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Is Sickle Cell Trait as Benign as is Usually Assumed?

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by

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Abstract

Introduction Sickle cell trait carriers may experience sickling events, which can cause severe health problems. Some sickle cell haplotypes contain genetic modifiers that are associated with increased levels of fetal hemoglobin, which is resistant to sickling. The aim of this study is to determine if sickle cell trait individuals who do not carry these modifiers are more likely to experience sickling episodes than those who do carry the modifiers.

Methods Participants were eligible for inclusion in this study if they were male, 18 years of age or older, a sickle cell trait carrier, and had previously played any level of organized football. Participants were recruited via Facebook, www.clinicaltrials.gov, e-mail, phone calls, and word of mouth. They were asked to complete a survey and return a buccal swab for genetic analysis to look for alleles associated with fetal hemoglobin persistence. To date, no genetic analyses have been run. Data from the surveys was analyzed using Fisher's Exact Test with the SAS 9.2 software.

Results Twenty participants were included in this phase of the study and all returned both the survey and buccal swab. Five of the 20 participants had been diagnosed with exertional sickling, 2 with heat illness, and 12 had experienced dehydration.

Conclusion Data in this study is purely observational, as no genetic analyses have been performed at this point. Early results indicate that the probability a player feels their muscle pain lasts longer than their peers' is greater among those who feel it takes their muscles longer to recover than their peers'.

Introduction

Sickle cell trait (Hb AS) is a hemoglobinopathy resulting from the sickle cell mutation. Sickle cell trait individuals inherit 1 A allele and 1 S allele and are often referred to as Hb AS carriers; 8% - 9% of the U.S. population are Hb AS carriers. Those with sickle cell disease inherit 2 sickle cell genes. Sickle cell trait carriers usually live a life free of the clinical problems associated with sickle cell disease. However, recent news reports point out that a number of sickle cell carrier athletes have unexpectedly died due to sickle cell crises. These articles note that there have been 22 sickle cell trait related deaths in athletes in the United States between 1980 and 2009; specifically, 18 deaths occurred among football players, 3 among basketball players, and 1 in a track athlete (Elseveth, 2013; George 2011; O'Connor et al., 2012). All abbreviations used throughout this paper can be found in Appendix 1.

Sickle Cell Anemia (Hb SS or Hb SS) is a severe disorder caused by the inheritance of 2 sickle cell genes. These individuals are referred to as sickle cell disease sufferers or homozygotes. In the United States (U.S.) alone approximately 72,000 people suffer from Hb SS (Anonymous, 2005). Research has had limited success in creating treatments to enhance quality of life for those suffering from Hb SS, though palliative methods have been used during the last 100 years (Wailoo & Pemberton, 2006). Recent advancements in medical care have increased life expectancy for individuals with Hb SS, including the discovery of the effects of fetal hemoglobin, which is perhaps the most important modifier of the clinical presentation

of Hb SS. Treatments that induce production of fetal hemoglobin (Hb F) show potential for preventive care and improved quality of life, though mechanisms underlying this approach are complex and require further investigation.

Sickle Cell Anemia: Environmental change and natural selection

The evolution of high frequencies of hemoglobin S is an area of study that benefits from a biocultural approach. The biocultural synthesis has looked beyond genetic evolution to identify possible causes for the spread and selection of the sickle cell gene as well as the factors behind the selective pressure. In 1976, Frank B. Livingstone succinctly wrote, "The simulation of clinal variation for the hemoglobin… show the kinds of ethnological, archeological, and demographic data that need to be collected to advance our understanding of human genetic change."

In 1949, Haldane proposed that heterozygotes for certain hemoglobinopathies were protected against malaria. In particular, he mentions the protective qualities of alpha-thalassemia. Other early papers discuss lower infection rates in Hb AS individuals and "sicklers". Shortly thereafter, in the late 1950's, Livingstone argued that Hb S was found in higher frequencies in populations who had adopted agricultural practices as opposed to those living in forested areas and who were hunters and gatherers. He concluded that the spread of Hb S mirrored the spread of agriculture due to commonly practiced slash and burn methods to clear areas in order to create fields. These methods affected the environment in myriad ways. The loss of trees not only served to increase the amount of sunlight reaching the ground, but also cleared away the humus created by the trees. The humus caused the soil to absorb water from frequent rainfalls quickly and efficiently; the lack of the humus caused

the ground to dry and harden, which in turn created standing pools of water from rainfall. Standing pools of water and adequate sunlight are 2 of the requirements for the growth of *Anopheles gambiae*, the mosquito vector for *Plasmodium falciparum*. Thus, the adoption of agriculture led to an increase in the mosquito population and an increase in malarial infection. Furthermore, Livingstone notes that spread of agriculture caused a population boom and humans became the most available source of food for the *Anopheles gambiae*. The increase in mosquitoes as well as humans allowed malaria to spread until it ultimately reached hyperendemicity (Gong et al., 2013; Livingstone, 1958; Livingstone, 1971; Livingstone, 1976; Weisenberg, 1967).

Though the exact mechanism of protection is unclear, the sickle mutation has been found to be protective against malaria in trait form. Due to this protection, selective pressures likely caused an increased prevalence of the sickle mutation in areas with historically endemic malaria. Erythrocytes infected with malaria may sickle more readily, leading to increased phagocytosis of infected cells. The sickle mutation may also cause red blood cells to present substances on the cell surface that bind to foreign substances, again resulting in increased phagocytosis of the Hb S cells. Additionally, it has been proposed that conditions within the Hb S erythrocyte are inhospitable to malarial infection, which can lead to reduced infection rates (Gong et al., 2013; Livingstone, 1971). Sickle cell trait carriers (Hb AS) are at a greater advantage for malaria protection with a reduced likelihood of poor clinical outcomes due to sickling. Unfortunately, Hb SS individuals may not see this protection and usually suffer from poor health and shortened lifespans due to their condition. On

average, women with Hb SS live to be approximately 48 years old, while men live to be about 42 years old (Platt et al., 1994).

Normal adult hemoglobin consists of 2 alpha chains and 2 beta chains. The 2 alpha chains are identical to each other and the 2 beta chains are also identical to each other. The alpha and beta chains combine to carry a heme, which is a nonprotein molecule that contains iron, which allows it to carry and oxygen molecule. In most normal adult and Hb F the alpha chains are identical and the hemoglobin differs by the second chain (ie. the gamma chain in place of the beta chain in Hb F). The allele that codes for the alpha chain is found at a high frequency in many populations and thus genotype AA is considered "normal" adult hemoglobin (Hb AA).

The sickle cell mutation is the result of a base substitution on the hemoglobin beta chain. The shift occurs when a glutamine is replaced by a valine at the sixth position on gene, which is found in the eleventh chromosome (Powars & Hiti, 1993; Maier-Redelsperger, 1998). This mutation causes the red blood cell to become hydrophobic and under certain conditions the cell will polymerize and become rigid. They usually last from 3 to 7 days, but can continue for months (Yale et al. 2000). The tissue and organ complications caused by VOCs contribute to the shortened lifespan expected in those with Hb SS. Once the cell has collapsed it looks like a sickle, which is where the name sickle cell anemia originates. It is largely accepted that at least five beta globin mutations arose in separate events and which were chosen by natural selection in response to malaria. To date, 31 variations of the beta gene have been found worldwide. These mutations have been divided into five haplotypes based on the restriction fragment length polymorphisms (RFLP)

surrounding the beta gene and named for the location where they were first discovered. The haplotype groupings are: Senegal, Benin, Central African Republic (CAR) (also known as Bantu (Neonato et al., 2000)), Indian/Arab (also known as Saudi Arabia/India), and Cameroon (occasionally identified as Asia). The three most common haplotypes in the United States and South America are CAR, Senegal, and Benin (Adekile et al., 2007; Mielke et al.; 2011; Powars & Hiti, 1993; Sheehan et al., 2013).

Clinical Presentation

In 1910, Dr. James Herrick was the first to describe the medical condition that came to be known as Hb SS (Pemberton, 2006). In 1904, a young man from Grenada requested treatment from Dr. Herrick for his symptoms, which included a cough, fever, jaundice, dizziness, weakness, anemia, chest complications, and a history of leg ulcers. Dr. Herrick found that his blood contained a "large number of thin, elongated, sickle-shape and crescent-shaped forms" (Herrick, 1910). While American doctors were largely unaware of the condition that came to be known as Hb SS, it appears that many West Africans knew of its existence and could accurately describe the symptoms. Africanus Horton provided the first written evidence of Hb SS in Africa in 1874, 36 years before Herrick named the condition (Anionwu & Atkin, 2001).

Acute chest syndrome (ACS) is a significant clinical complication of Hb SS and is the leading cause of death among Hb SS patients. The etiology of ACS is unclear and presentation is complex. It involves pleural effusion, fever, cough, multiple lobe involvement, leukocytosis, pleuritic chest pain, crackling noises when listening to the lungs, and infiltrates visible in chest x-rays (Vichinsky et al., 2000; Paul et al., 2011;

Castro et al., 1994). Vichinsky et al. (2000) found that 72% of patients who were found to have ACS were originally admitted to the hospital for a vaso-occlusive crisis (VOC). It is thought that ACS episodes are precipitated by a VOC, asthma, infection, and fat embolism (Vichinsky et al., 2000; Buchanan et al, 2005; Paul et al., 2011). Infection and fat embolism are the 2 most common causes of ACS; 1 study found that 30% of ACS cases were precipitated by bacterial infection (Paul et al., 2011).

Infection plays a significant role in sickle cell crises, especially in children. Between 6 months and 3 years of age normal spleen function virtually disappears in Hb SS children due to repeated splenic infarctions that cause the organ to shrink and in some cases to completely cease functioning. Since the spleen can no longer effectively filter blood the Hb SS individual is more susceptible to developing infection and then sepsis, which can lead to a rapid death. Due to the potential for health problems including bone and tissue infarction and an increased risk for hospitalization, Hb SS people are more susceptible to infection: bone infarction can create areas of bone that harbor bacteria, tissue infarction in the bowel may allow bacteria to enter the blood stream, and upper respiratory infections can end in an episode of ACS. Interestingly, 2 studies found that salmonella, a relatively common food-borne pathogen, is the root cause for almost half of the cases of acute osteomyelitis in Hb SS individuals. Furthermore, those who have been taking antibiotics intravenously for extended periods of time are also more likely to develop infections in hospital settings, particularly at catheter sites (Booth et al., 2010; Stacy et al., 1997).

In addition to autosplenectomy, cellular reactions within the body cause increased cell adhesion as well as red blood cell dehydration and deoxygenation. Red blood cell dehydration coupled with deoxygenation increases polymerization within the erythrocyte thereby increasing sickling. Increased adhesion and sickling can cause tissue ischemia, possibly resulting in a VOC. This is potentially more serious for infection involving the upper respiratory system, which could also cause an ACS episode (Booth et al., 2010).

Treatment

The search for treatment for Hb SS met with limited success during the mid-twentieth century. Treatments include parenteral analgesics when needed, blood transfusions, and bone marrow transplant (Buchanan et al., 2005;). As-needed pain management is relatively insufficient as an Hb SS treatment because it has no impact on future crises. Also, Hb SS patients who seek treatment at hospitals are often viewed as drug-seeking and receive inadequate pain management and care (Yale et al., 2000). Blood transfusions are a viable option as the goal is to introduce red blood cells without hemoglobin S (Hb S) in order to reduce the percentage of Hb SS carrying erythrocytes. However, transfusions for Hb SS need to be done frequently and can cause an iron overload in patients undergoing them regularly. This requires additional medication or treatment to remove the iron and reduce the risk for iron toxicity (Ware et al., 2011).

Neoplastic Treatment

Another option for Hb SS treatment are neoplastic treatments such as 5-azacytidine and hydroxyurea (HU), which are both ribonucleotide reductase

inhibitors. While the process is not fully understood, it has been shown that 5-azacytidine and hydroxyurea stimulate the production of Hb F in both children and adults by killing cycling cells and allowing gamma globin chains to be expressed (Voskaridou et al., 2010). Texiera et al. (2003) suggest that HU increases Hb F production by working "directly on late erythroid progenitors, reprogramming them to produce more Hb F." Increased levels of Hb F results in a larger percentage of F cells, which are simply erythrocytes containing some percentage of Hb F. Increased levels of Hb F and F cells have been shown to reduce the clinical severity of Hb SS (Enders et al., 2011; Vicari et al., 2005). As such, neoplastic treatments are currently the best preventative treatment option for Hb SS. Hydroxyurea has gained notoriety as the safer drug, and is the only pharmacologic treatment approved by the Federal Drug Administration to treat Hb SS (Green & Barral, 2011), because 5-azacytidine can cause bone marrow production to slow down or cease (Charache et al., 1995).

Vicari et al. (2005) followed 29 patients on HU treatment for an average of 30 months, but found that the increase in Hb F was only statistically significant for individuals with the CAR haplotype. Ware et al. (2011) compared the occurrence of stroke between Hb SS patients being treated with HU and traditional blood transfusions. Blood transfusion treatment resulted in a marginally greater decrease in recurrent strokes, and those not being treated by either method were twice as likely to suffer from recurrent strokes (Ware et al., 2011). In a double-blind trial involving 299 patients, Maier-Redelsperger et al. (1998) found that HU reduced the occurrence of ACS, painful crises, and the need for blood transfusions. The Multicenter Study of Hydroxyurea found that over the course of 2 ½ years the frequency of ACS and painful

crises dropped by almost 50% among those treated with HU (Paul et al., 2011). A nine-year study in Greece saw a reduction in hospital admissions, blood transfusions, ACS, and painful episodes for people taking HU (Voskaridou et al., 2010). Steinberg et al. (2010) noted a 40% reduction in mortality after just 1 year of HU treatment. Overall, it appears that HU treatment effectively reduces symptoms in most Hb SS children, but only about half of Hb SS adults. The results of HU are highly variable and warrant further study (Green & Barral, 2011).

The sickle cell mutation is a single nucleotide polymorphism capable of causing severe and painful symptoms, often resulting in a decreased quality of life and a shortened lifespan. Increased levels of Hb F have been shown to alleviate the clinical course of Hb SS in children and adults. Although three of the five Hb SS haplotypes are correlated with differing levels of Hb F, it is the genetic modifiers surrounding the sickle mutation that affect Hb F production that are most important in the study of differential health outcomes among those with Hb SS and Hb AS. Unfortunately, the production and distribution mechanisms for Hb F are poorly understood. The treatment of sickling episodes usually begins with fluid replacement and oxygenization (Kerle et al., 1996). The lack of a well-defined treatment plan for sickling crises can result in poor health outcomes, organ loss, and even death.

Sickle cell trait is a genetic mutation that often results in a benign clinical course. However, in some individuals it has the potential to cause significant episodes of adverse health and possibly death. Understanding the genetic differences between those experiencing ill health due to Hb AS and those who do not experience ill health is the key to determining why these events occur. Varying levels of Hb F in

Hb AS people is one of the genetic differences that may affect health outcomes among this group. Fetal hemoglobin levels are associated with genes carried in the Hb AS haplotype, suggesting that determining the haplotype carried may help identify those who should be monitored for sudden ill health resembling sickling events. Evidence for the association between Hb F, Hb AS, and sickling events is discussed in the systematic review presented in the next section, "Do Fetal Hemoglobin Levels Mitigate Clinical Outcomes in Hb SS and Hb AS Individuals?". Additionally, retrospective studies have shown that adverse health events are more common among Hb AS individuals who undergo strenuous exercise and exertion, particularly in relation to sports and military training. This evidence is discussed in the systematic review of exercise-related deaths in Hb AS beginning on page 37.

This study has been undertaken to provide evidence for further assessment of the association between genes that affect Hb F level and health outcomes in Hb AS athletes. We hypothesize that Hb AS athletes who do not carry genes associated with increased and/or persistent Hb F levels are more likely to have suffered ill health related to their mutation. This study is currently in its early stages of recruitment and data is not complete. The following information is descriptive in nature and is not appropriate for hypothesis testing.

Do Fetal Hemoglobin Levels Mitigate Clinical Outcomes in Hb SS and Hb AS Individuals?

Introduction for Fetal Hemoglobin Systematic Review

Hemoglobin is the substance contained within erythrocytes that carries oxygen to tissues through the body. Normal adult hemoglobin consists of 2 alpha chains (Hb A) and 2 beta chains (Hb B); however, hemoglobin is not restricted to these 2 chains as different types of hemoglobin are created during different life stages. The sickle cell mutation is a balancing polymorphism that was both advantageous and disadvantageous to survival at some point in time, resulting in the selection of the sickle mutation by the "watchdog of evolution" and its prevalence among some populations (Adekile & Huisman, 1993; Akinsheye et al., 2011; Enders et al., 2011; Madrigal, 2011; Miller, 2013; Olaniyi et al., 2010; Sankaran, et al., 2008; Stacy et al. 1997).

During the prenatal and infancy phases of life everyone creates Hb F, which is comprised of 2 alpha chains and 2 gamma chains (γ) that are "beta-like" (Sankaran et al., 2008). Fetuses and infants produce Hb F and shortly after birth a "fetal switch" takes place, which causes a decrease in the production of the Hb F, replacing them with adult Hb B chains. In non-anemic individuals this eventually results in a low level of Hb F. Normal adults usually have less than 1% Hb F in their total hemoglobin. Individuals with Hb SS carry 2 Hb A chains and 2 sickle hemoglobin chains (Hb SS) with varying levels of Hb F. People with Hb SS usually have higher levels of Hb F

throughout their lifetime (Adekile & Huisman, 1993; Akinsheye et al., 2011; Enders et al., 2011 Olaniyi et al., 2010; Sankaran, et al., 2008; Stacy et al. 1997).

Another hemoglobinopathy, known as hereditary persistence of fetal hemoglobin (HPFH) is present in some Hb SS and Hb AS individuals, which may be the reason they often have higher lifetime Hb F levels. Hereditary persistence of fetal hemoglobin can be deletional or nondeletional. In deletional HPFH, a section of genetic code is missing and results in Hb F production past infancy through the overexpression of both the $^{A}\gamma$ and $^{G}\gamma$ genes. In nondeletional HPFH, usually only one of these genes is overexpressed. There are multiple types of HPFH, but the 2 most common in blacks are HPFH-1 and HPFH-2, which are characterized by variable length deletions between 13 and 106kb of DNA. HPFH-1 and HPFH-2 carriers have similar mean corpuscular volumes (MCV) and Hb F levels between 20% and 28%. Deletional HPFH usually results in a pancellular Hb F distribution among F cells while nondeletional HPFH usually results in a heterozygous distribution pattern (Akinsheye et al., 2011; Enders et al., 2011; Forget, 1998; Ngo et al., 2011).

Hb F and F cells are 2 of the main modifiers of Hb SS presentation and severity. Hb F and F cells, along with other intracellular factors, may be resistant to Hb S polymerization depending on the percentage of Hb F present in the F cell. Therefore, erythrocytes containing Hb F are less likely to sickle. An erythrocyte that cannot sickle will either perform as normal or at decreased level, but will not become rigid and cause ischemia or infarction (Powars et al., 1989). Individuals with sickle cell anemia who have relatively high levels of Hb F have been shown to have a less severe clinical presentation, which can result in reduced morbidity and mortality (Akinsheye

et al., 2011; Enders et al., 2011; Ma et al., 2007; Maier-Redelsperger et al., 1998; Marcus et al., 1997; Murray et al., 1987; Ngo et al., 2011; Olaniyi et al., 2010; Powars & Hiti, 1993; Vicari et al., 2005; Ware et al., 2011). Multiple studies have found that any increase in Hb F is not necessarily sufficient to reduce clinical severity of Hb SS. It has been shown that in order to reduce morbidity and mortality in Hb SS the percentage of Hb F must be at least 15% of total hemoglobin, though the best results occur when more than 20% of the total hemoglobin is Hb F (Marcus et al., 1997; Powars et al., 1984). After a 17 ½ year study on the effectiveness of using hydroxyurea to increase Hb F levels, Steinberg et al. (2010) concluded that, "sufficiently high concentrations of Hb F evenly distributed among sickle erythrocytes can totally abrogate the disease pathophysiology."

Levels of Hb F persistence have been linked to the different Hb SS haplotypes. Specifically, the Senegal and Arab/Indian haplotypes have been found to have higher levels of Hb F and a generally mild clinical course of Hb SS. People who are homozygous for the Senegal haplotype are often found to have approximately 20% Hb F. The Benin haplotype has been shown to have intermediate levels of Hb F and a moderate clinical course. The CAR haplotype has the worst clinical course and the lowest amount of Hb F at approximately 5%. The linkage between Hb F, Hb SS haplotype, and clinical course is complicated in countries where people with African heritage are primarily found as result of the historical slave trade. As a result, these haplotypes were more common in certain countries and areas before global travel became more commonplace. Today, it is unknown how haplotypes are distributed throughout the U.S.; this is also due largely to the fact that Hb AS and Hb SS

haplotypes are rarely determined through genetic testing. Thus, there is no data regarding haplotype frequency and distribution through the United States. These Hb SS individuals are often heterozygous for 2 sickle haplotypes due to the admixture of genes found during and since slavery. Generally, individuals with at least 1 Senegal haplotype sickle cell gene can expect to have a moderate to mild clinic course with an onset of symptoms in adulthood. Individuals with at least 1 CAR haplotype sickle cell gene can expect a severe to moderate clinical course, with an increased level of soft tissue organ failure, osteonecrosis, and a shortened lifespan (Akinsheye et al., 2011; Powars et al., 1994; Powars & Hiti, 1993).

