/G (HbSS *n* = 1, HbSβ^+ *n* = 1); ET-B A/A (HbSS *n* = 1, HbSβ^+ *n* = 1), A/G (HbSS *n* = 1, HbSC n = 1, HbSβ^+ *n* = 2), and G/G (HbSS *n* = 8).

Figure 2.1 shows the genotypes for the ET-B SNP in 14 children with SCD in relation to health care utilization for pain over the previous 24 months as noted in the child's medical record, including hospitalizations, emergency room visits, and outpatient health care contacts. The figure shows a significant mean difference between the ET-B genotypes for health care utilization, with the GG genotype (*M* = 15.13, *SD* =7.77) being associated with higher health care utilization than the AG and AA subtypes (average of means = 2.38, average of *SD*s = 2.26), *t* (11) =3.55, *p* = .005. In contrast, The ET-A genotypes were not related to health care utilization in this sample. The findings from this study suggested that genetic variability in the ET-B receptor gene was a potential marker of health care utilization in children with SCD. The specific pattern of results also suggested a codominant model, in which each copy of the G allele conferred greater risk for pain.

Study Two: Aim and Hypothesis. The primary aim of Study Two was to replicate the results for the ET-B receptor using a larger sample of children with SCD, thereby establishing a novel marker of pain variability in SCD. The ET-B allele was examined in relation to health care utilization, with secondary exploratory analyses conducted for pain rate, intensity, and duration. Based on our preliminary data, the following hypothesis was made:

Hypothesis: The G allele will confer greater risk for health care utilization, such that the GG and AG genotypes will be associated with higher rate of utilization for pain than the AA genotype.

Note: Although the ET-B gene could have been examined in concert with the psychosocial variables in Study One, it was separated out due to the absence of prior research documenting this SNP as a predictor of pain in SCD.

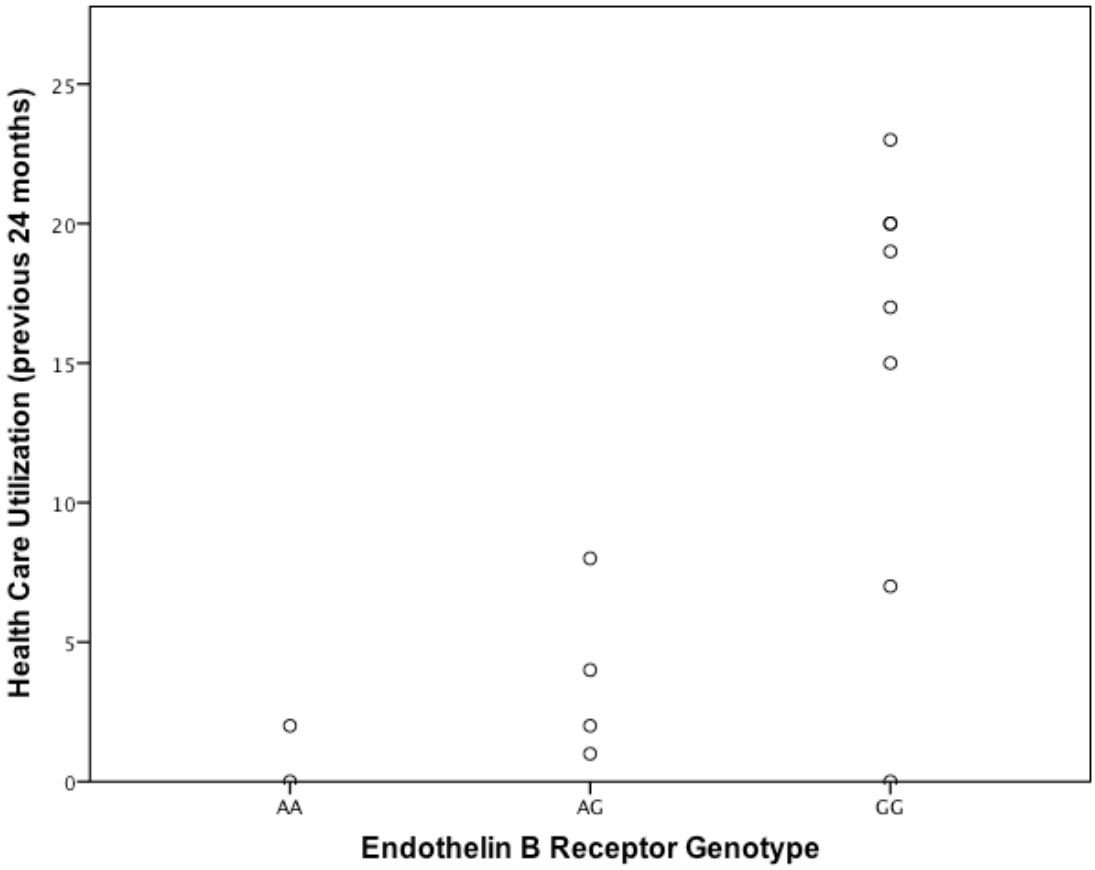

Figure 2.1.. Relationship between endothelin B receptor genotypes and health care utilization from preliminary data. This figure depicts the relationship between ET-B receptor genotypes (AA, AG, or GG) in 14 children with sickle cell disease and health care utilization over the previous 24 months as measured by the medical record.

CHAPTER 3

METHODS

3.1 Overall Approach

Study One and Two were undertaken concurrently using a cross-sectional design that included the collection of data from children with SCD and their caregivers during routine health visits. Children and caregivers completed measures that assessed demographic and psychosocial variables and pain history (Study One) and children provided a saliva sample for genetic analysis (Study Two). For psychosocial variables, children completed measures of coping and mood. Caregivers completed similar measures to assess coping and mood as well as a demographic questionnaire and a measure of family functioning. Information on additional demographic variables, medical status, pain, and laboratory markers of current disease status (e.g., fetal hemoglobin) were collected through medical record review.

3.2 Participants and Recruitment

Seventy-six children and youth with SCD, ages 8 to 21, and 70 caregivers participated in the studies (see Tables 3.1 and 3.2). This age range for children was chosen to maximize participation in the research while also considering the lowest age range that would allow for valid self-report data (our measures had been validated with children as young as age 8). Youth between the ages of 18 and 21 were given the choice of participating with or without a caregiver. This decision was made to maximize participation of this particular group of youth, who were more likely to attend

hematological visits without a caregiver. Five youth chose to participate without a caregiver.

Families were recruited during routine health visits at the Center for Children's Cancer and Blood Disorders (CCBD) at Palmetto Richland Children's Hospital in Columbia, SC. Study recruitment and enrollment occurred between April of 2012 and May of 2013. Eligibility for the study was determined by examining the child's medical chart. After the chart review, we consulted with Dr. Carla Roberts, the attending hematologist, to determine if there were any reasons to postpone approaching the family (e.g., acute medical or psychosocial concerns). Dr. Roberts asked the families about their interest in the study and an investigator followed up with the family after the visit or via phone to set up a time to complete the procedures (typically at the child's next scheduled hematological visit). During the study period, 126 families were approached and 6 were not interested. Reasons given included: not a good time for the family (n = 4), not enough time at appointments (n = 1), and no reason (n = 1). Forty-four families were interested, but did not participate. Reasons included the following: the family did not show up for their follow-up hematological visits (n = 19), the child was not scheduled to come back until after the study ended (n = 18), not enough time at child's hematological visit (n = 5), and acute psychosocial concerns (n = 2).

Inclusion and Exclusion Criteria. Eligible children must have received their primary hematological care through the CCBD. Children with major developmental disorders or overt neurologic disease resulting in severe cognitive limitations were excluded, as these conditions would limit the validity of the self-report data. Of the children eligible to participate by age, four percent were excluded due to cognitive or

developmental disorders. Participants could not be experiencing pain requiring the use of opioid-based drugs on the day of participation as this may have influenced their ability to complete self-report data on pain. If opioid drugs were used, children and their families were asked to participate at a later time or during their next routine visit.

Children undergoing transfusion therapy were excluded because transfusion therapy can alter the natural course of pain in SCD and these children represented a small minority of the overall population of children with SCD at the clinic (Miller, et al., 2001). Of the children eligible to participate by age, eight percent were excluded due to transfusion therapy. In contrast, children receiving hydroxyurea, a treatment that can also alter the natural course of pain in SCD, were not excluded as this treatment was much more prevalent in the clinic population (Strouse, et al., 2008). Caregivers of children treated with hydroxyurea were asked to provide additional detail about the child's pain history and the researcher documented treatment and pain history during the medical record review. In addition, the hematologist was consulted to provide ratings of therapeutic response to complement the medical record review.

3.3 Measures

Background Information. Caregivers completed a brief demographic questionnaire that requested information on the child' race and ethnicity as well as the caregiver's relation to the child, race, ethnicity, marital status, and education.

Pain and Disease Information. Information on the child's health status can be found in Table 3.1. Table 3.3 provides means and standard deviations for the pain outcomes.

Pain History Interview. The pain history interview is a modified version of the Structured Pain Interview (SPI) that was validated by Gil and colleagues (1991) in children and adolescents with SCD ages 7 to 18 years and their caregivers. The pain history interview assesses recent pain status and health care utilization through separate child and caregiver interviews. The interview also assesses family history of chronic health conditions, including pain conditions, for the caregiver interview. For recent pain status, pain rate and health care utilization (over the past 12 months), location, average duration (in days), and average intensity (on a scale from 0 to 10) were noted. For health care utilization, children and caregivers were asked about hospitalizations, emergency room visits, and outpatient health care contacts (e.g., calls or visits to the clinic for pain) over the past 12 months. For pain intensity, children and caregivers were shown a visual pain scale with numerical anchors from 0 to 10 along with three verbal anchors (0 = no pain, 5 = moderate pain, 10 = worst pain possible). For children currently taking hydroxyurea, caregivers were asked to provide additional ratings of pain rate, health care utilization, average duration, and average intensity for the year prior to the child taking hydroxyurea.

Gil and colleagues (1991, 1993) demonstrated moderate correspondence between child and caregiver reports on the SPI as well as temporal stability of the measure in caregivers of children with SCD. In the present sample, child and caregiver ratings were moderately associated for pain rate ($r = .32$, $p = .009$), intensity ($r = .30$, $p = .015$), and duration ($r = .31$, $p = .010$). Child and caregiver ratings were more strongly associated for health care utilization ($r = .66$, $p < .001$). There were no systematic differences in correspondence with caregiver reports between younger children (ages 8 to 12) and

adolescents (ages 13 and older). For younger children, the correlations with caregiver ratings were as follows: pain rate (r = .31), intensity (r = .46), duration (r = .31), and health care utilization (r = .65). For adolescents, the correlations with caregiver reports were as follows: pain rate (r = .33), intensity (r = .15), duration (r = .41), and health care utilization (r = .78). Given only moderate associations between most child and caregiver ratings, final ratings were averaged between reporters in an attempt to avoid over- or under-estimation of pain.

Medical Record Review. Medical record review was conducted using a structured coding method. Information was collected on the child's age, gender, sickle cell genotype, and the most recent laboratory blood results for hematocrit and fetal hemoglobin. Information was also collected on potential biological covariates, including the presence or absence of alpha thalassemia trait and the most recent laboratory blood results for white blood cells and platelets. At the CCBD, hematocrit, white blood cells, and platelets were routinely measured at each clinic visit. Fetal hemoglobin was measured every two years for children ages 8 to 21 until levels reach less than 10%. For pain history, documented hospitalizations, emergency room visits, and outpatient health care contacts (e.g., calls or visits to the clinic) for pain episodes over the previous 24 months were recorded. Although the time captured for medical record review differed from caregiver- and child-reported health care utilization in the pain history interview (i.e., 24 months versus 12 months), these measures were strongly related (r = .67).

For children taking hydroxyurea, laboratory values for hematocrit and fetal hemoglobin that were closest to the start date of treatment were recorded. In addition, documented hospitalizations, emergency room visits, and outpatient health care contacts

for pain episodes in the 24 months preceding the start of treatment were recorded. This information was used to obtain measures of the child's pre-hydroxyurea health status.

Hematologist Ratings of Hydroxyurea. For children taking hydroxyurea, the hematologist provided ratings of therapeutic response. This information was used descriptively to better understand the possible impact of hydroxyurea on the study outcomes. For therapeutic response, the hematologist reviewed the child's laboratory work since the start of treatment and provided a rating of good, partial, or no response to treatment. Children in the "partial response" category included those who had not yet reached a therapeutic dosage.

Psychological Variables: Child-Completed. Table 3.4 provides means, standard deviations, and internal consistency estimates for the psychosocial questionnaires.

Coping Strategies Questionnaire for Sickle Cell Disease (CSQ-SCD). The CSQ-SCD is an 80-item questionnaire that assesses the frequency of coping behaviors children use to deal with pain. The CSQ-SCD was validated by Gil and colleagues (1991) for children and adolescents with SCD ages 7 to 17. The CSQ-SCD includes 13 subscales with six items each, with each item rated on a 7-point Likert scale from 0 (never) to 6 (always) to indicate how often a strategy is used. Factor analysis has supported three broad subscales: Coping Attempts, Negative Thinking, and Passive-Adherence. Coping Attempts, which measures adaptive coping (e.g., distraction), and Negative Thinking, which measures maladaptive coping (e.g., catastrophic thinking), were the focus of the present study. Scores on the Coping Attempts scale ranged from 0 to 180, with higher scores representing higher coping attempts. Scores on the Negative Thinking Scale ranged from 0 to 144, with higher scores representing higher ratings of negative thinking.

In the current study, internal consistency was good to excellent for the Coping Attempts (α = .91) and Negative Thinking (α = .89) scales.

Positive and Negative Affect Scale (PANAS-C). The PANAS-C is a 27-item scale containing two subscales that assess positive and negative mood states. Children are asked to rate the extent to which they have felt different positive (e.g., happy, excited) and negative emotions (e.g., sad, upset) in the past week using a 5-point Likert scale ranging from 1 (very slightly or not at all) to 5 (extremely). Average ratings across all the positive and negative emotions were used to calculate overall Positive and Negative Mood scale scores. Scores ranged from 1 to 5, with lower scores representing lower ratings of positive or negative mood and higher scores representing higher ratings of positive or negative mood. The PANAS-C has been validated with children in grades 4 through 8 and has established convergent validity with measures of anxiety and depression (Laurent, et al., 1999). In the current study, internal consistency was good to excellent for the Positive (α = .90) and Negative Mood (α = .86) scales.

Social Variables: Caregiver-Completed.

Coping Strategies Questionnaire-Revised (CSQ-R). The CSQ-R is a revised 27-item version of the original Coping Strategies Questionnaire (CSQ) by Rosenstiel and Keefe (Rosenstiel & Keefe, 1983). The CSQ-R is considered to be a brief, parsimonious measure that captures the same constructs as the original CSQ. Each item on the CSQ-R is rated on a 7-point Likert scale from 0 (never) to 6 (always) to indicate how often a strategy is used. Caregivers reported how often they use coping strategies for their own pain (e.g., headache). Although the scale is considered to have six subscales, factor analysis suggests two broad factors: Active and Passive Coping. Active Coping refers to

adaptive coping behaviors (e.g., distraction) while Passive Coping refers to maladaptive coping behaviors (e.g., catastrophic thinking) for pain (Hastie, Riley, & Fillingim, 2004). Scores on the Active Coping scale ranged from 0 to 108, with higher scores representing higher ratings of active coping. Scores on the Passive Coping scale ranged from 0 to 54, with higher scores representing higher ratings of passive coping. These subscales are similar to the Coping Attempts and Negative Thinking subscales of the CSQ-SCD.

The CSQ-R has been validated in a large sample of healthy African-American adults (Hastie, et al., 2004). In the current study, internal consistency was good to excellent for the Active (α = .90) and Passive Coping (α = .77) scales. Caregivers who self-reported having a pain condition on the Pain History Interview produced lower ratings of active coping (M = 58.17, SD = 19.32) and higher ratings of passive coping (M = 28.56, SD = 10.35) versus caregivers without pain conditions (Active Coping: M = 59.40, SD = 18.18; Passive Coping: M = 25.52, SD = 6.97); however, these differences were not statistically significant ($p \geq .184$).

Positive and Negative Affect Scale - Revised (PANAS-X). The PANAS-X is a 20-item measure that contains two subscales that assess positive and negative mood states. Caregivers were asked to rate the extent to which they felt different positive (e.g., interested, excited) and negative emotions (e.g., scared, upset) in the past week using a 5-point Likert scale ranging from 1 (very slightly or not at all) to 5 (extremely). Average ratings across all the positive and negative emotions were used to calculate overall Positive and Negative Mood scale scores. Scores ranged from 1 to 5, with lower scores representing lower ratings of positive or negative mood and higher scores representing higher ratings of positive or negative mood. The PANAS-X has demonstrated temporal

stability as well as good convergent validity with measures of anxiety and depression (Watson, et al., 1988). In the current study, internal consistency was good for the Positive (α = .89) and Negative Mood (α = .87) scales.

McMaster Family Functioning Assessment Device (FAD). Caregivers reported on family functioning using the General Functioning (GF) subscale of the FAD. The GF is a 12-item subscale that assesses the degree to which a family functions well together as a unit. Caregivers rate the extent to which they strongly agree or disagree with 12 statements on a four-point Likert scale ranging from 1 (strongly agree) to 4 (strongly disagree) (Epstein, Baldwin, & Bishop, 1983). Scores are averaged across the 12 items to produce a GF score and range from 1 to 4, with *lower scores* representing *better family functioning*. The subscales of the FAD, including the GF subscale, are not substantially influenced by social desirability. The GF subscale has also established convergent validity with family variables thought to indicate familial dysfunction (e.g., caregiver mental health problems, spousal abuse, and alcohol abuse) (Byles, Byrne, Boyle, & Offord, 1988). Finally, this measure has shown very good psychometric qualities when used with African-American families, including families of children with SCD, with medium-to-large correlations with converging constructs (Guada, et al., 2010; Petrocelli, et al., 2003; Schatz, et al., 2008). In the current study, internal consistency was good (α = .83).

3.4 Procedures

All study procedures, including the consent and assent process, were conducted by the study investigators (A.M.S. or J.S.). Institutional Review Board approval was obtained from Palmetto Richland Hospital, with concurrent approval from the University

of South Carolina. Informed consent was obtained from all caregivers and assent was obtained from all children. Families were told that their choice to participate in the study would not impact their relationship with health care staff or their treatment at the CCBD.

Procedures for Questionnaires. Children and caregivers completed the demographic and psychosocial questionnaires as well as the structured pain interviews in a private clinic room. The pain history interview was conducted at the beginning of the session with the child and at the end of the session with the caregiver. Questions were read aloud to all children, with the exception of adolescents who indicated a preference for completing measures independently. For child questionnaires, one child did not complete the PANAS-C due to experimenter error. For caregiver questionnaires, one child's caregiver was unable to complete the questionnaires and the pain history interview due to an acute medical illness and two caregivers did not complete the Family Functioning scale by mistake. Attempts were made to contact these families via phone and mail to complete the forms, but were unsuccessful. For the caregiver who did not complete the pain history interview, only the child's ratings were used for recent pain status. Medical record reviews were conducted after the families' visit. Both electronic and paper records were reviewed. Hematologist ratings of therapeutic response for hydroxyurea were also completed after the child's visit.

Procedures for Saliva Collection, DNA Extraction, and Genotyping. Prior to completing the questionnaires, approximately 1.5mL of saliva was collected in a sterile container from each child. Samples were preserved within one hour of collection using 1 mL of DNAzol. The samples were then transported to a genetics laboratory, at which

point 20 uL Proteinase K was added for additional preservation. Samples were incubated overnight and refrigerated in a temperature-controlled cooling room until extraction.

Extraction was completed using an ethanol procedure. First, 750 uL of saliva was added to a 1.5 mL microcentrifuge tube and centrifuged at 13,000 rpm for 15 minutes. The supernatant was then transferred to a new 1.5 mL microcentrifuge tube and the pellet was discarded. Then, 750 uL of 100% ethanol was added to the supernatant and was gently mixed by inversion. The samples stood at room temperature for 10 minutes and were then centrifuged at 13,000 rpm for 3.5 minutes. The supernatant was removed, leaving the pellet, and 250 uL of 70% ethanol was then added. The supernatant was removed again and 150 uL of distilled water was added. The solution was vortexed for a few seconds and the suspended DNA was allowed to incubate for 24 hours. Extracted DNA was stored in temperature-controlled freezers until polymerase chain reaction (PCR) was performed.

A standard PCR was performed to amplify the DNA using the following oligonucleotide primers for the ET-B SNP: 5'AAGATTCTGTATGATATATACAAACT3' (forward for ET-B) and 5'ATTTCACAGGTCATTAGTGTAT3' (reverse for ET-B). The thermocylcer was programmed to the following settings: initial denature (94°C, 30 seconds), denature (94°C, 20 seconds), annealing (47 °C, 35 seconds), extension (65°C, 45 seconds), and final extension (65°C, 5 minutes) with a total of 35 cycles. Amplification was confirmed using 0.4% Agarose gel electrophoresis. Once confirmed, unpurified PCR product was sent for Sanger Sequencing provided by High Throughput Genomics Center (Seattle, WA). Two samples were unable to be reliably sequenced due to a low DNA yield,

resulting in 74 total samples that were used in the final analysis. Sequencing results were returned in the format of chromatograms and were interpreted using Sequencher software (Version 5.1). An example chromatogram and interpretation can be seen in Figure 3.1.

3.5 Statistical Analysis

Statistical analysis was completed using SPSS, Version 19. For all analyses below, pain features were assessed as follows. Pain rate was an average of total caregiver- and child-reported pain episodes over the previous 12 months, including those treated at home as well as those treated via health care utilization. Pain duration and intensity were an average of caregiver- and child-reported ratings for the previous 12 months. For health care utilization, two measures were used: a) an average of total caregiver- and child-reported health care utilization over the previous 12 months, and b) total health care utilization as measured by the medical record over the previous 24 months. Alpha was set to .05 for all analyses. Due to concerns about power, no corrections were made for multiple comparisons.

Study One: Analysis of Biopsychosocial Factors. For Study One Aims I and II, a multiple hierarchical regression approach was used to assess the contribution of biological factors, child psychological factors, and their interactions to pain variability, controlling for demographic and biological covariates (described below). Separate analyses were conducted for the full sample of 76 children and for the subpopulation of 52 children with HbSS and HbSβ^0 who had documented fetal hemoglobin levels. For the full sample, sickle cell subtype was used as the primary biological predictor. Sickle cell subtype was dichotomized into high (HbSS and HbSβ^0; n = 52) and low risk (HbSC and HbSβ^+; n = 24) groups using dummy codes for Aim I (i.e., 1, 0) and contrast codes for

Aim II (i.e., 1, -1). For the subsample of children with HbSS and HbSβ^0, hematocrit and fetal hemoglobin were used as the primary biological predictors. Separate analyses were conducted for pain rate, intensity, duration, and the two health care utilization outcomes. Both the full sample and subsample models were used to evaluate the hypotheses.

