2019

Association Of Sickle Cell Trait With Exertional Rhabdomyolysis And Atrial Fibrillation.

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ASSOCIATION OF SICKLE CELL TRAIT WITH EXERTIONAL
RHABDOMYOLYSIS AND ATRIAL FIBRILLATION.

A Thesis Presented

by

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to

The Faculty of the Graduate College

of

The University of Vermont

In Partial Fulfillment of the Requirements
for the Degree of Master of Science
Specializing in Clinical and Translational Studies

August, 2019

Defense Date: May 21, 2019
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ABSTRACT

Sickle cell trait (SCT), sickle cell disease's carrier status, is a common genetic variant found in many people of African, South Asian, Middle Eastern and Mediterranean descent. While overall considered a benign carrier status, it has been associated with an increased risk of several diseases, including exertional rhabdomyolysis (ER), and chronic kidney disease. While epidemiological evidence links SCT with ER, the actual pathophysiological mechanism less understood. Additionally, while there is an increased prevalence of atrial fibrillation (AF) documented in people with sickle cell disease, studies in individuals with SCT are lacking.

The objectives of this thesis are twofold: The first chapter is a literature review of studies to examine the physiological mechanisms linking SCT and exertional rhabdomyolysis. The second chapter is original research into the associations of SCT with AF.

The first chapter reviews studies that identify aggravating factors that may promote ER. It then reviews observed pathophysiological changes in people with SCT that may increase the risk of ER. It summarizes studies that assess mitigating factors that decrease the risk of ER. It then presents a postulated pathway of mechanisms that associate SCT with ER.

The second chapter uses data from African-American participants in the REasons for Geographic and Racial Differences in Stroke (REGARDS) study to assess the association of SCT with prevalent AF (by electrocardiogram or medical history) using logistic regression models adjusting for age, sex, income, education, history of stroke, myocardial infarction, diabetes, hypertension, and chronic kidney disease. In 10,409 participants with baseline ECG data and genotyping, 778 (7.5%) had SCT and 811 (7.8%) had prevalent AF. After adjusting for age, sex, education and income, SCT was associated with AF, OR 1.32 (95% CI 1.03-1.70). SCT remained associated with prevalent AF after adjusting for potential factors on the causal pathway such as hypertension and chronic kidney disease suggesting alternate mechanisms for the increased risk. SCT was associated with a higher prevalence of AF and a non-significantly higher incident AF over a 9.2 year period independent of AF risk factors.

CITATIONS

Material from this thesis has been accepted for publication in the Journal of Electrocardiology on April 16th, 2019 and published online in the following form:

Douce DR, M.D.; Soliman EZ, M.D., M.Sc, M.S.; Naik R M.D., M.H.Sc , Hyacinth HI MBBS, Ph.D,. MPH; Cushman M M.D., M.Sc; Winkler CA, Ph.D; Howard G, Dr.PH; Lange EM, Ph.D; Lange LA, Ph.D, Irvin MR, Ph.D, M.S.; Zakai NA ,M.D., M.Sc.. (2019). Association of Sickle Cell Trait with Atrial Fibrillation: the REGARDS Cohort . Journal of Electrocardiology, 55, 1-5. https://doi.org/10.1016/j.jelectrocard.2019.04.010

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CHAPTER 1: SICKLE CELL TRAIT AND EXERTIONAL RHABDOMYOLYSIS: A REVIEW OF THE KNOWN PATHOPHYSIOLOGICAL MECHANISMS

1.1. Introduction

Sickle Cell Trait (SCT) is the carrier condition in which individuals carry one abnormal allele of the hemoglobin beta-chain gene (encoding β-globin), hemoglobin S (HbS). SCT has a prevalence of 7.3 % among African Americans, 0.7% of Latinos, and 0.3% of Caucasians in the United States and 10-40% across equatorial Africa (Ojodu et al., 2014). As individuals with SCT carry one allele for one normal β-globin and one sickle hemoglobin, it is also termed hemoglobin AS (Hb AS). Sickle Cell Disease (SCD) occurs when an individual inherits two abnormal β-globin genes. Traditionally, SCT has been considered a benign condition and is known to be associated with a decreased risk of developing complications from falciparum malaria (Williams et al., 2005), and is prevalent in the same geographic distribution as falciparum malaria. There is however moderate to strong epidemiological evidence that now links SCT to an increased risk of developing chronic kidney disease, venous thromboembolism, and exertional rhabdomyolysis (R. P. Naik et al., 2018).