Within the last three decades it has become clear that Hb F levels can positively influence clinical outcomes in those with Hb SS. Unfortunately, not many studies have been conducted that assess the clinical differences between sickle cell haplotypes, other sickle hemoglobinopathies, and variation in Hb F levels. Recent studies have indicated that genetic modifiers surrounding the sickle cell mutation code for higher levels of Hb F in some sickle cell haplotypes. These studies, however, also include information pertaining to co-morbidity with other hemoglobinopathies, which can influence clinical outcomes of those with Hb SS. This systematic review was conducted in order to determine if Hb F levels above 20% are protective against sickling events in Hb SS individuals. This review fills a unique niche, as no prior systematic reviews on this topic exist.

Methods for Fetal Hemoglobin Systematic Review

Identification of literature for this review did not follow typical systematic review procedures. The author was not aware that she would need to write a

systematic review until she had already been collecting literature for a year, and thus she did not document the entire process. Early articles were either found through the Web of Knowledge Database or were provided to the author by co-investigators. The author has found the Web of Knowledge database to be the most efficient way to identify articles across disciplines.

The author had already obtained articles that were included in this review (found between September 2011-February 2013) before record keeping was initiated, which began in March 2013. Articles found during and after March 2013, were discovered through the Web of Knowledge Database using search terms including the phrases, “sickl*”, “cell”, “trait”, “fetal”, “hemoglobin”, “foetal”, “hemoglobin*”, and “haemoglobin*”. The author has found the Web of Knowledge database to be the most efficient way to identify articles across disciplines. The author has also found that Web of Knowledge is the most likely to provide full versions of the required article, and is the best source to find specific articles by title and author. Articles were eligible for inclusion if they were published in English during any time period, published within all health and science disciplines, and of any study types or design including, but not limited to, prospective and retrospective cohorts, case-control, randomized clinical trials, case series, case reports, and cross sectional. Commentaries, brief reports, and reviews that were not systematic reviews, were not eligible for inclusion as they would not provide data for the systematic review. Only studies on humans were eligible for inclusion in this review, as the sickle cell mutation does not occur within other species.

With all search results combined, 3,971 articles were originally identified. Many of these articles were not related to the topic of interest, which was identified by reading the abstract. Additionally, many articles were duplicates, which were identified while saving the articles in PDF format. Ultimately, 78 articles were reviewed for inclusion in the study. Four papers met all inclusion criteria. An additional article was identified through the citations of these articles. A total of 8 articles were included in this review. The specific search terms used can be found in Table 1. All tables and figures for this section can be found at the end of this chapter. A flow chart identifying the steps used to identify later articles is show in Figure 1. Studies were included in this review if they confirmed diagnosis of Hb AS or Hb SS and measured levels of Hb F regardless of study design. Studies were excluded from this review if they did not measure levels of Hb F or if they focused on the genetic modifiers influencing Hb F instead of clinical outcomes. Articles written at any time period were eligible for inclusion so long as they met the inclusion criteria. In total, 8 papers met the inclusion criteria and were selected for this review.

A scoring system to assess the quality of the papers was created based on the scoring system found in Dino et al.'s 2009 study. Papers were scored on a 22-point scale, as the quality of the papers over time made it difficult to create a more specific scoring system. The scores were used to assess the reliability and validity of the study, but did not affect their inclusion in this review. The scoring chart created for this review is available in Figure 2. A clear statement of the hypothesis and the use of a sample size under 50 were each worth 1 point. However, reporting a sample size over 50 was worth 2 points as was the representativeness of the target

population. A description of the inclusion and exclusion criteria for participants was worth 1 point. The inclusion or discussion of important variables, some of which were potential confounders, was worth up to 6 points. The following variables were worth 1 point each: Hb F levels, age, gender, α-thalassemia status, GP6D deficiency status, and hemoglobin C status. The description of statistical analyses used and the inclusion of a power calculation was worth 1 point. If statistical outcomes were reported the paper received 1 point, but 2 points were awarded for thorough statistical outcomes, such as the inclusion of a p-value or confidence interval. Papers received 1 point if they contained a clear statement regarding whether or not the final data supported the hypothesis. Scores for each paper can be found in Table 2.

Results for Fetal Hemoglobin Systematic Review

The majority of the studies (n = 7) did not differentiate between the types of Hb FP, with only 1 study creating separate groups for both HPFH-1 and HPFH-2. Within the 8 studies reviewed there was 1 randomized controlled trial, 1 case-control, 2 prospective cohorts, and 4 cross sectional designs. Four of the studies were conducted within the United States, 1 in France, 1 in Jamaica, 1 in Kuwait, and 1 was conducted with data from multiple international centers. All studies used data or participants from clinical settings including hospitals, SCD centers, hematology clinics, or longitudinal hematology studies. Four studies examined only pediatric data while the remaining 5 included participants of all ages. Only 2 of the 8 studies reported both p-values and confidence intervals for their findings, with 6 studies reporting p-values, and 1 study reporting only group means and standard deviations.

A summary table displaying the p-values, location, study designs, and confidence intervals where appropriate, can be found in Table 3.

Randomized Control Trial

In 2013, Sheehan et al. used data from the BABY HUG study, which was a placebo-controlled, double-blinded phase III randomized control trial sponsored by the National Heart, Lung, and Blood Institute (NHLBI) and the National Institute of Child Health and Human Development (NICHHD). This study looked at the effects of HU use in 190 children under the age of 4 years in order to look at the differential clinical outcomes associated with HPFH genetic modifiers and increased Hb F through the use of HU in relation to adverse clinical events in young children. To do this the investigators utilized 13 centers around the United States (U.S.) to record data on sickle cell haplotypes, Hb F modifiers, other hemoglobin disorders, and Hb F levels, among other variables. Patients were enrolled between the ages of 9 months and 18 months and followed for a period of 2 years. The study measured Hb F levels upon entry and exit to the study in both placebo and intervention groups. Using information that correlated severity of clinical outcome to sickle haplotype, the authors sorted the participants into favorable, intermediate, and unfavorable subgroups. However, this only took three of the five sickle haplotypes into consideration as data on clinical severity and haplotype is scarce (Sheehan et al., 2013).

Sheehan et al. (2013) also divided placebo and intervention groups by alpha-thalassemia status as infants with this disorder presented with substantial clinical differences when compared to those without the disorder. Upon entry to the study,

the Hb F level for all participants was approximately 26%, but was not statistically significant between alpha-thalassemia groups (p-value = 0.56). Upon exit, neither the placebo group nor the intervention group showed statistically significant differences in Hb F levels by alpha-thalassemia status. The authors found that among the placebo group the genetic modifiers examined were associated with a higher baseline Hb F level, but this was not seen upon exit from the study. Among the intervention group Hb F levels were higher regardless of the presence or absence of these genetic modifiers. Ultimately, Sheehan et al. (2013) state that infants within the favorable sickle haplotype group had higher baseline levels of Hb F, but that this did not persist throughout the study. Interestingly, this group also had higher rates of pain events than the beta-globin haplotype. They conclude that sickle haplotype does not influence response to HU and determining the haplotype is not necessary, as it does not help predict clinical outcomes for HB SS individuals (Sheehan et al., 2013).

Prospective Cohort

Powars et al. (1984) conducted a prospective cohort study to gather information on Hb F levels in Hb SS individuals with the belief that there may be a threshold level of Hb F that was indicative of increased risk, though they stated that their primary purpose was not to test a specific hypothesis. Instead, they conducted an observational study designed to collect data that could be used to help generate hypotheses in the future. The study recruited 272 participants of both sexes in the original cohort ranging in age from birth to 56 years. Data collection began in 1974, and participants were followed for an average of 11 years. Two hundred and sixteen

participants remained in the study after 11 years. It is unclear where and how participants were recruited (Powars et al., 1984).

The authors looked at the recurrent events of sickle cell crisis, chest syndrome, meningitis/septicemia, and hospitalizations as well as the fatal events of cerebrovascular accidents (CVA), and aseptic necrosis in relation to a range of Hb F levels. They found a linear incidence pattern for crisis, hospitalization, and chest syndrome among age-adjusted results for individuals who had 15% or greater Hb F levels. There was no pattern apparent among unadjusted data. Interestingly, participants who consistently had Hb F levels around 20% had lower incidence rates than those who consistently had levels with the 15 – 19% range. Meningistis/septicemia did not follow this pattern and the authors felt that it was due to the high occurrence rates among young children who have variable levels of Hb F. They state that it may also be due to the limited number of cases available for study. There were also a small number of aseptic necrosis and CVA cases available for analysis. Aseptic necrosis appeared to have an increased incidence among those with less than 10% Hb F while CVA had an increased rate among those with 15-19% Hb F. The authors chose 10% Hb F to be the threshold point for these events. Powars et al. (1984) found that among recurrent events, the risk ratio decreased with Hb F levels above 20%, and even more significantly among those with Hb F levels above 25%. Termination events had a decreased risk ratio beginning at 10%, but results showed that risk was not substantially less and was reduced only at intermediate levels. They conclude that there is no linear pattern associated with decreased risk for Hb F

levels, but that the data supports the idea that a threshold level exists whereby incidence of recurrent and fatal events is decreased (Powars et al., 1984).

Using data gathered from the multi-center French Study Group on Sickle Cell Disease, Maier-Redelsperger et al. (1998) investigated the association between the use of HU, myelotoxic events, and Hb F levels in a pediatric cohort. Males and females between the ages of 4 and 19 years were recruited for this study, resulting in a total sample size of 29. Participants must have had at least three sickle cell related pain events that required hospitalization within the last year in order to be eligible for the study. Participants were followed for an average of 22 months. Four participants were lost to follow-up due to a failure to respond to treatment (2), relocation (1), and development of systemic lupus (1). With the use of HU, the investigators found increased Hb F levels in all participants not lost to follow up. The authors also determine sickle haplotype for 24 participants from the original cohort and examined the association between haplotype and Hb F level. Nine of the patients were homozygous for the Benin haplotype, 8 were homozygous for the CAR haplotype, three were homozygous for the Senegal haplotype, three were heterozygous for the CAR and Benin haplotypes, and 1 was atypical (Maier-Redelsperger et al., 1998).

The investigators found that after three years with HU treatment Hb F levels increased between 1.5-fold and 16-fold among participants. They found variation among initial Hb F levels between haplotypes, but the difference between those levels and maximal levels after HU usage were not statistically significant. Additionally, there were no adverse events associated with HU use in this study. The authors state that the results suggest the use of HU may be more beneficial in

subadults than adults, but requires further study to examine its pleiotropic effects (Maier-Redelsperger et al., 1998).

Cross Sectional

Bhatnagar et al. (2013) conducted a cross sectional study using data from the Silent Infarct Transfusion (SIT) trial, conducted by the National Institute of Neurological Disorders and Stroke, and the Cooperative Study of Sickle Cell Disease (CSSCD) that is funded by the NHLBI. They collected data on VOC pain episodes and Hb F levels from one 2,220 participants: 456 from the SIT and trial and 764 from the CSSCD. Participants were both male and female and between the ages of 2 and 15 years. The authors looked at hematocrit levels, age, Hb F levels, and sex in relation to incidence of VOC pain crises. In the SIT trial hematocrit levels and age were strongly associated with increased pain episodes, with p-values of <0.0001 each. Sex and Hb F levels were not significantly associated with pain episodes, having p-values of 0.54 and 0.07 respectively. Similar results were found in the CSSCD study, with age and hematocrit levels having statistically significant p-values of 0.0016 and 0.019 respectively. Hb F level had a p-value of 0.15 while sex had a p-value of 0.67. Bhatnagar et al. (2013) then conducted a meta-analysis of the results from these 2 studies. In the meta-analysis age and hematocrit levels remained statistically significant and Hb F was also found to be associated with incidence of VOC pain episodes. Hematocrit and age both had p-values of <0.0001 and Hb F had a p-value of 0.02. Sex was not found to be strongly associated with pain episodes with a p-value of 0.46. The authors note that single-point measurements of Hb F may be a limitation of the study, but that Hb F levels in children of the ages included remain

fairly constant and thus were believed to be accurate representations. Additionally, pain episodes were recorded retrospectively in the SIT trial and therefore may not include data from all medical centers. They conclude that Hb F levels are associated with incidence of pain episodes and suggest that further research into this association is warranted (Bhatnagar et al., 2013).

A unique study cross-sectional study was conducted in Kuwait by Adekile et al. (2007) investigated Hb F levels among Kuwaiti people. Participants were recruited from hematology clinics at the Amiri and Mubarak hospitals, were of both genders, and ranged in age from 3 months to 60 years. A total of 149 participants were recruited for the study. Sickle cell haplotype was determined and only those carrying the Saudi Arabia/Indian (SAI) haplotype, either homozygously or heterzygously, were included. Of the 149 participants, only 4 were not homozygous for the SAI haplotype; these 4 were heterozygous for the SAI and Benin haplotypes.

The authors divided participants into age ranges of <5 years, ≥5 – 10 years, ≥ 10 to 15 years, and ≥ 15 years and found that mean Hb F levels were significantly different throughout these groupings with a p-value of <0.0001. They found that most patients in this population have Hb F levels between 20% and 30%. Fetal hemoglobin levels decreased from 1 year of age to 5 years of age, but then tended to remain fairly steady in this population. The authors believe these high Hb F levels are the reasons Kuwaiti people with Hb SS tend to have more mild clinical courses. This is especially evident in Kuwait children, who do not usually present with Hb SS symptoms until after 5 years of age when their Hb F levels have dropped below 30% (Adekile et al., 2007).

Marcus et al. (1997) used a cross sectional design and recruited patients with SCD from the Duke Pediatric Hematology Clinic that is within the Duke-UNC Comprehensive Sickle Cell Center in order to quantitate F-cells, which are cells containing any percentage of Hb F, among those with SCD. Two hundred and forty-two patients of both sexes between the ages of 2 and 19 ½ were recruited. Eight of the participants had Hb S/HPFH, 30 had Hb S and a beta-thalassemia, 74 had Hb SC, and 130 had Hb SS. Individuals with Hb SS were reported to have Hb F levels 10.4±6.3% while those with Hb S/HPFH had Hb F levels of 31.7±3.0, which was statistically significant with a p-value of <0.001. A significant correlation, with a p-value of <0.001, was also found between % F cell and hemoglobin concentration among Hb SS children. Marcus et al. (1997) note that the percentage of intracellular Hb F in F cells varies and is not related to the Hb F levels through the body. If the two were linked, it would be easier to predict Hb F or F cell levels from the measurement of the other. This is important because an understanding of the relationship between these two variables could benefit the treatment of SCD. Ultimately, the authors conclude that %Hb F and %F cells needs to be measured in all Hb SS individuals receiving clinical treatments such as hydroxyurea in order to more fully understand its interaction with other clinical parameters and thus the help determine more beneficial treatments for patients (Marcus et al., 1997).

A study conducted in Jamaica in the mid-1980's by Murray et al. (1987) compared the hematological characteristics of individuals with Hb SS, Hb S-HPFH, and Hb S-beta-thalassemia. The investigators recruited participants from those attending sickle cell clinics at the University Hospital of the West Indies in Kingston, Jamaica.

Participants were between the ages of 6 and 33 years and both male and female. There were a total of 27 participants included in the study. Identification of potential participants was based on diagnosis of the hemoglobinopathies in a parent or sibling. It is unclear whether all hemoglobinopathies were clinically confirmed in participants, but HPFH was confirmed through the testing for cord- blood in 6 of the participants, and 7 were confirmed through family study. However, Hb F levels were tested and only those with 20% or more Hb F were eligible to participate in the study. Beta-thalassemia was clearly differentiated from the other hemoglobinopathies by levels of HbA_2. Erythrocyte count provided a clear distinction between Hb S-HPFH and Hb SS, followed by Hb F levels though the authors state that the addition of Hb F levels to erythrocyte count was not substantial. In 3 of the 4 patients with Hb S-beta-thalassemia blood cell films showed irreversibly sickled cells. Irreversibly sickled cells were also present in 4 out of ten participants with Hb SS, but were not present in any of the 13 Hb S-HPFH participants. The authors conclude that it should be possible to differentiate between Hb S-HPFH and Hb SS with high levels of Hb F based on measurement of Hb F: Hb F ranges were only high enough in 2 of the Hb SS participants to overlap with Hb S-HPFH Hb F levels. They state that it should be possible to differentiate between Hb SS and Hb S-HPFH by the hematological features of red blood cell count and Hb F level, followed by reticulocyte count, total hemoglobin, and total bilirubin in most cases. Differentiation between those with Hb S beta-thalassemia should be possible through the measurement of HbA_2 levels. Limitations of this study include a small sample size. Clinical diagnoses were limited by the techniques available at the time of the study (Murray et al., 1987).

Case-Control

In 2011, Ngo et al. gathered data on Hb F levels among those with Hb SS. Participants included both sexes and ranged in age from newborn to 62.2. Using the Haemoglobin Diagnostic Reference Laboratory at Boston Medical Center the authors identified 28 participants with both types 1 and 2 HPFH. They also conducted a retrospective chart review to obtain data on longitudinal Hb F levels. They compared the results of their study to an external reference group using data from 4,085 in the CSSCD and also identified participants who carried the XmnI polymorphism that is associated with increased Hb F levels. There were 20 participants with HPFH-2 and 7 participants with HPFH-1. Within their group they found that increased levels of Hb F were associated with decreased sickle cell events. Additionally, the average Hb F level did not differ between HPFH types 1 and 2, having a p-value of 0.26. Using data from the CSSCD as a comparison for their study the investigators found that Hb F level for all ages and also for those over five years, MCV, haemoglobin levels, and reticulocyte level were all significantly increased in those with HPFH: p-values for each variable were <0.01. Ngo et al. (2011) also compared those with the XmnI polymorphism group to the CSSCD group and found that all variables except reticulocyte level were significantly increased, with all p-values again at <0.01. Reticulocyte level had a p-value of 0.09 in this group. The authors state that Hb F levels decreased among those with HPFH and the XmnI after infancy, but that the decline is not as steep as that seen among groups without these polymorphisms. They suggest that the regulatory mechanism for Hb F may differ among these groups. Ngo et al. conclude that Hb SS individuals with Hb F levels at or above 30% have fewer or

less severe sickling events and that a more thorough understanding of Hb F distribution and regulation may aid in the treatment of Hb SS individuals (Ngo et al., 2011).

Discussion for Fetal Hemoglobin Systematic Review

Hb F is a quantitative trait with great variation in the amount of Hb F produced in each haplotype. Among the numerous factors have been found to influence the phenotypic expressions of Hb F are gender, age, and genes located on different chromosomes. Chang et al. (1995) state, "within populations of SS patients that are relatively homogeneous for their ß-globin haplotypes, other variables must play a more dominant role in determining Hb F levels. Analyses of pedigrees with variable Hb F levels have indicated that Hb F is also influenced by genetic factors unlinked to the ß-globin region." They identified three DNA polymorphisms that modify Hb F productions: BCL11A, Hb S1L-MYB, and the beta globin locus. They found that Hb F levels were most strongly influenced by the F cell production locus located on the X chromosome, which indicates that females with Hb SS may have more mild clinical complications (Chang et al., 1995). Galarneau et al. (2010) studied the same three polymorphisms using a genome wide association study. They were able to link seven single nucleotide polymorphisms (SNPs) that independently affect Hb F production, but found that only the original three were statistically significant. They also found that the three previously identified SNPs were responsible for approximately 23 % to 30% of the variation in Hb F in Hb SS individuals (Galarneau et al., 2010; Higgs & Wood, 2008). In 2005, Sankaran et al. (2008) found that the BCL11A single nucleotide polymorphism actually works through epistasis by suppressing the production of

gamma chains for Hb F and may be responsible for the "fetal switch" that occurs shortly after birth. The phenotypic expression of Hb F by Hb SS individuals is variable and, according to Galarneau et al.'s (2010) work, illustrative of the central limit theorem.

Evidence from these studies indicate that levels of Hb F at 20% or greater results in better clinical outcomes for Hb SS individuals. Though many hemorheological features influence sickling in erythrocytes, the percentage of Hb F in each F cell is what controls whether or not the cell will sickle during vaso-occlusive events. It is not understood, however, what mechanism(s) control the intracellular distribution of Hb F within erythrocytes: levels may be distributed equally throughout cells or may vary between cells. Higher %Hb F levels thus increase the availability of Hb F for distribution and increase the likelihood of more mild clinical courses.

Strengths of this Review

Strengths of this study include its inclusion of studies conducted in multiple countries and across the last three decades. The inclusion of multiple study designs also benefits this review by helping to reveal and compensate for design limitations in each type.