For Aim I, the first step of all regression models contained demographic variables (age and gender). The second step contained biological variables (sickle cell risk group or hematocrit and fetal hemoglobin). The third step contained child psychological variables (coping and mood). The following biological covariates were also examined for the subsample of children with HbSS and HbSβ^0: alpha thalassemia trait, platelets, and white blood cells. These variables were added prior to step 2 if they had a correlation ≥ .20 with the pain outcomes. Hypotheses were examined by evaluating the ΔF statistic at each step and by comparison of effect sizes using R^2 values to understand the proportion of variance accounted for by biological and child psychological variables to different pain features. Hypothesis 1 was supported by a significant amount of variance accounted for by biological variables and/or an effect size of R^2 of .05 for pain rate and health care utilization. Hypothesis 2 was supported by a significant amount of variance accounted for by child psychological factors for pain intensity and health care utilization and/or effect sizes of .12 and .08, respectively. Analyses for pain duration were viewed as exploratory.

For Aim II, the order of variables entered into the model was the same; however, the final step contained the interaction terms for biological and child psychological variables. In addition, only child psychological variables that demonstrated a correlation ≥ .20 were examined as main effects and interactions with biological predictors. This approach led to a more parsimonious model and restricted the number of interactions to

only those likely to have a robust effect. In addition, age and gender were only included if they also met the .20 criterion, in order to conserve degrees of freedom. All continuous predictors were mean centered and sickle cell risk group was centered using contrast codes. Similar to Aim I, hypotheses were examined by focusing on both statistical significance (i.e., ΔF statistic) and effect sizes. Models with multiple interaction terms were evaluated to determine which terms were likely to be responsible for the effect (i.e., statistical significance or trend for one or more terms). If this was not apparent, all terms were followed up with simple slopes and graphical analysis. Hypothesis 3 was supported by the absence of a significant interaction and/or R^2 values of .01 or less for biological and child psychological factors. Hypothesis 4 was supported by significant interaction terms for biological and child psychological factors and/or R^2 values of .02 or higher and by subsequent graphical and statistical analysis supporting a synergistic or buffering effect.

For Aim III, the focus was on caregiver coping, mood, and family functioning in relation to child psychological variables and pain outcomes. Correlations were conducted to determine if mediation models were likely to be supported in future studies. Specifically, correlations were evaluated to determine whether the effects of the family environment on pain outcomes were likely to be mediated by child psychological variables. Hypothesis 5 was supported by statistically significant correlations between caregiver coping and mood and child coping and mood, with child coping and mood directly mirroring caregiver coping and mood. Hypothesis 6 was supported by statistically significant correlations between family functioning and child coping attempts and negative thinking. Hypothesis 7 was supported by statistically significant correlations

between caregiver coping, mood, family functioning and health care utilization for pain. For pain outcomes, the focus was on health care utilization, with additional exploratory analyses conducted with pain rate, intensity, and duration.

Study Two: Genetic Association Analysis. Allele frequencies were examined using chi-square analysis to establish Hardi-Weinberg equilibrium, a principle that is used to determine whether allele distributions are consistent with what would be expected in a population. Linear regression was then used to examine relationships between the ET-B genotypes and pain variability, with a primary focus on health care utilization. Additional exploratory analyses were examined between the ET-B genotypes and additional pain features (pain rate, intensity, and duration). A codominant model was used to analyze the data, consistent with preliminary data. A codominant model is also considered to be a parsimonious, yet powerful model for detecting associations for quantitative traits when the inheritance pattern has not been confirmed for a specific population (Lettre, Lange, & Hirschhorn, 2007). The codominant model resulted in two coefficients that were dummy coded (i.e., 1, 0) to compare the G/G and the A/G genotypes to the A/A genotype. Hypothesis 8 was supported if both coefficients suggested higher health care utilization for children with the G allele, with the G/G and A/G genotypes conferring greater risk for health care utilization for pain than the A/A genotype.

As with Study One, separate analyses were conducted for the full sample and the subset of children with HbSS and HbSβ^0. Biserial correlations were used to determine initial relationships. For both samples, age and gender were examined as potential covariates. In addition, hematocrit, fetal hemoglobin, alpha thalassemia trait, white blood

cells, and platelet count were considered as covariates for the subsample. Covariates with correlations ≥ .20 were included in the models. If no covariates met this criterion, correlations between the genotypes and pain outcomes were used in lieu of linear regression to simplify the results.

Table 3.1.
Demographic and Medical Characteristics of Children

Characteristics	***N* = 76**
Age (*M, SD*)	14.05, 3.26
Gender (*n*)	
Male	44
Female	32
Race* (*n*)	
African American	76
African International	1
White	1
American Indian/Alaska Native	3
Ethnicity (*n*)	
Hispanic, Latino, or Spanish Origin	2
Insurance Status	
Medicaid only	47
Medicaid and private insurance	13
Private insurance only	16
Sickle Cell Subtype (*n*)	
HbSS	48
HbSC	19
$HbS\beta^0$	4
$HbS\beta^+$	5
Presence alpha thalassemia trait (*n*)	8
Average hematocrit (*M, SD)*	27.51, 4.96
Average fetal hemoglobin (*M, SD)*	11.27, 9.82
Average white blood cells (*M, SD)*	9.51, 3.15
Average platelets (*M, SD)*	387.50, 183.57
Currently taking hydroxyurea (*n*)	40
Good therapeutic response	18
Partial therapeutic response	9
No therapeutic response	13

*Participants were able to mark multiple selections.

Table 3.2.
Demographic Characteristics of Caregivers

Characteristics (*n*)	***N* = 70**
Caregiver Relation to Child	
Mother	62
Father	4
Grandparent	4
Caregiver Race*	
African American	70
African International	1
White	1
American Indian/Alaska Native	5
Ethnicity	
Hispanic, Latino, or Spanish Origin	1
Caregiver Education	
Some high school	6
High school diploma	22
Some college	28
College degree	13
More than college degree	6
Marital Status*	
Single and never married	27
In a relationship/living with someone	3
Married	19
Separated	7
Divorced	7
Widowed	4
No response	3
Caregiver Health Status	
Caregiver with Pain Condition	18

*Participants were able to mark multiple selections.

Table 3.3.
Descriptive Information on Pain Outcomes

Pain Outcomes	***M, SD***
Caregiver and Child Report	
Pain rate (total previous 12 months)	7.52, 12.27
Average pain intensity (0 to 10; previous 12 months)	7.20, 1.70
Average pain duration (in days; previous 12 months)	2.67, 2.83
Health care utilization (previous 12 months)	
Total	6.25, 9.16
Hospitalizations	1.00, 1.81
Emergency Room Visits	1.48, 2.64
Outpatient Visits/Calls to Physician	4.24, 7.53
Medical Record	
Health care utilization (previous 24 months)	
Total	3.41, 4.02
Hospitalizations	1.64, 2.53
Emergency Room Visits	1.07, 1.80
Outpatient Visits/Calls to Physician	.70, 1.84

Table 3.4.
Descriptive Information on Psychosocial Variables for Children and Caregivers

Psychosocial Variables	α	***M, SD***
Child-Level Variables		
Coping Strategies Questionnaire for SCD (CSQ-SCD)		
Coping Attempts	.91	81.27, 31.09
Negative Thinking	.89	53.90, 25.62
Positive and Negative Affect Scale for Children (PANAS-C)		
Positive Mood	.90	3.48, .93
Negative Mood	.86	1.68, .58
Caregiver-Level Variables		
Coping Strategies Questionnaire – Revised (CSQ-R)		
Active Coping	.90	58.74, 18.77
Passive Coping	.77	25.77, 7.96
Positive and Negative Affect Scale – Revised (PANAS-X)		
Positive Mood	.89	3.75, .80
Negative Mood	.87	1.78, .70
Family-Level Variable		
McMaster Family Functioning Device		
General Functioning	.83	1.68, .44

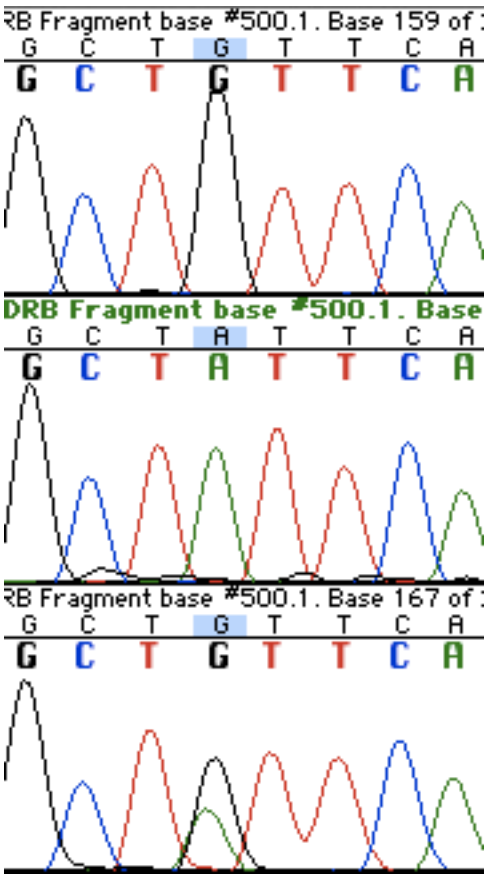

Figure 3.1. Example chromatogram demonstrating genotype results for the endothelin B receptor single nucleotide polymorphism. The single nucleotide polymorphism locus is highlighted in blue. A large, single black peak represents two copies of the G allele; a moderate, single green peak represents two copies of the A allele; and the combination of one black and one green peak overlapping represents the heterozygote AG group.

CHAPTER 4

STUDY ONE RESULTS

Study One examined the role of biological and psychosocial factors in relation to multiple pain outcomes in pediatric SCD. The following sections describe results for each of the primary aims. For each aim, additional analyses were completed to complement or address key limitations from the original analysis. These analyses are noted as "supplemental" and are described after the primary results of each aim.

4.1 Regression Assumptions

Regression assumes independence of errors, normality of residuals, absence of outliers, absence of multicollinearity, additivity, linearity, and homoscedasticity of residuals. When evaluating assumptions, three principles were prioritized: (a) maintaining the interpretability of the findings, in terms of the scaling of the variables, (b) identifying models with the highest degree of stability and concordance with model assumptions, and (c) using all of the data available to preserve power. The two primary issues identified for Study One were non-normality of residuals and the presence of outliers.

Normality of residuals was assessed via histograms of residuals for each model. Residual distributions for the models involving pain rate were consistently leptokurtic and the model for caregiver- and child-reported health care utilization was consistently leptokurtic and positively skewed. These deviations were likely due to non-normal distributions for both variables, which were positively skewed. Influential cases also

posed an issue for these specific pain features. Case diagnostics were used to examine the presence of influential cases for each predictor, using a DFBETA criterion of +/- 2 $\sqrt{k/n}$ (where *k* equals number of predictors and *n* equals sample size) for the full sample and a value of 1 for the subsample. The models for pain rate and caregiver- and child-reported health care utilization demonstrated the greatest number of influential cases, particularly for Aim One (ranging from 3 to 6 cases), with conflicting results obtained with the deletion of these cases individually.

Given concerns about non-normality of residuals and influential cases, these two pain features (pain rate and caregiver- and child-reported health care utilization) were log-transformed. The models with log-transformed outcomes were no longer influenced by individual cases. In addition, all of the models demonstrated normal residual distributions with the transformation. To aid in interpretation, significant coefficients and interactions were described using the original coefficients. The results tables include the original, untransformed and log transformed beta coefficients and standard errors.

In addition to these general issues with influential cases, there was a single influential case for pain duration (an 11 year-old girl with HbSC who self-reported 36.5 days for pain duration) that dramatically altered the results for the full sample models for Aim One and Two (this case was not included in the subsample analysis because of the child's sickle cell subtype). The child's average score with caregiver report (21.75) was far outside the mean of 2.67 for the sample, so the caregiver pain duration score (7) was used instead. The reason for this child's report of such a long duration is unclear, but it is possible that the child had difficulty discriminating between distinct episodes and instead grouped multiple pain episodes together.

Independence of errors was assessed using the Durbin-Watson statistic. Each model's Durbin-Watson statistic was compared to a statistical table with upper and lower limits for values based on *k* number of predictors and sample size. The focus of this assessment was on positive autocorrelation, which could have posed an issue for the models due to the presence of several sibling groups (9 sibling groups; $n = 19$). None of the models exceeded the lower limits for this statistic (Durbin-Watson ranged from 1.51 to 2.28); thus, independence of errors was assumed to be adequate.

For multicollinearity, correlations above .50 were presumed to present issues for the regression. Hematocrit and fetal hemoglobin exceeded this value for analyses involving the subsample of participants with HbSS and HbSβ^0 ($r = .58$). For Aim I, both variables were included together as the focus was on comparisons of cumulative effect size for biological factors. For Aim II, the primary analysis included these factors together to reduce the number of models examined; however, a supplemental analysis examining hematocrit and fetal hemoglobin in separate models was conducted to determine whether multicollinearity was an issue. Finally, residual plots were used to assess linearity and homoscedasticity; no curvilinear patterns or systematic deviations from homoscedasticity were identified.

4.2 Aim I: Compare the effect sizes of biological and psychological factors in relation to multiple pain outcomes in pediatric SCD

Tables 4.1 and 4.4 provide correlation tables for the covariates, predictors, and pain outcomes for the full sample and subsample of children with HbSS and HbSβ^0, respectively. Please note that the overall statistical significance of the full regression models is provided to give the reader a sense of how well the combination of biological

and psychological factors predicted each pain feature; however, the focus of the results is on the change in explained variance with each set of biological and psychological predictors (i.e., the incremental change in R^2 or ΔF statistic). This approach was taken because it provides more information about which predictors are most relevant to specific pain features, which may help with future research planning. In addition, please note that hypotheses 1 and 2 could be supported by the model demonstrating a significant amount of variance for the predictors or by meeting the hypothesized effect size estimate.

Full Sample. Table 4.2 provides multiple hierarchical regression results for pain rate, intensity, and duration. Table 4.3 provides results for both health care utilization outcomes.

Pain rate was log-transformed due to six influential cases that resulted in conflicting results. The log-transformed model demonstrated no influential cases and also conformed to the assumption of normality of residuals. Results are presented using the log-transformed results; however, significant coefficients are described using the original unstandardized beta coefficients, which are more interpretable. The overall regression model was significant, F (7, 67) = 2.31, p =. 036, with the model explaining 20% of the variance in pain rate overall. After controlling for age and gender, sickle cell risk group did not contribute a significant amount of variance to the model, ΔF (1, 71) = 1.52, p = .221, ΔR^2 = .02, and the R^2 was less than .05; thus, hypothesis 1 was not supported for pain rate. The addition of child psychological variables contributed a significant amount of variance to the model, ΔF (4, 67) = 3.28, p = .016, ΔR^2 = .16. Positive mood was also a significant individual predictor, t (1, 74) = -2.30, p = .025. Using results from the original, untransformed model, for every one-point increase in positive mood, there was a

3.01 decrease in pain rate. Negative thinking approached significance as an individual predictor, t (1, 74) = 1.94, p = .056.

For pain intensity, the overall regression model was not significant, F (7, 67) = 1.37, p = .234, with the model explaining 13% of variance overall. After controlling for age and gender, sickle cell risk group contributed a significant amount of variance to the model, ΔF (1, 71) = 4.33, p = .041, ΔR^2 = .06. According to the unstandardized beta coefficient, children with high-risk subtypes had a .89 higher pain intensity rating versus those with low-risk subtypes. The addition of child psychological variables did not contribute a significant amount of variance to the model, ΔF (4, 67) = 0.40, p = .807, ΔR^2 = .02, and the R^2 value was far below the hypothesized estimate of .12; thus, hypothesis 2 was not supported for pain intensity. For pain duration, the overall regression model was not significant, F (7, 67) = 0.91, p = .505, with the model explaining 9% of the variance overall. After controlling for age and gender, sickle cell risk group did not contribute a significant amount of variance to the model, ΔF (1, 70) = 0.01, p = .943, ΔR^2 = .00. The addition of child psychological variables also did not contribute a significant amount of variance to the model, ΔF (4, 67) = 1.55, p = .197, ΔR^2 = .09. Although the effect size was fairly large, there were no notable individual predictors.

Caregiver- and child-reported health care utilization was log-transformed due to three influential cases that resulted in conflicting results. The log-transformed model demonstrated no influential cases and also conformed to the assumption of normality of residuals. As with pain rate, results are presented using the log-transformed results; however, significant coefficients are described using the original unstandardized beta coefficients, which are more interpretable. For the caregiver- and child-reported health

care utilization variable, the overall regression model was significant, F (7, 67) = 2.39, p = .030, with the model explaining 20% of the variance overall. After controlling for age and gender, sickle cell risk group did not contribute a significant amount of variance to the model, ΔF (1, 71) = 2.35, p = .130, ΔR^2 = .03, and the R^2 value was lower than the hypothesized estimate of .05; thus, hypothesis 1 was not supported for caregiver- and child-reported health care utilization. The addition of child psychological variables contributed a significant amount of variance to the model, ΔF (4, 67) = 2.80, p = .033, ΔR^2 = .13, and the R^2 value was greater than .08, providing support for hypothesis 2 for caregiver- and child-reported health care utilization. Positive mood was a significant individual predictor of health care utilization, t (1, 74) = -2.33, p = .023. Using results from the original, untransformed model, for every one-point increase in positive mood, there was a 2.45 decrease in health care utilization.

For the medical record health care utilization variable, the overall regression model approached significance, F (7, 67) = 2.00, p = .069, with the model explaining 17% of the variance overall. After controlling for age and gender, sickle cell risk group approached significance as a contributor of variance to the model, ΔF (1, 71) = 3.59, p = .062, ΔR^2 = .05, and the R^2 value was .05; thus, hypothesis 1 for medical record health care utilization was supported. The addition of child psychological variables also approached significance as a contributor of variance to the model, ΔF (4, 67) = 2.10, p = .090, ΔR^2 = .10, and the R^2 value was greater than .08; thus, hypothesis 2 for medical record health care utilization was also supported. Although the overall effect of child psychological variables approached significance, there were no notable individual predictors.

Sickle Cell Anemia and Sickle Cell Beta-0-Thalassemia. Tables 4.5 and 4.6 provide multiple hierarchical regression results for pain rate, intensity, and duration. Table 4.7 provides results for both health care utilization outcomes.

Similar to the full sample model, pain rate was log-transformed due to three influential cases that resulted in conflicting results. The log-transformed model demonstrated no influential cases and also conformed to the assumption of normality of residuals. Results are presented using the log-transformed results. For pain rate, the overall regression model was not significant, F (9, 41) = 1.14, p = .361, with the model explaining 20% of the variance overall. After controlling for age, gender, and alpha thalassemia trait, the addition of hematocrit and fetal hemoglobin did not contribute a significant amount of variance to the model, ΔF (2, 45) = 1.00, p =. 376, ΔR^2 = .04, and the R^2 value was less than .05; thus, hypothesis 1 was not supported for pain rate. The addition of child psychological variables also did not contribute a significant amount of variance to the model, ΔF (4, 41) = 1.44, p =. 237, ΔR^2 = .11. Although the effect size was fairly large, there were no notable individual predictors.

For pain intensity, the overall regression model was not significant, F (9, 41) = 0.66, p = .739, with the model explaining 13% of the variance overall. After controlling for age, gender, and platelet count, the addition of hematocrit and fetal hemoglobin did not contribute a significant amount of variance to the model, ΔF (2, 45) = 1.69, p =. 196, ΔR^2 = .07; however, the R^2 value was fairly large and fetal hemoglobin approached significance as an individual predictor, t (1, 50) = -1.73, p = .090. The addition of child psychological variables did not contribute a significant amount of variance to the model,

ΔF (4, 41) = 0.07, p = .992, ΔR^2 = .01, and the R^2 value was far below the hypothesized estimate of .12; thus, hypothesis 2 was not supported for pain intensity.

For pain duration, the overall regression model was not significant, F (8, 42) = 0.90, p = .522, with the model explaining 15% of the variance overall. After controlling for age and gender, the addition of hematocrit and fetal hemoglobin approached significance in contributing variance to the model, ΔF (2, 46) = 2.60, p = .085, ΔR^2 = .10, and the R^2 value was fairly large. Fetal hemoglobin was significant as an individual predictor, t (1, 50) = -2.23, p = .031, and there was a trend for hematocrit as a significant predictor of pain duration, t (1, 50) = 1.67, p = .102. According to the unstandardized beta coefficient, for every one-percent increase in fetal hemoglobin, there was a .07 decrease in pain duration. The addition of child psychological variables did not contribute a significant amount of variance to the model, ΔF (4, 42) = 0.45, p = .771, ΔR^2 = .04.

Caregiver- and child-reported health care utilization was log-transformed due to four influential cases that resulted in conflicting results. The log-transformed model demonstrated no influential cases and also conformed to the assumption of normality of residuals. Results are presented using the log-transformed results. The overall regression model was not significant, F (8, 42) = 1.33, p = .256, with the model explaining 20% of the variance overall. After controlling for age and gender, the addition of hematocrit and fetal hemoglobin did not contribute significant variance to the model, ΔF (2, 46) = 1.14, p = .330, ΔR^2 = .05; however, the R^2 value was .05, providing support for hypothesis 1. The addition of child psychological variables also did not contribute a significant amount of variance to the model, ΔF (4, 42) = 1.99, p = .113, ΔR^2 = .15; however, the R^2 value was

higher than .08. Thus, hypothesis 2 was also supported for this outcome. Positive mood approached significance as an individual predictor of health care utilization, t (1, 50) = -1.73, p = .092.

For the medical record health care utilization variable, the overall regression model was significant, F (8, 42) = 2.46, p = .028, with the model explaining 32% of the variance overall. After controlling for age and gender, hematocrit and fetal hemoglobin did not contribute a significant amount of variance to the model, ΔF (2, 46) = 0.21, p = .811, ΔR^2 = .01, and the R^2 value was less than .05; thus, hypothesis 1 was not supported for medical record health care utilization. In contrast, the addition of child psychological variables contributed a significant amount of variance to the model, ΔF (2, 42) = 3.48, p = .015, ΔR^2 = .23, and the R^2 value easily exceeded .08; thus, hypothesis 2 was supported for this outcome. Positive mood was also a significant individual predictor of health care utilization, t (1, 50) = -2.09, p = .043. According to the unstandardized beta coefficient, for every one-point increase in positive mood, there was a 1.42 decrease in health care utilization.