The purpose of this review is to evaluate the current understanding of the pathophysiological mechanisms linking SCT and exertional rhabdomyolysis as well as the real and perceived implications for individuals with SCT. Individuals with SCT have historically been, and can currently be, excluded from sports and military service. Exertional rhabdomyolysis is on the postulated causal pathway between SCT and sudden

death, with a reported relative risk of sudden death of 28 in military recruits (Kark, Posey, Schumacher, & Ruehle, 1987) and 37 in NCAA athletes (Harmon, Drezner, Klossner, & Asif, 2012) for SCT individuals . However, recent epidemiological research with military personnel has documented a lower risk of exertional rhabdomyolysis with SCT (hazard ratio [HR], 1.54; 95% confidence interval [CI], 1.12 to 2.12), and finding no firm association between SCT and sudden death (HR, 0.99; 95% CI, 0.46 to 2.13) (Nelson et al., 2016). It is unclear if these discordant clinical observations are due to methodologic limitations or from the implementation of better preventative measures over time. An understanding of the mechanistic causes of SCT-associated rhabdomyolysis will help to make changes to decrease the risk of adverse events and better inform policy decisions like universal screening of SCT in NCAA athletes and service members.

The first association with SCT and its protective effects from complications of plasmodium falciparum Malaria was noted in the late 1940s when there were significant differences in the levels of parasitemia noted in individuals with and without SCT (Allison, 1954; Beet, 1946).

Multiple case reports and small case series were published documenting adverse clinical outcomes in SCT carriers, including splenic infarcts, hematuria and bacteriuria beginning in the 1960s and 1970s (Sears, 1978). It was noted at the time that there was no evidence for decreased overall survival and that properly controlled studies were lacking. Nevertheless, this led to SCT screening and exclusion from some forms of service in the

US military due to concerns about sudden death and developing complications at high altitude (Brodine & Uddin, 1977). These concerns were later rescinded.

Clinical research in the past 10 years has progressed as more rigorous, prospective cohort studies have been published. A recent systematic review noted that there are multiple studies published with a high level of evidence that there is an association with SCT and developing venous thromboemboli, proteinuria, chronic kidney disease, but only a moderate level strength of evidence associating exertional rhabdomyolysis with SCT and a low strength of evidence with sudden death (R. P. Naik et al., 2018).

Rhabdomyolysis is a life-threatening syndrome resulting from skeletal muscle breakdown and release of cellular contents into the circulatory system. This includes creatine kinase, myoglobin, and electrolytes including potassium (Visweswaran & Guntupalli, 1999). While there are multiple known causes of rhabdomyolysis, the final pathway leading to muscle breakdown and release of intracellular contents is the same. Muscles are reliant on adenosine triphosphate (ATP) to maintain low intracellular sodium and potassium levels. If ATP is depleted, these intracellular gradients cannot be maintained. High amounts of intracellular sodium and potassium in the cell lead to increased activity of proteolytic enzymes which release intracellular contents (Khan, 2009). Excessive muscle activity is thought to trigger rhabdomyolysis by depleting ATP so production cannot keep up with demand (Sharma, Winpenny, & Heymann, 1999). Trauma and burns which cause direct muscle injury, muscle ischemia, drugs and toxins, and genetic disorders affecting glycogen metabolism such as McArdle's disease, are also associated with rhabdomyolysis (Khan, 2009).

HbS is a variant on the β-globin gene that results from a substitution of valine for glutamic acid at the sixth amino acid position causing a missense mutation (Ashley-Koch, Yang, & Olney, 2000). HbS polymerizes when hemoglobin is in the deoxygenated state (Eaton & Hofrichter, 1990). Polymerization of HbS leads to decreased red blood cell flexibility, increased adhesion of red cells to vascular endothelium, as well as increased inflammation, coagulation and nitric oxide scavenging (Eaton & Bunn, 2017). The lifespan of RBCs containing HbS is shorter than RBCs without HbS, but varies significantly depending on the concentration of fetal hemoglobin (HbF) present (Franco et al., 1998). The extent to which HbS polymerizes correlates to the clinical and hemolytic severity in people with SCD (Brittenham, Schechter, & Noguchi, 1985).

1.1.1. Determinants of HBS percentage in SCT carriers.

Individuals with SCT typically have 35-40% HBS on hemoglobin electrophoresis (Angastiniotis et al., 2013). While there are equal numbers of genes present to produce equal amounts of HbA and HbS, HbS accounts for less than half of the total amount of hemoglobin present in carriers because HbS chains are less negatively charged and do not bind as readily to the α-subunit of hemoglobin (Forget & Bunn, 2013). The HbS percentage is further reduced when people with SCT also carry a 1 or 2 α-globin gene deletion (α -thalassemia silent carriers or α-thalassemia trait), conditions found in up to 30% of African-Americans (Steinberg & Embury, 1986). One study (Steinberg & Embury, 1986) showed progressive decreases in hemoglobin S percentage as the number of α-globulin gene deletions increased; those with 0, 1, or two α-globulin gene deletions had HbS percentages of 35-45%, 30-35% and 25-30% respectively.