Limitations of this Review

This study is limited in a number of ways. To begin, it may not be an accurate representation of the available literature since articles were only eligible for inclusion if they had been published in English. Although the search terms used were broad, they may not have revealed all articles eligible for inclusion in this study. Additionally, only the author reviewed articles for inclusion and scored them for

methodological quality. The use of multiple scorers may have resulted in the inclusion of different articles or the exclusion of ones reviewed. Reviewers with a high inter-rater reliability would have helped this review achieve higher validity and reproducibility. Another limitation is present in the populations chosen for this review, which may not accurately represent all individuals with SCT as many of samples were comprised of non-adults.

Limitations of Studies Included in this Review

Limitations of this study stem largely from the individual limitations posed by each study included in the review. The small sample of studies included is also potentially problematic as it may not fully represent all studies with data pertaining to Hb F levels in Hb SS individuals. Indeed, a number of studies were not included as their methods were unclear and the data presented was unintelligible.

Conclusion of Fetal Hemoglobin Systematic Review

Fetal hemoglobin persistence, its distribution, and mechanism regulating production levels warrant further research. This understanding would make the use of pharmaceuticals more beneficial by allowing medical care providers to understand the processes and likely outcomes before administering potentially toxic drugs to those already suffering ill health. It is clear that further research on the effects of Hb F on erythrocyte sickling is needed. This is especially important as SCD and Hb AS children age and their natural Hb F levels drop, decreasing their protection from sickling crises.

Based on the evidence in this review, increased fetal hemoglobin levels can positively influence the occurrence and severity of pain episodes in those with Hb SS

and Hb AS. However, the mechanisms controlling HPFH are poorly understood. Genes coding for Hb F persistence are beginning to be identified, but it is still unclear how these genes affect the levels of Hb F produced and why they vary through the life course. In addition, the distribution of Hb F through erythrocytes is often not uniform; mechanisms controlling the distribution process have not been identified. Given the ambiguity surrounding the protective measures provided by Hb F it is not possible to say exactly what levels are protective. Evidence provided herein suggests that high Hb F levels can be found among some haplotypes and that levels above 20-30% help mitigate the effects of sickling in Hb SS and Hb AS individuals.

Tables and Figures

Table 1. Search Terms Used to Identify Articles Potentially Eligible for Inclusion in this Review

Hb F Search Terms						
sickl*	cell	trait	fetal	foetal	haem*	hem*
sickl* and cell and trait and fetal and hem*						
sickl* and cell and trait and fetal and haem*						
sickl* and cell and trait and foetal						
sickl* and cell and fetal and hem*						
sickl* and cell and fetal and haem*						

Table 2. Methodological quality assessment of studies included in this review

Author	Year	Hypotheses (1 pt.)	Study Design (5 pts.)	Sampling Procedures (5 pts.)	Inclusion of Possible Confounders (6 pts.)	Statistical Analysis (4 pts.)	Results (1 pt.)	Final Score (max 22 pts.)	Final Score (%)
Adekile et al.	2007	1	1	5	5	2	1	15	68
Bhatnagar et al.	2013	1	1	5	3	3	1	14	82
Maier-Redelsperger et al.	1998	0	3	5	3	2	1	14	64
Marcus et al.	1997	0	1	4	4	1	0	10	59
Ngo et al.	2011	1	2	4	3	2	1	13	59
Powars et al.	1984	1	3	2	3	3	1	13	76
Sheehan et al.	2013	1	4	4	3	2	0	14	64
Murray et al.	1988	1	1	2	2	1	1	8	47

Table 3. Descriptive table of reported measures for included studies

Author	Year	Location	Sample Size (n)	# with HPFH	% F Cells	% HbF	Reported Measure	95% CI	p-value
Adekile et al.	2007	Kuwait	149				ANOVA = F = 7.8		<0.0001
Bhatnagar et al.	2013	U.S.	1220				Effect = -0.05	(-0.09, -0.01)	0.02
Maier-Redelsperger et al.	1998	France	29		24.4 ± 14.3	4.0 ± 3.4			0.005
Marcus et al.	1997	U.S.	242	8	100				0.001
Murray et al.	1988	Jamaica	27	13					
Ngo et al.	2011	U.S.	30			32.6 HPFH 1 31.0 HPFH 2			0.26
Powars et al.	1984	U.S.	272				Regression Coefficient = -0.379 Risk Ratio = 0.65	(-2.883, 2.125) (0.53, 0.80)	0.66
Sheehan et al.	2013	U.S.	190			26			0.56

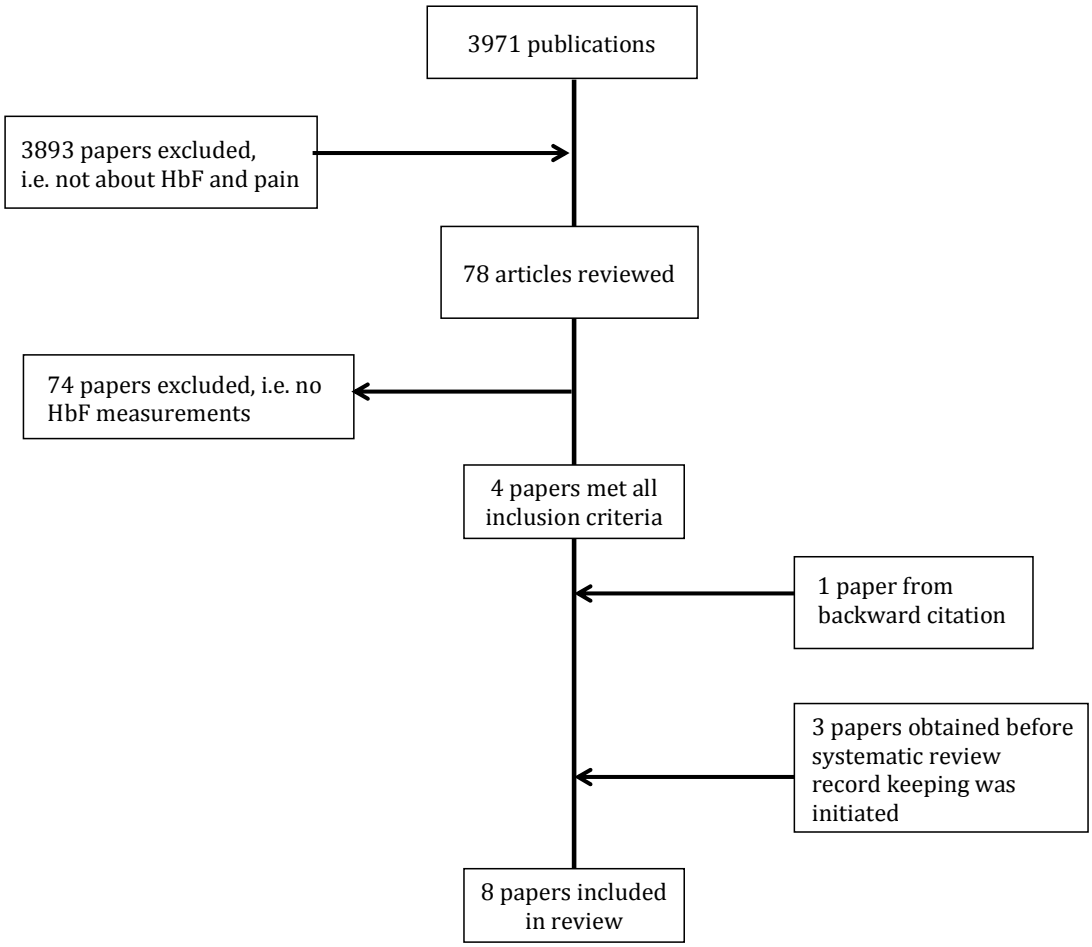

Figure 1. Flow chart detailing articles identified for possible inclusion in this review

Hypotheses (maximum possible = 1)

- The hypothesis/research question of the study not clearly described = 0
- The hypothesis/research question of the study clearly described = 1

Study Type (maximum possible = 5)

- Systematic Review = 5
- Randomized Control Trial = 4
- Cohort Study = 3
- Case-Control = 2
- Case Series OR cross sectional = 1

Sampling (maximum possible = 5)

- Sample Source and Size
  - Routine data = 0
  - Sample size less than 50 = 1
  - Sample size more than 50 = 2
- Sampling Representation (2)
  - Not representative of target population = 0
  - Representative of target population = 2
- Selection Criteria
  - Selection of cases/controls/population not well described = 0
  - Selection of cases/controls/population well described = 1

Methodology (maximum possible = 6)

- Inclusion of other variables:
  - Age = 1
  - Hb F = 1
  - Alpha-Thalassemia = 1
  - Gender = 1
  - Hb SC = 1
  - Hb S = 1

Statistical Analyses and Results (maximum possible = 5)

- Statistical Analysis (2)
  - Statistical methods not described = 0
  - Statistical methods clearly described = 1
- Statistical outcomes (2)
  - Statistical outcome not clearly reported = 0
  - Some statistical outcomes clearly reported (e.g. CI's but not P-values) = 1
  - Statistical outcomes clearly reported (e.g. CI's and P-values) = 2
- Results (1)
  - Research questions/hypotheses not clearly answered/tested = 0
  - Research questions/hypotheses clearly answered/tested = 1

Maximum Score = 22

Figure 2. Methodological quality scoring sheet used to assess the studies included in this review

Is Physical Exertion and/or Exercise a Risk Factor for Sudden Death Among Hb AS Carriers?

Introduction for Exercise Deaths Systematic Review

Recently, it has become clear that some Hb SS patients have more benign clinical profiles than others. If a sickling crisis is not stopped by prompt medical attention it may result in patient death. Fortunately, the genetic reason for such clinical differences has been well determined: although the mutation that alters the "normal" hemoglobin gene is the same in all patients, genetic modifiers surrounding the sickle gene influence the clinical course. Specifically, there are SNPS in several chromosomal areas that affect the production of fetal hemoglobin (Hb F). If higher than normal Hb F levels are produced, the clinical course is less severe; if Hb F is produced at average levels, the clinical course is more severe (Adekile et al., 2007; Akinsheye et al., 2011; Bailey et al., 1991; Bhatnagar, 2013; Charache & Conley; 1969; Ender et al., 2011; Forget, 1998; Franco et al., 2006; Jacob & Raper, 1958; Marcus et al., 1997; Ngo et al., 2011; Powars et al., 1984; Powars et al., 1989; Sheehan et al., 2013; Steinberg & Sebastiani, 2012; Whyte et al., 2013).

For several years, it was thought that the genetic material surrounding the beta gene, which is usually referred to as the beta-globin sickle haplotype or Hb S haplotype, was responsible for the different clinical manifestations of sickle cell anemia. However, it is clear now that Hb SS individuals who have a milder disease course have elevated levels of Hb F because their γ-globin gene remains active, while

it is inactivated soon after birth in most "normal" people. The higher levels of Hb F make up for the defective Hb S, leading to a milder clinical course (Bhatnagar et al. 2011; Farrell et al. 2011; Lettre et al. 2008). In addition, other hemoglobinopathies such as the thalassemias (which are themselves highly variable) modulate the clinical manifestation of sickle cell disease (Badens et al. 2011). In sum, the genetic basis to the differential clinical manifestation of sickle cell disease is best explained by the patient's SNPs at loci that affect fetal beta globin expression.

Clinical outcomes among those with SCD and Hb AS vary in severity. This is especially evident among Hb AS athletes, who sometimes suffer from ES or heat illness. Exertional sickling has often been confused with other causes of collapse among those who are exercising. Although symptoms differ, it is often mistaken for severe forms of heat illness. Symptoms of exertional sickling often include cramps that spread throughout the body, leg and/or low back pain, weakness, trouble catching one's breath, tightness in the chest, rapid breathing, or just not feeling "right" (Eichner, 2010). In many Hb AS individuals ES crises progress into muscle sickling, muscle infarction, and can result in rhabdomyolysis, which is the breakdown of muscle fibers (Kerle et al., 1996; Koppes, 1977; Rhabdomyolysis, 2013). When rhabdomyolysis occurs, myoglobin, which is a protein contained within the muscle, is released into the bloodstream. Myoglobin damages kidney cells and can cause kidney failure. If not treated quickly, rhabdomyolysis can cause severe clinical outcomes and possibly death. Among Hb AS athletes, death is often attributed, at least in part, to rhabdomyolysis and may contribute to incorrectly identified causes of death and therefore inaccurate Hb AS death rates.

Considering that the age category containing college students has a very low mortality rate, and that these are elite athletes in excellent physical health, these mortality numbers are rather high and should be cause for concern. As discussed in the introduction, there have been approximately 18 deaths due to Hb AS among athletes over the last few decades. Indeed, it is only recently that Hb AS has been acknowledged as a cause of death in the general population. Our work with all U.S. States which record this as cause of death indicates that the frequency of sickle-cell deaths is not as low as it was once assumed. We summarize these data in Table 4. All tables and figures for this section can be found at the end of this chapter. It is clear that states with high concentrations of African-derived populations and those with high altitudes have higher frequencies of Hb AS-related deaths. A map of deaths Hb AS deaths reported in this states can be found in Figure 3.

Sickle cell trait has also been listed as a cause of death in military personnel during strenuous physical training exercises. It is possible that outside conditions, such as higher temperatures, humidity, and elevation can increase the risk of having a sickling crisis. In addition to these conditions, intense physical training and conditions may exacerbate the sickling crisis and lead to severe or fatal health outcomes. This systematic review includes all available studies regarding death from or relating to Hb AS in athletes and military personnel in the U.S. in order to determine whether or not there is an increased risk of death in Hb AS individuals who engage in strenuous physical activities. This review fills a unique niche, as no prior systematic reviews on this topic exist.

Methods for Exercise Deaths Systematic Review

The author had already obtained three articles that were included in this review (found between September 2011-February 2013) before record keeping was initiated, which began in March 2013. Articles found during and after March 2013, were discovered through the Web of Knowledge Database using search terms including the phrases, "sickle", "cell", "trait", "athlete*", "exercis*", and "physical". Articles were eligible for inclusion if they used military or athlete populations, recorded Hb AS as a cause of death or related cause of death, and reported the participants' physical activity level preceding the crisis episode. Eligible articles were published in English during any time period, published within all health and science disciplines, and of any study type or design including, but not limited to, prospective and retrospective cohorts, case-control, randomized clinical trials, case series, case reports, and cross sectional. Commentaries, brief reports, and reviews that were not systematic reviews, were not eligible for inclusion as they would not provide data for the systematic review. Only studies on humans were eligible for inclusion in this review, as the sickle cell mutation does not occur within other species.

With all search results combined, 3,087 articles were originally identified. Many of the articles identified were not related to the topic of interest, which was identified by reading the abstract. Additionally, many articles were duplicates, which were identified while saving the articles in PDF format. From this, 55 articles matching the research aims of the author were reviewed for inclusion in this review. After reviewing these 55 papers, only 4 met all inclusion criteria, which was the included the reporting of data pertaining to episodes of pain or sudden death

associated with Hb AS status and the inclusion of data regarding whether or not the participant was engaged in physical exercise prior to the attack. Five additional articles were identified through the citations of articles already selected for this thesis. Two of these articles were requested through USF's Interlibrary Loan (ILL) program, but the ILL staff was unable to obtain copies of the articles. An additional article was identified through an article in *Scientific American* in which this study was mentioned. Studies were excluded from this review if they were case studies of a single patient, only discussed the topic generally without including data, or did not report outcomes of sickling crises or death. In sum, a total of 8 papers were included in this systematic review. The specific search terms used can be seen in Table 5. A flow chart identifying the steps used to identify later articles is shown in Figure 4.

A scoring system to assess quality of the papers was created based on the scoring system found in Dino et al.'s 2009 study. Papers were scored on a 17-point scale, as the quality of the papers over time made it difficult to create a more specific scoring system. The scores were used to assess the reliability and validity of the study, but did not affect their inclusion in this review. The scoring chart created for this review is available in Figure 5. A clear statement of the hypothesis and reporting a sample size under 50 was both worth 1 point. However, a paper was given 2 points if the sample size was over 50 participants. Representativeness of the target population was also worth 2 points. A description of the inclusion and exclusion criteria for participants was worth 1 point. The inclusion or discussion of important variables, some of which were potential confounders, was worth up to 6 points. The following variables were worth 1 point each: Hb F levels, age, gender, α-thalassemia

status, GP6D deficiency status, and hemoglobin C status. The description of statistical analyses used to analyze data and the inclusion of a power calculation was each worth 1 point. If statistical outcomes were vaguely reported the paper received 1 point, but 2 points were awarded for thorough statistical outcomes, such as the inclusion of a p-value or confidence interval. Papers received 1 point if they contained a clear statement regarding whether or not the final data supported the hypothesis. Scores for each paper can be found in Table 6.

Results for Exercise Deaths Systematic Review

All 8 papers reported studies that were conducted within the United States. Seven papers utilized retrospective cohort designs in order to gather their data and the remaining paper was a small retrospective case series. Five of the studies looked at athlete populations while the other three looked at members of the armed forces. Five of the studies gathered information on gender, though all studies included predominantly male data. Sickling crises and death related to Hb AS in athletes has only been recorded in 2 female athletes as far as the author has been able find. Six studies recorded data on race, but only differentiate between Caucasian and African American (AA) identification, which does not take the Hb AS rate 0.5% among those of Hispanic/Latino descent into account (Tarini et al., 2012). Measures of association varied between the studies, with statistical outcomes vaguely reported on the whole. A summary table displaying measures of association, confidence intervals, and p-values where appropriate can be found in Table 7.

Retrospective Cohort Studies in Athletes

Harris et al. (2012) conducted a retrospective cohort study on Hb AS related deaths in competitive athletes. Participants were identified through a forensic database registry at the Minneapolis Heart Institute Foundation (MHIF). This data set was created in order to compile data on death among young athletes playing organized sports. The investigators gathered past data and also collected data Participants were included in the study if they were 39 years of age or younger at the time of death and if they were participating "in organized team or individual sports requiring regular competition, and placing a premium on excellence and achievement" at the time of death. The database was used to identify sudden deaths in athletes between 1980 and 2010, resulting in 2,642 participants. Of these, there were only 23 deaths related to sickle cell trait and all but 2 occurred in males. These 23 participants were between the ages of 12 and 22 years and made up 3.3% of the total African American study population. Nineteen of the deaths were associated with football, 3 with basketball, and 1 with track. Confirmation of Hb AS status was done through hemoglobin electrophoresis, solubility testing, or both. These tests were conducted either premortem or during autopsy.

The authors also included data on environmental conditions during which death occurred. Twenty-two of the participants died during training or conditioning while 1 died during competition. Seventeen of the deaths occurred between June and October, 2 deaths occurred at high altitude, and 20 happened while temperatures were 80° Fahrenheit or higher. Four of the athletes had structural cardiac abnormalities, but these were determined not to be the major or primary cause of

death. Rhabdomyolysis was found in 11 of these deaths, while it was only determined to be the cause of death in 5 of the remaining 2,619 deaths in the registry (Harris et al., 2012).

Harris et al. (2012) state that Hb AS deaths were more common among AA at 100% vs. 37% (p-value <0.001). Their data show that these 23 participants are 7% of the total 271 AA football players in the registry, which reflects the overall AA Hb AS carrier status rate of 8% in the U.S. population. However, the results used for this conclusion are slightly flawed, as they actually represent just over 8% of the AA football players in the registry. Unfortunately, they do not make it clear which comparison group they used to draw this conclusion. They also note that Hb AS status was not known for all deaths found in the in the registry due to privacy restrictions and may have resulted in the lack of inclusion of all possible Hb AS deaths. Harris et al. (2012) note that this may over-represent the risk of Hb AS related death as many more AA in the US should suffer from adverse health outcomes if these rates held true in the general population. They suggest that a subset of Hb AS carriers is at a higher risk due to currently unidentified environmental, physiological, and genetic factors. Ultimately, the authors conclude that Hb AS is a risk during athletic competition and should be treated as such (Harris et al., 2012).

Boden et al. (2013) also utilized a retrospective cohort study to look at all fatalities among college and high school football players. Participants were identified through reports of fatal episodes during organized school-sponsored sporting events gathered from national newspaper clippings, athletic trainers, national and state athletic organizations, and athletic coaches from July 1990 through June 2010. The

authors classified injuries as occurring from trauma sustained during training, conditioning, and competition as a result of cervical fracture, cardiac failure, sickle cell trait, pulmonary embolism, asthma, brain injury, intra-abdominal injury, or heat illness. The authors used a questionnaire to collect information weight, height, participation level, timing of injury, and position played. They also gathered information on the weather on the days of deaths where Hb AS and heat death occurred. They found a total of 243 deaths; all decedents were male (Boden et al., 2013).

Boden et al. reported 11 Hb AS deaths, all of which occurred during training and conditioning, specifically during agility and sprinting drills. These deaths were all found to occur between 1999/2000 and 2009/2010. Interestingly, the authors state that risk of death from Hb AS complications during the second decade of the study was 9.7 times higher than that during the first decade. This was a counterintuitive finding because, according to their data, all Hb AS related deaths occurred during the second decade of the study. Temperatures on the day of death ranged from 44°F to 93°F, with humidity between 50% and 100%. Altitude was also highly variable, ranging from 48 to 2,116 feet above sea level. Eight of the deaths occurred in southern states, 2 in mid-western states, and the location of 1 death was unknown. The authors found that in all types of deaths the odds ratio for death was higher among college level athletes than high school athletes with an odds ratio for college fatalities to high school fatalities of 66.6 (Boden et al, 2013).