Supplemental Analyses: Pre-Hydroxyurea Relationships with Biological Risk Factors. The majority of children (n = 40) with HbSS and HbSβ^0 were treated with hydroxyurea at the time of the study. Due to the possibility of hydroxyurea influencing both the hematological variables (hematocrit and fetal hemoglobin) and pain outcomes, it is possible that the effect of biological risk on pain outcomes was attenuated. Thus, these relationships were evaluated again using information from caregivers and the medical record regarding pre-hydroxyurea values for biological variables and pain outcomes. For the full sample, correlations were conducted between sickle cell risk group and pain

outcomes, with the pre-hydroxyurea pain outcomes used for children taking hydroxyurea at the time of the study (see Table 4.8). The correlation values were used to calculate R^2 values for sickle cell risk group and each pain outcome. For the subsample of children with HbSS and HbSβ^0, pre-hydroxyurea measures of hematocrit and fetal hemoglobin and pain outcomes were used and the variables were entered into a regression model to calculate R^2 values (see Table 4.9).

Pre-Hydroxyurea Analysis for Sickle Cell Risk Group (Full Sample). For pain rate, the R^2 value for sickle cell risk group was .03, which is higher than the original analysis, but still below the hypothesized value of .05 and not statistically significant; thus, similar to the original analysis, hypothesis 1 was not supported. For pain intensity, the R^2 value was .11, which is higher than the original analysis. Similar to the original analysis, the correlation was statistically significant. For pain duration, the R^2 value was less than .01, which is lower than the original analysis. For caregiver- and child-reported health care utilization, the R^2 value was .06, which is higher than the original analysis and, in contrast to Aim 1, does support hypothesis 1. Finally, for medical record health care utilization, the R^2 value was also .06, which is close to the original analysis and similarly supports hypothesis 1.

Pre-Hydroxyurea Analysis for Hematocrit and Fetal Hemoglobin (Sickle Cell Anemia and Sickle Cell Beta-0-Thalassemia). For pain rate, the R^2 value was .04 for hematocrit and fetal hemoglobin, which is larger than the original analysis; however, this value is still under the hypothesized value of .05. Thus, similar to Aim I, hypothesis 1 was not supported. For pain intensity, the R^2 for hematocrit and fetal hemoglobin was .14, which is higher than the primary analysis. In addition, fetal hemoglobin was a significant

individual predictor. Although the value is higher, the results were fairly similar to the original analysis described in Aim I. For pain duration, the R^2 value was .09 for hematocrit and fetal hemoglobin, which is similar to the original analysis. For caregiver- and child-reported health care utilization, the R^2 value was .03, which is lower than the original analysis. Finally, for medical record health care utilization, the R^2 value of .07 for hematocrit and fetal hemoglobin was higher than the original analysis and, in contrast to the results for Aim 1, supports hypothesis 1.

4.3 Aim II: Explore interrelationships among biological and psychological factors

For Aim II, age and gender were included for the model for medical record health care utilization in the subsample because these variables met the ≥ .20 correlation criterion. These variables were excluded from other models in order to conserve degrees of freedom. As noted previously, in order to reduce the number of interactions examined, psychological variables were selected for models if they had a correlation ≥ .20. For interactions, please note that interaction terms were pursued based on statistical significance or an effect size greater than R^2 = .02. This approach was taken to evaluate interactions that may be meaningful despite having a small effect size.

Full Sample. Table 4.10 provides results for multiple hierarchical regressions for pain rate and duration with the selected variables and their interactions. Table 4.11 provides results for the health care utilization variables. For pain intensity, none of the psychological predictors demonstrated a correlation ≥ .20; thus, interactions were not evaluated for this pain outcome.

In order to be consistent with the model from Aim I, pain rate was log-transformed. Results are presented using the log-transformed results. For this model, in

addition to sickle cell risk group, negative thinking and positive mood were entered into the model along with two interaction terms for each child psychological variable with sickle cell risk group. The overall regression model was significant, F (5, 69) = 3.07, p =. 015, with the model explaining 18% of the variance overall; however, the interaction terms did not contribute a significant amount of variance, ΔF (1, 73) = 0.23, p = .795, ΔR^2 < .01, and the R^2 value was less than .01. For main effects, sickle cell risk group did not contribute a significant amount of variance to the model, ΔF (1, 73) = 2.19, p = .143, ΔR^2 = .03. The addition of negative thinking and positive affect contributed a significant amount of variance to the model, ΔF (2, 71) = 6.35, p =. 003, ΔR^2 = .15. Negative thinking was a significant individual predictor, t (1, 74) = 2.55, p = .013. Positive mood was also a significant individual predictor, t (1, 74) = -2.06, p = .043. These results provide support for hypothesis 3 (an additive effect) for pain rate.

For pain duration, in addition to sickle cell risk group, positive mood was entered into the model along with one interaction term for positive mood with sickle cell type group. The overall regression model was not significant, F (3, 71) = 1.75, p = .164, with the model explaining 7% of the variance overall. The addition of the interaction term did not contribute a significant amount of variance to the model, ΔF (1, 71) = 2.32, p = .133, ΔR^2 = .03; however, the R^2 value was greater than .01.

This interaction was probed further via graphical analysis and simple slopes. As shown in Figure 4.1, for children with low-risk subtypes, higher positive mood was related to lower pain duration (B = -.80), whereas the slope was relatively flat for those with high-risk subtypes (B = -.10). For main effects, sickle cell risk group did not contribute a significant amount of variance to the model, ΔF (1, 73) = 0.01, p = .945, ΔR^2

= .00. The addition of positive mood approached significance as a contributor of variance to the model, ΔF (1, 72) = 2.89, p = .094, ΔR^2 = .04. Although these results suggest an interactive effect, they were not in the hypothesized direction for hypothesis 4.

In order to be consistent with the model from Aim One, caregiver- and child-reported health care utilization was log-transformed. Results are presented using the log-transformed results; however, for interactions, simple slopes were calculated using the original unstandardized beta coefficients. For this model, in addition to sickle cell risk group, negative thinking and positive mood were entered into the model along with two interactions terms for each psychological variable with sickle cell risk group. The overall regression model was significant, F (5, 69) = 3.91, p = .004, with the model explaining 22% of the variance overall. The interaction terms did not contribute a significant amount of variance to the model, ΔF (2, 69) = 1.22, p = .302, ΔR^2 = .03; however, the R^2 value was greater than .01.

These interactions were probed further via graphical analysis and simple slopes. As shown in Figures 4.2 and 4.3, the difference between children with high- versus low-risk subtypes was a change in slope, such that children with low-risk subtypes had a stronger relationships between negative thinking (B = .08) and positive mood (B = -2.56) with health care utilization versus children with high-risk subtypes (Negative Thinking: B = .05; Positive Mood: B = -2.14). For main effects, sickle cell risk type contributed a significant amount of variance to the model, ΔF (1, 73) = 3.85, p = .054, ΔR^2 = .05. The addition of child psychological variables also contributed a significant amount of variance to the model, ΔF (2, 71) = 6.30, p = .003, ΔR^2 = .14, and positive mood was a

significant individual predictor, t (1, 74) = -2.95, p = .004. Although these results suggest an interactive effect, they were not in the hypothesized direction for hypothesis 4.

For the medical record health care utilization variable, in addition to sickle cell risk group, negative thinking and positive mood were entered into the model along with two interactions terms for each psychological variable with sickle cell risk group. The overall regression model was significant, F (5, 69) = 2.95, p = .018, with the model explaining 18% of the variance overall. The interaction terms did not contribute a significant amount of variance to the model, ΔF (2, 69) = 1.59, p = .211, ΔR^2 = .04; however, the R^2 was greater than .01.

These interactions were probed further via graphical analysis and simple slopes. As shown in Figures 4.4 and 4.5, the difference between children with high- versus low-risk subtypes was a change in slope, such that children with high-risk subtypes had stronger relationships between negative thinking (B = .05) and positive mood (B = -1.70) with health care utilization versus children with low-risk subtypes (Negative Thinking: B = .01; Positive Mood: B = -.30). For main effects, sickle cell risk group approached significance as a contributor of variance to the model, ΔF (1, 73) = 3.45, p = .067, ΔR^2 = .05. The addition of child psychological variables contributed a significant amount of variance to the model, ΔF (2, 71) = 3.82, p = .027, ΔR^2 = .09. Positive mood was also a significant predictor of health care utilization, t (1, 74) = -2.14, p = .036. These results suggest an interactive effect in the hypothesized direction, supporting hypothesis 4.

Sickle Cell Anemia and Sickle Cell Beta-0-Thalassemia. Tables 4.12 and 4.13 provide multiple hierarchical regression results for pain rate and both health care

utilization outcomes. For pain intensity and duration, none of the psychological variables reached a correlation value of ≥ .20, so these models were not examined.

In order to be consistent with the model from Aim I, results for pain rate are presented using the log-transformed variable. For this model, two interaction terms were added for negative thinking and hematocrit and negative thinking and fetal hemoglobin. The overall regression model was not significant, F (6, 44) = 1.18, p = .332, with the model explaining 14% of the variance overall. The addition of interaction terms did not contribute a significant amount of variance to the model, ΔF (2, 44) = 0.11, p = .893, ΔR^2 < .01, and the R^2 was less than .01. For main effects, after controlling for alpha thalassemia trait, the addition of hematocrit and fetal hemoglobin did not contribute a significant amount of variance to the model, ΔF (2, 47) = 1.06, p = .356, ΔR^2 = .04. The addition of negative thinking also did not contribute a significant amount of variance to the model, ΔF (1, 46) = 2.57, p = .115, ΔR^2 = .05. These results support an additive effect (hypothesis 3) for pain rate.

In order to be consistent with the model from Aim I, results for caregiver- and child-reported health care utilization are presented using the log-transformed variable; however, for interactions, simple slopes were calculated using the original unstandardized beta coefficients. For the caregiver- and child-reported health care utilization variable, two interaction terms were added for positive mood and hematocrit and positive mood and fetal hemoglobin. The overall regression model was significant, F (5, 45) = 2.44, p = .048, with the model explaining 21% of the variance overall. The addition of interaction terms did not contribute a significant amount of variance to the model, ΔF (2, 48) = 1.53, p = .227, ΔR^2 = .05; however, the R^2 was greater than .01.

These interactions were probed further using graphical analysis and simple slopes calculated at the 25th and 75th percentiles of hematocrit and fetal hemoglobin. As shown in Figures 4.6 and 4.7, the difference between children with high versus low levels of hematocrit and fetal hemoglobin was a change in slope, such that children with low levels of hematocrit (B = -5.36) and fetal hemoglobin (B = -3.20) had stronger relationships between positive mood and health care utilization versus those with high levels of hematocrit (B = -1.40) and fetal hemoglobin (B = -2.22). For main effects, the addition of hematocrit and fetal hemoglobin did not contribute a significant amount of variance to the model, ΔF (2, 48) = 1.24, p = .298, ΔR^2 = .05. The addition of positive mood was a significant contributor of variance to the model, ΔF (1, 47) = 6.20, p = .016, ΔR^2 = .11. Although these results suggest an interactive effect, the direction of effects is unclear in terms of biological risk (see Discussion).

For medical record health care utilization, six interaction terms were added for coping attempts, positive mood, and negative mood with hematocrit and fetal hemoglobin. The overall regression model approached significance, F (13, 37) = 1.94, p = .057, with the model explaining 41% of the variance overall. Age and gender were included as covariates. The addition of interaction terms did not contribute a significant amount of variance, ΔF (6, 37) = 1.05, p = .408, ΔR^2 = .10; however, the R^2 value was greater than .01 and the interaction term for positive mood and hematocrit approached significance, t (1, 49) = 1.93, p = .062.

This interaction was probed further using graphical analysis and simple slopes calculated at the 25th and 75th percentiles of hematocrit. As shown in Figure 4.8, the difference between children with high versus low levels of hematocrit was a change in

slope, such that children with low levels of hematocrit had a stronger relationship between positive mood and health care utilization (B = -3.57) versus those with high levels of hematocrit (B = -0.60). For main effects, the addition of hematocrit and fetal hemoglobin did not contribute a significant amount of variance to the model, ΔF (2, 46) = 0.21, p = .811, ΔR^2 = .02. In contrast, the addition of child psychological variables did contribute a significant amount of variance to the model, ΔF (3, 43) = 4.33, p = .009, ΔR^2 = .21. Positive mood was also a significant individual predictor, t (1, 49) = -2.33, p = .024, and negative thinking approached significance as an individual predictor, t (1, 49) = 1.82, p = .076. Although these results suggest an interactive effect, the direction of effects is unclear in terms of biological risk (see Discussion).

Supplemental Analysis: Examining Separate Models for Hematocrit and Fetal Hemoglobin. Due to concerns about multicollinearity between hematocrit and fetal hemoglobin, the same models above for the subsample were analyzed again with these variables entered individually into separate models (see Tables 4.14 and 4.15). Consistent with the original model, the models did not suggest an interaction between hematocrit or fetal hemoglobin with negative thinking (R^2 for interactions = .00 for both models) for pain rate. Similarly, the models continued to suggest that the strongest potential interaction effect for medical record health care utilization was between hematocrit and positive mood. For caregiver- and child-reported health care utilization, the models continued to suggest potential interactions for both hematocrit and fetal hemoglobin and positive mood, consistent with the original model; however, of note is that each of these interaction terms approached significance when entered into separate models, whereas neither term approached significance when combined in the original model. This finding

suggests that multicollinearity likely attenuated the effects of the interaction terms in the original model for caregiver- and child-reported health care utilization.

4.4 Aim III: Examine relationships between child psychological variables, caregiver psychological variables, family functioning, and pain outcomes

Table 4.16 provides correlation results for the association of child age and gender, and child, caregiver, and family variables. Table 4.17 provides correlations results for the association of caregiver and family variables with pain outcomes. Age and gender were examined to determine their potential use as covariates in future studies.

Age and Gender, Caregiver Psychological Variables, and Family Functioning. Age was significantly related to caregiver negative mood, with older child age related to higher caregiver negative mood. Gender was significantly related to family functioning, such that girls had poorer ratings of family functioning versus boys.

Trends. Trends were observed between age and caregiver positive mood and family functioning, with older child age related to lower caregiver positive mood and poorer family functioning.

Child and Caregiver Psychological Variables and Family Functioning. Child negative thinking was significantly associated with family functioning, with higher child negative thinking associated with poorer family functioning. This result provides support for hypothesis 6, specific to child negative thinking.

Trends. A trend was observed between child negative thinking and caregiver passive coping and negative mood, such that higher child negative thinking was related to higher caregiver passive coping and negative mood. In addition, a trend was found for child positive mood and caregiver active coping and positive mood, with higher child

positive mood associated with higher caregiver active coping and positive mood. These results provide preliminary support for hypothesis 5 specific to caregiver passive coping and positive mood, which directly mirrored similar constructs in children.

Caregiver Psychological Variables, Family Functioning, and Pain Outcomes. For health care utilization, caregiver passive coping was significantly related to medical record health care utilization, with higher passive coping associated with higher health care utilization. Caregiver negative mood was also significantly medical record health care utilization, with higher caregiver negative mood associated with health care utilization. These findings provide support for hypothesis 7, specific to caregiver passive coping and negative mood. Additional exploratory analyses with other pain features suggested that caregiver negative mood was significantly related to pain duration, with higher negative mood associated with longer pain duration.

Trends. A trend was observed between caregiver active coping and medical record health care utilization, with *higher* active coping associated with higher health care utilization. In addition, a trend was observed for caregiver passive coping and caregiver- and child-reported health care utilization, with higher passive coping associated with higher health care utilization. The latter result provides additional support for hypothesis 7. Additional exploratory analyses with other pain features suggested that caregiver passive coping was also associated with pain duration, with higher caregiver passive coping associated with longer pain duration.

Results Summary: Future Mediation Analysis. The above relationships were compared to the original relationships from Table 4.1 for child psychological variables and pain outcomes for the full sample. The most probable relationships in support of

future mediation were for child negative thinking as a mediator of caregiver passive coping and negative mood in predicting health care utilization (as measured by the medical record). These relationships are depicted in Figure 4.9. Using Fritz and Mackinnon (2007), a sample size was estimated to detect the mediated effect. This reference suggested that 148 to 196 participants would be needed to detect the mediated effect, depending on the statistical method used.

Supplemental Analysis: Direct Effects of Caregiver Variables on Pain Outcomes. Although the primary analyses were focused on uncovering possible mediation or indirect effects of caregiver variables, it is also possible that caregiver variables had direct effects on pain outcomes. Thus, a supplemental analysis was conducted to determine whether caregiver variables had direct effects on pain outcomes after controlling for biological and child psychological variables. Three models were chosen, one for pain duration and one for each of the health care utilization variables. For child and caregiver psychological variables, only those variables demonstrating a correlation of ≥ .20 were chosen. In addition, due to the large correlation between caregiver passive coping and caregiver negative mood, only one variable was chosen for the final models to avoid issues of multicollinearity. The variable with the higher correlation was chosen in these circumstances.

Results for the supplemental analysis can be found in Table 4.18. For pain duration, the overall regression model was significant, F (3, 65) = 2.83, p = .045, with the model explaining 12% of the variance overall. Above and beyond sickle cell risk group and child positive mood, the addition of caregiver negative mood accounted for a significant amount of variance, ΔF (1, 65) = 5.35, p = .024, ΔR^2 = .07. According to the

unstandardized beta coefficient, for every one-point change in caregiver negative mood, there was a .68 increase in pain duration. For caregiver- and child-reported health care utilization, the overall regression model was significant, F (4, 64) = 4.59, p = .003, with the model explaining 22% of the variance overall. Above and beyond sickle cell risk group and child psychological variables, caregiver passive coping did not contribute a significant amount of variance to the model, ΔF (1, 64) = 2.24, p = .140, ΔR^2 = .03. Finally, for the model for medical record health care utilization, the overall regression model was significant, F (5, 63) = 4.25, p = .002, with the model explaining 25% of the variance overall. Above and beyond sickle cell risk group and child psychological variables, the addition of caregiver active and passive coping accounted for a significant amount of variance, ΔF (2, 63) = 4.33, p = .017, ΔR^2 = .10. There were trends for caregiver active, t (1, 68) = 1.80, p = .076, and passive coping, t (1, 68) = 1.71, p = .092, as individual predictors of health care utilization.

Table 4.1.
Full Sample: Correlations between Covariates, Predictors, and Pain Outcomes

Variable	**1.**	**2.**	**3.**	**4.**	**5.**	**6.**	**7.**	**8.**	**9.**	**10.**	**11.**	**12.**
1. Age	-											
2. Gender	.13	-										
3. Sickle Cell Risk Group	.19	-.24*	-									
4. Coping Attempts	-.25*	-.07	-.03	-								
5. Negative Thinking	.14	-.26*	.28*	.25*	-							
6. Positive Mood	-.16	-.06	.07	.36**	-.13	-						
7. Negative Mood	-.02	-.26*	-.02	.31**	.42***	-.21†	-					
8. Pain Rate	.01	-.02	.00	.11	.32**	-.21†	.13	-				
9. Pain Intensity	.11	-.11	.25*	.03	.21†	-.01	.03	-.02	-			
10. Pain Duration	.03	.03	-.01	-.14	.13	-.20†	.14	.08	.15	-		
11. HC Utilization (Caregiver and Child)	.08	-.13	.14	-.04	.22†	-.25*	.15	.53***	.24*	.18	-	
12. HC Utilization (Medical Record)	.15	.07	.23*	-.11	.25*	-.25*	.11	.38**	.25*	.14	.67***	-

†$p < .10$ *$p < .05$ **$p < .01$ ***$p < .001$ HC = Health Care

Table 4.2.

Full Sample: Multiple Hierarchical Regressions Predicting Pain Rate, Intensity, and Duration

Variable	*B*	*B SE*	*β*	ΔR^2	Total R^2
Outcome: Pain Rate					
Demographics				.02	.02
Age	.03/.01	.46/.02	.03		
Gender	-.50/-.12	2.96/.11	-.13		
Biological Variable				.02	.04
Sickle Cell Risk Group	-.44/.15	3.31/.12	.15		
Psychological Variables				.16*	.20
Coping Attempts	.05/.00	.06/.00	.13		
Negative Thinking	.16/.01†	.07/.00	.26		
Positive Mood	-3.01/-.15*	1.77/.06	-.30		
Negative Mood	-1.95/-.04	2.92/.11	-.06		
Outcome: Pain Intensity					
Demographics				.05	.05
Age	.09	.06	.18		
Gender	-.55	.39	-.16		
Biological Variable				.06*	.10
Sickle Cell Risk Group	.89*	.43	.25		
Psychological Variables				.02	.13
Coping Attempts	.00	.01	.05		
Negative Thinking	.01	.01	.15		
Positive Mood	-.03	.25	-.02		
Negative Mood	-.21	.41	-.07		
Outcome: Pain Duration					
Demographics				.00	.00
Age	.02	.07	.03		
Gender	.09	.44	.02		
Biological Variable				.00	.00
Sickle Cell Risk Group	-.04	.50	-.01		
Psychological Variables				.09	.09
Coping Attempts	-.01	.01	-.21		
Negative Thinking	.01	.01	.15		
Positive Mood	-.14	.28	-.07		
Negative Mood	.49	.46	.15		

Note. Pain rate was log-transformed to generate the final results. The original untransformed unstandardized beta coefficients and standard errors are presented alongside the final values. The untransformed values are first, followed by the log-transformed values. Values are separated by a slash.
†$p < .10$ *$p < .05$

Table 4.3.
Full Sample: Multiple Hierarchical Regressions Predicting Health Care Utilization Outcomes

Variable	*B*	*B SE*	*β*	ΔR^2	Total R^2
Outcome: Health Care Utilization (Caregiver and Child Report)					
Demographics				.04	.04
Age	.30/.02	.34/.02	.14		
Gender	-2.70/-.14	2.18/.11	-.15		
Biological Variable				.03	.07
Sickle Cell Risk Group	1.91/.19	2.43/.12	.18		
Psychological Variables				.13*	.20
Coping Attempts	.01/.00	.04/.00	-.02		
Negative Thinking	.04/.00	.05/.00	.13		
Positive Mood	-2.45/-.15*	1.35/.07	-.30		
Negative Mood	.33/.05	2.23/.11	.06		
Outcome: Health Care Utilization (Medical Record)					
Demographics				.02	.02
Age	.15	.15	.12		
Gender	.54	.95	.07		
Biological Variable				.05†	.07
Sickle Cell Risk Group	1.98†	1.04	.23		
Psychological Variables				.10†	.17
Coping Attempts	-.01	.02	-.11		
Negative Thinking	.03	.02	.21		
Positive Mood	-.73	.57	-.17		
Negative Mood	.53	.94	.08		

Note. Caregiver- and child-reported health care utilization was log-transformed to generate the final results. The original untransformed unstandardized beta coefficients and standard errors are presented alongside the final values. The untransformed values are first, followed by the log-transformed values. Values are separated by a slash.
†$p < .10$ *$p < .05$

Table 4.4.