1.2. Postulated causal pathway linking SCT and ER

The mechanism by which exertional rhabdomyolysis in SCT carriers occurs is not clear but is thought to be due to a combination of factors that increase the risk of muscle microvascular occlusions and muscle ischemia. Factors associated with exertion that increase this risk include metabolic acidosis, local hypoxemia around the capillaries of exercising muscles as well as high intensity exercise. A combination of these factors in turn causes increased viscosity of blood, release of inflammatory and vascular adhesion molecules, vascular remodeling and oxidative stress. These physiological changes are in turn thought to cause microvascular occlusions that lead to muscle ischemia, triggering ATP depletion, and rhabdomyolysis. Mitigating factors include degree of physical
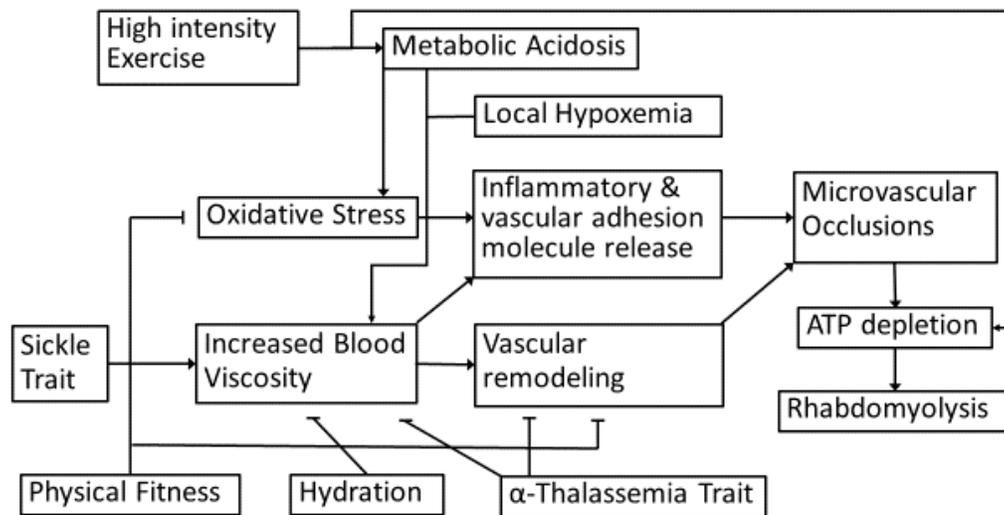
Figure 1: Postulated mechanism of sickle trait and rhabdomyolysis

fitness, the presence of alpha thalassemia trait, and adequate hydration. These mechanisms are shown in figure 1.

It is not clear if cells need to be in a sickled state per se in order to cause endothelial damage and capillary occlusion (Eichner, 2010) or if damage from RBC sickling is only one of several mechanisms that lead to microvascular occlusions and ischemia (Tripette et al., 2013). When blood from SCT carriers collected at rest was exposed to acidic and deoxygenated *in vitro* environments to a similar degree to that seen in strenuous exercise, a significantly increased RBC rigidity was detected that further increased in an acidic environment relative to control blood even though no sickling was observed (Xu et al., 2016).

1.2.1. Aggravating factors

1.2.1.1. Hypoxemia

A study of otherwise healthy SCT carriers and controls underwent arterial and venous blood sampling before and after performing maximal arm crank exercises, once at an altitude of 1,270 meters ($PiO2 = 127$ mmHg), and again at a simulated level of 4000 meters ($PiO2 = 85$ mmHg). Even though there was a wide range of sickling, a significant increase was found in the percentage of sickled cells in venous blood drawn from the arm performing exercise. There was no association between exercise performance and degree of sickling, and no significant difference in pH recorded at or after peak exercise between the different simulated altitudes., There was, however, a significant increase in sickling in blood from an exercising limb at 1,270 m and even more sickling in the limb at the

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simulated 4000 m elevation, suggesting that differences in PiO2 rather than pH

contributed to sickling (Martin, Weisman, Zeballos, & Stephenson, 1989).

1.2.1.2. Acidosis

In contrast, there is also evidence that metabolic acidosis independently

contributes to increased RBC rigidity. An *in vitro* study (Xu et al., 2016) used a

microfluidic system that was capable of controlling oxygen and pH levels separately to

study RBC rigidity. They measured the degree of RBC stiffness by measuring the

change in RBC shape during and after shear stress was applied. They found that at

baseline, RBCs from healthy SCT volunteers were stiffer than those from controls, but

the stiffness increased further when RBC samples were measured under acidic

environments meant to match the local pH level of strenuous exercise. Of note, the

degree of rigidity in this study did not change under deoxygenated states; even in the

acidic environment, decreasing the local oxygen level did not change the level of

stiffness.

1.2.1.3. Intensive exercise

Intensive exercise can increase the blood viscosity in both healthy individuals and

SCT carriers. A study (Philippe Connes et al., 2006) measured blood viscosity, RBC

rigidity and hematocrit in both healthy subjects and SCT carriers during intense

exercising using a cycle ergometer conducted to maximal oxygen uptake (100% VO_2

max) and 110% VO_2 max. They found that SCT carriers had both elevated levels of

blood viscosity and RBC rigidity at baseline. Viscosity increased in both groups with

maximal and supramaximal exercise, and the increase in viscosity was maintained in

SCT carriers and continually stayed higher with exercise relative to controls. There was

no difference in plasma viscosity (as opposed to total blood viscosity) between the two

groups. RBC rigidity did not change with or without exercise.