Boden et al. (2013) state that it is not clear why the odds ratio for Hb AS related deaths among college level football players is so much higher than odds ratio

for high school level players, but speculate that this may reflect higher rates of AA players at the college level, more intense training in college, Hb AS awareness and diagnosis, or unknown factors. They found that average temperatures and humidity were lower during fatal events for Hb AS than for heat related deaths, indicating that these factors may not be as important in the development of Hb AS crises. They also state that Hb AS athletes had lower average BMI's than those dying from heat related causes. At the time of death, the participants had core body temperatures under 105°F, pain in the low back and buttocks, and a rapid development of sickling symptoms without warning signs. The authors note that their study is limited by the use of information from the public domain, underreporting of high school and college football deaths, and the lack of specific information on some of the fatalities. The authors conclude by supporting the Hb AS testing of athletes and suggest that 5 of the Hb AS deaths may have been prevented had the coaches, trainers, and athletes been aware of the athletes' Hb AS status (Boden et al., 2013).

A similar retrospective cohort study conducted by Harmon et al. (2012) looked at Hb AS-related sudden deaths among National Collegiate Athletic Association (NCAA) football players and discussed different screening methods and their cost-effectiveness. Participants were found using a database of athlete deaths that contains data from both the Parent Heart Watch (PHW) database and the NCAA Resolutions list. The NCAA resolutions list is voluntary and the PHW database is updated through weekly internet searches to identify reports of sudden death and sickle cell anemia Hb SS in young people. All participants were male and died between January 2004 and December 2008. The authors cross-referenced NCCA

deaths found in the PHW database with the NCAA resolutions list and created a single database for their own use. Any missing information was obtained through contact with athletic directors, athletic trainers, coroners, sports information directors, and Internet searches. Demographic information was found in other NCAA reports (Harmon et al., 2012).

The authors found that there were 7,432 NCAA football athletes who carried Hb AS between 2004 and 2008. There were 7,102 Hb AS carriers among black athletes and 331 among non-black athletes. The authors estimated that approximately 7% of black NCAA athletes had Hb AS, while approximately 0.16% of non-black athletes carried Hb AS. There were 12 exertional deaths, 5 of which were Hb AS related deaths, representing 42% of the exertional deaths. Harmon et al. (2012) found that among football players of all divisions the relative risk of exertional death among those with Hb AS compared to those without Hb AS was 29. Among division 1 football players, this was even higher at 37. The authors found that Hb AS is associated with an increased risk of death in football players and argue that in order to prevent death in Hb AS carriers, especially those who play college level football, strategies to prevent and treat Hb AS crises are needed (Harmon et al., 2012). They suggest, "screening to identify athletes followed by education, targeted modifications of training and conditioning, an acclimatization schedule, cessation of activity with any symptoms, and allowing Hb AS athletes to set their own pace" (Harmon et al., 2012).

In 1995, VanCamp et al. used a retrospective cohort design to examine nontraumatic deaths among high school and college athletes. The National Center for Catastrophic Sports Injury Research (NCCSIR) data was searched for nontraumatic

deaths occurring between July 1983 and June 1993, as well as a news clipping service. The authors defined nontraumatic deaths as those with symptoms beginning during or within 1 hour following high school or college sporting events. One hundred and sixty non-traumatic deaths were identified and complete autopsy data was available for 126 of the deaths. Seven of the 160 deaths were found to be from rhabdomyolysis and sickle cell trait, accounting for 5% of the non-traumatic deaths. Six of these deaths occurred in males and 1 in a female; all were AA (VanCamp et al., 1995).

Limitations to this study include underreporting of non-traumatic sports deaths occurring in the off-season and not related to athletics. Additionally, autopsies were performed by multiple medical examiners, which could introduce interobserver biases. Lastly, the authors twice state that 136 were used for the purpose of this study while 137 are reported in their tables. The authors state that the 7 deaths due to rhabdomyolysis associated with Hb AS were not expected as Hb AS (at this time) was believed to be a benign condition that did not influence health outcomes. They discuss recent studies reporting that Hb AS may be a key component in the development of exertional rhabdomyolysis in athletes, especially in well-muscled Hb AS athletes performing maximal exercises during preseason conditioning (VanCamp et al., 1995).

The last retrospective cohort study included in this review was undertaken by Maron et al. in 1996. Maron collected cases of sudden death in athletes beginning in 1985. Data were also collected from the cardiovascular pathology registry of Baylor University Medial Center, news stories, the NCCSIR, and through reports and contacts with high schools and colleges. Participants were included in the review if they were

under the age of 35 years, a competitive athlete, had available autopsy information, and showed no use of drugs in toxicological screenings after death. The authors identified 158 deaths for inclusion from 1985 through 1995. Twenty-four participants died from noncardiovascular conditions. Among those dying from cardiovascular causes there were 12 deaths in individuals under 14 years, 83 in high school athletes, 30 in college athletes, and nine in professional athletes. Hypertrophic cardiomyopathy (HCM), which is when the heart muscle becomes thick, was found in 48 individuals and was the leading cause of death in this study. In 6 of these 48 deaths there were comorbidities that contributed to death: while only 1 of these deaths was associated with Hb AS. The authors do not discuss the role of Hb AS in sudden death. They state that of the 130 cases who had individual cardiac evaluations and standard preparticipation screenings only 2 of the cases were denied eligibility while only 6% of the cases were correctly diagnosed. They conclude that preparticipation screening needs to be more rigorous in order to identify potential health risks and prevent sudden death in athletes.

Retrospective Case-Series in Military Personnel

The earliest study included in this review was conducted by Jones et al. in 1970. It is a small retrospective case-series examining four deaths among African American military recruits at 1 training facility between March 1968 and February 1969. These deaths were the only four that occurred among African Americans at this facility during this time period. All participants were reportedly healthy and in good athletic shape before reporting for basic training. This particular training facility was located at an altitude of 4,060 feet above sea level. Three cases were 21 years old

and 1 was 19 years old. The recruits who were 21 years old all collapsed during their first day of training. The 19-year-old recruit collapsed during his 21st day of training. Sickle cell status was confirmed upon autopsy in all cases. Case 1 collapsed, but regained consciousness at the hospital where he complained of shortness of breath and faintness. Within the hour he became hypotensive and combative and then lost consciousness. He was treated for hyperkalemia and hypotension, but did not improve and died 24 hours after his initial collapse. Case 2 fell ill while running 1 mile and was resuscitated once he arrived at the medical clinic. He also became hypotensive and died 25 hours after he collapsed. In the third case the recruit complained of faintness, but continued participating in a 20-yard low crawl whereupon he passed out. He was pronounced dead upon arrival at the medical clinic. The fourth recruit complained of numbness in his legs and faintness. He lost consciousness while running around the barracks, but regained consciousness and complained of leg weakness and pain. He became apneic and died 8 hours after his initial collapse. The authors proposed that the recruits all entered into sickling crises, which became fatal. The authors believe the crises were caused by the arrival at a high altitude and moderate-to-severe exercise despite the recruits apparent good health. Jones et al. (1970) conclude that under certain circumstances Hb AS can be a risk factor for sudden death (Jones et al., 1970).

Retrospective Cohort Studies in Military Personnel

The first study discussing Hb AS and sudden death during physical exertion in military recruits was conducted by Kark et al. in 1987. The retrospective cohort study was conducted to determine risk of Hb AS and age on sudden death among similar

populations: recruits with Hb AS and those without. Participants were all enlisted recruits between the ages of 17 and 34 years during the years of January 1, 1977 through December 31, 1981. Data on the recruits was gathered from the Defense Manpower Data Center and Accession Operations in the Department of Defense. Mortality data were identified through morgue logs, patient administration logs and files, and autopsy files from 17 hospitals serving 15 basic training centers. Any participants who were transferred to civilian facilities were tracked through patient logs and outside records (Kark et al., 1987).

Recruits were divided into those with Hb AS and those without Hb AS as well as black and non-black groups. Recruits were also grouped by unexplained and explained sudden death or non-sudden death. In total, 62 deaths were identified. Among all Hb AS recruits there were 12 unexplained sudden deaths 1 explained sudden death in black recruits, and no sudden deaths either explained or unexplained in non-black recruits. Among those without Hb AS there were 5 unexplained and explained sudden deaths each in black recruits, and in non-black recruits without Hb AS there were 11 unexplained sudden deaths and 8 explained sudden deaths. Non-sudden deaths only occurred in 3 black recruits without Hb AS and in 17 non-black recruits without Hb AS. The unexplained sudden deaths of 13 black recruits with Hb AS were all exercise related. Additionally, of the 42 sudden deaths in all groups, 40 of them were exercise related (Kark et al., 1987).

The authors found the unexplained sudden death rate among Hb AS recruits to be 32.2 and among non-Hb AS recruits to be 1.2, resulting in a relative risk of 27.6 with a wide confidence interval of (9, 100) that was statistically significant with a p-

value < 0.001. All sudden deaths among Hb AS carriers compared to all sudden death among non-Hb AS carriers resulted in a relative risk of 15 with a confidence interval of (6, 38) and a p-value of <0.001. Interestingly, the authors then subdivided these groups by age into the ranges of 17 to 18, 19 to 20, 21 to 22, 23 to 25, 26 to 30, and 31 to 34. Using these groupings Kark et al. (1987) found an increasing trend of death among Hb AS recruits as age increased. This trend proved to be statistically significant with a p-value of 0.04. There were no deaths among Hb AS carriers between the ages of 31 and 34 years, but the second oldest grouping had a relative risk of 95.1 with the youngest grouping having a relative risk of 12.9. The authors note that this is due to an increasing rate of death as age increases while the pool of sudden unexplained death remained the same (Kark et al., 1987).

Kark et al. (1987) suggested that their results may not be generalizable to populations of athletes, those with advanced military training, or heavy-labor workers, and state that previous research has not examined large enough study populations to determine whether higher death rates occur in Hb AS carriers. The authors found that Hb AS is not a risk factor for explained sudden death or non-sudden death, but conclude that it is a risk factor for exertion-induced unexplained sudden death among military recruits (Kark et al., 1987).

The most recent study regarding Hb AS in military personnel was also a retrospective cohort study and was conducted by Eckart et al. in 2004. The authors' basic research question was to determine causes of nontraumatic death in military recruits. Participants were identified through the Department of Defense Recruit Mortality Registry, which has records for every recruit death and autopsy.

Nontraumatic deaths were eligible for inclusion in this study if they were classified as idiopathic or from exertional heat illness, cardiac, exercise-related, vascular, or asthma and occurred at a military training site before initial training was completed and while the recruit had an enlisted status in the armed forces. The study spanned 25 years and included deaths occurring from 1977 through 2001. A death was considered sudden if symptoms began and became fatal within 1 hour. Of the 277 deaths identified by the authors, only 126 were included in the study. Participants were between the ages of 19 and 35 years and included 15 females and 111 males. The authors found 3 non-cardiac deaths from sickle cell crises. However, there were 12 exercise related deaths in which the decedent was an Hb AS carrier. The authors state that Hb AS is associated with idiopathic sudden death when compared with other causes of death, resulting in a p-value of <0.001. Ultimately, Eckart et al. (2004) found that Hb AS was associated with 27% of idiopathic sudden deaths, which is similar to data found by Kark et al. (1987). However, as the study encompassed the recruit deaths used in Kark et al.'s (1987) study, the authors argue that it is not additional supporting evidence of the association (Eckart et al., 2004).

Discussion for Exercise Deaths Systematic Review

Sickle cell trait is usually a benevolent condition that is not associated with adverse clinical manifestations in the general public or in the athlete population. Indeed, many Hb AS carriers have perfectly normal lives which allow them to engage in strenuous exercise and even military service. However, it has become clear that the trait sometimes results in adverse health events during athletic or military training. This review was undertaken to gather information on Hb AS deaths among

military and athlete populations and determine whether or not Hb AS individuals who engage in strenuous physical activities face an increased risk of death. Evidence in this review showed that there were Hb AS related deaths in every sample, but that these were very rare. This does suggest, however, that carrying Hb AS may result in an increased risk of ill health and possibly death during or immediately following strenuous physical activities. The results of this review suggest that further research on the topic is warranted as not all Hb AS individuals face this increased risk and it is important to determine which environmental and genetic factors aide in this increased risk.

Although the genetic reason for the different clinical outcomes of sickle cell anemic patients is now better understood (different SNPs modify fetal hemoglobin production and result in different disease courses), the possibility that different SNPs may be the reason why some Hb AS carriers have a mild outcome while others suffer from sickling events has not been well explored. An excellent recent review on the physiological response of Hb AS carriers during exercise asks why some Hb AS individuals collapse under stress, but does not raise the possibility that differences in these SNPs may explain these events (Connes et al. 2008).

Research on physical exertion among Hb AS individuals has recently focused on athletes for two reasons. First, a number of sudden deaths have made media headlines over the last decade (Elseveth, 2013; George, 2011), and second, the armed forces have had protocols in place to identify and protect Hb AS individuals. The U.S. Navy, Air Force, and Marines currently screen for Hb AS after accession. The army screened for Hb AS until 1991, but no longer does. Instead, it now restricts

individuals going into specialized placements by testing their hematocrit levels. These individuals are only screened for Hb AS and alpha-thalassemia if test results indicate they are anemic. The Air Force tests Hb S levels among recruits with Hb AS and does not allow individuals who have levels higher than 45% of total hemoglobin to remain in the service. Sickle cell trait individuals with less than 45% Hb S are allowed to choose whether to leave the Air Force or remain as enlisted military personnel. The Navy, by far, has the most proactive approach to Hb AS. Naval recruits with Hb AS are given a red wristband to indicate that they are only allowed to perform light duty activities until their hemoglobin levels have been tested. Once tested, those with Hb S levels above 45% are issued a reddish orange belt to wear while engaging in strenuous physical activities as well as red dog tags. They are also counseled regarding risk of sickling crisis at high altitudes and without proper hydration and taught the symptoms of exertion-induced sickling. However, Hb AS recruits are not counseled on the risk of exercise related sudden death (Mitchell, 2007; O'Connor et al., 2012).

Strengths of this Review

A major strength of this study is its inclusion of studies with large sample sizes. These populations are also the best representation of Hb AS deaths in the U.S. over the last few decades as Hb AS was not added to the international classification of diseases index (ICD) as a cause of death until relatively recently and deaths in the general population may have been misclassified (Anonymous, 2011). Additionally, the inclusion of articles published during any point in time allowed for more complete inclusion of articles pertinent to this review.

Limitations of this Review

This study is limited in a number of ways. To begin, it may not be an accurate representation of the available literature since articles were only eligible for inclusion if they had been published in English. Although the search terms used were broad, they may not have revealed all articles eligible for inclusion in this study. Additionally, only the author reviewed articles for inclusion and scored them for methodological quality. The use of multiple scorers may have resulted in the inclusion of different articles or the exclusion of ones reviewed. Reviewers with a high inter-rater reliability would have helped this review achieve higher validity and reproducibility. Articles that were not available for inclusion in this review are also a limitation. The populations chosen for this review presents another limitation; military and sports populations are comprised of primarily very physically active and physically fit individuals who tend to be in their late teens to early 30's. As such, they may not accurately represent individuals with SCT and the risks they face with different lifestyles. Further, many individuals in this study were male and thus both genders were not equally represented. Gender may be a confounder that this review did not take into consideration.

Limitations of the Studies Included in this Review

Limitations of this study stem largely from the individual limitations posed by each study included in the review. The small sample of studies included is also potentially problematic as it does not fully represent all sudden death from Hb AS in the U.S. In fact, this is impossible due to differential diagnostic procedures in place throughout the U.S. Furthermore, a deoxygenated state occurs upon death, which

can result in sickling. This may skew results in both directions as some medical examiners dismiss this as a result of death while other may list it as the cause of death.

Conclusion of Exercise Deaths Systematic Review

Sickle cell trait has been linked to ill health and sudden death in carriers. It is evident that it can no longer be considered a benign condition that does not affect health outcomes. While not all Hb AS carriers experience ill health, they should be aware of their status and the fact that they may suffer adverse health events. These events may be mild or severe and should be treated appropriately by medical personnel to minimize the amount of harm suffered by the patient. Medical care providers, athletic trainers, and coaches should be educated on the risks and symptoms so that Hb AS carriers can be treated properly upon collapse and increase their chance of surviving a sickling crisis without lasting health effects.

It is almost as if Hb AS athletes have been medically ignored; they are healthy college athletes who might 1 day collapse and die. It is obvious that not all Hb AS individuals are the same, and that athletic departments need to be aware that some are at greater risk than others. Though many studies have examined hemorheological changes between those with hemoglobinopathies and those without, it is still not well understood what precipitates sickling changes in those with Hb AS. Sickle cell trait is associated with an increased risk of sudden death during and immediately following exercise. It is time that it is recognized as a cause of poor health, a risk factor for sudden death, and as a significant burden for some carriers.

Tables and Figures

Table 4. Count of Hb AS as a Reported Cause of Death in all U.S. States between 2000-2011*[$]

Alabama	3
Alaska	1
Arizona	2
Arkansas	does not record SCT as a COD
California	does not record SCT as a COD
Colorado	13
Connecticut	1
Delaware	no data
Florida	10
Georgia	no data
Hawaii	no data
Idaho	0
Illinois	2
Indiana	2
Iowa	1
Kentucky	0
Kansas	0
Louisiana	no data
Maine	0
Maryland	1
Massachusetts	1
Michigan	does not record SCT as a COD
Minnesota	3
Mississippi	3
Missouri	does not record SCT as a COD
Montana	0
Nebraska	0
Nevada	0
New Hampshire	1
New Jersey	4
New Mexico	0
New York	4
New York City	3
North Carolina	5
North Dakota	0
Ohio	no data
Oklahoma	1
Oregon	does not record SCT as a COD
Pennsylvania	no data
Rhode Island	no data
South Carolina	does not record SCT as a COD
South Dakota	0
Tennessee	2
Texas	6
Utah	0
Vermont	0
Virginia	4
Washington	1
Washington, D.C.	0
West Virginia	0
Wisconsin	no data
Wyoming	0
Total	74

*Not all states record deaths from SCT.

[$]Data was not available for the full time period in every state.