Sickle Cell Anemia and Sickle Cell Beta-0-Thalassemia Subtypes: Correlations between Covariates, Predictors, and Pain Outcomes

Variable	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.
1. Age	-															
2. Gender	.18	-														
3. Hematocrit	-.04	-.16	-													
4. Fetal Hemoglobin	-.19	-.30*	.58***	-												
5. White Blood Cells	-.14	-.02	-.35*	-.45**	-											
6. Platelets	-.03	.01	.00	-.07	.01	-										
7. Alpha Thalassemia	-.03	.11	.04	-.19	.32*	-.21	-									
8. Coping Attempts	-.31*	-.04	.13	.24†	.06	.06	.05	-								
9. Negative Thinking	.02	-.11	.01	.05	.18	-.04	.23	.26†	-							
10. Positive Mood	-.19	-.07	.09	.18	-.20	-.25†	.11	.34*	-.07	-						
11. Negative Mood	-.12	-.27†	.06	.19	.19	.14	.10	.33*	.36**	-.08	-					
12. Pain Rate	.05	.05	.05	.05	.01	-.08	.31*	.09	.30*	-.11	.11	-				
13. Pain Intensity	.00	-.02	.09	-.15	.03	.21	-.03	.02	.07	-.07	.01	-.14	-			
14. Pain Duration	-.08	.04	.06	-.20	.18	.08	.13	-.16	.04	-.05	-.04	.11	.18	-		
15. HC Utilization (Caregiver and Child)	.12	-.10	.13	.02	-.08	.09	-.02	-.05	.13	-.20	.16	.40*	.22	.26†	-	
16. HC Utilization (Medical Record)	.22	.22	.04	-.02	-.11	.13	.06	-.21	.23	-.37**	.11	.27*	.21	.29*	.62***	-

†$p < .10$ *$p < .05$ **$p < .01$ ***$p < .001$ HC = Health Care

Table 4.5.
Sickle Cell Anemia and Beta-0-Thalassemia Subtypes: Multiple Hierarchical Regressions Predicting Pain Rate and Intensity

Variable	*B*	*B SE*	*β*	ΔR^2	Total R^2
Outcome: Pain Rate					
Demographics				.00	.00
Age	.15/-.01	.57/.02	-.04		
Gender	1.00/.00	3.51/.12	.00		
Biological Covariate				.05	.05
Alpha thalassemia	10.25/.24	4.54/.16	.21		
Biological Variables				.04	.09
Hematocrit	-.17/.03	.58/.02	.22		
Fetal hemoglobin	.21/.00	.23/.01	-.03		
Psychological Variables				.11	.20
Coping Attempts	.03/.00	.07/.00	.00		
Negative Thinking	.10/.00	.08/.00	.17		
Positive Mood	-2.50/-.12	2.27/.08	-.25		
Negative Mood	-.94/.05	3.32/.11	.08		
Outcome: Pain Intensity					
Demographics				.00	.00
Age	-.02	.08	-.04		
Gender	.03	.48	.01		
Biological Covariate				.05	.06
Platelet count	.00	.00	.23		
Biological Variables				.07	.12
Hematocrit	.12	.08	.26		
Fetal hemoglobin	-.05†	.03	-.31		
Psychological Variables				.01	.13
Coping Attempts	.00	.01	.03		
Negative Thinking	.00	.01	.06		
Positive Mood	-.06	.33	-.03		
Negative Mood	-.08	.47	-.03		

Note. Pain rate was log-transformed to generate the final results. The original untransformed unstandardized beta coefficients and standard errors are presented alongside the final values. The untransformed values are first, followed by the log-transformed values. Values are separated by a slash.
†$p < .10$

Table 4.6.
Sickle Cell Anemia and Beta-0-Thalassemia Subtypes: Multiple Hierarchical Regressions Predicting Pain Duration

Variable	***B***	***B SE***	***β***	ΔR^2	**Total R^2**
Outcome: Pain Duration					
Demographics				.01	.01
Age	-.05	.08	-.09		
Gender	.19	.47	.06		
Biological Variables				.10†	.11
Hematocrit	.13†	.08	.29		
Fetal hemoglobin	-.07*	.03	-.40		
Psychological Variables				.04	.15
Coping Attempts	-.01	.01	-.22		
Negative Thinking	.01	.01	.11		
Positive Mood	.07	.30	.04		
Negative Mood	.07	.45	.03		

†$p < .10$ *$p < .05$

Table 4.7.
Sickle Cell Anemia and Beta-0-Thalassemia Subtypes: Multiple Hierarchical Regressions Predicting Health Care Utilization Outcomes

Variable	*B*	*B SE*	*β*	ΔR^2	Total R^2
Outcome: Health Care Utilization (Caregiver and Child Report)					
Demographics				.00	.00
Age	.28/.01	.44/.02	.06		
Gender	-1.60/.00	2.73/.13	.00		
Biological Variables				.05	.05
Hematocrit	.45/.03	.46/.02	.26		
Fetal hemoglobin	-.08/-.01	.18/.01	-.19		
Psychological Variables				.15	.20
Coping Attempts	-.01/.00	.06/.00	-.17		
Negative Thinking	.01/.00	.06/.00	.00		
Positive Mood	-2.29/-.14†	1.81/.08	-.27		
Negative Mood	2.14/.16	2.68/.12	.21		
Outcome: Health Care Utilization (Medical Record)					
Demographics				.09	.09
Age	.18	.18	.14		
Gender	1.84	1.13	.23		
Biological Variables				.01	.10
Hematocrit	.06	.19	.05		
Fetal hemoglobin	.02	.08	.06		
Psychological Variables				.23*	.32
Coping Attempts	-.03	.02	-.23		
Negative Thinking	.04	.02	.21		
Positive Mood	-1.42*	.68	-.30		
Negative Mood	.98	1.01	.15		

Note. Caregiver- and child-reported health care utilization was log-transformed to generate the final results. The original untransformed unstandardized beta coefficients and standard errors are presented alongside the final values. The untransformed values are first, followed by the log-transformed values. Values are separated by a slash.
†$p < .10$ *$p < .05$

Table 4.8.
Supplemental Analysis for the Full Sample: Pre-Hydroxyurea Correlations between Sickle Cell Risk Group and Multiple Pain Outcomes

Variable	**1.**	**2.**	**3.**	**4.**	**5.**	**6.**
1. Sickle Cell Risk Group	-					
2. Pain Rate	.16	-				
3. Pain Intensity	.33**	.05	-			
4. Pain Duration	.03	.01	.21†	-		
5. Health Care Utilization (Caregiver and Child)	.25*	.49***	.44***	.19†	-	
6. Health Care Utilization (Medical Record	.25*	.59***	.20†	.10	.38**	-

†$p < .10$ *$p < .05$ **$p < .01$ ***$p < .001$

Table 4.9.
Supplemental Analysis for Sickle Cell Anemia and Beta-0-Thalassemia Subtypes: Pre-Hydroxyurea Hematocrit and Fetal Hemoglobin Predicting Multiple Pain Outcomes

Variable	***B***	***B SE***	***β***	**Total R^2**
Outcome: Pain Rate				
Hematocrit	-1.06	.87	-.22	.04
Fetal Hemoglobin	.27	.32	.15	
Outcome: Pain Intensity				
Hematocrit	-.18	.11	-.27	.14*
Fetal Hemoglobin	.11*	.04	.44	
Outcome: Pain Duration				
Hematocrit	.25†	.13	.34	.09
Fetal Hemoglobin	-.07	.05	-.27	
Outcome: Health Care Utilization (Caregiver and Child Report)				
Hematocrit	-1.11	1.11	-.18	.04
Fetal Hemoglobin	.50	.40	.22	
Outcome: Health Care Utilization (Medical Record)				
Hematocrit	.31†	.18	.30	.07
Fetal Hemoglobin	-.03	.06	-.08	

†$p < .10$ *$p < .05$

Table 4.10.
Full Sample: Multiple Hierarchical Regressions Predicting Pain Rate and Duration with Interactions

Variable	*B*	*B SE*	*β*	ΔR^2	Total R^2
Outcome: Pain Rate					
Biological Variable				.03	.03
Sickle Cell Risk Group	-.13/.08	1.56/.06	.17		
Psychological Variables				.15**	.18
Negative Thinking	.15/.01*	.06/.00	.29		
Positive Mood	-2.09/-.11*	1.49/.05	-.23		
Interactions				.01	.18
SC Risk Group x Negative Thinking	.01/.00	.06/.00	-.06		
SC Risk Group x Positive Mood	1.16/.02	1.52/.06	.04		
Outcome: Pain Duration					
Biological Variable				.00	.00
Sickle Cell Risk Group	-.02	.23	-.01		
Psychological Variable				.04†	.04
Positive Mood	-.39†	.23	-.20		
Interaction				.03	.07
SCD Risk Group x Positive Mood	.35	.23	.18		

Note. Pain rate was log-transformed to generate the final results. The original untransformed unstandardized beta coefficients and standard errors are presented alongside the final values. The untransformed values are first, followed by the log-transformed values. Values are separated by a slash. †$p < .10$ *$p < .05$ **$p < .01$ SC = sickle cell

Table 4.11.
Full Sample: Multiple Hierarchical Regressions Predicting Health Care Utilization Outcomes with Interactions

Variable	*B*	*B SE*	*β*	ΔR^2	Total R^2
Outcome: Health Care Utilization (Caregiver and Child Report)					
Biological Variable				.05*	.05
Sickle Cell Risk Group	1.36/.11*	1.15/.06	.22		
Psychological Variables				.14**	.19
Negative Thinking	.06/.00	.04/.00	.17		
Positive Mood	-2.37/-.16**	1.13/.06	-.32		
Interactions				.03	.22
SCD Risk Group x Negative Thinking	-.02/.00	.05/.00	-.19		
SCD Risk Group x Positive Mood	.21/-.02	1.18/.06	-.03		
Outcome: Health Care Utilization (Medical Record)					
Biological Variable				.05†	.05
Sickle Cell Risk Group	.92†	.50	.21		
Psychological Variables				.09*	.14
Negative Thinking	.03	.02	.16		
Positive Mood	-1.04*	.49	-.24		
Interactions				.04	.18
SCD Risk Group x Negative Thinking	.01	.02	.06		
SCD Risk Group x Positive Mood	-.78	.50	-.18		

Note. Caregiver- and child-reported health care utilization was log-transformed to generate the final results. The original untransformed unstandardized beta coefficients and standard errors are presented alongside the final values. The untransformed values are first, followed by the log-transformed values. Values are separated by a slash.

†$p < .10$ *$p < .05$ **$p < .01$ SCD = sickle cell disease

Table 4.12.
Sickle Cell Anemia and Beta-0-Thalassemia Subtypes: Multiple Hierarchical Regression Predicting Pain Rate with Interactions

Variable	***B***	***B SE***	***β***	**ΔR^2**	**Total R^2**
Outcome: Pain Rate					
Biological Covariate				.05	.05
Alpha thalassemia	10.24/.23	4.43/.15	.21		
Biological Variables				.04	.09
Hematocrit	-.14/.02	.57/.02	.22		
Fetal hemoglobin	.17/.00	.22/.01	-.03		
Psychological Variables				.05	.14
Negative Thinking	.12/.00	.07/.00	.23		
Interactions				.00	.14
Hct x Negative Thinking	-.06/.00	.03/.00	-.08		
FetHgb x Negative Thinking	.01/.00	.01/.00	.02		

Note. Pain rate was log-transformed to generate the final results. The original untransformed unstandardized beta coefficients and standard errors are presented alongside the final values. The untransformed values are first, followed by the log-transformed values. Values are separated by a slash. Hct = hematocrit Fet Hgb = fetal hemoglobin

Table 4.13.
Sickle Cell Anemia and Beta-0-Thalassemia Subtypes: Multiple Hierarchical Regressions Predicting Health Care Utilization Outcomes with Interactions

Variable	*B*	*B SE*	*β*	ΔR^2	Total R^2
Outcome: Health Care Utilization (Caregiver and Child Report)					
Biological Variables				.05	.05
Hematocrit	.46/.03	.45/.02	.27		
Fetal hemoglobin	-.08/-.01	.17/.01	-.20		
Psychological Variables				.11*	.16
Positive Mood	-2.67/-.18*	1.57/.07	-.34		
Interactions				.05	.21
Hct x Positive Mood	.85/.02	.70/.03	.16		
Fet Hgb x Positive Mood	.06/.01	.22/.01	.13		
Outcome: Health Care Utilization (Medical Record)					
Demographics				.09	.09
Age	.18	.18	.14		
Gender	1.84	1.13	.23		
Biological Variables				.01	.09
Hematocrit	.06	.19	.05		
Fetal hemoglobin	.02	.08	.06		
Psychological Variables				.21*	.30
Coping Attempts	-.02	.02	-.18		
Negative Thinking	.04†	.02	.25		
Positive Mood	-1.55*	.67	-.33		
Interactions				.10	.41
Hct x Coping Attempts	.00	.01	.05		
FetHgb x Coping Attempts	.00	.00	-.01		
Hct x Negative Thinking	.00	.01	.06		
FetHgb x Negative Thinking	.00	.00	.00		
Hct x Positive Mood	.64†	.33	.53		
FetHgb x Positive Mood	-.08	.10	-.18		

Note. Caregiver- and child-reported health care utilization was log-transformed to generate the final results. The original untransformed unstandardized beta coefficients and standard errors are presented alongside the final values. The untransformed values are first, followed by the log-transformed values. Values are separated by a slash.
†$p < .10$ *$p < .05$ Hct = hematocrit FetHgb = fetal hemoglobin

Table 4.14.
Supplemental Analysis for Sickle Cell Anemia and Beta-0-Thalassemia Subtypes: Multiple Hierarchical Regressions Predicting Pain Rate and Health Care Utilization Outcomes (Hematocrit only)

Variable	*B*	*B SE*	*β*	ΔR^2	Total R^2
Outcome: Pain Rate					
Biological Covariate				.04	.04
Alpha thalassemia	10.19/.23	4.38/.15	.21		
Biological Variables				.04	.08
Hematocrit	.13/.02	.45/.02	.20		
Psychological Variables				.05	.14†
Negative Thinking	.12/.00†	.07/.00	.23		
Interactions				.00	.14
Hct x Negative Thinking	-.05/.00	.02/.00	-.06		
Outcome: Health Care Utilization (Caregiver and Child Report)					
Biological Variables				.02	.02
Hematocrit	.35/.02	.37/.02	.15		
Psychological Variables				.10	.13*
Positive Mood	-2.38/-.18*	1.57/.07	-.32		
Interactions				.04	.17
Hct x Positive Mood	.99/.04	.50/.02	.26		
Outcome: Health Care Utilization (Medical Record)					
Demographics				.08	.08
Age	.25	.18	.19		
Gender	1.53	1.13	.19		
Biological Variables				.01	.09
Hematocrit	.09	.16	.08		
Psychological Variables				.18*	.28
Coping Attempts	-.02	.02	-.18		
Negative Thinking	.05*	.02	.28		
Positive Mood	-1.31†	.67	-.27		
Interactions				.08	.36
Hct x Coping Attempts	.00	.01	.01		
Hct x Negative Thinking	.00	.01	.06		
Hct x Positive Mood	.44†	.23	.36		

Note. Pain rate and caregiver- and child-reported health care utilization were log-transformed to generate the final results. The original untransformed unstandardized beta coefficients and standard errors are presented alongside the final values. The untransformed values are first, followed by the log-transformed values. Values are separated by a slash. †$p < .10$ *$p < .05$ Hct = hematocrit

Table 4.15.
Supplemental Analysis for Sickle Cell Anemia and Beta-0-Thalassemia Subtypes: Multiple Hierarchical Regressions Predicting Pain Rate and Health Care Utilization Outcomes (Fetal Hemoglobin only)

Variable	*B*	*B SE*	*β*	ΔR^2	Total R^2
Outcome: Pain Rate					
Biological Covariate				.05	.05
Alpha thalassemia	10.24/.23	4.43/.15	.21		
Biological Variables				.01	.06
Fetal Hemoglobin	.14/.00	.17/.01	.11		
Psychological Variables				.04	.10
Negative Thinking	.12/.00	.07/.00	.21		
Interactions				.00	.10
FetHgb x Negative Thinking	.00/.00	.01/.00	-.02		
Outcome: Health Care Utilization (Caregiver and Child Report)					
Biological Variables				.00	.00
Fetal Hemoglobin	.02/.00	.14/.01	-.04		
Psychological Variables				.12*	.12
Positive Mood	-2.71/-.18*	1.57/.07	-.34		
Interactions				.05†	.17
FetHgb x Positive Mood	.26/.01†	.15/.01	.23		
Outcome: Health Care Utilization (Medical Record)					
Demographics				.09	.09
Age	.18	.18	.14		
Gender	1.84	1.13	.23		
Biological Variables				.01	.09
Fetal Hemoglobin	.04	.06	.09		
Psychological Variables				.21*	.30
Coping Attempts	-.02	.02	-.18		
Negative Thinking	.04†	.02	.25		
Positive Mood	-1.56*	.66	-.33		
Interactions				.03	.33
FetHgb x Coping Attempts	.00	.00	.03		
FetHgb x Negative Thinking	.01	.00	.08		
FetHgb x Positive Mood	.06	.07	.13		

Note. Pain rate and caregiver- and child-reported health care utilization were log-transformed to generate the final results. The original untransformed unstandardized beta coefficients and standard errors are presented alongside the final values. The untransformed values are first, followed by the log-transformed values. Values are separated by a slash. †$p < .10$ *$p < .05$ FetHgb = fetal hemoglobin

Table 4.16.
Correlations between Child and Caregiver Psychological and Family Variables

Variable	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.
1. Age	-										
2. Gender	.13	-									
3. Child Coping Attempts	-.25*	-.07	-								
4. Child Negative Thinking	.14	-.26*	.25*	-							
5. Child Positive Mood	-.16	-.06	.36**	-.13	-						
6. Child Negative Mood	-.02	-.26*	.31**	.42***	-.21†	-					
7. Caregiver Active Coping	-.12	.19	.17	.07	.22†	-.01	-				
8. Caregiver Passive Coping	.17	.02	.03	.21†	-.10	.05	.27*	-			
9. Caregiver Positive Mood	-.23†	.14	.03	-.16	.21†	-.08	.19	-.20†	-		
10. Caregiver Negative Mood	.32**	.08	-.06	.23†	-.19	.06	.02	.62***	-.49***	-	
11. Family Functioning	.21†	-.27*	-.03	.26*	-.10	.07	-.11	.27*	-.58***	.32**	-

†$p < .10$ *$p < .05$ **$p < .01$ ***$p < .001$

Table 4.17.
Correlations between Caregiver Psychological and Family Variables and Child Pain Outcomes

Variable	**1.**	**2.**	**3.**	**4.**	**5.**	**6.**	**7.**	**8.**	**9.**	**10.**
1. Caregiver Active Coping	-									
2. Caregiver Passive Coping	.27*	-								
3. Caregiver Positive Mood	.19	-.20†	-							
4. Caregiver Negative Mood	.02	.62***	-.49***	-						
5. Family Functioning	-.11	.27*	-.58***	.32**	-					
6. Child Pain Rate	.15	.16	-.12	.05	.03	-				
7. Child Pain Intensity	.04	-.07	.05	.02	.04	.15	-			
8. Child Pain Duration	-.07	.20†	-.06	.28*	.01	.11	.15	-		
9. Child Health Care Utilization (Caregiver and Child Report)	.11	.22†	-.12	.05	-.02	.74***	.24*	.18	-	
10. Child Health Care Utilization (Medical Record)	.21†	.31**	.04	.25*	-.09	.48***	.25*	.14	.67***	-

†$p < .10$ *$p < .05$ **$p < .01$ ***$p < .001$

Table 4.18.
Supplemental Analysis: Direct Effects of Biological and Child and Caregiver Psychological Variables in Relation to Pain Duration and Health Care Utilization

Variable	*B*	*B SE*	*β*	ΔR^2	Total R^2
Outcome: Pain Duration					
Biological Variables				.02	.02
Sickle Cell Risk Group	.51	.45	.14		
Child Psychological Variables				.02	.04
Positive Mood	-.29	.22	-.15		
Caregiver Psychological Variable				.07*	.12
Negative Mood	.68*	.29	.28		
Outcome: Health Care Utilization (Caregiver and Child Report)					
Biological Variable				.06*	.06
Sickle Cell Risk Group	1.53/.12*	1.24/.06	.24		
Child Psychological Variables				.14**	.20
Negative Thinking	.06/.00	.04/.00	.18		
Positive Mood	-2.46/-.16**	1.21/.06	-.31		
Caregiver Psychological Variable				.03	.22
Passive Coping	.21/.01	.14/.01	.17		
Outcome: Health Care Utilization (Medical Record)					
Biological Variable				.04	.04
Sickle Cell Risk Group	1.69	1.07	.19		
Child Psychological Variables				.11*	.15
Negative Thinking	.03	.02	.17		
Positive Mood	-1.22*	.52	-.27		
Caregiver Psychological				.10*	.25
Variables	.05†	.03	.21		
Active Coping	.10†	.06	.20		
Passive Coping					

Note. Caregiver- and child-reported health care utilization was log-transformed to generate the final results. The original untransformed unstandardized beta coefficients and standard errors are presented alongside the final values. The untransformed values are first, followed by the log-transformed values. Values are separated by a slash.
†$p < .10$ *$p < .05$ **$p < .01$

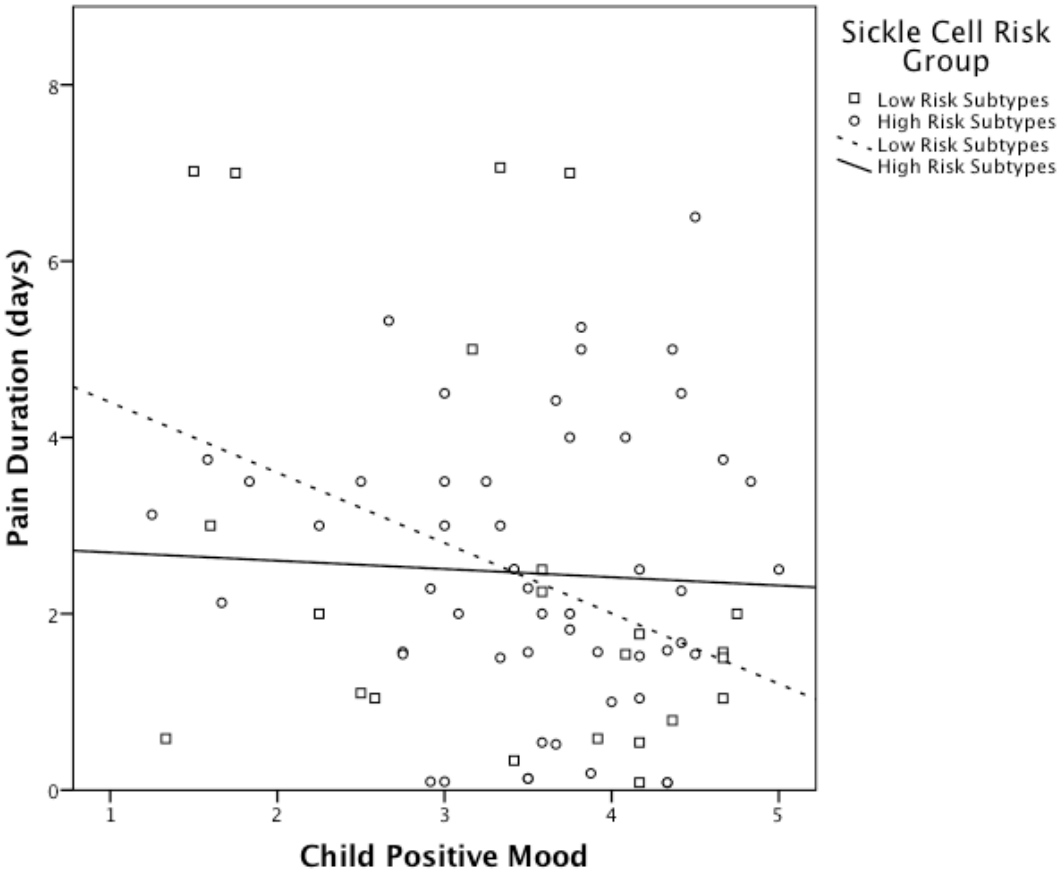

Figure 4.1. The relationship between positive mood and pain duration is dependent on sickle cell risk group. Children with low-risk subtypes demonstrated a stronger, negative relationship between positive mood and pain duration while this relationship was relatively weaker for children with high-risk subtypes.