1.2.2. Observed pathologic changes

1.2.2.1. Increased blood viscosity

Blood viscosity in SCT carriers increases with exercise. A study (Diaw et al.,

2014) involving 11 otherwise healthy male SCT carriers and 11 controls showed that

while blood viscosity in SCT carriers was higher at baseline relative to controls, during

vigorous exercise with and without access to water, the SCT carriers' blood viscosity

decreased below resting values to the same level as controls, whereas when exercising

without water, blood viscosity rose over baseline.

1.2.2.2. Oxidative stress.

Reduced nitric oxide (NO) availability and dysregulated NO homeostasis is

known to play a role in the development of SCD complications (Kato, Gladwin, &

Steinberg, 2007). In SCD, hemolyzed RBCs release intravascular hemoglobin which

consumes NO, decreasing the availability of NO in the endothelium which in turn can

reduce vascular relaxation, increase platelet activation and expression of cell adhesion

molecules. While SCT carriers do not experience hemolysis to the extent that people

with SCD have, autoxidation also occurs with HbS molecules of SCT carriers which can

also contribute to oxidative stress (Barodka et al., 2014).

Though exercise in SCT is not associated with hemolysis, one study (Fasmall io et al., 2012) noted that there were increased markers of oxidative stress in subjects with SCT relative to normal controls. Interestingly, in subjects with both SCT and alpha thalassemia, these effects were mitigated, with no significant changes in markers of oxidative stress compared to people without SCT with or without α-thalassemia.

Another method to measure oxidative stress is to measure concentrations of the heme degradation products oxyhemoglobin and methemoglobin (Nagababu, Fabry, Nagel, & Rifkind, 2008). One study (Barodka et al., 2014) evaluated levels of heme degradation products and levels of RBC deformability among four groups of children and young adults: those with SCT, those with SCD who were not currently suffering a sickle cell crisis, those currently in crisis, and healthy controls. The groups with SCT and SCD all had significantly higher levels of heme degradation products and significantly lower levels of deformability compared to healthy controls, with the patients in sickle cell crisis being the most markedly abnormal, followed by the other patients with SCD currently in a steady state, followed by the group with SCT. α-thalassemia status was not evaluated.

In contrast, a separate study (Tripette, Connes, et al., 2010) measuring adhesion molecules, oxidative stress and nitric oxide markers after exercise in sickle cell trait carriers with age-matched controls (and excluding people with α -thalassemia) did not show any differences in markers of oxidative stress.

1.2.2.3. Increased RBC adhesion and release of inflammatory molecules.

Cellular adhesion interactions are known to be augmented with epinephrine release in people with SCD (Hines et al., 2003). A study by Maciaszek et al(Maciaszek, Andemariam, Huber, & Lykotrafitis, 2012) found that epinephrine increases the frequency and strength of adhesion events between adhesion molecules on RBCs of SCT carriers via BCAM/Lu and Intercellular adhesion molecule 4 (ICAM-4). BCAM/Lu, also known as Lutheran group and basal cell adhesion molecule, antigens are present on RBCs and adhere to the endothelial basement membrane (El Nemer et al., 2007). ICAM-4 is also found on RBCs and had an increased frequency and strength of adhesion in SCT RBCs relative to wild-type controls. Of note, in the study, areas of increased adhesion were able to be spatially mapped on the surface of RBCs, and adhesion molecules were noted to be more heterozygously distributed on the hemoglobin of SCT carriers relative to normal controls. This led the authors to speculate that HbS may disrupt the structure of the RBC membrane resulting in aggregation when exposed to epinephrine.

There are significantly higher levels of vascular adhesion molecules in SCT carriers with low amounts of physical activity compared to physically active SCT carriers as well as controls when undergoing strenuous exercise. Aufradet et al (Aufradet et al., 2010) performed a study measuring levels of soluble vascular cell adhesion molecule-1 (sVCAM), P-selectin and other markers of inflammation in a group of 32 subjects, half of whom were SCT carriers. Half of the SCT carriers exercised regularly and half had sedentary lifestyles. They performed measurements of these markers before and after exercising, and found that while other inflammatory markers were not significantly different between groups, the sedentary SCT carrier group had significantly higher levels

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of sVCAM relative to the other three groups: active SCT carriers, active controls and sedentary controls, none of whom differed significantly from each other.

A similar study evaluated levels of adhesion molecules in athletes with SCT with α-thalassemia trait, athletes with SCT but no without α-thalassemia trait, and athletes with neither SCT or α-thalassemia. Athletes who had SCT but no α-thalassemia had higher levels of sVCAM-1 at baseline. All three groups had significant increases in sVCAM-1 with exercise, but the elevated levels persisted longer during recovery in people with SCT and no α-thalassemia. Participants with both SCT and α-thalassemia had similar levels to the control group (Monchanin et al., 2008).