Table 5. Search Terms Used to Identify Articles Potentially Eligible for Inclusion in this Review

Search Terms Used					
sickl*	cell	trait	athlet*	exercis*	physical
sickl* and cell and trait and athlet*					
sickl* and cell and trait and exercis*					
sickl* and cell and trait and physical					
sickl* and cell and athlet*					
sickle and cell and exercis*					
sickl* and cell and physical					

Table 6. Methodological quality assessment of studies included in this review

Author	Year	Hypotheses (1 pt.)	Study Design (5 pts.)	Sampling Procedures (3 pts.)	Inclusion of Possible Confounders (3 pts.)	Statistical Analysis (4 pts.)	Results (1 pt.)	Final Score (max 17 pts.)	Final Score (%)
Eckart et al.	2004	1	3	3	3	3	1	14	82
Harmon et al.	2012	1	3	3	1	1	1	10	59
Boden et al.	2013	1	3	3	2	1	1	11	65
Harris et al.	2012	1	3	3	3	2	1	13	76
Van Camp et al.	1995	1	3	3	3	2	1	13	76
Kark et al.	1987	1	3	3	3	4	1	15	88
Maron et al.	1996	1	3	3	3	1	1	12	71
Jones et al.	1970	1	1	2	3	0	1	8	47

Table 7. Descriptive table of reported measures for included studies

Author	Year	Location	Study Sample (n)	# SCT Deaths	Odds Ratio	Relative Risk	95% CI	p-value	Other Measure Reported
Boden et al.	2013	U.S.	243	11	66.6 [ξ]	9.7 [δ]	(14.4, 308)		
Eckart et al.	2004	U.S.	277	15				<0.001	
Harmon et al.	2013	U.S.	273	5		15			
Harris et al.	2012	U.S.	2462	23					
Jones	1970	U.S.	N/A	4					
Kark et al.	1987	U.S	62	13					31 deaths/100000 people [§]
Maron et al.	1996	U.S.	158	1					
Van Camp et al.	1995	U.S.	160	7					

[§] Risk of sudden unexplained death attributable to SCT

[δ] Comparing 2nd decade of study to 1st decade of study

[ξ] Comparing college level deaths to high school deaths

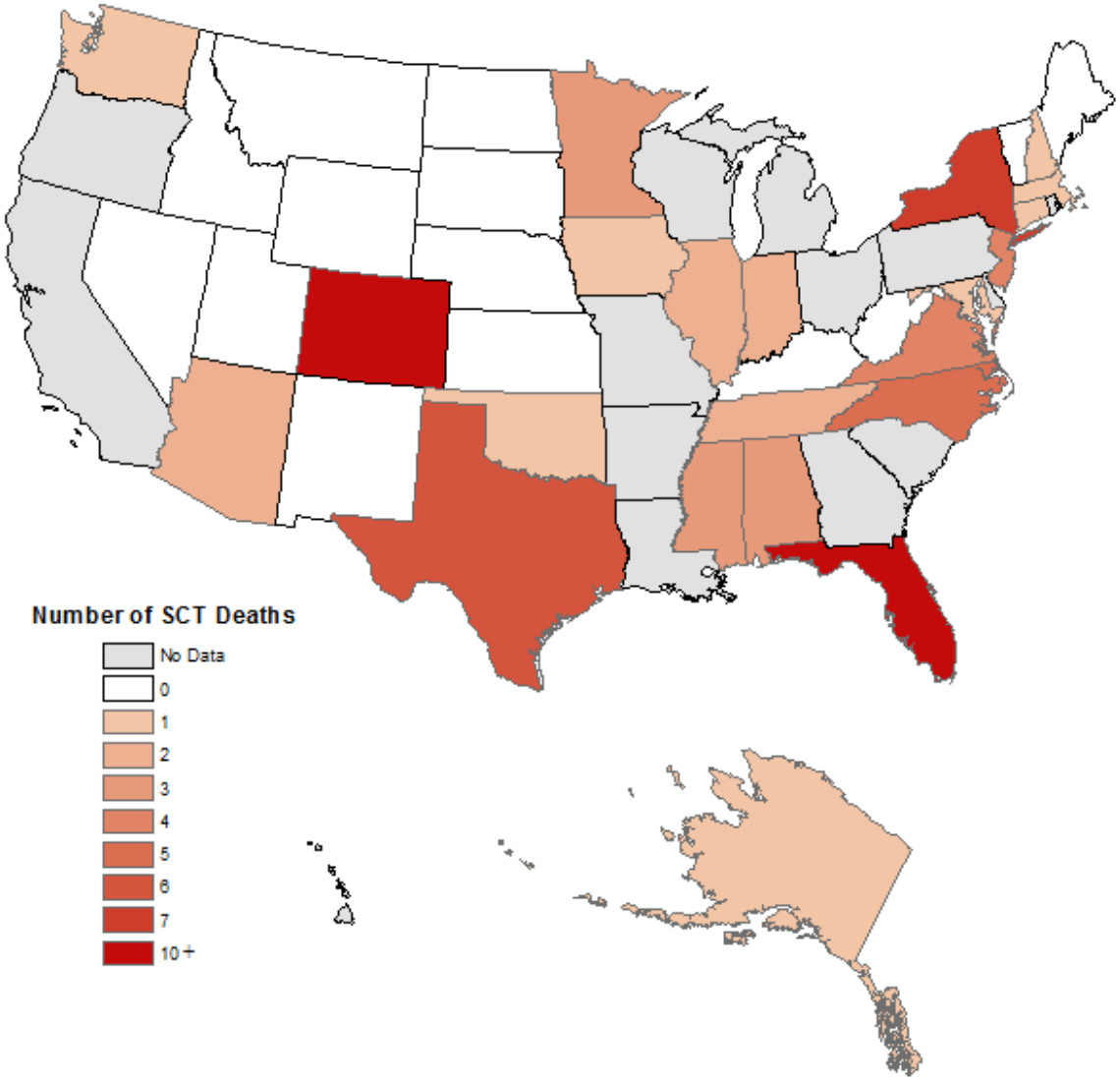

Figure 3. Geospatial representation of Hb AS related deaths by U.S. state*
*All deaths occurred between 1999 and 2010.

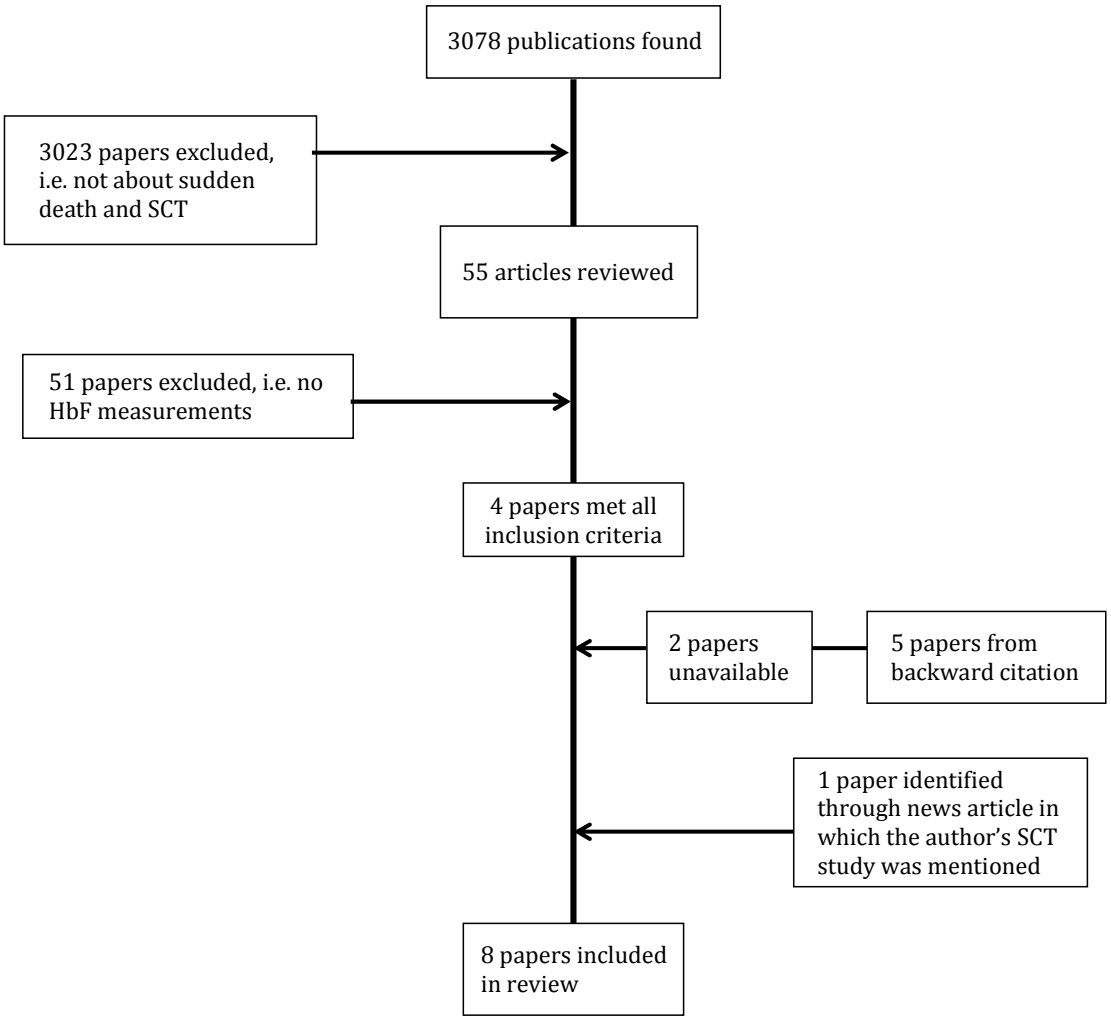

Figure 4. Flow chart detailing articles identified for inclusion this review

Hypotheses (maximum possible = 1)

- The hypothesis/research question of the study not clearly described = 0
- The hypothesis/research question of the study clearly described = 1

Study Type (maximum possible = 5)

- Systematic Review = 5
- Randomized Control Trial = 4
- Cohort Study = 3
- Case-Control = 2
- Case Series OR Cross Sectional = 1

Sampling (maximum possible = 3)

- Sample Source and Size
  - Routine data = 0
  - Sample size less than 50 = 1
  - Sample size more than 50 = 2
- Selection Criteria
  - Selection of cases/controls/population not well described = 0
  - Selection of cases/controls/population well described = 1

Methodology (maximum possible = 3)

- Inclusion of other variables:
  - Age = 1
  - Race = 1
  - Hb AS Status = 1

Statistical Analyses and Results (maximum possible = 5)

- Statistical Analysis (2)
  - Statistical methods not described = 0
  - Statistical methods clearly described = 1
- Statistical outcomes (2)
  - Statistical outcome not clearly reported = 0
  - Some statistical outcomes clearly reported (e.g. CI's but not P-values) = 1
  - Statistical outcomes clearly reported (e.g. CI's and P-values) = 2
- Results (1)
  - Research questions/hypotheses not clearly answered/tested = 0
  - Research questions/hypotheses clearly answered/tested = 1

Maximum Score = 17

Figure 5. Methodological quality scoring sheet used to assess the studies included in this review

Methods

Recruitment

Participants were eligible for inclusion in this study if they were over the age of 18, male, had tested positive for Hb AS, and had played organized football at some point in their lives. Participants were recruited via Facebook, Twitter, e-mail, phone calls, and word of mouth. During the first phase of recruitment a Facebook page for Hb AS carriers was created. Through the page the authors "friended" multiple sickle cell community, awareness, and advocacy groups. The also used this medium to communicate with football groups in order to inform them about the study. No participants were found through these means, though many people shared personal experiences of pain and sickling crises with the author that were used as supporting evidence for our study. The website address can be found in Appendix 2. No participants or information was gathered through twitter. The second phase of recruitment consisted of e-mails sent out on the behalf of the authors to team physicians for collegiate football players containing a recruitment letter and promotional flyer. The third phase of recruitment included phone calls to all Division 1 NCAA football teams. The next phase included phone calls to Division II and III schools. The fifth phase of recruitment consisted of e-mails to all historically black colleges and universities (HBCUs). All phases of recruitment were ongoing and overlapped once they were initiated.

In early summer 2013, the author was able to register the study on the website www.ClincialTrails.gov, which provided a small measure of exposure for the study. In July of 2013, the author connected with a team physician at the University of Washington, Dr. Kim Harmon, who had recently published a paper on the risk of Hb AS in football athletes. Dr. Harmon sent e-mails on the authors' behalf to trainers and team physician in the Pacific-12 Conference. Data for this thesis is in phase 1 of the study and is purely descriptive.

In July and August of 2013, the authors of this paper we able to partner with the Sickle Cell Foundation, who have agreed to help with recruitment for phase 2 of this study. With their assistance, the authors believe that they can recruit a total of 100 participants. All participants from phases 1 and 2 will undergo genetic testing to determine whether or not they carry SNPs associated with increased levels of Hb F and their Hb S haplotype. Using this information the authors hope to examine whether or not Hb S haplotype and genetic modifiers for HPFH and high levels of Hb F are associated with better and worse health outcomes.

Phase 1 - Survey Data

Packets containing all data collection materials and consent forms were mailed to participants once they agreed to participate in the study. Data for phase 1 consisted of a set of clinical variables gathered through a survey completed by the participant. A copy of the survey can be found in Appendix 3. Data were collected on the variables of age, height, weight, football position played, whether or not the athlete felt he maintained adequate hydration during training and conditioning, where or not he had experienced sickling crises, heat illness, or dehydration, and

location of these episodes. Participants were given the option of providing their medical records to our team clinician, Dr. Eduardo Gonzalez, though at this juncture no medical records have been received.

Phase 2 – Genetic Analyses

Participants collected or will collect a genetic sample via a buccal swab and send the sample directly to the School of Human Evolution & Social Change at Arizona State University. There, Dr. Anne Stone will oversee all genetic typing. She states,

> DNA will be extracted following standard procedures, and SNPs involved in fetal beta globin regulation will be typed (Wajcman and Riou 2009). DNA will be extracted using standard phenol-chloroform method (Sambrook and Russell 2001). The DNA will then be used to assess three SNPs that have been linked to fetal globin production: Rs4671393 in BCL11A, Rs9402686 in HB S1L-MYB, and rs7482144 downstream of HBG1 (Galarneau et al. 2010). For each SNP, the polymerase chain reaction (PCR) will be used to amplify the DNA containing the SNP. For rs7482144, the primers and PCR conditions used are as previously published (Sutton et al. 1989), while new primers were designed for the other two SNP regions. The PCR products containing Rs4671393 and rs7482144 are digested with the restriction enzymes BtsIMutI and Xmn1, respectively, to detect the presence or absence of the SNP. For both restriction digests, the products will be visualized on a 2% agarose gel with a 1 kb ladder size standard. The PCR products containing Rs9402686 in the HB S1L-MYB gene region are sequenced in the ASU core laboratory using the ABI 3100 sequencer. Sequence data will be analyzed using SeqMan Pro in the Lasergene core suite (DNAstar).

Results from the DNA typing will be sent to the author, who will correlate the results with the surveys collected from participants and will be analyzed by the team clinician, Dr. Eduardo Gonzalez. Fisher's exact test will be used to test associations between haplotypes, SNPs, and health outcomes. Fisher's exact test is particularly well designed to deal with low-frequency events such as we anticipate in this project and will be used to test the hypothesis that SNPs associated with production of Hb F influence health outcomes. We will perform a logistic regression analysis in which the

dependent variable is the dichotomous variable "presence of adverse health outcomes" and "absence of adverse health outcomes". During phase 1 the predictive model will include only clinical variables. During phase 2 the predictive model will be expanded to include clinical variables and SNP genotypes. Odds ratios comparing the occurrence of adverse health outcomes between SNPs associated with the production of Hb F will be calculated.

Statistical Analyses

Given the small sample size, the author was only able to run Fisher's exact tests to compare survey responses. Analyses were run using SAS 9.3. All SAS codes used can be found in Appendix 4.

Results

Facebook

Initial results were gathered through personal and public communications on a Facebook page the author created titled "Carriers of Sickle Cell Trait". Communication with 5 individuals from the U.S., Uganda, and the United Kingdom (U.K.) add support to the proposition that Hb AS carriers suffer poorly explained ill health. A woman in the U.S. reported that she is physically active, rides her bike 3 to 4 miles every other day and walks the same distance 3 to 4 times a week, but has unexplained pain in her arms and legs in cold weather, especially during the winter. A different woman in the U.S. states that she suffers from bone pain, particularly in her back, which becomes so strong she is often hospitalized and put on prescription painkillers. Another Hb AS individual in the U.S. informed the author that he suffers from body pain frequently, but his doctors are unable to explain it. He has asked his doctors about a connection to his Hb AS status, but they have dismissed it and told him that it does not cause health issues. A woman from the U.K. and her 2 sons are all Hb AS carriers. Both of her sons suffer frequent pain and fatigue. Sometimes her youngest son cannot get out of bed due to the pain, but doctors have been unable to explain it. During an emergency room visit for 1 of her sons she informed the doctor that her son had Hb AS and he responded by simply stating that they were not black. The woman has had unexplained health issues involving her spleen and liver. Interestingly, she also reports pain in her joints during cold weather. Lastly, a

Ugandan man reports that he has had unexplained pain for years. He has undergone 50 diagnostic tests in an effort to find the source of his symptoms, but the only thing he has tested positive for has been Hb AS (Personal Communications, 2012; Personal Communications, 2013).

Surveys

Between October 2012 and June 2013, 14 participants were recruiting for the study. An additional 6 participants have been recruited between August and September 2013, thanks to the help of Dr. Kim Harmon. All were included in the data used for this thesis. The author received 20 surveys while 21 samples were received at the SHEMC. Participant number 4 returned a sample to the lab, but did not return a survey. The vial containing his sample was, however, empty upon receipt leaving 20 participants. The data set containing deindentified responses can be found in Appendix 5.

Fourteen of the participants felt that they maintained a safe and healthy level of hydration during training, 3 participants felt that they did not, 1 participant did not know whether or not he kept this level, and 2 participants did not answer. Fourteen participants felt they were able to maintain an adequate level of hydration during athletic competitions, 4 felt they were unable to maintain an adequate level, and 2 participants did not respond. Twelve of the participants reported that they had unhealthy dehydration (UD) at some point, while 8 reported that they had not experienced UD. Symptoms reported by the 12 participants who responded positively to UD include: dry, sticky mouth, sleepiness or being tired, thirst and/or extreme thirst, decreased urine output, headache, dizziness or being lightheaded, rapid

breathing, and rapid heartbeat. Among these participants the frequency of UD varied from 1 to 100 experiences. One participant responded that he felt unusually dehydrated 10% of the time and 1 responded that it happened 2 to 3 times a week. Unusual dehydration was experienced in Pennsylvania, New York, South Carolina, Washington, California, Florida, and Colorado. Two participants responded that they had not experienced UD at any point, but 1 indicated that he had experienced dizziness or lightheadedness twice and the other replied that he did not know what symptoms he had experienced. It is unclear if they experienced symptoms, but were unaware that they had been dehydrated, or did not read the instructions clearly and skip this question as instructed when answering "no".

Two participants had been diagnosed with heat illness (HI) at some point, 17 participants had never been diagnosed with HI, and 1 participant did not answer. The 2 participants who had experienced HI had each been diagnosed during early season training. One participant who had not experienced HI replied that he had been diagnosed once, and another responded that he did not know how many times he had been diagnosed. The 2 participants who had HI experienced symptoms of hard, tense muscles, weakness, fatigue, headache, excessive thirst, and agitation. Three participants who had not been diagnosed with HI reported that they had experienced similar symptoms or that they did not know or preferred not to answer what symptoms they had experienced. One participant with HI replied that the intensity of his workout immediately preceding their episode of HI was very hard while the other preferred not to answer. Four participants indicated the intensity of their workout before diagnosis with HI, but stated that they had not ever been diagnosed with HI.

The participants who had been diagnosed stated that the episodes occurred in Washington and South Carolina. Two other participants who had not had HI reported that they had experienced episodes in Washington (this participant reported he had experienced HI twice in this location) and Colorado, while 2 other reported that they preferred not to answer where they had experienced HI.

Section four of the survey asked personal opinion questions about the participants' experiences in comparison with their peers. Fifteen respondents said that they did not feel their muscle pain lasted for longer periods of time than their peers', 2 responded that they did feel their pain lasted longer, and 3 did not answer. Four participants felt that it took their muscles longer to recover from workouts or training than their peers', 12 felt this was not the case, 3 participants did not answer, and 1 preferred not to answer. When asked if they felt they experienced higher levels of muscular pain in comparison to their peers, 13 participants responded that they did not feel this was the case, 2 felt they did experience higher levels of pain, 2 did not know, and 3 did not answer. The 2 respondents who felt they had experienced more pain than their peers indicated that this pain was mild or moderate in comparison. Three participants who did not feel they had higher levels of pain indicated that they did not know or preferred not to answer their level of pain in comparison to their peers'.

Five of the 20 participants reported that they had been diagnosed with exertional sickling (ES), with the remaining 15 responding that they had never been diagnosed with ES. The 5 participants with ES reported symptoms of leg pain or weakness, low back pain or weakness, muscle cramping that spread throughout their

body, and rapid breathing. One participant preferred not to answer, and 1 participant who had not been diagnosed with ES also indicated that he preferred not to report what symptoms he had. The 5 participants who responded yes indicated that they had experienced ES 1, 3, 7, 8, and a "few" times. These episodes occurred in South Carolina (4 of the 5 participants) and Washington.

Respondents were also asked what symptoms they had experienced during workouts or training from a compiled list of all symptoms from HI, US, and ES. Eighteen of the participants responded to this question while 2 chose not to answer. Among the symptoms experienced were: a dry, sticky mouth, sleepiness or tiredness, thirst or extreme thirst, rapid breathing, fatigue, decreased sweating, breathlessness, low back pain or weakness, decreased urine output, headache, dizziness or lightheadedness, low blood pressure, rapid heartbeat, hard or tense muscles, nausea or vomiting, fainting, agitation, weakness, confusion or anxiety, hot, flushed, or dry skin, blood in urine or stool, increased body temperature, convulsions or seizures, severe or debilitating heat cramps, cold and clammy skin, leg pain or weakness, and muscle cramping that spread throughout the body. One participant indicated that he preferred not to answer this question, did not know what he had experienced, and had experienced "other" symptoms. He did not indicate what these "other" symptoms were, as we had requested if choosing this option. These symptoms were experienced in South Carolina, Pennsylvania, Florida, California, New York, Washington, and Colorado. One of the participants who chose not to answer indicated that he did not know where he had not experienced symptoms. Two

participants each responded that they did not know where they had experienced symptoms or preferred not to answer.

The remaining questions on the survey dealt with demographic information such as height, weight, biological background, racial self-identification, whether they smoked or had asthma, whether they had any hemoglobinopathies besides Hb AS, average resting blood pressure, and the position they played on their football team. One participant did not answer any demographic questions. Nineteen participants weighed between 115 pounds and 345 pounds, with a mean weight 201 pounds. However, 17 of the participants weighed within a 106 pound range, from 157 263 lbs. Participants' height ranged between 61 and 78 inches, with a mean height of 71.72 inches. All participants were over the age of 18, with an age range of 18 to 24 years with an average of age of 19.9 years. None of the 19 participants who responded reported that they had asthma; 18 stated that they did not smoke, and 1 indicated that he preferred not to answer if he smoked. Only 1 participant knew his resting blood pressure, which was 120/80 mmHg. Nineteen participants responded that they did not have any other hemoglobinopathies besides Hb AS.