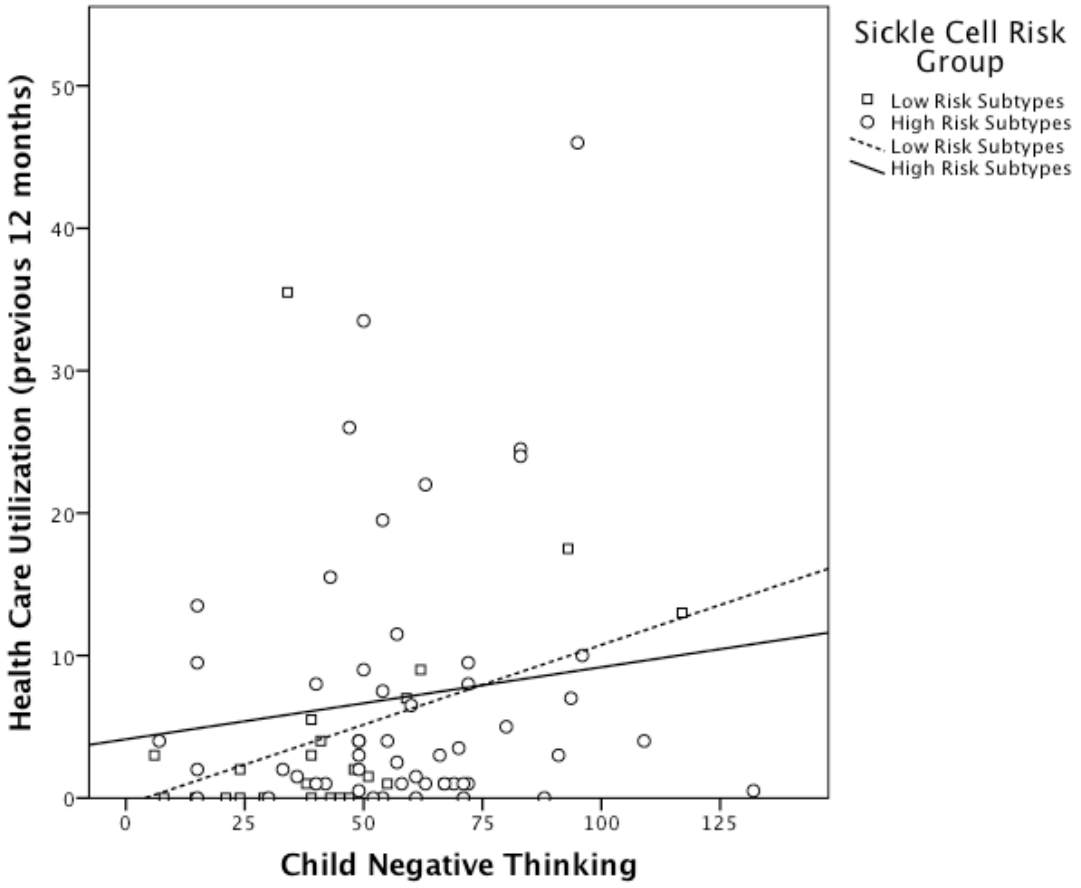

Figure 4.2. The relationship between negative thinking and caregiver- and child-reported health care utilization is dependent on sickle cell risk group. Children with low-risk subtypes demonstrated a stronger, positive relationship between negative thinking and health care utilization over the previous 12 months as measured by caregiver and child report. This relationship was relatively weaker for children with high-risk subtypes.

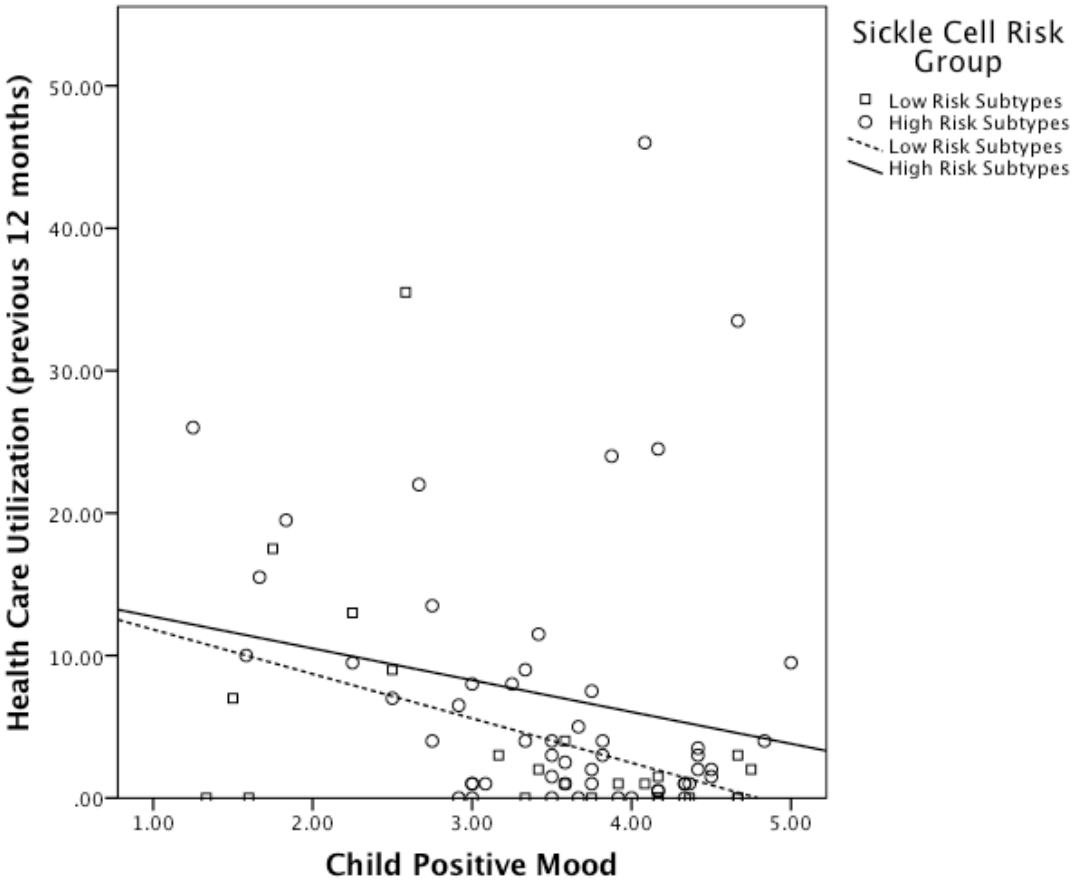

Figure 4.3. The relationship between positive mood and caregiver- and child-reported health care utilization is dependent on sickle cell risk group. Children with low-risk subtypes demonstrated a stronger, negative relationship between positive mood and health care utilization over the previous 12 months as measured by caregiver and child report. This relationship was relatively weaker for children with high-risk subtypes.

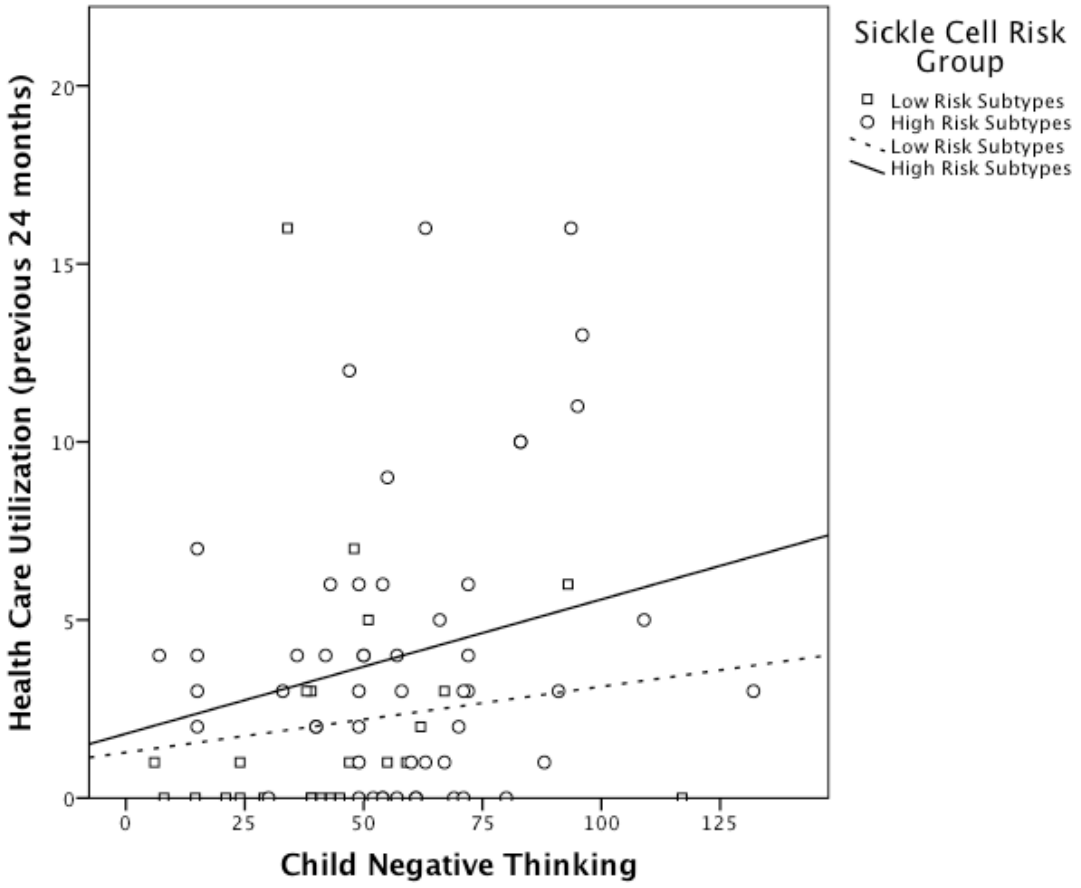

Figure 4.4. The relationship between negative thinking and medical record health care utilization is dependent on sickle cell risk group. Children with high-risk subtypes demonstrated a stronger, positive relationship between negative thinking and health care utilization over the previous 24 months as measured by the medical record. This relationship was relatively weaker for children with low-risk subtypes.

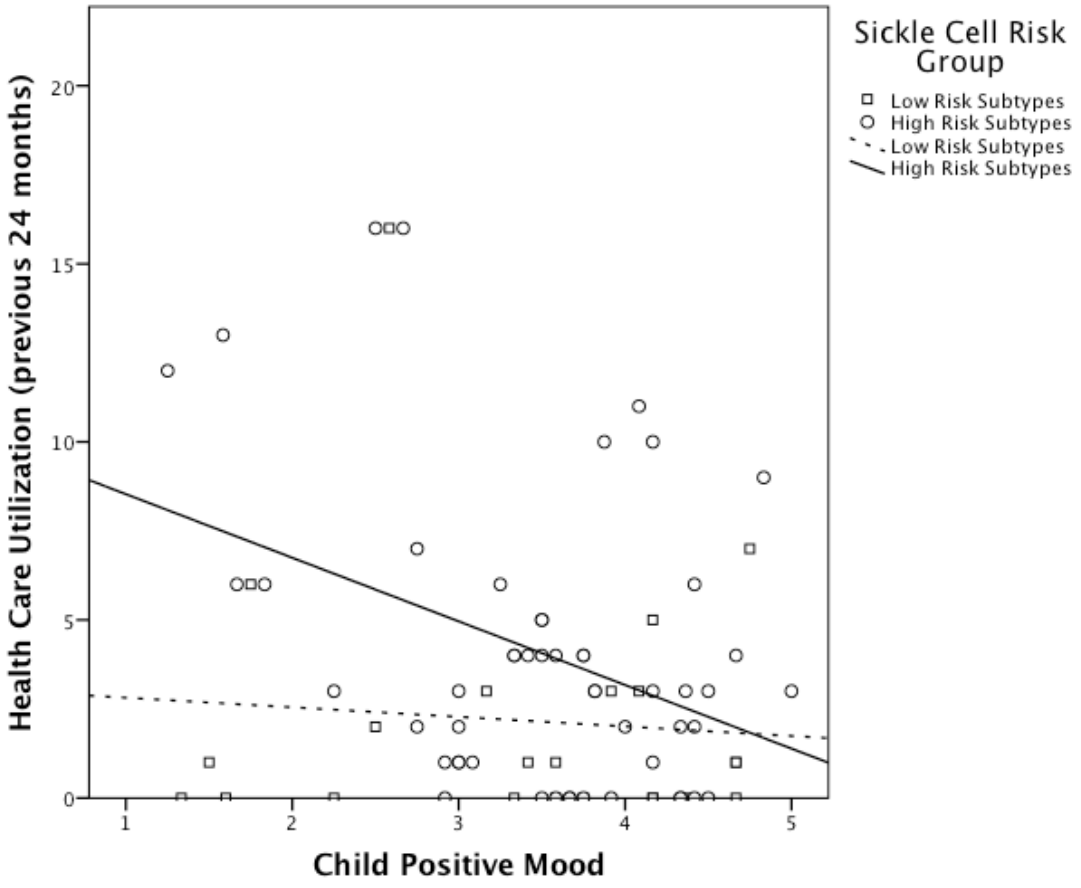

Figure 4.5. The relationship between positive mood and medical record health care utilization is dependent on sickle cell risk group. Children with high-risk subtypes demonstrated a stronger, negative relationship between positive mood and health care utilization over the previous 24 months as measured by the medical record. This relationship was relatively weaker for children with low-risk subtypes.

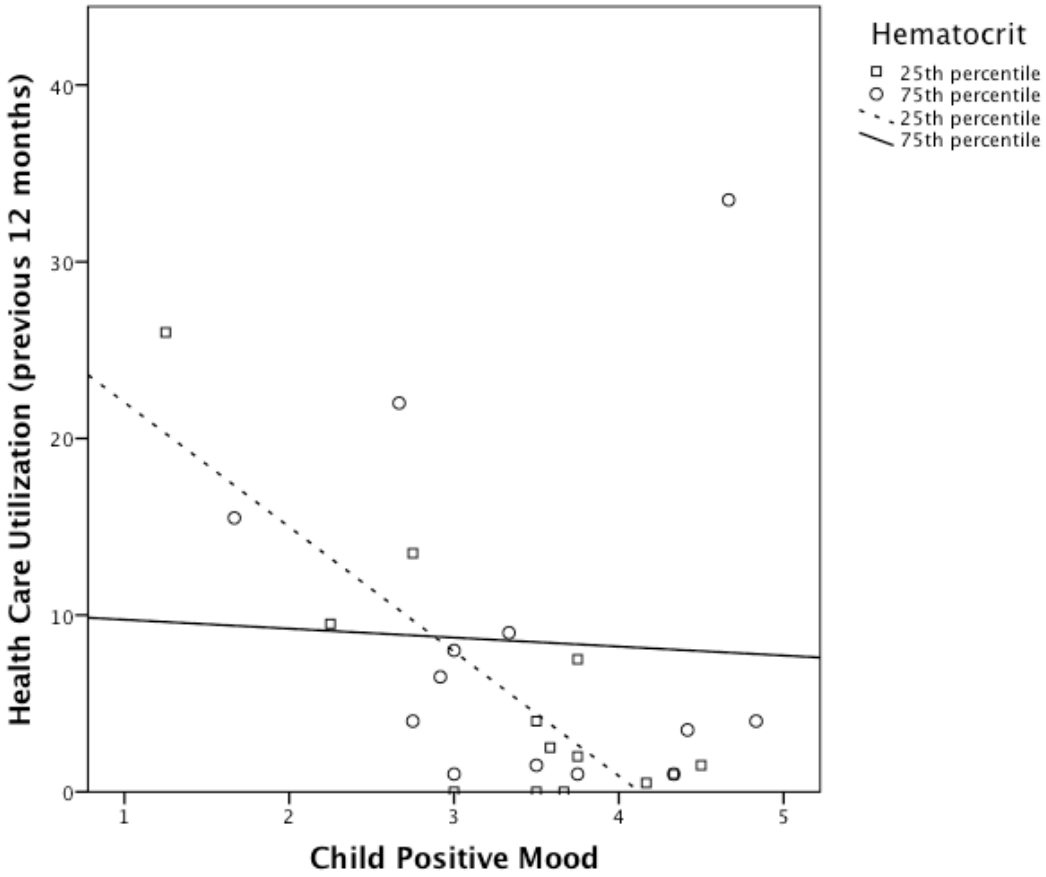

Figure 4.6. The relationship between positive mood and caregiver- and child-reported health care utilization is dependent on hematocrit. Children with lower hematocrit demonstrated a stronger, negative relationship between positive mood and health care utilization over the previous 12 months as measured by caregiver and child report. This relationship was relatively weaker for children with higher hematocrit.

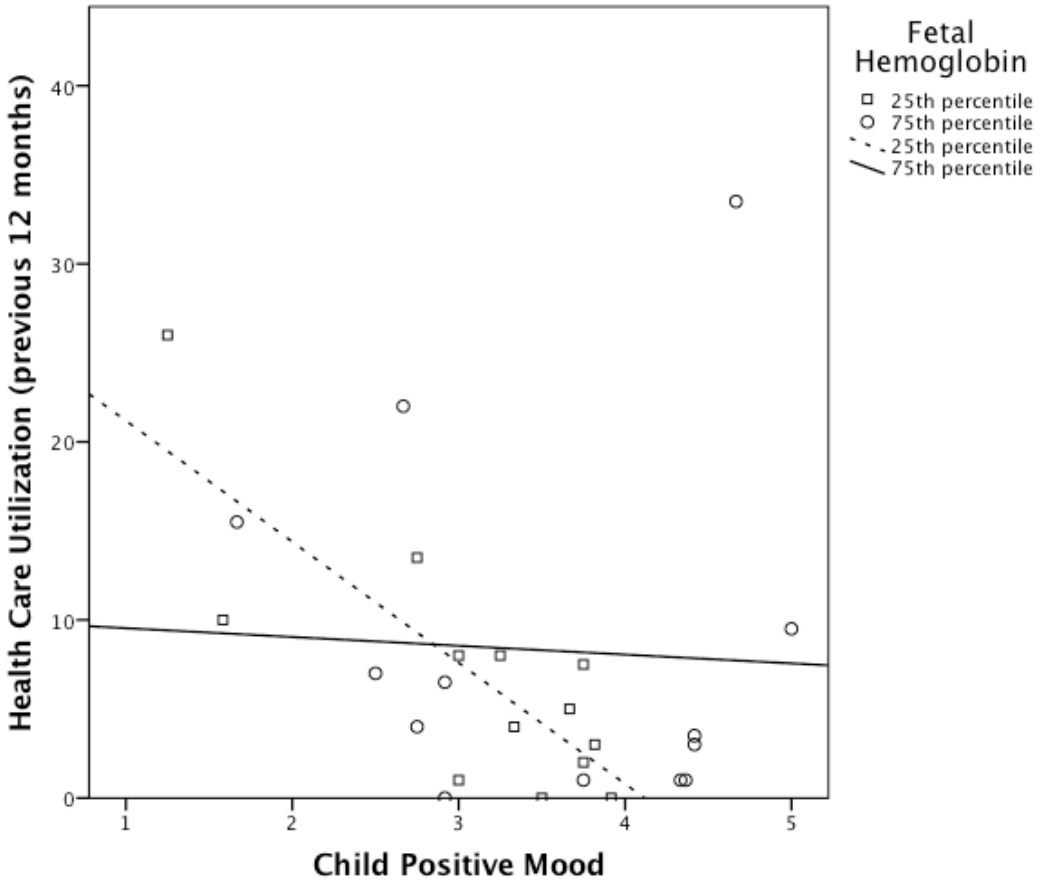

Figure 4.7. The relationship between positive mood and caregiver- and child-reported health care utilization is dependent on fetal hemoglobin. Children with lower fetal hemoglobin demonstrated a stronger, negative relationship between positive mood and health care utilization over the previous 12 months as measured by caregiver and child report. This relationship was relatively weaker for children with higher fetal hemoglobin.