1.2.2.4. Vascular remodeling.

SCT carriers have a different skeletal muscle capillary structure compared to fitness-matched controls. In a study by Vincent et al of 30 Cameroonian volunteers, 10 of whom had normal hemoglobin, 5 with alpha thalassemia, 6 with SCT carrier status and 9 with both SCT and α-thalassemia, significant differences were noted between groups (L. Vincent et al., 2010). SCT carriers had a lower capillary density, and lower capillary tortuosity. SCT carriers also had a significantly increased number of wider capillaries. Participants with both SCT and α thalassemia trait had a significantly higher capillary tortuosity relative to SCT carriers without α thalassemia. The study also measured various endothelial markers and noted no differences in levels of soluble ICAM-1 (sICAM-1), sVCAM-1, sE-selectin, sP-selectin, IL-8, or IL-10 between SCT carriers and

controls. A limitation of this study was that the participants were sedentary and blood samples were not collected during exercise.

In a separate study by the same group, the researchers found that capillary density increased with exercise in SCT carriers, however the differences between SCT carriers and controls remained despite this increase (Lucile Vincent et al., 2012).

1.2.2.5. Microvascular occlusions.

Increased RBC rigidity, increased viscosity, RBC adhesion, inflammation and vascular remodeling are all pathologic changes thought to contribute to microvascular occlusions. There is also evidence that there are higher levels of coagulation activation and inflammation markers in individuals with SCT. A study of individuals from Saudi Arabia showed significantly elevated levels of D-dimer and lower levels of protein C, protein S, and fibrinogen in individuals with SCT relative to normal controls (Adam et al., 2008). D-dimer levels were also elevated in African American SCT carriers in the Jackson Health Study relative to African American participants without SCT, with a median D-dimer concentration of 0.55 μg/mL compared to 0.38 μg/mL (Rakhi P. Naik et al., 2016).

However, actually observing microvascular occlusions in SCT carriers has been only rarely reported, and even then, only in organs other than muscles. Anzalone et al. reported the autopsy results of a 19 year old male SCT carrier who died after collapsing during college football training who had focal packing of sickled red blood cells in the spleen (Anzalone et al., 2010). Wirthwein et al. reported autopsies of three young SCT

carriers who died following physical exertion, and they noted occlusions in the microvasculature of the heart, kidneys, and spleen (Wirthwein, Spotswood, Barnard, & Prahlow, 2001).

In contrast, in studies of SCD, microvascular occlusions have been observed in multiple settings: in autopsy specimens (Niraimathi, Kar, Jacob, & Basu, 2016), biopsy specimens of SCD patients of both the microvasculature (Lipowsky, Sheikh, & Katz, 1987), and larger vessels (Stockman, Nigro, Mishkin, & Oski, 1972), and in mouse models (Kaul, Fabry, & Nagel, 1989; Kaul, Finnegan, & Barabino, 2009; Manwani & Frenette, 2013).

There is a well-documented association of venous thromboembolism risk with SCT (Austin et al., 2007). However, it is not clear if the mechanisms and risk factors that would trigger a venous thromboembolism would also cause microvascular occlusions.

1.2.3. Mitigating factors

1.2.3.1. Physical fitness

Markers of oxidative stress associated with exercise was decreased in SCT carriers that exercise regularly relative to inactive SCT carriers. A study by Chirico et al. (Chirico et al., 2012) studied levels of malondialdehyde (MDA, a byproduct of lipid peroxidation and a marker of oxidative stress), antioxidant enzymes (superoxide dysmutase, gludathione peroxidase, and catalase) as well as markers of nitric oxide metabolism among SCT carriers that were sedentary, SCT carriers that played sports at least 8 hours a week, and normal controls who exercised regularly or were sedentary.

The study observed that anti-oxidants and products of nitric oxide metabolism were significantly increased in active SCT carriers. The greatest magnitude of difference was from changes in plasma MDA. There was a significant increase in MDA in the sedentary SCT participants during and after exercise, whereas none of the other subgroups experienced any significant changes from baseline

1.2.3.2. Adequate hydration

Adequate hydration is important in mitigating the effects of heavy exercise at least in part by decreasing blood viscosity. A study by Tripette et al (Tripette, Loko, et al., 2010) evaluated 12 SCT carriers and 12 controls and had them exercise for 40 minutes of submaximal exercise. Both the SCT carrier group and the control group performed the same exercise twice, once with water offered ad libitum and again without water. Both blood viscosity and RBC rigidity were elevated in SCT carriers at baseline relative to controls, but viscosity and RBC rigidity both decreased to the same level as the control group when the exercise was performed while hydrated. The non-SCT control group who did not hydrate during exercise did not experience any change in RBC rigidity and had only a non-significant increase in blood viscosity with exercise and this decreased to baseline in the recovery phase.

1.2.3.3. Alpha Thalassemia.

In addition to have having a lower hemoglobin S percentage as mentioned above, people who co-inherit both a single or double loss of an α-globin gene and SCT have fewer physiological abnormalities than do SCT carriers without any degree of α

thalassemia, as seen in the study on capillary tortuosity as by (L. Vincent et al., 2010) and levels of adhesion molecules (Monchanin et al., 2008). MDA levels in the study by Chirico et al (Chirico et al., 2012) were higher in SCT carriers without α-thalassemia after undergoing strenuous exercise, as well as a significantly lower change in nitrogen oxide metabolism from baseline.