Five participants play(ed) safety, 2 cornerback, 3 outside linebacker, 2 defensive end, 2 wide receiver, and 1 each defensive tackle, tight end, quarterback, and fullback/running back. One safety also played cornerback, and 1 defensive end also played tight end. Seven participants did not know their biological background, 2 reported that their family was from the Middle East and North Africa, 3 said that they were of North American of African descent, 3 stated that they were of Caribbean of African descent, and 5 did not respond. One respondent who indicated that he was

Caribbean of African descent also marked that his biological background included both Caribbean of Northern and Southern European descent. When asked how they self-identify racially, 17 participants indicated that they say they are "Black, African American, or Negro", 1 replied that he is "Cuban", and 2 did not respond. One of the "Black, African American, or Negro" respondents also identifies as "American Indian or Alaska Native", and the "Cuban" also identifies as "White".

Of the 12 participants who experienced UD, only 1 of them was also diagnosed with HI. Two of these 12 were also diagnosed with ES. One of the participants diagnosed with HI was also diagnosed with exertional sickling. None of the participants reported experiencing all 3 health issues. These results can be found in Table 8. All tables and figures for this section can be found at the end of this chapter The frequency of ES, UD, and HI were compared with each other and with the variables adequate level of hydration (ALHY), safe and healthy level of hydration (SHLHY), muscle pain severity in comparison to peers (COMPPN), recovery time in comparison with peers (COMPRECV), and muscle pain duration in comparison to peers (COMPMPD) in individuals who experienced ES, UD, and HI. To determine whether or not there were associations between the variables SHLHY, ALHY, COMPPN, COMPRECV, and COMPMPD they were all compared using Fisher's Exact Tes

Given the small sample size, no significant results were expected. However, of the 20 tests run, the comparison of COMPRECV by COMPMPD produced a statistically significant right-tailed p-value $\geq$ F of 0.0049. The results of this test indicate that the probability of the player feeling his muscle pain lasts longer than his peers' is greater among those who feel it takes their muscles longer to recover than their peers' than

among those who do not feel it takes their muscles longer to recover than their peers. In other words, players who feel that their muscles take longer to recover compared to their peers were significantly more likely to feel their muscle pain lasts longer than their peers. This result can be found in Table 9. Results from all tests can be found in Appendix 6.

Tables

Table 8. Participant Responses to UD, HI, and ES

Study ID	Unhealthy Dehydration	Diagnosed with Heat Illness	Exertional Sickling
1	2	2	1
2	2	2	1
3	2	1	1
4			
5	2	2	2
6			
7	1	2	2
8	1	2	2
9	2	2	2
12	2	2	2
11	1	2	2
12	1	2	2
13	1	2	2
14			
15			
16			
17	2	2	2
18			
19			
22	1	2	1
21			
22	1	2	1
23	1	1	2
24	1	.	2
25	1	2	2
26	1	2	2
27	1	2	2
28	2	2	2

individual did not participate

. missing data

Table 9. COMPRECV by COMPMPD

Table of COMPRECV by COMPMPD				Cell (1,1) Frequency F	4	Left-sided Pr ≤ F	1	Right-sided Pr≥ F	0.0049	Table Probability (P)	0.0049	Two-sided Pr ≤ P	0.0049
	COMPMPD												
COMPRECV		1	0	Total									
1		4	2	6									
0		0	12	12									
Total		4	14										

Discussion

Genetic Analyses of Hb F

Hemoglobin requires 2 α-globin amino acid chains and 2 ß-globin amino acid chains. The beta chain is encoded by a cluster of genes on chromosome 11 that are each turned on during specific stages of development (Fritsch et al. 1979; Mahajan et al. 2007). During embryonic development the epsilon gene (Hb E1) is active, which causes 2 pairs of identical chains. The chains created by the epsilon gene are alpha-like and beta-like. During fetal development the 2 gamma genes (Hb G2 and Hb G1) are active and after birth 2 adult genes, beta (Hb B) and a minor delta (Hb D) gene are active. Eventually, the Hb D chain is replaced by an adult alpha chain (Hb A), resulting in the "normal" adult phenotype Hb AA, as discussed in the introduction. The chains created throughout development function similarly to the adult alpha and beta chains in order to ensure smooth transition to adult hemoglobin. As the successive genes are turned on, the genes active during the earlier stage are turned off. While each beta globin gene is rather short, the beta globin cluster is ~60 kb long and has a recombination hotspot in the midst of it (just 5' of the Hb B gene) (Antonarakis et al. 1982; Wall et al. 2003). The diversity in the region has been assayed using 6-15 RFLP sites spread over 45-60 kb (e. g. Lapoumeroulie et al. 1992; Pagnier et al. 1984) as well as through resequencing (Galarneau et al. 2010). Among those with Hb SS, more benign clinical profiles were initially linked to specific haplotypes in the beta-globin cluster (Nagel et al. 1985), but have recently been

linked to specific SNPs that influence Hb F expression (Galarneau et al. 2010; Uda et al. 2008). Specifically, three SNPs that have been linked to higher expression of the fetal (HBG1 and HBG2) genes in the ß-globin gene cluster will be investigated. These SNPs include Rs4671393 in BCL11A, Rs9402686 in HB S1L-MYB, and rs7482144 downstream of HBG1 (Akinsheye et al. 2011; Platt et al. 1994; Powars et al. 1984). Ender et al., 2011; Green & Barrall, 2011; Sankaran et al., 2008; Sheehan et al. 2013; Stone, 2013; Thein et al., 2007).

Hb F Level Differences among Hb AS Haplotypes

For many years, better clinical outcomes among SCD individuals were thought to be tied to the sickle haplotype they carried. Sickle cell disease individuals homozygous for a haplotype showed that there was stratification in Hb F levels and clinical outcomes among four of the five haplotypes. Specifically, the Saudi Arabia/India (SAI) haplotype had the highest Hb F levels and the best outcomes, followed by Senegal, Benin, and Bantu. Ngo et al. (2011) report that the SAI and Senegal haplotypes usually have Hb F levels between 11% and 21%. In comparison, Maier-Redelsperger et al. (1998) report that those with the Senegal haplotype usually have Hb F levels of 5.5% $\pm$ 0.8%, those with the Bantu haplotype have Hb F levels of 4.3% $\pm$ 4.1%, and those with the Benin haplotype have Hb F levels of 3.3% $\pm$ 1.6% (Maier-Redelsperger et al., 1998; Ngo et al., 2011; Steinberg & Sebastiani, 2012; Texiera, 2003;).

Fetal hemoglobin may be the most important modifier of clinical outcomes in HB AS and SCD. Higher Hb F levels are associated with fewer and less severe pain episodes in Hb SS individuals. This is due to the fact that increased Hb F production

compensates for lost Hb A production and helps minimize the number of cells that can sickle in response to stressors. Levels of Hb F in Hb AS carriers are generally between 15% and 35%. Presence of fetal hemoglobin itself is not enough to mitigate sickling symptoms, and studies have not been able to determine exact levels of Hb F that are protective. Levels of Hb F over 20% to 30% have been shown to be the most protective. Non-F cells survive for approximately 2 weeks while F cells survive for approximately 6 weeks. Additionally, F cells with higher Hb F levels survive longer than their counterparts with lower Hb F levels and those with higher F cell levels have lower non-F cell survival rates. This differential erythrocyte survival rate can lead to increased Hb F levels. Lower Hb F levels are tied to painful crises and acute chest syndrome. Unfortunately, high Hb F levels can also cause ill health and are associated with silent cerebral infarcts, splenic sequestration, and dactylitis (inflammation of an entire finger or 2, which can be painful) (Adekile et al., 2007; Akinsheye et al., 2011; Bailey et al., 1991; Bhatnagar, 2013; Charache & Conley; 1969; Ender et al., 2011; Forget, 1998; Franco et al., 2006; Jacob & Raper, 1958; Marcus et al., 1997; Ngo et al., 2011; Powars et al., 1984; Powars et al., 1989; Sheehan et al., 2013; Steinberg & Sebastiani, 2012; Whyte et al., 2013).

Levels of Hb F are influenced by many factors including, but not limited to, transcription factors, genetic elements linked to the ß-globin gene cluster, age, α-globin gene number, ß-globin (Hb AS) haplotypes, chromosome remodeling activities, and kinetics of erythroid cell differentiation. It is also somehow linked to the X chromosome, as females often have higher levels than males. In addition, Hb F levels are affected by comorbidity with other hemoglobinopathies such as alpha thalassemia

(Adekile et al., 2007; Adekile & Huisman, 1993; Ma et al., 2007; Marcus et al., 2007). An early study by Chang et al. (1995) found that the F cell production locus (FCP) located at Xp22.2.-22.3 was the most important genetic factor influence Hb F levels among SCD individuals. However, they state that their study was unable to account for 50% of Hb F production variation.

Hemoglobin Levels as Sickling Event Modifiers

Sickling episodes among Hb AS individuals may be influenced by their Hb S levels. Kennedy et al. (1985) found that among military personnel there were no differences in Hb S level for certain outcomes, but that hematological characteristics differed among some groups. Participants were divided into groups of Hb S $\leq$ 30, 30 $\leq$ Hb S < 35, 35 $\leq$ Hb S $\leq$ 40, and Hb S > 40. Statistically significant differences were found between the groups on the variables of age ($p = 0.01$), mean corpuscular volume ($p = 0.01$), and hemoglobin concentration ($p = 0.002$). Age was found to be associated with outcomes of hypertension ($p = 0.000$), myocardial infarction ($p = 0.0002$), and, CVA ($p = 0.0003$). However, the authors state that no relationship was found between Hb S level and vaso-occlusive crisis. The authors suggest that a larger study be conducted to rule out the possibility that relatively rare outcomes such as thrombophlebitis, pulmonary embolism, and hematuria were not associated with Hb S level simply due to chance. Kennedy et al. (19855) conclude, "Regardless of Hb S level, this and other studies suggest that Hb AS in black men is a benign condition"(Kennedy et al., 1985).

Sickling and Pathophysiological Differences During Exercise

The association between physical exertion, Hb AS, and sudden death remains unclear. It does appear that intensity and speed of workouts, especially maximal exertion exercise, and inconsistent training can precipitate a sickling crisis, with sickling levels usually involving between 1% and 25% of erythrocytes. Studies have been conducted on physiological responses in Hb AS individuals in comparison to Hb AA individuals and also those with Hb AS and alpha thalassemia. These studies have included hemorheological and oxygen saturation responses, but have provided contradictory results. An early study by Weisman et al. (1988) looked at cardiopulmonary and gas exchange responses in young and healthy Hb AS individuals. The study showed no statistically significant differences in cardiopulmonary response among Hb AA and Hb S individuals at rest, peak incremental testing, or steady-state exercise testing. In fact, the only difference the authors observed was in the amount of oxygen consumed between groups during testing. Monchanin et al. (2005) also conducted a study where they found maximal exercise performance did not differ between Hb AS and HbAA individuals. However, they do state that Hb AS carriers are more likely to suffer hemorheological alterations than their peers. Gozal et al. (1992) conducted a study to determine whether or not repeated bouts of short, maximal exertion affected their exercise performance. They concluded that among highly trained Hb AS individuals repeated anaerobic and aerobic maximal exercise exertion did not affect exercise performance or recovery, though Hb AS participants were found to have lower blood lactate levels. Chirico et al. (2012), on the other hand, tested oxidative stress among Hb AS and Hb AA at rest and post-exercise. They found

no difference in oxidative stress between the groups, but sate that after exercising Hb AS carriers experience higher levels of oxidative stress, especially among those who do not train regularly (Chirico et al., 2012; Connes et al., 2008; Eichner, 1993 & Eichner, 2010; Ferster & Eichner; 2012; Kerle and Nishimura, 1996; Mitchell, 2007; Monchanin et al., 2005; Ramirez et al., 1976; Weisman et al., 1988).

Although Hb AS is often considered a benign condition, studies have shown that it can result in adverse health outcomes. In 1978, Dr. David Sears compiled literature regarding diseases associated with Hb AS. Literature shows that there is likely an increased risk for bacteriuria and pyelonephritis during pregnancy, glaucoma secondary to hyphema, renal medullary cancer, venous thromboembolism, hematuria, splenic infarction at high altitude, bacteriuria, and hyposthenuria with Hb AS comorbidity. The author suggests that Hb AS may also be associated with avascular necrosis of bone, complicated migraine, proliferative retinopathy, increased fertility, abnormal physical properties of stored red cells, reduced urinary filtration fraction, decreased birth weight of babies born to mothers with Hb AS, intravascular sickling with strenuous exertion, and delayed skeletal maturation (Eichner, 1993; Kerle and Nishimura, 1996; Rodgers, 1988; Sears, 1978; Tsaras et al., 2009).

Factors that may increase the risk of sudden sickling events in Hb AS individuals include dehydration, increased intercurrent infection, hypothermia, temperature, altitude, acidosis, increased blood viscosity, exercise intensity, and local hypoxia. Studies have shown that even at sea level, maximal exertion exercise results in sickling, though it is usually involves less than 1% of erythrocytes. Conversely, Martin et al. (1989) showed that at an altitude of 1,270 meters above sea level sickling can

occur at rest, increases during exercise, and increases significantly under conditions replicating 4,000 meters above sea level. According to Loosemore et al. (2012), sickling deaths are often the results on sepsis due to functional hypersplenism, acute end-organ damage, heat stroke, disseminated intravascular coagulation, VOC, cardiac arrhythmia, multiorgan failure, renal failure, or a combination of these events (Eichner, 2010; Loosemore, 2012; Martin et al., 1989; Mitchell, 2007; Ramirez et al., 1976; Tsaras et al., 2009).

Several studies suggest that Hb AS is related to increased risk of sudden exertional death (Chirico et al., 2012; Loosemore et al., 2012; Mitchell, 2007; Ramirez et al., 1976). Since 2000, there have been 16 deaths among NCAA Division I football players, all of which occurred during conditioning and none of which occurred during game play. Although Hb AS only affects 3% to 4% of the NCAA Division I football players, exertional sickling was responsible for 10 of these 16 deaths. These deaths produce a 16 to 21 fold excess risk of death in this group. As Eichner (1993), poignantly states, "Athletes – with Hb AS or not – should train wisely, stay hydrated, heed environmental stress and early symptoms, and never charge recklessly into heroic exercise. It's not worth dying to make the team."

NCAA and Mandatory Testing for Hb AS

In 2010, the NCAA made Hb AS testing mandatory for all Division I athletes. This program was the result of a lawsuit filed by the family of Dale Lloyd, Jr., a 19 year old who was a freshman football player at Rice University. Lloyd experienced a sickling crisis associated with exertional rhabdomyolysis and died in 2006. The screening program, which is the largest instituted since 1970's, became a hotbed for

debate, with many people arguing that it was unnecessarily discriminatory. Many feared that the discrimination found in earlier screening initiatives would be inadvertently replicated under NCAA screening. Sickle cell trait carriers in the military were restricted from certain duties, and Hb AS screening among the general public led to insurance discrimination among carriers. In addition to this, early programs propagated misinformation regarding the presence and outcomes associated with SCD and Hb AS. It has also been suggested that this testing will pave the way for future genetic testing and potential discrimination. Students can opt out of testing by providing earlier test results or signing a waiver releasing their university and the NCAA from all legal liability in case of Hb AS-related adverse clinical outcomes. Many feel that the screening program is merely a legal tactic to avoid future lawsuits. Interestingly, although all newborns in all 50 states are tested for Hb AS, these results may never reach the parent or are not well-explained. If parents are given the results they are often told the condition is benign and are not counseled on potential health risks (Bonham et al., 2010; Koopmans et al., 2011; O'Connor et al., 2012; NCCA Research Committee, 2012; Tarini et al., 2012).

Since 2012, the NCAA has made Hb AS-testing mandatory in all divisions, though players may still opt of out screening. Those testing positive for Hb AS are offered genetic counseling so that they may make more informed health choices regarding their athletic pursuits, personal health, and family planning. A study by Tarini et al. (2012) estimated that among NCAA Division I schools, this screening program would prevent 7 deaths over a ten year period; at a ratio of 1 death prevented per every 141,181 student athletes screened. The authors discuss the prevention strategies

suggested by the NCAA, but note that these strategies are difficult to enforce, especially given the "pressure to play" atmosphere present in sports (Bonham et al., 2010; Koopmans et al., 2011; O'Connor et al., 2012; NCCA Research Committee, 2012; Tarini et al., 2012).

Koopmans et al. (2011) conducted an interesting study surveying pediatrician's attitudes and concerns regarding Hb AS testing in athletes. Most respondents were male and Caucasian, with less than half aware that the NCAA had made Hb AS testing mandatory for Division I athletes. The authors found that mandatory screening was not supported as a whole, but that many preferred screening based on type of sport played and race/ethnicity. Some participants expressed worry that carriers would face discrimination of some kind based on their status. Only 42% respondents felt that college level athletes would face discrimination, 38% felt professional athletes would face it, and 43% and 49% felt carriers would face discrimination through health insurance or life insurance respectively. Seventy-five percent of the pediatricians felt that the athlete or his/her parents should be allowed to sign a waiver in order to decline screening (Koopsmans et al, 2011).

A similar study among sports medicine providers found that 90% of the respondents were aware of the testing policy. Forty-one percent were in favor of screening all athletes for Hb AS while 76% supported selective screening based on race/ethnicity. Male physicians favored selective screening more than female physicians. The majority of participants felt the athlete or his or her parent should be able to opt of the screening program. Twenty-five percent of respondents felt the athlete would face discrimination at all playing levels, while 40% felt it was a

possibility. Rates were similar among physicians regarding insurance discrimination with 37% feeling they would definitely be discriminated against and 41% feeling they might be in regards to health insurance, and 39% thinking they would definitely face discrimination, and 42% thinking they may face discrimination in obtaining life insurance (Achaya et al., 2011).

Guidelines for Prevention & Treatment of Exertional Sickling

The National Athletic Trainer's Association (NATA) provides guidelines for the recognition and treatment of ES crises. They suggest that all coaches, parents, and trainers be educated regarding signs and symptoms of ES, allowing a longer recovery time between conditioning repetitions and excluding Hb AS athletes from performance tests based on environmental and physical factors. In addition, they suggest placing an emphasis on adequate hydration, refraining from exercise if the athlete is ill, controlling asthma, and adjusting work-rest cycles based on environmental heat stress. Upon presentation of an ES they recommend immediate removal from the activity, administration of high flow oxygen, cooling the athlete, and ~~the~~ monitoring of vital signs. They also suggest having emergency equipment on hand to deal with explosive rhabdomyolysis and its associated metabolic complications. NATA lists differences between ES and HI, that include muscle twinges, excruciating and specific pain, and contracted and hard muscles in HI, compared to no twinges, generalized pain, muscle weakness, and normal muscle presentation in ES (Anderson et al., 2012).

Sickle cell trait testing in athletes faces many challenges. It is controversial, tests can be inaccurate and expensive, risks are not clearly relayed to carriers, many

students choose not to participate, and the risk of discrimination of any kind against carriers is high. Medical care providers, coaches, trainers, Hb AS athletes, and the athletes' families face a difficult ethical dilemma: does the risk of discrimination outweigh the price of 1 life saved? Guidelines regarding prevention and treatment of ES may help reduce the risk of sickling crises among athletes, but guidelines are not enforceable. A more complete understanding of the genetic and environmental factors that precipitate sickling crises is crucial in the effort to protect Hb AS individuals while maintaining their privacy and respecting their personal choices.

Suggestions for Education and Medical Treatment of Hb AS Individuals

No evidence exists regarding treatment to increase standing Hb F levels among Hb AS carriers. As Hb AS is often a benign mutation no clinical trials have been conducted to examine the use of neoplastic drugs such as hydroxyurea. These drugs can cause severe side effects so their use on those who have historically been considered healthy individuals has not been warranted. However, new evidence suggesting that not all Hb AS carriers have a favorable clinical course may someday prompt the study of preventative treatment, which may include increasing Hb F levels. Additionally, given the controversy surrounding mandatory testing for Hb AS it is highly probably that arguments surrounding the testing and use of drugs that may enhance the performance of Hb AS athletes will arise. Overall, the author feels that it is not appropriate to investigate the use of drugs that increase Hb F levels on Hb AS carriers until we better understand the risk factors and causes for their health disparities.

Public health campaigns to educate Hb AS carriers and medical care providers about their mutation and its potential for ill health are justified at this time. Educating medical care providers about the possibility for adverse clinical courses in Hb AS carriers will help providers administer appropriate care. Campaigns could also be used to encourage providers to appropriately educate carriers and their families so that they better understand the potential health risks.

Newborn screening panels test for sickle cell disease, and sickle cell trait by proxy, in 41 U.S. states and also the District of Columbia (Lane, 2001). Despite this mandatory testing, many Hb AS carriers do not know that they carry the gene. This may due to a lack of communication between health care professionals and parents or parents and children as it is generally thought not to cause health problems. Thus, further studies examining the attitudes surrounding Hb AS in health care professionals and those with SCD or Hb AS should be conducted. Broadly, future investigations should examine the delivery method of testing results to care providers, if and how these providers convey results to parents, whether providers believe these results are medically important, why they do or do not inform parents of the results, and how parents interpret and treat these results. Challenging and changing beliefs about the Hb AS mutation and health are important in the effort to promote practices supporting the overall wellbeing of individuals who carry Hb AS.