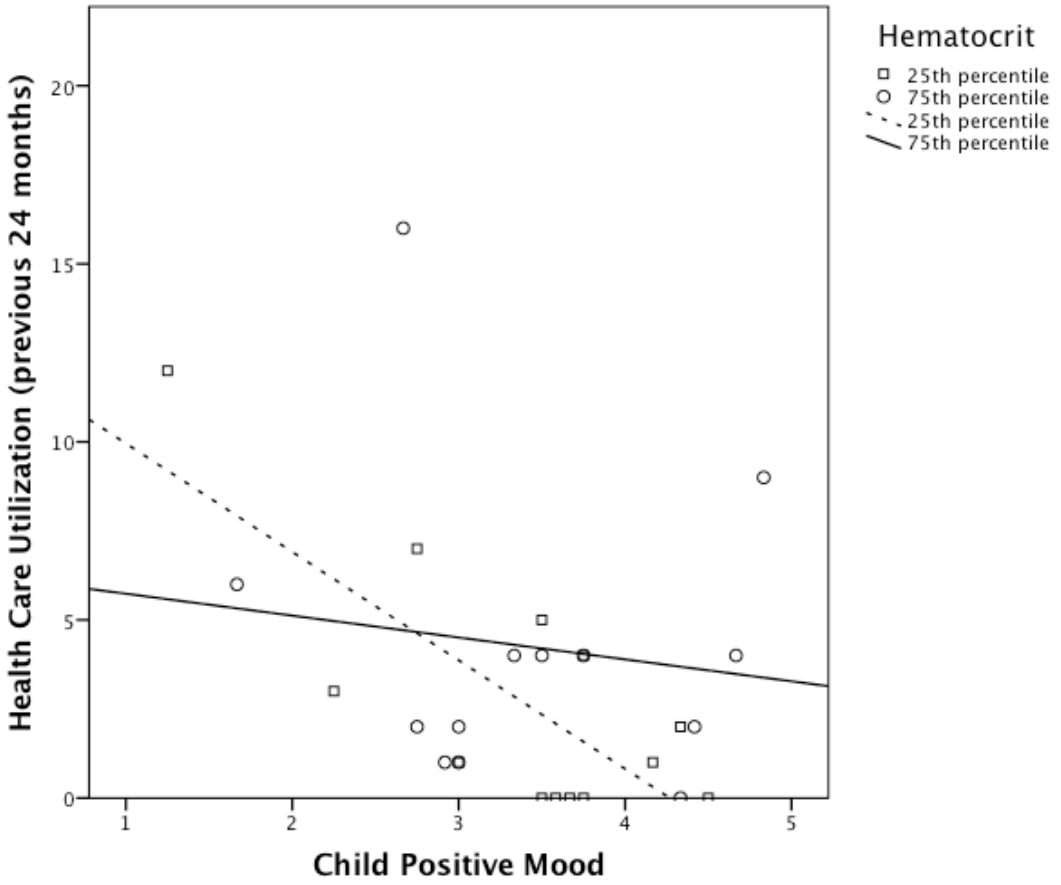

Figure 4.8. The relationship between positive mood and medical record health care utilization is dependent on hematocrit. Children with lower hematocrit demonstrated a stronger, negative relationship between positive mood and health care utilization over the previous 24 months as measured by the medical record. This relationship was relatively weaker for children with higher hematocrit.

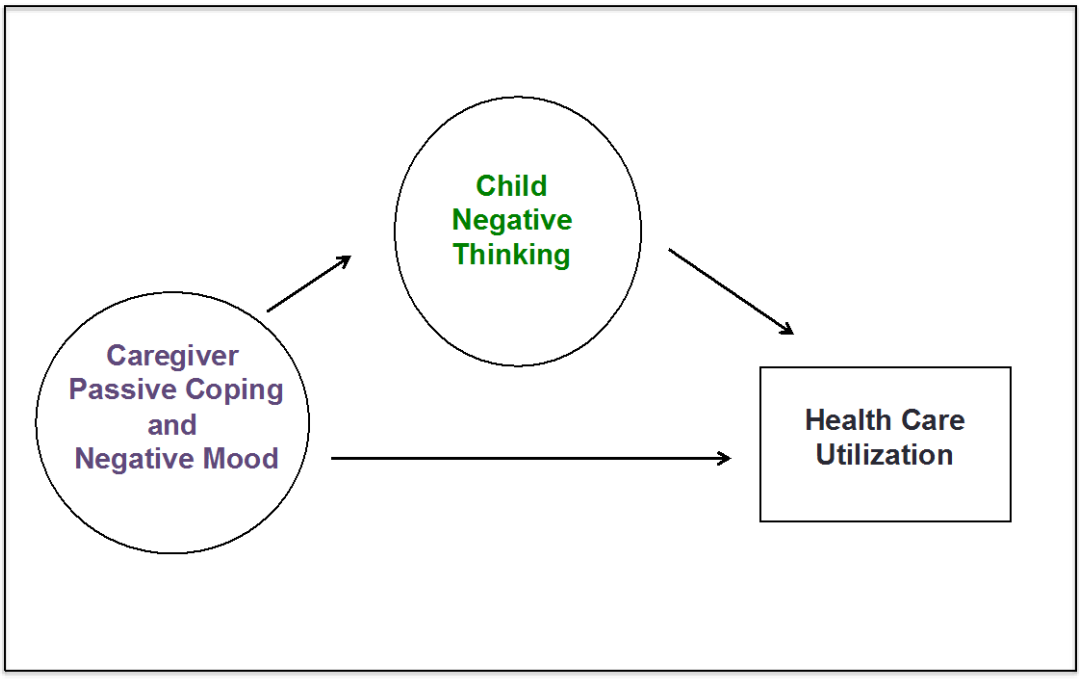

Figure 4.9. Proposed mediation model for future studies assessing child psychological variables as mediators of caregiver psychological variables and health care utilization. The proposed model, based on correlations results, suggests that the effects of caregiver passive coping and negative mood may be mediated by child negative thinking in predicting health care utilization for pain.

CHAPTER 5

STUDY TWO RESULTS

Study Two examined a novel genetic marker of pain in pediatric SCD. As with Study One, the primary results are described below followed by supplemental analyses designed to complement or address key limitations of the original analysis.

5.1 Allele Distributions

In both the full sample and subsample of children with HbSS and HbSβ^0, the distribution for the A allele was .45 and for the G allele was .55. These allele frequencies were in accordance with Hardi-Weinberg equilibrium, χ^2 (1) = 0.74, $p > .05$. The allele frequencies observed were also comparable to those found in a large sample of African Americans listed in the National Institutes of Health SNP database (.44 for A allele and .56 for G allele; $N = 122$).

5.2 Regression Assumptions

Similar to the analysis for Study One, the two primary issues identified for regression assumptions in Study Two were non-normality of residuals and the presence of influential cases for pain rate and caregiver- and child-reported health care utilization. Thus, the same modifications were made to the data, including log-transformation of these outcome variables and presentation of the results using both the original, untransformed and log-transformed beta coefficients. Independence of errors was assumed for the models, as the Durbin-Watson statistic did not exceed lower limits for this statistic (Durbin-Watson ranged from 1.61 to 2.25). Multicollinearity for the two

coefficients representing genotype comparisons was expected and no other correlations between the covariates and primary predictors exceeded .50; thus, this assumption was assumed to be adequate. No curvilinear patterns or systematic deviations from homoscedasticity were identified on residual plots.

5.3 Primary Aim Results

Full Sample. Table 5.1 provides biserial correlations to demonstrate relationships between the ET-B genotypes and pain features for the full sample. Table 5.2 provides means and standard deviations by genotype for each pain outcome to aid in interpretation. Neither age nor gender had a correlation of .20 or higher with any pain outcomes; thus, correlations between the ET-B genotypes and pain features were used in lieu of linear regression. The correlations between ET-B genotypes and caregiver- and child-reported health care utilization were not significant. For medical record health care utilization, the correlation comparing the A/G versus the A/A genotypes approached significance (r = .22, p = .065); however, the correlation comparing the G/G and A/A genotypes was not significant nor was it in the expected direction (r = -.15, p = .194). Thus, hypothesis 8 regarding the association of ET-B genotype and health care utilization was not supported for either health care utilization outcome.

In terms of additional pain features, the correlation comparing the A/G versus the A/A genotype was significant for pain duration (r = .27, p = .022); however, the correlation comparing the G/G and A/A genotypes was not significant nor was it in the expected direction (r = -.11, p = .345). Cumulatively, these results do not lend consistent support for the G allele conferring greater risk than the A allele.

Sickle Cell Anemia and Sickle Cell Beta-0-Thalassemia. Table 5.3 provides biserial correlations to demonstrate the relationships between the ET-B genotypes and pain features for the subsample of children with HbSS and HbSβ^0. Table 5.5 provides means and standard deviations by genotype for each pain outcome to aid in interpretation. There were no covariates for caregiver- and child-reported health care utilization that demonstrated correlations ≥ .20, and neither of the correlations with the ET-B genotypes was significant. For medical record health care utilization, age and gender were entered into a linear regression model with both of the ET-B coefficients (Table 5.4). The overall regression model was not significant, F (4, 45) = 1.28, p = .292, with the model accounting for 10% of the variance overall. After controlling for age and gender, the ET-B coefficients did not contribute a significant amount of variance to the model, ΔF (2, 45) = 0.49, p = .614, ΔR^2 = .02. Thus, similar to the full sample, hypothesis 8 was not supported for health care utilization.

Exploratory models were examined for additional pain features. For pain rate, alpha thalassemia trait was entered as a covariate with the ET-B genotypes. The overall regression model was not significant, F (3, 46) = 0.43, p = .733, with the model explaining 3% of the variance overall. After controlling for alpha thalassemia trait, the ET-B coefficients did not contribute a significant amount of variance to the model, ΔF (2, 46) = 0.58, p = .563, ΔR^2 = .02. For pain intensity, platelet count was entered as a covariate with the ET-B genotypes. The overall regression model was not significant, F (3, 46) = 0.81, p = .497, with the model explaining 5% of the variance overall. After controlling for platelet count, the ET-B genotypes did not contribute a significant amount of variance to the model, ΔF (2, 46) = 0.32, p = .725, ΔR^2 = .01.

Finally, for pain duration, fetal hemoglobin was entered as a covariate with the ET-B genotypes. The overall regression model was significant, F (4, 44) = 2.65, p = .046, with the model explaining 19% of the variance in pain duration overall. After controlling for fetal hemoglobin, the ET-B genotypes approached significance as a contributor of variance to the model, ΔF (2, 45) = 2.89, p = .066, ΔR^2 = .11. The beta coefficient comparing the A/G to the A/A genotype was significant as an individual predictor of pain duration, t (1, 47) = 2.39, p = .021. The beta coefficient comparing the G/G to the A/A genotype was not significant though it was in the expected direction, t (1, 47) = 1.33, p = .191. Cumulatively, these results do not lend consistent support for the G allele conferring greater risk than the A allele, though it is noted that the largest observed effect for the G allele was found with pain duration.

Supplemental Analysis: Pre-Hydroxyurea Pain Measures. As with Study One, supplemental analyses were conducted to determine whether hydroxyurea may have altered the pain outcomes in a manner that would attenuate the effects of the ET-B genotypes. Biserial correlations between the ET-B genotypes and pre-hydroxyurea pain outcomes were used to determine if the strength and direction of associations differed from the original analysis (see Tables 5.6 and 5.8 for correlations and Tables 5.7 and 5.9 for means and standard deviations).

For the full sample, the association between the ET-B correlation comparing the G/G versus the A/A genotype approached significance for medical record health care utilization (r = -.21, p = .080); however, it was in the opposite direction to what was hypothesized. A similar effect was found for medical record health care utilization in the subsample of children with HbSS and HbSβ^0 (r = -.22, p = .135); however, it was in also

in the wrong direction. There were no other notable changes in correlations for either the full sample or subsample.

Supplemental Analysis: Alternate Pain Measures. Supplemental analyses were also conducted to determine whether alternative pain measures would have resulted in more robust effects with the ET-B genotypes. Two methods were used in this analysis. First, dichotomous variables were created for both health care utilization outcomes and pain rate and compared to the ET-B genotypes. The original, untransformed variables were dummy coded into 0 (no pain) and 1 (at least one pain episode). These specific variables were used because the continuous distributions demonstrated the most non-normal patterns characterized by a positive skew with several values of 0. In addition, a previous genetic association study had dichotomized pain in this manner in a pediatric SCD population; thus, there was precedent for using this approach (Chaar, et al., 2006). Second, the health care utilization outcomes were analyzed again using only emergency room visits and hospitalizations, which tend to be associated with severe pain episodes, and this variable was analyzed both as a continuous and dichotomous variable.

Chi-square analysis and biserial correlations were used to examine these exploratory relationships (Tables 5.10 and 5.11). For the full sample, there were significant relationships for correlations comparing the A/G to the A/A genotype for the dichotomous versions of medical record health care utilization (r = .29, p = .013) and emergency room visits and hospitalizations (r = .27, p = .019). In addition, this same association approached significance with the dichotomous version of caregiver- and child-reported health care utilization (r = .21, p = .077) and was significant for caregiver- and child- reported emergency room visits and hospitalizations (r = .35, p = .002).

However, as with previous analyses demonstrating this effect, the correlations comparing the G/G and A/A genotype were not significant for these outcomes nor were they in the expected directions (*r* ranged from -.13 to -.20; *p* ranged from .090 to .327).

For the subsample of children with HbSS and HbSβ^0, the correlation comparing the A/G to the A/A genotype approached significance for the dichotomous version of caregiver- and child-reported emergency room visits and hospitalizations ($r = .28$, $p = .052$); however, the correlation comparing the G/G and A/A genotype was not significant or in the expected direction ($r = -.03$, $p = .850$).

Table 5.1.
Full Sample: Biserial Correlations between Endothelin B Receptor Genotypes, Covariates, and Pain Outcomes

Variable	1.	2.	3.	4.	5.	6.	7.	8.	9.
1. ET-B (G/G vs. A/A)	-								
2. ET-B (A/G vs. A/A)	-.62***	-							
3. Age	-.01	-.04	-						
4. Gender	-.06	-.01	.13	-					
5. Pain Rate	-.06	.10	.01	-.02	-				
6. Pain Intensity	-.01	-.03	.11	-.11	-.02	-			
7. Pain Duration	-.11	.27*	.03	.03	.08	.15	-		
8. Health Care Utilization (Caregiver and Child)	.05	.08	.08	-.13	.53***	.24*	.18	-	
9. Health Care Utilization (Medical Record)	-.15	.22†	.15	.07	.38**	.25*	.14	.67***	-

†p < .10 *p < .05 ***p < .001 ET-B = Endothelin B Receptor

Table 5.2.
Full Sample: Mean Differences in Pain Outcomes by the Endothelin B Receptor Genotypes

Pain Outcome	Overall *M (SD)*	A/A *M (SD)*	A/G *M (SD)*	G/G *M (SD)*
N	*N* = 74	*n* = 17	*n* = 33	*n* = 24
Pain Rate	6.63 (9.25)	5.79 (8.13)	7.64 (11.05)	5.81 (7.28)
Pain Intensity	7.24 (1.68)	7.38 (1.28)	7.18 (1.63)	7.21 (2.04)
Pain Duration	2.49 (1.86)	1.85 (2.11)	3.04 (1.91)	2.20 (1.42)
Health Care Utilization (Caregiver and Child Report)	6.36 (9.26)	3.85 (6.31)	7.15 (8.39)	7.04 (11.85)
Health Care Utilization (Medical Record)	3.39 (4.07)	2.76 (3.33)	4.36 (4.72)	2.50 (3.40)

Table 5.3.
Sickle Cell Anemia and Sickle Cell Beta-0-Thalassemia Subtypes: Biserial Correlations between Endothelin B Receptor Genotypes, Covariates, and Pain Outcomes

Variable	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.
1. ET-B (G/G vs. A/A)	-													
2. ET-B (A/G vs. A/A)	-.63***	-												
3. Age	.10	.06	-											
4. Gender	-.17	.12	.18	-										
5. Alpha Thalassemia	-.15	.09	-.03	.11	-									
6. Hematocrit	-.02	.01	-.04	-.16	.04	-								
7. Fetal Hemoglobin	-.02	-.02	-.19	.30*	-.19	.58***	-							
8. White Blood Cells	-.18	.21	-.14	-.02	.32*	-.35*	-.45**	-						
9. Platelets	-.17	.10	-.03	.01	-.21	.00	-.07	.01	-					
10. Pain Rate	.13	-.12	.05	.05	.31*	.05	.05	.01	-.08	-				
11. Pain Intensity	.07	-.02	.00	-.02	-.03	.09	-.15	.03	.21	-.14	-			
12. Pain Duration	-.03	.28*	-.08	.04	.13	.06	-.20	.18	.08	.11	.18	-		
13. HC Utilization (Caregiver and Child)	.18	-.07	.12	-.10	-.02	.13	.02	-.08	.09	.40*	.22	.26†	-	
14. HC Utilization (Medical Record)	-.12	.17	.22	.22	.06	.04	-.02	-.11	.13	.27*	.21	.29*	.62***	-

*p < .05 ***p < .001 HC = Health Care ET-B = Endothelin B Receptor

Table 5.4.
Sickle Cell Anemia and Sickle Cell Beta-0-Thalassemia Subtypes: Multiple Hierarchical Regressions for Endothelin B Receptor Genotypes Predicting Pain Outcomes

Variable	*B*	*B SE*	*β*	ΔR^2	Total R^2
Outcome: Pain Rate					
Covariate				.01	.01
Alpha Thalassemia	.64/.04	1.35/.08	.08		
ET-B Genotypes				.02	.03
G/G vs. A/A	1.60/.16	2.64/.15	.20		
A/G vs. A/A	-.66/.10	2.45/.14	.14		
Outcome: Pain Intensity					
Covariate				.04	.04
Platelet Count	.00	.00	.19		
ET-B Genotypes				.01	.05
G/G vs. A/A	.49	.64	.14		
A/G vs. A/A	.17	.59	.05		
Outcome: Pain Duration					
Covariate				.04	.04
Fetal Hemoglobin	-.03	.02	-.20		
ET-B Genotypes				.11†	.15
G/G vs. A/A	.82	.62	.24		
A/G vs. A/A	1.39*	.58	.42		
Outcome: Health Care Utilization (Medical Record)					
Covariates				.08	.08
Age	.25	.19	.19		
Gender	1.55	1.17	.19		
ET-B Genotypes				.02	.10
G/G vs. A/A	-.29	1.66	-.03		
A/G vs. A/A	.98	1.53	.12		

Note. Pain rate was log-transformed to generate the final results. The original untransformed unstandardized beta coefficients and standard errors are presented alongside the final values. The untransformed values are first, followed by the log-transformed values. Values are separated by a slash.
†$p < .10$ *$p < .05$

Table 5.5.
Sickle Cell Anemia and Sickle Cell Beta-0-Thalassemia Subtypes: Mean Differences in Pain Outcomes by Endothelin B Receptor Genotypes

Pain Outcome	**Overall** ***M (SD)***	**A/A** ***M (SD)***	**A/G** ***M (SD)***	**G/G** ***M (SD)***
N	*N* = 50	*n* = 11	*n* = 23	*n* = 16
Pain Rate	6.18 (6.57)	6.05 (7.48)	5.37 (4.79)	7.44 (8.22)
Pain Intensity	7.56 (1.60)	7.36 (1.45)	7.52 (1.56)	7.72 (1.83)
Pain Duration	2.48 (1.63)	1.56 (1.97)	2.96 (1.49)	2.42 (1.35)
Health Care Utilization (Caregiver and Child Report)	7.29 (9.74)	5.27 (7.51)	6.52 (7.03)	9.78 (13.76)
Health Care Utilization (Medical Record)	4.02 (4.17)	3.45 (3.75)	4.78 (4.60)	3.13 (3.84)

Table 5.6.
Supplemental Analysis for the Full Sample: Pre-Hydroxyurea Biserial Correlations between Endothelin B Receptor Genotypes and Pain Outcomes

Variable	1.	2.	3.	4.	5.	6.	7.
1. ET-B (G/G vs. A/A)	-						
2. ET-B (A/G vs. A/A)	-.62***	-					
3. Pain Rate	-.05	.06	-				
4. Pain Intensity	-.13	.00	.05	-			
5. Pain Duration	-.16	.09	.01	.21†	-		
6. Health Care Utilization (Caregiver and Child)	-.09	.12	.49***	.44***	.19†	-	
7. Health Care Utilization (Medical Record)	-.21†	.16	.59***	.20†	.10	.38**	-

†$p < .10$ ***$p < .001$ ET-B = Endothelin B Receptor

Table 5.7.
Supplemental Analysis for the Full Sample: Pre-Hydroxyurea Mean Differences in Pain Outcomes by Endothelin B Receptor Genotypes

Pain Outcome	**Overall** *M (SD)*	**A/A** *M (SD)*	**A/G** *M (SD)*	**G/G** *M (SD)*
N	*N* = 72	*n* = 17	*n* = 32	*n* = 23
Pain Rate	8.78 (13.83)	8.35 (11.68)	9.77 (16.61)	7.72 (11.25)
Pain Intensity	7.53 (2.05)	8.06 (2.01)	7.53 (1.74)	7.14 (2.46)
Pain Duration	3.65 (3.52)	4.09 (4.67)	3.99 (3.81)	2.84 (1.63)
Health Care Utilization (Caregiver and Child Report)	8.51 (15.73)	7.38 (12.00)	10.55 (19.91)	6.43 (10.81)
Health Care Utilization (Medical Record)	2.20 (2.71)	2.44 (3.27)	2.66 (2.78)	1.36 (2.01)

Table 5.8.
Supplemental Analysis for Sickle Cell Anemia and Beta-0-Thalassemia Subtypes: Pre-Hydroxyurea Biserial Correlations between Endothelin B Receptor Genotypes and Pain Outcomes

Variable	1.	2.	3.	4.	5.	6.	7.
1. ET-B (G/G vs. A/A)	-						
2. ET-B (A/G vs. A/A)	-.63***	-					
3. Pain Rate	.01	.00	-				
4. Pain Intensity	-.02	-.07	-.05	-			
5. Pain Duration	-.11	-.09	-.14	.18	-		
6. Health Care Utilization (Caregiver and Child)	-.07	.08	.46**	.46**	.16	-	
7. Health Care Utilization (Medical Record)	-.22	.16	.60***	.12	-.02	.34*	-