1.3. Conclusion

Under certain circumstances, SCT can provoke rhabdomyolysis. The most commonly postulated mechanism by which this happens is via changes to the red blood cell that increase adhesion, blood viscosity, and the activity of inflammatory molecules. This is thought to lead to vascular and endothelial changes which ultimately increase the risk of micro-occlusions in the musculature which lead to muscle necrosis and release of intracellular components. Mitigating this is the presence of α-thalassemia trait, physical fitness, and adequate hydration which appear to downregulate his process.

Arguing against this mechanism is the paucity of data demonstrating actual microvascular occlusions in SCT carriers as well as more recent vigorous epidemiological data suggesting that the association of exertional rhabdomyolysis with SCT is lower than was once thought (Nelson et al., 2016). An alternative explanation for the association of SCT and exertional rhabdomyolysis is the co-inheritance of other genetic abnormalities with SCT, such as glucose -6-phosphate dehydrogenase deficiency or differences in intra-cellular calcium receptors (P. Connes, Harmon, & Bergeron, 2013).

More research is needed into the pathophysiology of rhabdomyolysis in SCT carriers, specifically in the development of microvascular obstruction or muscle ischemia. The recent characterization of a mouse model of SCT may be helpful with this (Zappia et al., 2017) given the ethical problems this would pose in testing human volunteers. The fact that the exact mechanism remains difficult to prove may be good news for SCT carriers as it suggests that exertional rhabdomyolysis is a rare event, and supports the American Society of Hematology's recommendations against universal SCT testing in athletic participation (Thompson, 2013).

CHAPTER 2: ASSOCIATION OF SICKLE CELL TRAIT WITH ATRIAL FIBRILLATION: THE REGARDS COHORT

2.1. Introduction

Sickle cell trait (SCT) is the heterozygous, purportedly asymptomatic carrier state for sickle cell disease (SCD) and is present in approximately 8% of African-Americans (Ojodu et al., 2014). SCD profoundly affects individuals' quality of life and leads to painful vaso-occlusive crises and progressive disability leading to early death. Once thought to be a benign condition (Motulsky, 1973), SCT is associated with an increased risk of chronic kidney disease (CKD) (R. P. Naik et al., 2014; R. P. Naik & Haywood, 2015), and other medical conditions (Austin et al., 2007; Davis, Mostofi, & Sesterhenn, 1995; Harmon et al., 2012; Kark et al., 1987; Tsaras, Owusu-Ansah, Boateng, & Amoateng-Adjepong, 2009). Previous studies have shown no evidence to date that SCT leads to a lower life expectancy (Ashcroft & Desai, 1976; Stark, Janerich, & Jereb, 1980), or stroke (H. I. Hyacinth, Carty, Seals, & et al., 2018), and there are mixed data on the association with coronary artery disease (Bucknor, Goo, & Coppolino, 2014; Hyacinth I Hyacinth et al., 2016; Nelson et al., 2016). Prior studies have not examined associations between arrhythmias such as atrial fibrillation (AF) and SCT.

AF has been documented in SCD patients in multiple studies (Bode-Thomas, Hyacinth, Ogunkunle, & Omotoso, 2011; Garadah, Gabani, Alawi, & Abu-Taleb, 2011; Holloman, Johnson, & Haywood, 1987; Upadhya et al., 2013). In contrast to SCD, there

are no similar studies documenting AF in SCT carriers. Cardiac problems in people with SCT have focused on athletes and sudden cardiac death (Key, Connes, & Derebail, 2015; Tsaras et al., 2009). We evaluated the association with SCT and AF as AF is a common condition with an increasing prevalence (Piccini et al., 2012) especially as the population ages and is a risk factor for ischemic stroke and heart failure (Zoni-Berisso, Lercari, Carazza, & Domenicucci, 2014).

Naik et al. demonstrated an association of SCT with an increased risk of CKD in several cohorts, including the REasons for Geographic and Racial Differences in Stroke (REGARDS) study (R. P. Naik et al., 2014; R. P. Naik & Haywood, 2015). CKD and hypertension are known risk factors for AF (Watanabe et al., 2009), and SCT may be associated with AF by other poorly defined mechanisms beyond simply exacerbating other known risk factors. Thus we hypothesized that SCT would be associated with an increased risk of prevalent and incident AF in African-Americans.

2.2. Methods

2.2.1. Study Cohort

REGARDS recruited 30,239 black and white men and women over 45 years old from 2003-2007, with the principal aim of identifying causes of racial and regional disparities in stroke incidence and mortality in the United States. The detailed study design has been published (Howard et al., 2005). REGARDS recruited individuals from a commercially available list of U.S. residents. Participants were excluded if they could not speak English, had a self-reported race other than black or white, were on a waiting

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list for a nursing home, or had active cancer in the past year. Using a computer-assisted

telephone interview, interviewers obtained demographic information and a cardiovascular

medical history, including a physician diagnosis of AF. Consent was obtained initially on

the telephone and subsequently in writing during an in-person evaluation 3-4 weeks later.