Recruitment Difficulties

This study faced a number of limitations. First and foremost was the incredible difficulty found in recruiting participants. Student athletes are a particularly well-protected group of athletes who are held to privacy standards above and beyond

normal college students. Often, their schools control their use of personal media as it can directly reflect on the school itself (Yuksel, 2012). As such, it is impossible to contact student athletes directly. In fact, the author was only able to speak with 1 student athlete directly and he attended her university. He did not, however, actually participate in the study. These programs and students are so well shielded that the author was unable to gain access to a football practice at her own university in order to observe the types of conditioning drills associated with football practice despite multiple attempts on her behalf by a team physician.

In addition to these issues, more than 1 athletic trainer informed the author that Hb AS athletes often do not understand the medical implications of their carrier status; some athletes are not even aware that it is a genetic condition and have asked if they must be retested year after year. Also, student athletes generally lead rather busy lives between training and attending college and many did not have the time to participate. Additionally, the author was surprised to learn that the athletes had the option to sign a waiver and not participate in the screening program. It came to light that many athletic trainers and coaches have their entire team sign the waiver and no Hb AS testing is performed for that college or university (Anonymous, 2013).

Limitations

Selection and information biases were present in this study. Volunteer bias was a large concern to this study since it was difficult to recruit participants and seems likely that those who were willing to participate did so for reasons known only to themselves. Participants in this study may differ from participants who were not eligible for inclusion based on important variables such as participation in organized

sports. Participants in this study also represent a very healthy segment of the Hb AS population and may not accurately represent all Hb AS symptoms and health outcomes. Consent bias is possible, as a number of potential participants received participation kits, but chose not to participate. Bias was also evident from missing data as many participants did not answer all questions on their surveys. Additionally, the study may not be an accurate representation of the available literature since articles were only reviewed if they had been published in English.

Information bias was present in the forms of recall and social desirability biases. Participants were likely to answer questions in a way that minimized adverse health events in order to protect their student athlete status. They also may not accurately recall adverse clinical outcomes that happened many years prior to participation and may not have been diagnosed correctly, if at all, at that time.

Strengths

This study benefitted from the possible inclusion of all Hb AS collegiate football players regardless of age, race, and location.

Phase 2 – Expected Results

Phase 2 of this study is expected to both increase the sample and to return results on SNPs associated with increased levels of Hb F as well as sickle haplotype. This data will be used to determine whether or not there is an association between Hb F levels, haplotype, and adverse health outcomes. We hypothesize that SNPs associated with worse clinical outcomes in SCD individuals are those carried by Hb AS individuals who experience poor health.

Conclusion

The sickle mutation can cause mild to severe ill health among Hb SS and Hb AS individuals. Sickle cell trait is associated with increased risk of exertional sickling and death in individuals performing intense physical exercise. Although other environmental, genetic, and hemorheological characteristics influence the extent and severity of the sickling crisis, Hb AS is nonetheless a risk factor for adverse clinical outcomes. High levels of Hb F in circulating erythrocytes may mitigate the extent of the sickling crisis since Hb F is resistant to polymerization. Guidelines to help prevent sickling crises have been produced, but it is not possible to ensure that they are enforced.

The risk of sudden death among Hb AS individuals has been overlooked and underestimated. That any sudden deaths should occur in athletes in peak physical condition during the prime of their lives is alarming. Efforts to prevent these deaths through education and research has waxed and waned since the 1970's, resulting in a poorly understood set of clinical variables that affect the occurrence of exertional sickling. New research into genetic modifiers that result in higher than normal Hb F levels has offered renewed hope into the study Hb SS and Hb AS health outcomes, but has not progressed to the point where it can be used to alleviate symptoms. The research presented herein, and the research that will be undertaken in the second phase of this study has the potential to benefit a neglected group of individuals. The results of this study may ultimately help health care providers, coaches, athletic

trainers, and Hb AS individuals understand why some present with mild to severe health events while others do not. This information may also be used to create more detailed guidelines regarding health risks and prevention in Hb AS carriers.

Funding

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Conflicts of Interest

The author has no competing interests.

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Appendix 1. University of South Florida Institutional Review Board Approval Letter

USF
UNIVERSITY OF SOUTH FLORIDA

RESEARCH INTEGRITY AND COMPLIANCE
Institutional Review Boards, FWA No. 00001669
12901 Bruce B. Downs Blvd., MDC035 • Tampa, FL 33612-4799
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4/30/2013

Carroll Flansburg
Anthropology
12770 University Club Dr
Apt 104
Tampa, FL 33612

RE: **Full Board Approval for Initial Review**
IRB#: Pro00012688
Title: Is Sickle Cell Trait as Benign as is Usually Assumed?

Study Approval Period: 4/25/2013 to 4/25/2014

Dear Ms. Flansburg:

On 4/25/2013, the Institutional Review Board (IRB) reviewed and **APPROVED** the above application and all documents outlined below.

Approved Item(s):
Protocol Document(s):
SCT Study Protocol v4 4.30.13.docx

Consent/Assent Document(s)*:
SCT Consent Form 4.28.13.docx.pdf
SCT Genetic Addendum 4.28.13.docx.pdf

*Please use only the official IRB stamped informed consent/assent document(s) found under the "Attachments" tab. Please note, these consent/assent document(s) are only valid during the approval period indicated at the top of the form(s).

Please note, the board approved the study to be reviewed under expedited category 9 in the future.

As the principal investigator of this study, it is your responsibility to conduct this study in accordance with IRB policies and procedures and as approved by the IRB. Any changes to the approved research must be submitted to the IRB for review and approval by an amendment.

We appreciate your dedication to the ethical conduct of human subject research at the University of South Florida and your continued commitment to human research protections. If you have any questions regarding this matter, please call 813-974-5638.

Sincerely,

Jose Montero, M.D., Chairperson
USF Institutional Review Board

Appendix 2. Abbreviations Used Throughout this Paper

Abbreviation	Meaning
AA	African Americans
ACS	Acute Chest Syndrome
ALHY	Adequate Level of Hydration
CAR	Central African Republic
COD	Cause of Death
COMPMPD	Muslce Pain Duration in Comparison with Peers
COMPPN	Muscle Pain Severity in Comparison with Peers
COMPRECV	Recovery Time in Comparison with Peers
CSSCD	Cooperative Study of Sickle Cell Disease
CVA	Cerebrovascular Accident
DNA	Deoxyribonucleic Acid
ES	Exertional Sickling
FCP	F Cell Production Locus
Hb A	Alpha Hemoglobin Chains
Hb AA	Normal Adult Hemoglobin
Hb AS	Sickle Cell Trait Hemoglobin
Hb B	Beta Hemoglobin Chains
Hb D	Minor Delta Hemoglobin
Hb F	Fetal Hemoglobin
Hb G	Gamma Hemoglobin
Hb SS	SickleCell Hemoglobin
HBCUs	Historically Black Colleges and Universities
HCM	Hypertrophic Cardiomyopathy
HI	Heat Illness
HPFH	Hereditary Persistence of Fetal Hemoglobin
HU	Hydroxyurea
ICD	International Classification of Diseases Index
MCV	Mean Corpuscual Volume
MHIF	Minneapolis Heart Institute Foundation
NATA	National Athletic Trainer's Association
NCAA	National Collegiate Athletic Association
NCCSIR	National Center for Catastrophic Sports Injury Research
NHBLI	National Heart, Lung, and Blood Institute
NICHHD	National Institute of Child Health and Human Development

PHW	Parent Heart Watch
RFLP	Restriction Fragment Length Polymorphism
SAI	Saudi Arabia/Indian Haplotype
SCD	Sickle Cell Disease
SHEMC	School of Human Evolution & Molecular Change
SHLHY	Safe and Healthy Level of Hydration
SIT	Silent Infarct Transfusion
SNPs	Single Nucleotide Polymorphisms
U.K.	United Kingdom
U.S.	United States
UD	Unhealthy Dehydration
VOC	Vaso-occlusive Crisis

Appendix 3. Carriers of Sickle Cell Trait – Facebook page

Carriers of Sickle Cell Trait:

https://www.facebook.com/pages/Carriers-of-Sickle-Cell-Trait/150929204998069

Appendix 4. Sickle Cell Trait in Athletes Survey

Sickle Cell Trait in Athletes

Please give us your contact information so that we can contact you if we need to:

First Name_____ Last Name _____

Date of Birth (mm/dd/yyyy):_____/_____/____

Mailing Address: _____

City _____ State _____ Zip Code _____

E-mail: _____

Phone Number With Area Code: (_____)_____

This survey has five sections. Each section will have instructions on how to answer the questions in that section. Throughout this survey sickle cell trait is referred to as HB AS. The survey should take approximately 15-30 minutes to complete. If you have any questions please call 802.585.1054 and ask for Carroll Flansburg. Your answers will be kept private and you will not be identified in our study.

Thank you for your participation in this study! With your help we hope to be able to allow athletes like yourself to make more informed health decisions.

YOU CAN ONLY TAKE PART IN THIS STUDY IF YOU HAVE HAD A BLOOD TEST AND BEEN TOLD BY A MEDICAL PROFESSIONAL THAT YOU CARRY SICKLE CELL TRAIT

Section I - Your Experience(s) with Dehydration:

Q1. Have you ever felt unusually dehydrated? Place a ✓ or an X in the box next to your answers.

Yes	☐	Go to Q2
No	☐	Skip to Section II
Prefer not to answer	☐	
Do not know	☐	

Q2. If yes to Q1, place a ✓ or an X in the box next to your symptoms. You may mark as many as you need to.

Dry, sticky mouth	☐
Sleepiness or tired	☐
Thirst/Extreme Thirst	☐
Decreased urine output	☐
Headache	☐
Dizziness or lightheadedness	☐
Lack of sweating	☐
Rapid Breathing	☐
Low Blood Pressure	☐
Rapid Heartbeat	☐
Dry skin that doesn't "bounce back" when pinched	☐
Prefer not to answer	☐
Do not know	☐
Other	☐

Describe your symptoms in the space below.

If "other" please describe your symptoms here:

_____

_____

Q3. Please write the number of times you have experienced these symptoms: _____

Q4. Please list the name of the city and the state where you experienced these symptoms. If it has happened more than once please list all locations by city and state. Place a ✓ or an X in the box next to your answers if you don't know or would prefer not to answer.

Do not know	☐
Prefer not to answer	☐

City _____ State _____

City _____ State _____

City _____ State _____

Q5. Do you feel that you keep a safe and healthy level of hydration during training (do you drink enough liquids to stay healthy)? Place a ✓ or an X in the box next to your answers.

Yes.....1	☐
No.....2	☐
Prefer not to answer.....88	☐
Do not know.....99	☐

Q6. Do you feel that you keep an adequate level of hydration during athletic competitions (do you drink enough liquids to stay healthy)? Place a ✓ or an X in the box next to your answers.

Yes.....1	☐
No.....2	☐
Prefer not to answer.....88	☐
Do not know.....99	☐

Section II: Your Experience(s) with Heat Illness Diagnoses:

Q7. Have you ever been told you had "heat illness" by the athletic department staff or medical personnel? Place a ✓ or an X in the box next to your answers. If you mark "no", "prefer not to answer", or "don't know" please skip to Section III.

Yes.....1	☐	Go to Q8
No.....2	☐	Skip to Section V
Prefer not to answer.....88	☐	
Do not know.....99	☐	

Q8. If you marked "yes" to Q7, please tell us at what point in the season you were diagnosed with "heat illness". Please note, we are NOT asking about off-season training. Place a ✓ or an X in the box to mark your answer. You may mark more than one box if you need to.

Yes.....1	☐
No.....2	☐
Prefer not to answer.....88	☐
Do not know.....99	☐

Q9. What was the intensity of your work out right before you were diagnosed with "heat illness"? Place a ✓ or an X in the box next to your answers. You may mark more than one box if you need to.

No exertion at all.....1	
Extremely light.....2	
Very light.....3	
Light.....4	
Somewhat hard.....5	
Hard.....6	
Very hard.....7	
Extremely hard.....8	
Maximal exertion.....9	
Prefer not to answer.....88	
Do not know.....99	

Q10. If you were told you had "heat illness" by the athletic department staff or medical personnel please place a ✓ or an X in the box next to your symptoms. Please mark all of your symptoms.

Hard, tense muscles.....1	
Fatigue.....2	
Nausea and/or vomiting.....3	
Headaches.....4	
Excessive Thirst.....5	
Fainting.....6	
Agitation.....7	
Weakness.....8	
Confusion or anxiety.....9	
Hot, flushed or dry skin.....10	
Decreased sweating.....11	
Breathlessness.....12	
Lower amounts of urine.....13	
Blood in urine or stool.....14	
Increased body temperature.....15	
Convulsions or seizures.....16	
Confusion.....17	
Heat cramps that are severe or sometimes disabling.....18	
Drenching sweats.....19	
Cold and clammy skin.....20	
Prefer not to answer.....88	
Do not know.....99	

Q11. Please list the name of the city and the state where you were when you were diagnosed with "heat illness". If it has happened more than once please list all locations by city and state. Place a ✓ or an X in the box next to your answers if you would prefer not to answer or do not know.

Prefer not to answer.....99 ☐
Do not know.....88 ☐

City _____ State _____
City _____ State _____
City _____ State _____

Section IV - Your Workouts:

Q12. Compared with your teammates, do you feel that your muscle pain lasts for longer periods of time? Place a ✓ or an X in the box next to your answer.

Yes.....1 ☐
No.....2 ☐
Prefer not to answer.....88 ☐
Do not know.....99 ☐

Q13. Compared with your teammates, do you feel that your muscles take longer to recover from workouts or training? Place a ✓ or an X in the box next to your answer.

Yes.....1 ☐
No.....2 ☐
Prefer not to answer.....88 ☐
Do not know.....99 ☐

Q14. Compared to your teammates, do you feel that you experience high amounts of muscular pain? Place a ✓ or an X in the box next to your answer.

Yes.....1 ☐ Go to Q15
No.....2 ☐
Prefer not to answer.....88 ☐ Skip to Section V
Do not know.....99 ☐

Q15. If you marked "yes" to Q14, please tell us how severe your pain was. Place a ✓ or an X in the box next to your answer.

Mild.....1	☐
Moderate.....2	☐
Severe.....3	☐
Causes you to be unable to perform normal activities.....4	☐
Prefer not to answer.....88	☐
Do not know.....99	☐

Section V – Your Experiences with Exertional Sickling:

Q16. Have you ever been told you had "exertional sickling" by the athletic department staff or medical personnel? Place a ✓ or an X in the box next to your answer.

Yes.....1	☐	Go to Q17
No.....2	☐	Skip to Section VI
Prefer not to answer.....88	☐	
Do not know.....99	☐	

Q17. If you answered yes to Q16 please place a ✓ or an X in the box next to your symptoms. Please mark all your symptoms.

Leg pain or weakness.....1	☐
Low back pain or weakness.....2	☐
Muscle cramping that spread throughout the body.....3	☐
Rapid breathing.....4	☐
Prefer not to answer.....88	☐
Do not know.....99	☐

Q18. Please write the number of times you have experienced these symptoms: _____

Q19. Where were you when you were diagnosed with "exertional sickling"? Please list the name of the city and the state. If it has happened more than once please list all locations by city and state. Place a ✓ or an X in the box next to your answers if you don't know or would prefer not to answer.

Prefer not to answer.....88	☐
Do not know.....99	☐

City _____ State _____
City _____ State _____
City _____ State _____

Section VI – Your Background Information:

Q20. How much do you currently weigh in pounds? ☐☐☐ lbs

Q21. What is your ☐☐ height? ft ☐☐ in

Q22. Do you smoke? Place a ✓ or an X in the box to mark your answer.

Yes	1
No	2
Prefer not to answer	88
Do not know	99

Q23. Do you have asthma? Place a ✓ or an X in the box to mark your answer.

Yes	1
No	2
Prefer not to answer	88
Do not know	99

Q24. What position do you play on your football team? Place a ✓ or an X in the box to mark your answer. You may mark all that apply.

Safety	1
Cornerback	2
Outside Linebacker	3
Defensive End	4
Defensive Tackle	5
Middle Linebacker	6
Wide Receiver	7
Offensive Tackle	8
Offensive Guard	9
Center	10
Tight End	11
Quarterback	12
Fullback/Running Back	13
Halfback/Running Back	14
Prefer not to answer	88
Do not know	99

Q25. If you know anything about the background of your biological family, please tell us where they came from. Place a ✓ or an X in the box to mark your answer. You may mark all that apply.

Northern Europe.....1	
Southern Europe.....2	
Middle East and North Africa.....3	
Pre-partition India (Sri-Lanka, India, Pakistan, Tibet).....4	
South East Asia (Thailand, Vietnam, etc.).....5	
China.....6	
Central and Eastern Europe (including all former Soviet republics).....7	
Melanesia, Micronesia, Polynesia and Australia.....8	
Latin American of African descent.....9	
Latin American of Amerindian descent.....10	
Latin American of Northern European descent.....11	
Latin American of Southern European descent.....12	
North American of Amerindian descent.....13	
North American of Northern European descent.....14	
North American of Southern European descent.....15	
North American of African descent.....16	
Caribbean of African descent.....17	
Caribbean of Amerindian descent.....18	
Caribbean of Northern European descent.....19	
Caribbean of Southern European descent.....20	
Caribbean of East Asian descent.....21	
Prefer not to answer.....88	
Do not know.....99	

Q26. How do you identify yourself? Place a ✓ or an X in the box to mark your answer. You may mark all that apply.

Hispanic, Latino, or Spanish origin....1	☐
Mexican, Mexican American, or Chicano....2	☐
Puerto Rican....3	☐
Cuban....4	☐
White....5	☐
Black, African American, or Negro....6	☐
American Indian or Alaska Native....7	☐
Asian Indian....8	☐
Japanese....9	☐
Native Hawaiian....10	☐
Chinese....11	☐
Korean....12	☐
Guamanian or Chamorro....13	☐
Filipino....14	☐
Vietnamese....15	☐
Samoan....16	☐
Other Asian....17	☐
Other Pacific Islander....18	☐
Prefer not to Answer....88	☐
Do not know....99	

Q27. Do you have any other blood disorders besides Sickle Cell Trait? (An example of another blood disorder is a thalassemia.) Answer "yes" ONLY if you have had a blood test and been told that you do have another blood disorder. Answer "no" only if you do not know if you have had any such blood tests and you do not know if you do or do not carry any of these disorders. Place a ✓ or an X in the box to mark your answer.

Yes....1	☐	Go to Q28
No....2	☐	} Go to Q29
Prefer not to answer....88	☐	
Do not know....99	☐	

Q28. If yes, what blood disorder besides Sickle Cell Trait do you have? _____

Q29. Average resting blood pressure (please leave blank if you do not know):

_____ / _____

Section VII: Your Experiences with Ill Health During Training or Workouts:

Q30. Please mark all symptoms you have experienced during training or workouts. Place a ✓ or an X in the box next to your answer. You may mark all that apply.

Dry, sticky mouth.....1	
Sleepiness or tiredness.....2	
Thirst/Extreme Thirst.....3	
Decreased urine output.....4	
Headache.....5	
Dizziness or lightheadedness.....6	
Rapid Breathing 7	
Low Blood Pressure.....8	
Rapid Heartbeat 9	
Dry skin that doesn't "bounce back" when pinched.....10	
Hard, tense muscles.....11	
Fatigue.....12	
Nausea and/or vomiting.....13	
Headaches.....14	
Fainting.....15	
Agitation.....16	
Weakness.....17	
Confusion or anxiety.....18	
Hot, flushed or dry skin.....19	
Decreased sweating.....20	
Breathlessness.....21	
Lower amounts of urine.....22	
Blood in urine or stool.....23	
Increased body temperature.....24	
Convulsions or seizures.....25	
Heat cramps that are severe or sometimes disabling.....26	
Drenching sweats.....27	
Cold and clammy skin.....28	
Leg pain or weakness.....29	
Low back pain or weakness.....30	
Muscle cramping that spread throughout the bod.....31	
Prefer not to answer.....88	
Do not know.....99	
Other.....32	

If "other" please describe your symptoms here:

Describe your symptoms in the space below.

Q31. Where were you when you experienced these symptoms? Please list the name of the city and the state. If it has happened more than once please list all locations by city and state. Place a ✓ or an X in the box next to your answers if you don't know or would prefer not to answer.

Prefer not to answer.....88 ☐
Do not know.....99 ☐

City _____ State _____
City _____ State _____
City _____ State _____

THANK YOU FOR YOUR PARTICIPATION!