*$p < .05$ **$p < .01$ ***$p < .001$ ET-B = Endothelin B Receptor

Table 5.9.
Supplemental Analysis for Sickle Cell Anemia and Beta-0-Thalassemia Subtypes: Pre-Hydroxyurea Mean Differences in Pain Outcomes by Endothelin B Receptor Genotypes

Pain Outcome	**Overall** ***M (SD)***	**A/A** ***M (SD)***	**A/G** ***M (SD)***	**G/G** ***M (SD)***
N	*N* = 49	*n* = 11	*n* = 23	*n* = 15
Pain Rate	10.31 (15.29)	10.00 (12.65)	10.30 (18.31)	10.57 (12.92)
Pain Intensity	8.03 (1.89)	8.41 (2.35)	7.89 (1.56)	7.96 (2.11)
Pain Duration	3.73 (3.01)	5.02 (5.40)	3.44 (2.05)	3.23 (1.41)
Health Care Utilization (Caregiver and Child Report)	11.30 (18.38)	10.73 (13.94)	12.89 (23.29)	9.27 (12.57)
Health Care Utilization (Medical Record)	2.65 (3.02)	3.00 (3.81)	3.14 (3.01)	1.64 (2.31)

Table 5.10.
Supplemental Analysis for the Full Sample: Biserial Correlations and Chi-Square Associations for Endothelin B Receptor Genotypes with Alternative Pain Measures

Variable	1.	2.	3.	4.	5.	6.	7.	8.	9.
1. ET-B (G/G vs. A/A)	-								
2. ET-B (A/G vs. A/A)	-.62***	-							
3. Pain Rate (dichotomous)	-.08	.19	-						
4. Health Care Utilization (Caregiver and Child dichotomous)	-.14	.16	76***	-					
5. Health Care Utilization (Medical Record dichotomous)	.07	.10	.31**	.30**	-				
6. Emergency Room/Hospitalizations (Caregiver and Child)	-.13	.21†	.37**	.40***	.75***	-			
7. Emergency Room/Hospitalizations (Medical Record)	-.18	.29*	.34**	.52***	.36**	.60***	-		
8. Emergency Room/Hospitalizations (Caregiver and Child dichotomous)	-.12	.35**	.51***	.52***	.60***	.72***	.64***	-	
9. Emergency Room/Hospitalizations (Medical Record dichotomous)	-.20†	.27*	.38**	.56***	.40***	.62***	.94***	.70***	-

†$p < .10$ *$p < .05$ **$p < .01$ ***$p < .001$ ET-B = Endothelin B Receptor

Table 5.11.
Supplemental Analysis for Sickle Cell Anemia and Beta-0-Thalassemia Subtypes: Biserial Correlations and Chi-Square Associations for Endothelin B Genotypes with Alternative Pain Measures

Variable	1.	2.	3.	4.	5.	6.	7.	8.	9.
1. ET-B (G/G vs. A/A)	-								
2. ET-B (A/G vs. A/A)	.36**	-							
3. Pain Rate (dichotomous)	.09	.31*	-						
4. Health Care Utilization (Caregiver and Child dichotomous)	-.09	.20	.83***	-					
5. Health Care Utilization (Medical Record dichotomous)	-.15	.18	.31*	.49***	-				
6. Emergency Room/Hospitalizations (Caregiver and Child)	-.02	.10	.27†	.32*	.40**	-			
7. Emergency Room/Hospitalizations (Medical Record)	-.11	.00	.28*	.35*	.50***	.80***	-		
8. Emergency Room/Hospitalizations (Caregiver and Child dichotomous)	-.12	.27†	.57***	.68***	.68***	.47***	.50***	-	
9. Emergency Room/Hospitalizations (Medical Record dichotomous)	-.15	.18	.31*	.49***	1.00***	.40**	.50***	.68***	

†$p < .10$ *$p < .05$ **$p < .01$ ***$p < .001$ ET-B = Endothelin B Receptor

CHAPTER 6

DISCUSSION

The current project focused on two studies that had a common goal of explaining pain heterogeneity in pediatric SCD. The first study sought to examine an integrated, biopsychosocial model of pain while the second study sought to examine a novel genetic marker of pain in pediatric SCD. The sections below will discuss each of these aspects of the project separately, including limitations that are specific to each aim. A separate section is provided afterwards that discusses general limitations that cut across the studies. The document concludes with a discussion of clinical implications and future research directions.

6.1 Study One: Examine an Integrated, Biopsychosocial Model of Pain in Pediatric SCD

Study One examined a biopsychosocial model of pain through three aims. The first two aims focused on understanding the contribution of child biological and psychological predictors to multiple pain features, with a focus on understanding the relative effect sizes of these predictors (Aim I) and determining whether these factors worked in an additive versus an interactive manner (Aim II). The third aim sought to understand the role of caregiver and family factors in relation to child psychological factors and multiple pain features. Consistent with the biopsychosocial model, the findings suggest that a combination of biological, psychological, and social factors are necessary for understanding pain variability in SCD.

Aim I: Compare the effect sizes of biological and psychological factors in relation to multiple pain outcomes in pediatric SCD. Aim I focused on examining the relative effect sizes of biological and child psychological factors in explaining variability in multiple pain features. The results suggested that both biological and psychological variables are important for understanding pain variability; however, the relative effect sizes varied according to specific pain features. The most consistent finding suggested that biological factors played a stronger role in explaining average pain intensity versus child psychological factors. In contrast, both biological and child psychological factors were found to be important for explaining pain rate and health care utilization. These results and limitations specific to Aim I are described below.

For pain intensity, biological factors were found to explain more variance than child psychological factors in the models for both the full sample and the subsample of patients with HbSS and HbSβ^0. Specifically, the range for R^2 was .06 to .07 for biological variables versus .01 to .02 for child psychological variables. This finding was in direct contrast to hypothesis 2, which predicted that child psychological factors would have a moderate effect size (R^2 = .12) in relation to pain intensity and which made no prediction for biological factors. This hypothesis was based on previous daily diary studies that have found significant relationships between positive and negative mood and pain intensity in children and adults with SCD (Gil, et al., 2003; Gil, et al., 2004). Two prior cross-sectional studies were unable to find a significant relationship between coping strategies and pain intensity, suggesting that methodological differences may have accounted for the discrepancy in findings (Anie, Steptoe, Ball, Dick, & Smalling, 2002; Gil, et al., 1991). Specifically, child psychological factors may play a greater role in

immediate reporting of pain intensity that is captured in daily diaries versus average retrospective ratings over a period of time. It is notable that no previous studies have examined biological risk factors in relation to this particular pain outcome (as shown in Table 1.2). The current findings suggest that pain intensity, when averaged over time, may be an important clinical outcome for biomedical studies.

In contrast, for pain rate and health care utilization, both biological and child psychological factors were found to be important, with some models suggesting that child psychological factors accounted for greater variance than biological factors. For pain rate, the range for R^2 was .11 to .16 for psychological variables versus .02 to .04 for biological variables (sickle cell risk group and hematocrit and fetal hemoglobin, respectively). If this estimate included the biological covariate (i.e., alpha thalassemia trait) from the subsample of children with HbSS and HbSβ^0, the larger end of the range increases to .09 for biological variables, which is somewhat more comparable to psychological factors. Positive mood was also found to be a significant individual predictor of pain rate and there was a trend for negative thinking in the full sample. Of note is that prior studies have not found significant associations between child psychological factors and pain rate, though these studies predominantly focused on coping strategies rather than mood (Anie, et al., 2002; Gil, et al., 1991). The present study suggests that both biological and child psychological factors may be relevant for this particular pain outcome.

For health care utilization, both caregiver and child report and medical record variables were considered. The results suggested a fairly large discrepancy in effect size between child psychological and biological factors, with R^2 ranging from .10 to .23 for psychological factors versus .01 to .05 for biological factors. Considering additional

biological variables from models in the subsample (i.e., alpha thalassemia, platelet count) raises the effect size range to .09 to .12 for biological variables, which is somewhat more comparable to psychological variables. These results consistently supported hypothesis 2, which anticipated a moderate effect size for psychological factors (R^2 = .08), and partially supported hypothesis 1, which anticipated a small to moderate effect size for biological factors in predicting health care utilization (R^2 = .05). These findings are in line with previous research suggesting that both biological and child psychological factors play a role in health care utilization for pain in SCD (Anie, et al., 2002; Gil, et al., 1991).

Although no primary hypotheses were made for pain duration, this outcome was evaluated as a relevant aspect of the pain experience in pediatric SCD. The most notable finding for pain duration was for the subsample of children with HbSS and HbSβ^0, for whom biological factors approached significance as a contributor of variance (R^2 = .10). Fetal hemoglobin also emerged as a significant predictor in this model. For psychological variables, the R^2 ranged from .04 to .09; however, there were no notable individual predictors. These findings are consistent with previous work that has failed to find significant associations between coping strategies and pain duration in pediatric SCD (Anie, et al., 2002; Gil, et al., 1991). The present study replicated this finding for coping strategies and also failed to find an association between mood and pain duration. It is also notable that previous biomedical studies have not focused on pain duration (see Table 1.2). Similar to pain intensity, this variable may be an important clinical outcome in biomedical studies for children with HbSS and HbSβ^0. Cumulatively, these findings suggest that biological factors play a stronger role versus child psychological factors in predicting pain duration for children with SCD.

In terms of limitations, there were two caveats specific to Aim I that should be noted. First, there were more psychological than biological predictors in the models; thus, there may have been greater opportunity to accumulate explained variance for psychological variables in the models for Aim I. The models for Aim II (described in greater detail below), which restricted the number of psychological variables, were compared to Aim I to assess this hypothesis. This comparison suggested that the effect size for psychological variables may have been inflated for the full sample model for pain duration, which had an R^2 estimated at .04 for psychological variables in Aim II (versus R^2 = .09 in the model for Aim I). Similarly, the effect size for psychological variables may have been inflated for the model of caregiver- and child-reported health care utilization with the subsample of children with HbSS and HbSβ^0, which had an R^2 estimated at .11 for psychological variables in Aim II (versus R^2 = .15 in the model for Aim I).

Another limitation to these findings was that several children (n = 40) were taking hydroxyurea at the time of the study, which may have attenuated the effects of biological variables. Based on the supplemental analysis that was conducted with pre-hydroxyurea ratings, the effect size for medical record health care utilization was likely attenuated (pre-hydroxyurea R^2 estimate = .03 to .07 versus .01 to .05), with the pre-hydroxyurea results suggesting more consistent support for a small to moderate effect size for biological factors (i.e., sickle cell risk group and hematocrit and fetal hemoglobin), consistent with hypothesis 1. The effect size for pain intensity may also have been attenuated for biological variables (pre-hydroxyurea R^2 estimate = .11 to .14 versus .06 to .07), though this result does not affect the interpretation above. Finally, the estimates for

pain rate and duration were not substantially altered and do not affect the interpretation of results.

In conclusion, the results from Aim I suggest that both biological and child psychological factors are important for understanding pain variability in pediatric SCD. In addition to replicating previous findings for pain duration and health care utilization, the work from Aim I also provided additional information regarding the role of biological risk factors in relation to average pain intensity and duration and the role of psychological factors in relation to pain rate. Aim II, described in the next section, was designed to complement these findings by determining whether biological and psychological factors should be viewed in an additive or interactive manner, a question that has not been addressed in prior studies.

Aim II: Explore interrelationships among biological and psychological factors. The focus of Aim II was to determine whether biological and child psychological factors worked in an additive or interactive manner in relation to multiple pain features. For interaction effects, a particular focus was placed on identifying synergistic or buffering effects for child psychological factors and biological risk. Due to the small sample size and concerns about power, both statistical significance and effect sizes were used to determine the likelihood of an interactive effect being present in the sample. Given these limitations, the results should be viewed as preliminary findings that can inform future work. The most consistent finding was an additive effect for pain rate. The models also demonstrated an interaction effect for health care utilization; however, the results were inconsistent across models and difficult to interpret. These results and limitations specific to Aim II are described below.

For pain rate, the results for the full sample and subsample of children with HbSS and HbSβ^0 consistently demonstrated an additive effect, with the interaction effects accounting for minimal variance (less than 1%). This finding suggests that biological and child psychological factors may play independent roles in explaining variability in pain rate. This result also suggests that psychological factors may not moderate biological risk; thus, psychosocial interventions may be less helpful for altering pain rate if children are not presenting with risk factors, such as high levels of negative thinking or low levels of positive mood. This finding is consistent with a view of pain that considers biological and psychological factors as independent aspects of the pain experience (Edwards, et al., 2005).

It is notable that pain rate demonstrated an additive effect whereas health care utilization demonstrated an interactive effect (see below), as these outcome variables demonstrated moderate to large correlations with one another (r = .27 to .53, Tables 4.1 and 4.4). Although related, these variables have important distinctions that should be noted. Specifically, pain rate is meant to capture episodes managed both at home and via health care contact; thus, this variable likely captured both mild and severe episodes of pain. In contrast, health care utilization was more likely to capture only severe episodes that required medical attention. As noted in the introduction, health care utilization is also a complex construct that involves both pain and additional factors, such as decision making (Reese & Smith, 1997). These differences may partially account for the discrepancy in findings.

For health care utilization, all of the models demonstrated potential interactive effects, as indicated by effect sizes greater than 1% for the interaction terms (R^2 range of

.03 to .10). Also, the subsample of children with HbSS and HbSβ^0 demonstrated larger effect sizes for the interaction terms for health care utilization versus the full sample. The direction of the interaction effects was inconsistent for the health care utilization variables in the full sample. In addition, although the results were more consistent for the subsample, the findings were difficult to interpret due to characteristics of the sample, including the high use of hydroxyurea and its effects on hematocrit and fetal hemoglobin. The following paragraphs describe these issues and possible interpretations.

For the full sample, the results were conflicting, with the model for caregiver- and child-reported health care utilization suggesting an interaction in which child psychological factors had a stronger relationship with health care utilization for children with low- versus high-risk subtypes and the model for medical record health care utilization suggesting the opposite. The reason for this discrepancy is unclear as these models were essentially identical in terms of variables. These outcome variables were also highly correlated, suggesting that they are largely capturing the same construct.

An evaluation of the components of the health care utilization variables (see Table 3.4) did suggest a discrepancy in the number of doctor visits and calls that comprise these variables, with the caregiver- and child-reported variable comprising more of these types of visits compared to the medical record variable. Thus, although related, the caregiver- and child-reported variable may have represented broader utilization whereas the medical record variable may have been more representative of utilization required for severe episodes. It is possible that psychological factors played a larger role in influencing broader forms of utilization for the low- versus high-risk subtypes of SCD (as shown by the caregiver and child report variable) whereas these factors played a larger role in

influencing utilization requiring hospitalization or emergency department visits in the high- versus low-risk subtypes (as shown by the medical record variable). Although possible, these explanations must also be tempered by the possibility of Type I error.

For the subsample, the models suggested that psychological factors played a stronger role in children who had lower hematocrit (both models) and fetal hemoglobin levels (caregiver and child report). The original hypotheses regarding biological risk factors assumed that higher hematocrit and lower fetal hemoglobin would be indicative of higher biological risk, a finding that is based on the largest epidemiological study of children and adults with SCD (Platt, et al., 1991). However, characteristics of the sample, including the high rate of hydroxyurea treatment and the focus on a pediatric population, make these findings somewhat difficult to interpret. In order to evaluate these findings, the following approach was undertaken: (a) examine the main effects of hematocrit and fetal hemoglobin, (b) determine how hydroxyurea may have impacted the findings, and (c) present information from the literature regarding possible alternative explanations.

First, the main effects of hematocrit and fetal hemoglobin were examined. Hematocrit demonstrated a positive association with health care utilization for both models, suggesting that higher hematocrit was associated with higher health care utilization overall. Fetal hemoglobin demonstrated less consistent relationships, with the caregiver- and child-reported model suggesting a negative association and the medical record model suggesting a positive association with health care utilization. These results are inconsistent for fetal hemoglobin, but suggest that higher hematocrit may have represented higher biological risk in the sample.

The effects of hydroxyurea were also examined using information on hydroxyurea status and therapeutic response (see Table 6.1). The majority of children with HbSS and HbSβ^0 were taking hydroxyurea at the time of the study (77% of the subsample), which is known to increase both hematocrit and fetal hemoglobin (Rosse, Narla, Petz, & Steinberg, 2000). Several of these children had also established a partial or good therapeutic response (n = 28, 54% of the subsample), meaning that they had demonstrated a positive lab response that included higher hematocrit and fetal hemoglobin levels. Notably, examination of mean differences in health care utilization based on hydroxyurea status suggested that those taking hydroxyurea had similar levels of hematocrit and higher levels of fetal hemoglobin, but had *higher* rates of health care utilization versus those not taking hydroxyurea. For those with a partial or good response, children had similar levels of hematocrit and higher levels of fetal hemoglobin, but either demonstrated similar (caregiver and child report variable) or *higher* rates of health care utilization (medical record variable) versus those with no response.

These findings are not surprising as children taking hydroxyurea are likely to have the highest rate of health care utilization of the sample; however, the results suggest that hydroxyurea may have increased hematocrit and fetal hemoglobin in these children without necessarily lowering their rate of health care utilization relative to other children in the sample. Thus, these findings support higher hematocrit and fetal hemoglobin as being indicative of higher biological risk in the present sample of children, as the result of these children being a higher risk group of patients in terms of pain, but also having artificially high levels of hematocrit and fetal hemoglobin due to hydroxyurea.

Finally, studies specific to pediatric populations were reexamined to determine alternative explanations for the findings. There has been inconsistency in the literature regarding the role of hematocrit in pediatric populations, with some studies supporting the original epidemiological investigation by Platt and colleagues (1991) and others suggesting that *lower* hematocrit is indicative of higher biological risk. A daily diary study with a small sample of children with SCD by Dampier and colleagues (2004) was able to replicate the findings of the original epidemiological study, with higher hematocrit associated with higher pain rate. In contrast, in a large epidemiological study specific to children with SCD, lower hemoglobin, of which hematocrit is a derivative, was associated with greater disease severity as measured by a combination of adverse events, including pain rate (Miller, et al., 2000). Hargrave and colleagues (2003) also found a similar relationship between lower hematocrit and higher rates of health care utilization for pain in smaller sample of children with SCD. This literature supports the possibility of lower hematocrit being associated with higher biological risk.

Collectively, these findings do not provide a clear interpretation of the findings, particularly for fetal hemoglobin. However, based on the evaluation of main effects in the final models and the results evaluating group differences in hematocrit by hydroxyurea treatment status, there is more support for higher hematocrit being indicative of higher biological risk for this specific sample of children. Thus, in these models, child psychological factors may have had a stronger relationship with health care utilization for those with lower rather than higher biological risk in the subsample.

Considering these findings together, it is notable that three of the four models suggested that child psychological factors had a stronger relationship with health care

utilization for children with lower rather than higher biological risk. There are multiple interpretations for this finding. First, the results may suggest that psychological factors are more likely to alter pain in children with low to moderate risk, including children with low risk subtypes and those with milder disease risk in the high risk subtypes; however, there may be a population of children with high biological risk who require health care utilization for pain and whose pain is less affected by psychological factors. Although this finding was not in the hypothesized direction, it is still consistent with modern theoretical models of pain, which suggest that pain is the result of interplay between biological and psychological risk factors (Edwards, Campbell, Jamison, & Wiech, 2009; Keefe & France, 1999; Melzack, 1999). Alternatively, this finding may suggest that psychological factors play a greater role in decision-making for health care use in children with low to moderate biological risk versus those with higher biological risk. The latter interpretation would be supported by theoretical models specific to health care utilization, which suggest that the decision to use health care in SCD is the product of multiple factors, including disease status and pain-related distress (Reese & Smith, 1997).

In terms of additional pain outcomes, interaction effects were not examined for pain intensity due to small effect sizes for psychological variables. For pain duration, results for the full sample suggested an interaction effect in which psychological factors had a stronger effect for children with low- versus high-risk subtypes. This finding was not replicated for the subsample of children with HbSS and HbSβ^0, suggesting that psychological factors differentiated pain duration between high- and low-risk subtypes, but did not differentiate pain duration within the high risk group. This finding is

consistent with the above findings and suggests that child psychological factors may alter the effects of lower, but not higher biological risk for pain duration.

As with Aim I, a primary limitation of Aim II was the high use of hydroxyurea in the subsample, which interfered with establishing a firm interpretation of how the biological risk factors were impacting health care utilization in the subsample of children with HbSS and HbSβ^0. In addition, due to the small sample size and limited power, it was difficult to establish which interaction terms were most important to investigate in the models. Specifically, only one model (medical record health care utilization for the subsample) demonstrated a clear interaction term (hematocrit and positive mood) that was driving the result.

The unstandardized beta coefficients were provided in the results to better describe the potential interaction effects. The results for positive mood, in particular, suggested that interactions accounting for the lowest effect sizes may have been less clinically meaningful. For example, for the full sample model with caregiver- and child-reported health care utilization, the difference in slope for positive mood between children with high- versus low-risk subtypes was about half of one health care visit. In contrast, for the subsample model with medical record health care utilization, which had the largest effect size for interaction terms, the difference in slope for positive mood between children with higher versus lower hematocrit was close to three health care visits. Thus, while a larger sample would be beneficial for replicating these results and establishing statistical significance, the clinical meaningfulness of the effects is also important. Future studies may consider powering results to detect a small to moderate effect size.

In conclusion, the results from Aim II provided support for both additive and interaction relationships between biological and child psychological risk factors depending on specific pain features. Pain rate demonstrated a consistent additive model whereas health care utilization demonstrated a consistent pattern of interactive models. Pain duration also demonstrated a potential interactive model with the full sample. Across interactive models, there was more support for child psychological factors playing a stronger role in children with lower as opposed to higher biological risk.

Aim III: Examine relationships between child psychological variables, caregiver psychological variables, family functioning, and pain outcomes. The focus of Aim III was to examine the impact of caregiver and family variables on child psychological variables and multiple pain features. A particular emphasis was placed on establishing possible mediators that could inform future family-based intervention efforts. The results supported the potential of mediation analysis to uncover important family processes that impact pain outcomes in children with SCD. The results also provided additional information that may have utility for family-based work, even in the absence of mediation analysis. These results and limitations specific to Aim III are described below.

In terms of possible mediation, the findings suggested that child negative thinking could be a mediator of the relationship between caregiver passive coping and negative mood with health care utilization. In other words, caregiver passive coping and negative mood may result in higher negative thinking in the child that ultimately leads to greater health care utilization. Unfortunately, the results suggested that a large sample would be needed to conduct a powered mediation analysis to detect this relationship, with

an estimated 148 to 196 children and caregivers needed (Fritz & Mackinnon, 2007). Although mediation analysis would be ideal for informing family-based intervention work, the remaining findings from the study along with previous literature provide strong evidence for the importance of including caregivers in pain management interventions for children.