During the visit, staff obtained blood and urine samples, medication history, and

performed a resting ECG. ECGs were centrally interpreted for factors including prevalent

AF. Participants were followed every 6 months by telephone for possible stroke and

other outcomes (Soliman et al., 2012).   A second in-home visit was performed an

average of 9.2 years later among available REGARDS participants, where participants

gave their health history and a second ECG was performed, and similarly interpreted for

AF.

SCT status was determined by direct genotyping for rs334 using TaqMan®

(Hyacinth I Hyacinth et al., 2016; R. P. Naik & Derebail, 2017).

Genotyping for SCT was not performed on participants who self-reported as

white. For this analysis, REGARDS participants were excluded if they did not self-

identify as black, did not have stored DNA or consent for genetics research, or if they had

hemoglobin SS (SCD) or hemoglobin SC disease (a related sickling hemoglobinopathy).

REGARDS was approved by the institutional review boards of all participating

institutions and was conducted according to the provisions of the Declaration of Helsinki.

2.2.2. Outcomes

For the cross-sectional analysis, as previously reported (Soliman et al., 2011), AF was defined at baseline as ECG evidence of AF at the time of the in-home physical exam or as a self-reported physician diagnosis of AF. For the incident AF analysis, incident AF was defined as a self-reported physician diagnosis of AF or ECG evidence of AF in those free of AF by ECG or self-report at baseline and was assessed in the form of a 2nd in-home visit.

2.2.3. Definitions

Age was specified as age at the time of entry into the REGARDS cohort. Income was categorized as <$20,000, $20,000-34,999, $35,000-74,999, or ≥$75,000 annually, and education as less than high- school, high-school graduate, some college, and college and above. Cardiovascular disease (CVD) was defined as a self-reported physician diagnosis of prior myocardial infarction or stroke. Hypertension was defined as the use of antihypertensive medications, or a resting in-home systolic blood pressure reading of ≥140 mmHg. Diabetes was defined as a prior self-reported physician diagnosis of diabetes, or the use of oral medications or insulin, excluding gestational diabetes. CKD was stratified into four groups (stage 0-1, 2, 3, 4+) according to the Kidney Disease: Improving Global Outcomes 2012 Clinical Practice Guidelines (Levey et al., 2011) incorporating estimated glomerular filtration rate from the CKD-EPI equation (Levey & Stevens, 2010) and urine albumin concentration.

2.2.4. Statistical analysis

Baseline characteristics were compared by SCT status using chi-square analysis and t-tests for continuous variables. Staged logistic regression models were used to calculate odds ratios (ORs) of AF by presence of SCT. Covariates were added in three steps:

Model 1: adjusted for age, sex, income, and education

Model 2: Model 1 + body mass index, history of CVD, and diabetes

Model 3: Model 2 + CKD and hypertension

The purpose of Model 3 was to test for possible mediation of the association by CKD and hypertension, given the known association of SCT with CKD (R. P. Naik et al., 2014). In addition to model 3, to control for population substructure, 10 principal components (PC) of genetic ancestry were generated in Eigenstrat (Tran et al., 2015) and added to Model 3. PC of genetic ancestry were measured in a case-control study and available in 67 percent of African-American REGARDS participants. As PCs were not available in all individuals, we assessed the association of SCT with AF only in those with PCs of ancestry available, excluding those without data. Additionally, a sensitivity analysis using electrocardiogram documented AF only was performed. Unadjusted baseline associations were also compared using Chi square analysis. All two-sided p values < 0.05 were considered significant.

The above analyses were repeated in those who had a 2nd home visit by REGARDS investigators who were free of AF at the initial visit to determine the OR of incident AF based on AF status at the second examination. Given that it is not possible to

estimate the precise timing of developing AF based on ECG evidence seen on the 2nd

home visit, cox proportional hazard models could not be used

All analysis was performed using STATA v 14.1 (StataCorp. 2015. *Stata*

Statistical Software: Release 14. College Station, TX: StataCorp LP.)