Appendix 5. SAS Code

```
libname HB AS "s:\HB AS";
proc import datafile = 's:\HB AS\HB ASDatacopy.xls'
DBMS = xls OUT = HB ASData;
run;

/*Missing Value Counts*/

proc means data=HB ASData nmiss n;
run;

/*Contents*/

proc contents data=HB ASData;
run;

/*Fisher's Exact Tests*/

data fisher;
input UNDHY EXSCKL count;
datalines;
1 1 2
1 0 10
0 1 3
0 0 5
;
run;

proc freq data=fisher order=data;
weight count;
tables UNDHY*EXSCKL;
exact fisher;
run;


data fisher1;
input UNDHY DHI count;
datalines;
1 1 1
1 0 10
0 1 1
0 0 7
;
run;

proc freq data=fisher1 order=data;
weight count;
tables UNDHY*DHI;
```

```
exact fisher;
run;

data fisher2;
input DHI EXSCKL count;
datalines;
1 1 1
1 0 1
0 1 4
0 0 13
;
run;

proc freq data=fisher2 order=data;
weight count;
tables DHI*EXSCKL;
exact fisher;
run;

data fisher3;
input COMPMPD UNDHY count;
datalines;
1 1 2
1 0 0
0 1 9
0 0 6
;
run;

proc freq data=fisher3 order=data;
weight count;
tables COMPMPD*UNDHY;
exact fisher;
run;

data fisher4;
input COMPRECV UNDHY count;
datalines;
1 1 4
1 0 0
0 1 7
0 0 5
;
run;

proc freq data=fisher4 order=data;
weight count;
tables COMPRECV*UNDHY;
exact fisher;
run;

data fisher5;
input COMPPN UNDHY count;
datalines;
1 1 2
1 0 0
```

```
0 1 7
0 0 6
;
run;

proc freq data=fisher5 order=data;
weight count;
tables COMPPN*UNDHY;
exact fisher;
run;

data fisher6;
input COMPRECV COMPMPD count;
datalines;
1 1 4
1 0 2
0 1 0
0 0 12
;
run;

proc freq data=fisher6 order=data;
weight count;
tables COMPRECV*COMPMPD;
exact fisher;
run;

data fisher7;
input COMPPN COMPMPD count;
datalines;
1 1 1
1 0 1
0 1 0
0 0 13
;
run;

proc freq data=fisher7 order=data;
weight count;
tables COMPPN*COMPMPD;
exact fisher;
run;

data fisher8;
input COMPMPD DHI count;
datalines;
1 1 1
1 0 1
0 1 1
0 0 13
;
run;

proc freq data=fisher8 order=data;
weight count;
tables COMPMPD*DHI;
```

```
exact fisher;
run;

data fisher9;
input COMPRECV DHI count;
datalines;
1 1 1
1 0 3
0 1 1
0 0 10
;
run;

proc freq data=fisher9 order=data;
weight count;
tables COMPRECV*DHI;
exact fisher;
run;

data fisher10;
input COMPPN DHI count;
datalines;
1 1 0
1 0 2
0 1 1
0 0 11
;
run;

proc freq data=fisher10 order=data;
weight count;
tables COMPPN*DHI;
exact fisher;
run;

data fisher11;
input SHLHY UNDHY count;
datalines;
1 1 9
1 0 5
0 1 3
0 0 0
;
run;

proc freq data=fisher11 order=data;
weight count;
tables SHLHY*UNDHY;
exact fisher;
run;

data fisher12;
input ALHY UNDHY count;
datalines;
1 1 8
1 0 6
```

```
0 1 4
0 0 0
;
run;

proc freq data=fisher12 order=data;
weight count;
tables ALHY*UNDHY;
exact fisher;
run;

data fisher13;
input SHLHY DHI count;
datalines;
1 1 1
1 0 12
0 1 1
0 0 2
;
run;

proc freq data=fisher13 order=data;
weight count;
tables SHLHY*DHI;
exact fisher;
run;

data fisher14;
input ALHY UNDHY count;
datalines;
1 1 1
1 0 12
0 1 1
0 0 3
;
run;

proc freq data=fisher14 order=data;
weight count;
tables ALHY*UNDHY;
exact fisher;
run;

data fisher15;
input SHLHY EXSCKL count;
datalines;
1 1 3
1 0 11
0 1 1
0 0 2
;
run;

proc freq data=fisher15 order=data;
weight count;
tables SHLHY*EXSCKL;
```

```
exact fisher;
run;

data fisher16;
input ALHY EXSCKL count;
datalines;
1 1 3
1 0 11
0 1 1
0 0 3
;
run;

proc freq data=fisher16 order=data;
weight count;
tables ALHY*EXSCKL;
exact fisher;
run;

data fisher17;
input COMPMPD EXSCKL count;
datalines;
1 1 0
1 0 2
0 1 4
0 0 11
;
run;

proc freq data=fisher17 order=data;
weight count;
tables COMPMPD*EXSCKL;
exact fisher;
run;

data fisher18;
input COMPRECV EXSCKL count;
datalines;
1 1 1
1 0 3
0 1 3
0 0 9
;
run;

proc freq data=fisher18 order=data;
weight count;
tables COMPRECV*EXSCKL;
exact fisher;
run;

data fisher19;
input COMPPN EXSCKL count;
datalines;
1 1 1
1 0 1
```

```
0 1 3
0 0 10
;
run;

proc freq data=fisher19 order=data;
weight count;
tables COMPPN*EXSCKL;
exact fisher;
run;
```

Appendix 6. Deidentified Data Set

StudyID	UNDHY	YUNDHY	YUNDHYA	YUNDHYB	YUNDHYC	YUNDHYD	YUNDHYE	YUNDHYF	$ YUNDHYT	$ YUNDHYL	$ YUNDHYLA	$ YUNDHYLB	SHLHY	ALHY	DHI	DHIT	IWKOUT	DHISYMP	DHISYMPA	DHISYMPB	$ DHIL
1	2															2					
2	2															2					
3	2													1	1	1	1	7	2	4	8 Columbia, SC
4																					
5	2																				
6																2					
7	1	1	2	3	8				10%	Oxford, PA	Miami, FL			1	1	2					
8	1	1	2	3	4	8			100	Union City, CA	Hayward, CA	Oakland, CA		1	2	2					
9	2	99							88					1	1	2		99	99		88
10	2													1	1	2					
11	1	6							1	Oxford, PA				1	1	2	99	88	88		88
12	1	1	5	6	8	10			2	Bloomingburg, NY				2	2	2					
13	1	1	3	6					1	Lincoln University, PA				1	1	2					
14																					
15																					
16																					
17	2	6							2		99			99	1	2					
18																					
19																					
20	1	3	6	8	10				4	Columbia, SC	Charleston, SC			2	2	2					
21																					
22	1	2	3						2	Seattle, WA	Seattle, WA			1	1	2		5	2		Seattle, WA
23	1	1	2	3	6	8	10		2-3times a week	88, 99				2	2	1	1	88	1	2	5 Seattle, WA
24	1	2	3	6	8	10			4	Seattle, WA				1	1						
25	1	1	2	3	5	6	8	10	20	Seattle, WA	Seattle, WA	Seattle, WA		1	1	2	1	8			
26	1	1	3	5					5	Aurora, CO	Boulder, CO			1	1	2					
27	1	1	6						1	Boulder, CO				1	1	2					Boulder, Co
28	2													1	1	2					

no participant response received

$ DHILA	COMPMPD	COMPRECV	COMPPN	COMPPNS	EXSCKL	EXSCKLS	EXSCKLSA	EXSCKLSB	EXSCKLSC	$ EXSCKLT	$ EXSCKLL	$ EXSCKLLA	WEIGHTLB	HEIGHTIN	SMOKE	ASTHMA	PSTN	PSTNA	BIOBCK	BIOBCKA	BIOBCKB	SELFID	
						1	2	4			8	Columbia, SC Beaufort, SC	187	71	2	2	1	2	16				6
	2	2	2			1	2				7	Columbia, SC Manning, SC	232	66	2	2	11		99				6
	2	2	2			1	1	2	3	4 few		Columbia, SC	175	74	2	2	2		3				6
						2							197	73	2	2	1		17				6
	2	2	99			2							157	71	88	2	12		16				6
	1	1	1	2		2																	
	2	2	2	99		2							345	78	2	2	5		99				6
	2	99	2			2							260	72	2	2	13						6
	2	2	2	88		2	88				88		263	61	2	1	4	11	99				6
						2							196	74	2	2	7		99				6
	2	2	2			2							180	75	2	2	1		17				6
	2	2	2			2							220	72	2	2	3		99				6
	2	1	1	1		1	2	4			3	Columbia, SC Charleston, SC	217	74	2	2	3		16				6
Seattle, WA	2	2	2			1	88				1	Seattle, WA	190	70	2	2	1						6
	1	1	99	99		2							185	72.75	2	2	2		99				6
	2	2	2			2							204	74	2	2	7		99				6
	2	2	2			2							218	72	2	2	1		17		19	20	3
	2	1	2			2							115	65	2	2							6
	2	2	2			2							242	75	2	2	4		3				
	2	2	2			2							197	73	2	2	3						6

SELFIDA	OTHRBD	$ AVGRBP	TRNGSM	TRNGSMA	TRNGSMB	TRNGSMC	TRNGSMD	TRNGSME	TRNGSMF	TRNGSMG	TRNGSMH	TRNGSMI	TRNGSMJ	TRNGSMK	TRNGSML	TRNGSMM	TRNGSMN	TRNGSMO	TRNGSMP	TRNGSMQ	$ TRNGSML
		2	1	2	7	12	21	22	30												Columbia, SC
		2	30																		Columbia, SC
		2	3	5	6	7	11	17													Columbia, SC
		2																			
		2	1	2	7	12	17	29													Oxford, PA
			1	2	3	4	11	12	14	17	21	25	26	28	30						Berkely, CA
		2																			99
		2	3	23	26																Queens, NY
		2	88	99	32																88
		2	1	2	5	6	7	9	11	12	20	26	29								Bloomingburg, NY
		2	1																		99
		2	1	6	12																88
		2	3	6	7	8	9	12	15	17	20	23	29								Columbia, SC
		2	1	2	7	13	26														Seattle, WA
		2	1	2	3	6	7	9	11	12	17	30	31								99
		2	1	3	6	7	9	12	14	16	23	26	28								Seattle, WA
	5	2	120/80	1	2	3	6	7	9	12	13	14	17	18	19	21	24	26	29	30	31 Seattle, WA
	7	2		1	3	5	7														Aurora, CO
		2		1	6	7	8														Boulder, CO
		2		1																	Boulder, CO

Appendix 7. SAS Results

The SAS System

The MEANS Procedure

Variable	Label	N Miss	N
StudyID	StudyID	0	28
UNDHY	UNDHY	8	20
YUNDHY	YUNDHY	14	14
YUNDHYA	YUNDHYA	17	11
YUNDHYB	YUNDHYB	19	9
YUNDHYC	YUNDHYC	21	7
YUNDHYD	YUNDHYD	23	5
YUNDHYE	YUNDHYE	26	2
YUNDHYF	YUNDHYF	27	1
SHLHY	SHLHY	10	18
ALHY	ALHY	10	18
DHI	DHI	9	19
DHIT	DHIT	24	4
IWKOUT	IWKOUT	22	6
DHISYMP	DHISYMP	23	5
DHISYMPA	DHISYMPA	26	2
DHISYMPB	DHISYMPB	26	2
COMPMPD	COMPMPD	11	17
COMPRECV	COMPRECV	11	17
COMPPN	COMPPN	11	17
COMPPNS	COMPPNS	23	5
EXSCKL	EXSCKL	8	20
EXSCKLS	EXSCKLS	22	6
EXSCKLSA	EXSCKLSA	25	3
EXSCKLSB	EXSCKLSB	27	1
EXSCKLSC	EXSCKLSC	27	1
WEIGHTLB	WEIGHTLB	9	19
HEIGHTIN	HEIGHTIN	9	19
SMOKE	SMOKE	9	19
ASTHMA	ASTHMA	9	19
PSTN	PSTN	10	18
PSTNA	PSTNA	26	2
BIOBCK	BIOBCK	13	15
BIOBCKA	BIOBCKA	27	1
BIOBCKB	BIOBCKB	27	1
SELFID	SELFID	10	18
SELFIDA	SELFIDA	26	2
OTHRBD	OTHRBD	9	19
TRNGSM	TRNGSM	10	18
TRNGSMA	TRNGSMA	13	15
TRNGSMB	TRNGSMB	13	15
TRNGSMC	TRNGSMC	16	12
TRNGSMD	TRNGSMD	18	10
TRNGSME	TRNGSME	19	9
TRNGSMF	TRNGSMF	21	7
TRNGSMG	TRNGSMG	22	6
TRNGSMH	TRNGSMH	22	6
TRNGSMI	TRNGSMI	22	6
TRNGSMJ	TRNGSMJ	22	6
TRNGSMK	TRNGSMK	26	2
TRNGSML	TRNGSML	26	2
TRNGSMM	TRNGSMM	27	1

8	YUNDHYE	Num	8	BEST12.		YUNDHYE
9	YUNDHYF	Num	8	BEST12.		YUNDHYF
48	__AVGRBP	Char	10	$10.	$10.	$ AVGRBP
22	__DHIL	Char	14	$14.	$14.	$ DHIL
23	__DHILA	Char	12	$12.	$12.	$ DHILA
34	__EXSCKLL	Char	14	$14.	$14.	$ EXSCKLL
35	__EXSCKLLA	Char	14	$14.	$14.	$ EXSCKLLA
33	__EXSCKLT	Char	14	$14.	$14.	$ EXSCKLT
67	__TRNGSML	Char	16	$16.	$16.	$ TRNGSML
68	__TRNGSMLA	Char	22	$22.	$22.	$ TRNGSMLA
69	__TRNGSMLB	Char	11	$11.	$11.	$ TRNGSMLB
11	__YUNDHYL	Char	22	$22.	$22.	$ YUNDHYL
12	__YUNDHYLA	Char	14	$14.	$14.	$ YUNDHYLA
13	__YUNDHYLB	Char	11	$11.	$11.	$ YUNDHYLB
10	__YUNDHYT	Char	15	$15.	$15.	$ YUNDHYT

The SAS System

The CONTENTS Procedure

Data Set Name	WORK.SCTDATA	**Observations**	28
Member Type	DATA	**Variables**	69
Engine	V9	**Indexes**	0
Created	Saturday, October 12, 2013 12:10:40 PM	**Observation Length**	640
Last Modified	Saturday, October 12, 2013 12:10:40 PM	**Deleted Observations**	0
Protection		**Compressed**	NO
Data Set Type		**Sorted**	NO
Label			
Data Representation	WINDOWS_64		
Encoding	wlatin1 Western (Windows)		

Engine/Host Dependent Information

Data Set Page Size	16384
Number of Data Set Pages	2
First Data Page	1
Max Obs per Page	25
Obs in First Data Page	12
Number of Data Set Repairs	0
Filename	C:\Users\cflansbu\AppData\Local\Temp\3\SAS Temporary Files_TD7668_XA063_\sctdata.sas7bdat
Release Created	9.0301M1
Host Created	X64_ES08R2

Alphabetic List of Variables and Attributes

#	Variable	Type	Len	Format	Informat	Label
15	ALHY	Num	8	BEST12.		ALHY
39	ASTHMA	Num	8	BEST10.		ASTHMA
42	BIOBCK	Num	8	BEST10.		BIOBCK
43	BIOBCKA	Num	8	BEST10.		BIOBCKA
44	BIOBCKB	Num	8	BEST10.		BIOBCKB
24	COMPMPD	Num	8	BEST10.		COMPMPD
26	COMPPN	Num	8	BEST12.		COMPPN
27	COMPPNS	Num	8	BEST12.		COMPPNS
25	COMPRECV	Num	8	BEST12.		COMPRECV
16	DHI	Num	8	BEST12.		DHI
19	DHISYMP	Num	8	BEST12.		DHISYMP
20	DHISYMPA	Num	8	BEST12.		DHISYMPA
21	DHISYMPB	Num	8	BEST12.		DHISYMPB
17	DHIT	Num	8	BEST12		DHIT

28	EXSCKL	Num	8	BEST12.		EXSCKL
29	EXSCKLS	Num	8	BEST12.		EXSCKLS
30	EXSCKLSA	Num	8	BEST12.		EXSCKLSA
31	EXSCKLSB	Num	8	BEST12.		EXSCKLSB
32	EXSCKLSC	Num	8	BEST12.		EXSCKLSC
37	HEIGHTIN	Num	8	BEST10.		HEIGHTIN
18	IWKOUT	Num	8	BEST12.		IWKOUT
47	OTHRBD	Num	8	BEST10.		OTHRBD
40	PSTN	Num	8	BEST10.		PSTN
41	PSTNA	Num	8	BEST10.		PSTNA
45	SELFID	Num	8	BEST10.		SELFID
46	SELFIDA	Num	8	BEST10.		SELFIDA
14	SHLHY	Num	8	BEST12.		SHLHY
38	SMOKE	Num	8	BEST10.		SMOKE
1	StudyID	Num	8	BEST12.		StudyID
49	TRNGSM	Num	8	BEST12.		TRNGSM
50	TRNGSMA	Num	8	BEST12.		TRNGSMA
51	TRNGSMB	Num	8	BEST12.		TRNGSMB
52	TRNGSMC	Num	8	BEST12.		TRNGSMC
53	TRNGSMD	Num	8	BEST12.		TRNGSMD
54	TRNGSME	Num	8	BEST12.		TRNGSME
55	TRNGSMF	Num	8	BEST12.		TRNGSMF
56	TRNGSMG	Num	8	BEST12.		TRNGSMG
57	TRNGSMH	Num	8	BEST12.		TRNGSMH
58	TRNGSMI	Num	8	BEST12.		TRNGSMI
59	TRNGSMJ	Num	8	BEST12.		TRNGSMJ
60	TRNGSMK	Num	8	BEST12.		TRNGSMK
61	TRNGSML	Num	8	BEST12.		TRNGSML
62	TRNGSMM	Num	8	BEST12.		TRNGSMM
63	TRNGSMN	Num	8	BEST12.		TRNGSMN
64	TRNGSMO	Num	8	BEST12.		TRNGSMO
65	TRNGSMP	Num	8	BEST12.		TRNGSMP
66	TRNGSMQ	Num	8	BEST12.		TRNGSMQ
2	UNDHY	Num	8	BEST10.		UNDHY
36	WEIGHTLB	Num	8	BEST10.		WEIGHTLB
3	YUNDHY	Num	8	BEST12.		YUNDHY
4	YUNDHYA	Num	8	BEST12.		YUNDHYA
5	YUNDHYB	Num	8	BEST12.		YUNDHYB
6	YUNDHYC	Num	8	BEST12.		YUNDHYC
7	YUNDHYD	Num	8	BEST12.		YUNDHYD

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The SAS System

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Frequency Percent Row Pct Col Pct	Table of UNDHY by EXSCKL		
	EXSCKL		
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1	2	10	12
	10.00	50.00	60.00
	16.67	83.33	
	40.00	66.67	
0	3	5	8
	15.00	25.00	40.00
	37.50	62.50	
	60.00	33.33	
Total	5	15	20
	25.00	75.00	100.00

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Mantel-Haenszel Chi-Square	1	1.0556	0.3042
Phi Coefficient		-0.2357	
Contingency Coefficient		0.2294	
Cramer's V		-0.2357	

WARNING: 50% of the cells have expected counts less than 5. Chi-Square may not be a valid test.

Fisher's Exact Test	
Cell (1,1) Frequency (F)	2
Left-sided Pr <= F	0.2962
Right-sided Pr >= F	0.9422
Table Probability (P)	0.2384
Two-sided Pr <= P	0.3473

Sample Size = 20

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Table Probability (P)	0.2384
Two-sided Pr <= P	0.3473

Sample Size = 20

The SAS System

The FREQ Procedure

Frequency
Percent
Row Pct
Col Pct

Table of UNDHY by EXSCKL

UNDHY	EXSCKL 1	EXSCKL 0	Total
1	2	10	12
	10.00	50.00	60.00
	16.67	83.33	
	40.00	66.67	
0	3	5	8
	15.00	25.00	40.00
	37.50	62.50	
	60.00	33.33	
Total	5	15	20
	25.00	75.00	100.00

Statistics for Table of UNDHY by EXSCKL

Statistic	DF	Value	Prob
Chi-Square	1	1.1111	0.2918
Likelihood Ratio Chi-Square	1	1.0949	0.2954
Continuity Adj. Chi-Square	1	0.2778	0.5982
Mantel-Haenszel Chi-Square	1	1.0556	0.3042
Phi Coefficient		-0.2357	
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	15.00	25.00	40.00
	37.50	62.50	
	60.00	33.33	
Total	5	15	20
	25.00	75.00	100.00

Statistics for Table of UNDHY by EXSCKL

Statistic	DF	Value	Prob
Chi-Square	1	1.1111	0.2918
Likelihood Ratio Chi-Square	1	1.0949	0.2954
Continuity Adj. Chi-Square	1	0.2778	0.5982
Mantel-Haenszel Chi-Square	1	1.0556	0.3042
Phi Coefficient		-0.2357	
Contingency Coefficient		0.2294	
Cramer's V		-0.2357	

WARNING: 50% of the cells have expected counts less than 5. Chi-Square may not be a valid test.

Fisher's Exact Test	
Cell (1,1) Frequency (F)	2
Left-sided Pr <= F	0.2962
Right-sided Pr >= F	0.9422
Table Probability (P)	0.2384
Two-sided Pr <= P	0.3473

Sample Size = 20

The SAS System

The FREQ Procedure

Frequency
Percent
Row Pct
Col Pct

Table of UNDHY by EXSCKL

UNDHY	EXSCKL 1	EXSCKL 0	Total
1	2	10	12
	10.00	50.00	60.00
	16.67	83.33	
	40.00	66.67	
0	3	5	8
	15.00	25.00	40.00
	37.50	62.50	
	60.00	33.33	
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