First, the correlation analysis replicated the finding that child coping tends to mirror caregiver coping. This was particularly apparent for trends that supported relationships between child negative thinking and caregiver passive coping as well as child and caregiver positive mood. This finding is consistent with several previous studies suggesting the importance of caregivers in modeling and teaching coping strategies for pain (Brown, et al., 1993; Gil, et al., 1991; Kliewer & Lewis, 1995; Lutz, et al., 2004). Second, findings from the present study suggest that caregiver psychological factors may have both indirect (i.e., mediated) and direct effects on pain outcomes. Specifically, the findings from the supplemental analysis suggested that higher caregiver negative mood may be directly associated with pain duration and that higher caregiver active and passive coping may be directly associated with health care utilization. Health care utilization, in particular, is a construct that is likely to be influenced by both the child's health status and caregiver decision-making in SCD (Reese & Smith, 1997). These findings suggest the importance of caregivers in both the development of coping strategies for pain and in the direct management of pain.

One unusual finding from the correlation analysis was that caregiver active and passive coping were positively associated. This finding suggests that caregivers may use a combination of both active and passive coping strategies, rather than relying on one or

the other. Of note is that higher caregiver active coping was related to *higher* health care utilization. Children similarly showed a positive association between coping attempts and negative thinking; however, a higher level of coping attempts was related to lower health care utilization. Of note is that caregiver active coping was weakly and negatively related to child coping attempts, suggesting that children were less likely to mirror this type of caregiver coping. One possible interpretation for these findings is that children may be less likely to engage in coping attempts if their caregiver is actively addressing their pain and vice versa; however, in order to experience a reduction in health care utilization, the child may need to be directly engaged in active coping attempts. This interpretation would be consistent with a previous study on family processes in SCD, which suggested that certain family processes may encourage dependence on the caregiver rather than independent coping in the child (Kliewer & Lewis, 1995).

Finally, family functioning was not related to any of the pain outcomes, though it was related to child negative thinking as well as caregiver passive coping, positive mood, and negative mood. This variable may not have established relationships with pain outcomes because it is a more distal variable compared to child or caregiver psychological factors. This variable also seemed to overlap with several of the caregiver variables and was completed by caregivers, suggesting that it may simply represent another manifestation of caregiver psychological status in the present study. One limitation of Aim III was the absence of child report of family functioning. Due to the concerns about the time required to complete the study measures, children were not asked to complete a parallel version of the family functioning scale. The scale used was also meant to capture broad aspects of family functioning rather than specific family

dynamics. Future studies may consider adding the child's perspective on family functioning or using the full version of the scale to obtain a more nuanced picture of family functioning.

In conclusion, the results from the study provided preliminary support for the use of mediation analysis in future studies; however, a large sample size would be needed to conduct an appropriately powered analysis. Although this work would be informative, the results from the study replicate previous research findings and suggest the importance of caregiver factors in the development of coping strategies for pain. In addition, the results suggest that specific caregiver psychological factors, such as negative mood and passive coping, may directly impact pain outcomes in SCD. Thus, even in the absence of additional mediation analyses, it is clear that caregivers contribute to the variability observed in pain outcomes for children with SCD.

6.2 Study Two: Examine a Novel Genetic Marker of Pain in Pediatric SCD

The focus of Study Two was to establish a novel genetic marker of pain in pediatric SCD by specifically focusing on the ET-B SNP (rs5351). Multiple analyses were completed in an attempt to replicate a preliminary study suggesting that the G allele of this SNP was related to greater pain severity versus the A allele in children with SCD; however, none of the models replicated this relationship. A summary of the findings as well as limitations and alternative explanations for the results are described below.

The primary model examined was for health care utilization, with exploratory analyses completed for additional pain outcomes. The models for medical record health care utilization and pain duration in the full sample were able to demonstrate that the AG genotype group had higher utilization for pain than the AA genotype group; however, the

models were unable to demonstrate a similar relationship between the GG and AA group, which is necessary for replicating the codominant model described in the preliminary results. The latter relationship, in particular, should have demonstrated the greatest difference in health care utilization, as children with the GG genotype would be assumed to have the greatest risk for pain. In contrast, some models suggested that the AA genotype group had *higher* health care utilization than the GG genotype group. The model for pain duration in the subsample was able to demonstrate these relationships in the expected directions; however, the difference between the GG and AA genotypes in the regression models was smaller than that of the AG and AA genotypes, which again runs counter to the preliminary data.

Additional analyses were completed with pre-hydroxyurea pain outcomes, in order to determine whether hydroxyurea may have attenuated the relationship between the ET-B genotypes and pain outcomes. These models were generally similar to the original results, with several models demonstrating a difference in pain severity between the AG and AA genotypes; however, the difference between the GG and AA genotypes was either weaker or not in the expected direction. In addition, alternative pain outcomes were used to determine whether the genotypes distinguished between categories of children (i.e., children with or without any health care utilization for pain) or to determine whether the genotypes distinguished between children with and without more severe pain requiring hospitalization or emergency room visits. These analyses resulted in similar findings to the primary models.

The means between groups were also evaluated to help interpret the analyses. One unusual finding that appeared was an underdominant effect, in which the heterozygote

AG group demonstrated greater pain severity versus both the AA and GG genotypes. For example, this relationship can be seen in the models for medical record health care utilization and pain duration in both the full sample and subsample of children. This effect is unusual and has not been demonstrated in previous studies with the ET-B SNP (Dong, et al., 2004; Iemitsu, et al., 2006); thus, it seems unlikely to be meaningful in the present study.

There are a few alternative explanations for these findings that should be noted. The first possibility is that there is an effect with the ET-B SNP that could not be detected in the sample, either due to the relatively small sample size or due to other factors related to the sample (i.e., the use of hydroxyurea). The hypothesized pattern of mean differences can be observed in the subsample for the caregiver- and child-reported health care utilization variable and for the pre-hydroxyurea pain rate variable; however, this pattern is absent from all other pain outcomes, despite the large number of models examined. Thus, it seems unlikely that this relationship would be consistently detected in a larger sample. The use of hydroxyurea may have also altered the natural course of pain in the sample and may have attenuated pain in children who would have otherwise demonstrated the highest pain ratings. On the other hand, the results from Aim II in Study One suggested that children taking hydroxyurea continued to demonstrate higher rates of health care utilization than other children. In addition, the pre-hydroxyurea analyses were not significantly different from the primary models.

Another explanation relates to the finding of an underdominant effect for some of the pain outcomes. An underdominant effect is an unusual finding that may be indicative of issues with admixture in the sample. Admixture can occur when different populations

interbreed, resulting in different haplotypes or combinations of genes that are inherited (Deng, 2001). Admixture has been discussed as a potential issue for genetic studies in SCD. Specifically, the sickle cell beta globin mutation that results SCD developed independently in different ethnic groups in Africa and other parts of the world. Although these ethnic groups all carry the same mutation that causes SCD, they may inherit other genes that alter disease course. For example, a haplotype from Senegal has been associated with fewer pain episodes and hospitalizations than other groups. In the United States, it is widely recognized that African-American patients with SCD likely represent a combination of ethnic groups, resulting in admixture (Steinberg, 2005). Although many genetic studies assume that ethnic groups will be randomly distributed into different genotype groups for the SNP under consideration, this issue remains a possibility (Deng, 2001).

A third explanation is that the relationship in the preliminary data was the result of SCD genotype effects rather than the ET-B SNP. In the preliminary data, it is notable that the GG genotype group was only comprised of children with HbSS, a high-risk subtype, whereas the other groups were comprised of a combination of SCD genotypes. Thus, it is possible that the preliminary data was demonstrating a SCD subtype effect rather than an effect of the ET-B SNP. Finally, the association in the preliminary data may have been found due to chance and was simply not replicated in a larger sample. Replication has been a widely discussed issue for genetic association studies (Cardon & Bell, 2001).

In conclusion, the present study was unable to replicate preliminary data suggesting that the G allele of the ET-B SNP is associated with greater pain severity

versus the A allele. Although several explanations were provided to explain the results, the most parsimonious and likely explanations are that the relationship in the preliminary date was the result of SCD genotype differences or was due to chance, though admixture could have been a possible confounding factor in the study.

Table 6.1.
Supplemental Analysis: Mean Differences in Hematocrit, Fetal Hemoglobin, and Health Care Utilization based on Hydroxyurea Status and Therapeutic Response

Treatment Status	**Hematocrit**	**Fetal Hemoglobin**	**Health Care Utilization (Caregiver and Child)**	**Health Care Utilization (Medical Record)**
Hydroxyurea Status	*M (SD)*	*M (SD)*	*M (SD)*	*M (SD)*
Taking hydroxyurea	25.11 (3.89)	14.30 (9.90)	8.55 (10.51)	4.73 (4.38)
Not taking hydroxyurea	25.94 (2.93)	9.73 (8.44)	2.25 (1.62)	1.67 (1.37)
Therapeutic Response				
Good or partial response	25.71 (3.39)	17.83 (9.29)	8.44 (11.93)	5.11 (4.94)
No response	23.85 (4.65)	7.24 (7.03)	8.77 (7.11)	3.93 (2.90)

CHAPTER 7

GENERAL LIMITATIONS

The results noted above should be considered in the context of general limitations that cut across multiple aims of the study. The following issues are described below: (a) the use of a cross-sectional approach and causality, (b) pain measurement, 3) sample size and power, and (c) generalizability.

The use of a cross-sectional approach is a key limitation of the study that limits inferences that can be made regarding causality. This limitation is particularly important when considering the results from Study One. Specifically, previous authors have noted the possibility that child and caregiver psychological factors are a consequence rather than a predictor of severe pain (Gil, et al., 2003; Gil, et al., 2004). There has been minimal research examining temporal precedence in SCD; however, one prior study by Gil and colleagues (2003) suggested that pain intensity, in particular, may be a precipitant rather than a consequence of mood changes in adolescents with SCD. Similar research has yet to be conducted with other pain features; thus, it is unclear whether these findings would apply to pain rate or health care utilization, which demonstrated the most consistent effects with child and caregiver psychological factors.

In the present study, one method for identifying temporal precedence would be to examine whether biological and psychological risk factors are associated. Sickle cell genotype, in particular, can be assumed to temporally precede the development of coping strategies or mood changes in pediatric SCD. As shown in demonstrated a moderate

association with SCD genotype, such that children with high-risk subtypes reported higher levels of negative thinking versus those with low-risk subtypes. Thus, this particular child psychological factor may have represented a consequence rather than a predictor of pain severity. Of note is that the most consistent child psychological predictor of pain in the study, positive mood, did not demonstrate a significant relationship with SCD genotype. Although it is not possible to confirm causality between pain and psychosocial factors in the present study, modern conceptualizations of pain suggest that these factors are likely to have bidirectional relationships with one another (Keefe & France, 1999). Future work in this area, particularly with the use of daily diaries, may provide more specific information about temporal precedence and causality.

A second limitation of the study is the manner in which pain was measured. In the present study, child and caregiver reports and medical records were used to measure pain features. For child and caregiver reports, one particularly important limitation is recall bias (Shiffman, Stone, & Hufford, 2008). Children and their caregivers generally demonstrated only moderate associations in their reports of pain. For this reason, child and caregiver ratings were averaged to avoid over- or underestimation of pain. Despite this approach, it is likely that the retrospective reports contained measurement error due to inaccuracies in recalling health information over the previous year. This issue likely posed an even greater issue for the pre-hydroxyurea analysis, which required varying amounts of time for caregiver recall in order to establish pre-hydroxyurea pain ratings.

In addition, previous research has suggested that the psychological state of an individual can bias retrospective reporting of events, including for children who are asked to recall pain (Van Den Brink, Bandell-Hoekstra, & Huijer Abu-Saad, 2001) Thus, it is

possible that child and caregiver psychological factors were associated with pain outcomes because of reporting bias rather than their direct influence on pain. It is notable that the largest effect size for child psychological factors was observed for health care utilization as measured by the medical record. While this measure is partially based on caregiver reports in the medical chart, it is also based on documented health care visits, which would not be influenced by reporting bias. It is also notable that psychological factors were not consistently related to all pain outcomes. Thus, if reporting bias did exist based on psychological status, the bias did not affect pain outcomes equally.

The use of medical record reviews also has specific limitations that should be noted, including potential issues with missing information and difficulty interpreting information in the chart (Gearing, Mian, Barber, & Ickowicz, 2006). The medical chart measure of health care utilization was strongly related to the child and caregiver ratings; however, the medical record variable resulted in similar or *lower* ratings of health care utilization versus child and caregiver reports, despite representing twice as much time. This finding suggests that the medical record may have been missing information or that children and caregivers were overestimating their utilization.

An alternative approach to measuring pain is the use of prospective daily diaries, which are considered the gold standard for measuring pain. Although the use of pain diaries has clear advantages over retrospective recall, it is unclear how much time would have been needed in order to obtain a representative assessment of pain in the present study. For example, many children with SCD go for weeks or months without pain and then may have periods of more frequent pain. In addition, given the sample size desired

and resources available for the study, the collection of daily diaries over a long period of time would not have been feasible.

Another key limitation to the study was the relatively small sample size and issues related to power. Post hoc power analysis suggested that a sample of 76 participants was powered to detect an effect size equivalent to an R^2 of .10. The sample of 52 children with HbSS and HbSβ^0 was powered to detect an R^2 of .14. Issues related to power were most problematic for detecting statistical significance for the biological predictors and the interaction terms. These issues were anticipated in the study and the use of effect size estimates and unstandardized beta coefficients was used to determine the meaningfulness of the results. For the interaction results, this analysis suggested that models with small effect sizes may have been less likely to be clinically meaningful versus models with moderate effect sizes. Thus, future studies may consider powering studies to detect an effect size between small and moderate, which is more likely to be clinically meaningful. Power may also have been an issue for the genetic analysis; however, even with a larger sample, the direction of findings would not have been in the expected direction.

A final consideration is the generalizability of the study. The study sample had very good representations of SCD genotypes, with the frequencies closely approximating those found in the overall population of patients with SCD (i.e., approximately 63% HbSS, 25% HbSC, and 11% for both HbSβ^+ and HbSβ^0); however, other characteristics of the sample may be less likely to generalize to other children. First, all of the children in the study must have been receiving care through a comprehensive sickle cell center. Thus, children with milder disease presentations who chose not to receive comprehensive sickle cell care or those who were unable to attend routine hematological visits are less

likely to be represented in the study. Second, over half of the children in the study were taking hydroxyurea at the time of the study and these children varied in their treatment status in terms of whether they had established a therapeutic response. Although the use of hydroxyurea has increased in recent years, the prevalence of this treatment in the overall population of children with SCD is unclear. It is likely that children taking hydroxyurea were overrepresented in the present study due to our recruitment approach. Specifically, children taking hydroxyurea typically attend routine clinic visits every two months, as opposed to every 6-12 months for routine health maintenance visits; thus, children taking hydroxyurea had more frequent clinic visits that made recruitment more likely. As noted previously, the use of hydroxyurea primarily affected the effect size and interpretation of hematocrit and fetal hemoglobin. The interaction results, in particular, are likely to be specific to the present sample.

In conclusion, the results of this study should be considered with the above limitations in mind. In particular, alternative explanations related to causality and reporting bias should be considered when evaluating the findings. In addition, the extent to which the findings are relevant for other pediatric SCD populations should be considered, both in terms of clinical meaningfulness and generalizability. The final sections below discuss clinical implications and future research directions, considering the interpretations from the discussion and limitations sections.

CHAPTER 8

CLINICAL IMPLICATIONS

The findings from the present study provide information that may have utility for future intervention planning. In particular, the findings have implications for structuring interventions, incorporating caregivers, and using biomarkers in prevention efforts. These areas are discussed below.

As noted in the introduction, the biopsychosocial model has the potential to inform integrated biomedical and behavioral treatments for pain. The present study provides information that may be helpful in determining who is most likely to benefit from these interventions and the changes in pain outcomes that can be expected. Specifically, the results from Study One suggest that a combined medical and behavioral approach to treatment may be most beneficial in altering pain rate and health care utilization. For pain rate, the preliminary results consistently found an additive model, suggesting that an integrated approach is most likely to alter pain for children presenting with psychological risk factors for this particular outcome. For health care utilization, the results found an interactive model and suggested that children with low to moderate disease risk may be most likely to experience a reduction in health care utilization using an integrated approach. In contrast, children with very high disease risk may not experience changes in health care utilization with the addition of a behavioral component.

It is important to note that these findings are specific to pain rather than functional

outcomes. Many children, particularly those at high disease risk, may still benefit from a combined medical and behavioral approach to pain that targets *functional consequences*, including depression, anxiety, and poor quality of life (Barakat, et al., 2007; Burlew, et al., 2000; Chen, et al., 2004). The results from the present study were designed to complement this work by also highlighting the changes that may occur using an integrated approach to address both pain and functional outcomes.

The present study also has implications for incorporating caregivers into treatment. The present findings, along with previous work, suggest that caregivers may play both indirect and direct roles in modifying their children's pain (Logan, et al., 2002; Lutz, et al., 2004; Mitchell, et al., 2007). Unfortunately, existing work has not demonstrated clear efficacy for incorporating family members into treatment (Anie & Green, 2012; Chen, et al., 2004); however, it is clear that caregivers are important to consider in intervention planning. In addition to demonstrating efficacy, future intervention work may need to evaluate the effects of incorporating caregivers into a largely child-focused intervention (an indirect approach), which is what the majority of previous intervention work has done, versus targeting the behaviors and parenting practices of caregivers themselves (a direct approach). Also, future intervention work may consider a more preventative approach to pain that provides psychoeducation and training in behavioral strategies to caregivers for children early in life.

Finally, both Study One and Two incorporated biomarkers in an attempt to predict pain outcomes, with Study One focused on laboratory markers and Study Two focused on a novel genetic marker. The use of biomarkers remains the only method for trying to predict future disease risk early in life in SCD; however, both studies highlighted

important limitations to this work, including small effect sizes for biomarkers and difficulty with replication. In addition, as seen in the present study, the use of hydroxyurea may limit the ability of existing laboratory markers to predict future pain. Although previous studies have excluded children receiving hydroxyurea, this approach may become less feasible over time as this treatment approach becomes more common. These limitations suggest that this work may not be immediately impactful in terms of prevention efforts and that it will take time to develop models that have clinical utility for predicting pain. At the same time, the identification of biomarkers continues to have utility for developing novel treatment approaches; thus, even in the absence of a prevention framework, this work continues to be an important aspect of developing new medical approaches for pain.

This section has highlighted some of the clinical implications that may arise from pursuing an integrated, interdisciplinary approach to pain in pediatric SCD. The following section describes future research directions that would be helpful in continuing to inform this work.

CHAPTER 9

FUTURE RESEARCH DIRECTIONS

The present study poses several important research directions for studying pain in pediatric SCD. This section highlights three broad issues that would particularly benefit from additional research: (a) understanding developmental aspects of SCD, (b) improving pain measures in SCD, and (c) refining the biopsychosocial model.

The current study emphasized work on children in order to inform a discussion of early prevention and intervention efforts for pain and to provide information that could specifically address issues pertinent to a pediatric population. One finding that emerged from this project is the importance of understanding how biopsychosocial constructs operate in children and how these factors change over the course of development. In terms of biomedical factors, it is notable that there is inconsistency regarding the role of hematocrit for pain in children with SCD, particularly since the original epidemiological findings for hematocrit occurred over 20 years ago (Platt, et al., 1991). Given this inconsistency, it would be helpful for researchers to reevaluate the role of hematocrit in existing data sets of children with SCD or for new research to be conducted evaluating the impact of hematocrit over the course of childhood in SCD. This work would be particularly useful in determining whether hematocrit is a meaningful marker to use for predicting pain severity in children with SCD.

In terms of psychosocial factors, one unique component of childhood is that children change over time from being predominantly reliant on caregivers to becoming

independent in managing their pain. Previous developmental research has been helpful in identifying important developmental periods for the stabilization of coping strategies in children with SCD (Gil, et al., 1993). It would be helpful to have similar research conducted with caregivers to better understand their role in the management of pain over time, including during infancy and early childhood. This work may help to inform caregiver- and family-based interventions for pain in pediatric SCD (Anie & Green, 2012; Chen, et al., 2004).

The present study also highlighted limitations to current measurement tools in pediatric SCD and the need for additional research in this area. In particular, future research would be helpful in identifying how different measurement approaches influence results for pain in SCD. For example, it would be helpful to know the concordance of daily diaries and retrospective ratings of pain in SCD and whether the concordance varies by child age. Similarly, it would be useful to know if a patient's psychological state is likely to impact their ratings. Finally, it would be beneficial to know how different definitions of pain in SCD influence results, such as the impact of including home-based episodes of pain versus focusing on just health care utilization. Although inferences can be made for some of these research questions, having more specific information on the strengths and weaknesses for these tools in SCD could help to inform the strategies that researchers use for measuring pain and how limitations of specific measures impact the findings.

Finally, previous researchers have noted the importance of refining the biopsychosocial model for pain conditions (Keefe & France, 1999). A particular strength of the current study was the examination of how biological and psychosocial factors work

together to impact pain in pediatric SCD, which had not been the focus of any prior studies. The current study also incorporated multiple psychosocial factors (i.e., child coping and mood) in the same model. Future work is needed both to replicate the findings from this study and to further clarify how biological and psychosocial factors work together in this condition. In particular, research on temporal precedence would be beneficial. In addition, it would be helpful to identify other psychosocial constructs that may be relevant for this condition based on literature in other pain populations. For example, mood has received widespread attention in the pain literature, but has been less widely studied in SCD, despite being one of the more consistent psychological predictors in the present project (Keefe, et al., 2005). Additional psychological constructs, such as self-efficacy, may benefit from additional attention in studies of pain in SCD (Turk & Okifuji, 2002).

To conclude, the present project integrated biomedical and psychosocial approaches to understand pain heterogeneity in pediatric SCD. The project contributed several new findings to the literature on pediatric SCD, but also highlighted the complexity of this condition. In particular, the project suggests that pain in pediatric SCD may be best viewed in terms of both a developmental and biopsychosocial perspective. In addition, pain in this condition must be viewed in the context of changes in medical care, such as the increasing use of hydroxyurea, which may fundamentally alter relationships between biological risk and pain outcomes. It is clear that future work on pain in pediatric SCD will require interdisciplinary approaches that are able to refine existing models and fit novel predictors in the context of a complicated and changing disease.

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