2.3. Results

There were 10,433 African-American REGARDS participants who had

genotyping for SCT as well as ECG and complete medical history (83% of the 12,514

African Americans in the study). Ten individuals homozygous for hemoglobin SS (sickle

cell anemia) (0.09%) and 14 (0.13%) with hemoglobin SC disease were excluded from

the analysis. There were 778 participants with SCT (7.5%). Figure 2 outlines how many

participants were excluded or had missing data at each stage of the analysis.

| 10,433 participants with genotyping for sickle cell trait and a baseline electrocardiogram | | 10,409 participants with data for model 1 | | 10,295 participants with data for model 2 | | 10,241 participants with data for model 3 |

Approximately 21% withdrew from study, 12% died, 21% not assessed for other reasons

24 participants excluded who had sickle cell anemia or Hb SC disease

80 participants excluded who had missing BMI data, 34 excluded with missing diabetes data

54 participants excluded who had missing data on kidney function

4836 participants available for 2nd in-home visit

4,836 participants with data for model 1

4,795 participants with data for model 2

4,764 participants with data for model 3

29 participants excluded who had missing BMI data, 12 excluded with missing diabetes data

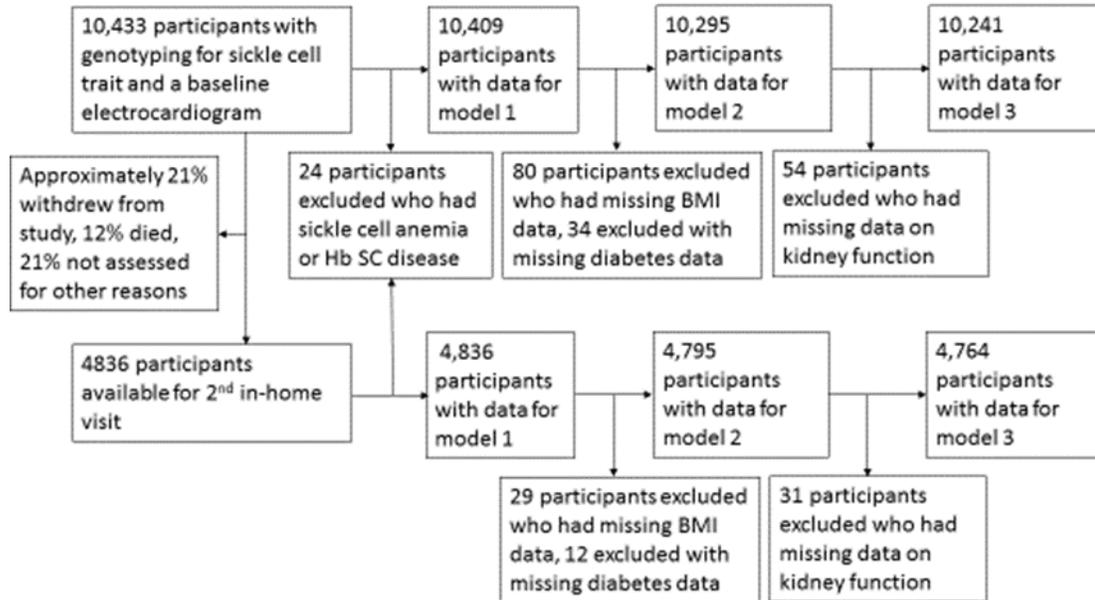31 participants excluded who had missing data on kidney function

Figure 2: Summary of participant exclusions and missing data

Overall, 811 (7.8%) of these participants had either a medical history and/or ECG evidence of AF at baseline. Table 1 summarizes the baseline characteristics of participants with and without SCT.

Table 1: Baseline characteristics of African American REGARDS participants by Sickle Cell Trait Status

	No Sickle Cell Trait	Sickle Cell Trait	p-value
Mean Age (SD)	64 (9)	63.5 (9)	0.14
Female	61%	64%	0.10
Income < $20,000/year	38.9%	40.9%	0.28
Less than high school education	19.7%	19.2%	0.84
Mean BMI (SD)	30.8 (6.7)	30.7 (6.8)	0.72
History of cardiovascular disease	13.7%	13.5%	0.85
Diabetes	30.3%	32.2%	0.25
Stage 4 or higher chronic kidney disease	1.4%	2.9%	0.001
Hypertension	66%	63%	0.07
Mean Systolic Blood Pressure (SD)	131 (17)	132 (17.7)	0.26
Atrial Fibrillation	7.3%	9.5%	0.02

The mean baseline age was 64 years (range 45-96). Twice as many participants with SCT had stage 4 kidney disease by KDIGO criteria (2.9% vs 1.4% for people with and without SCT respectively, p=0.001).

Table 2 outlines the main results of the cross sectional analysis. Overall, SCT was associated with an approximately 1.3-fold increased odds of AF in all models (lower limit of the 95% CI >1.0). In Model 1 (the demographic model) the OR (95% CI) was 1.32 (1.03-1.70). Further adjustment for AF risk factors (Model 2) or for CKD and hypertension (Model 3) did not attenuate or strengthen the association of SCT with AF.

Table 2 Odds Ratios (ORs) and 95% Confidence Intervals (CIs) of prevalent atrial fibrillation with sickle cell trait

	N	N with prevalent AF	OR (95% CI)
Model 1	10,409	811	1.32 (1.03, 1.70)
Model 2	10,295	797	1.33 (1.03, 1.71)
Model 3	10,241	793	1.32 (1.02, 1.70)
Model 1: adjustment for age, sex, income and education. Model 2: Model 1 + adjustment for body mass index, cardiovascular disease history, diabetes. Model 3: Model 2 + adjustment for chronic kidney disease and hypertension.			

At the second in-home visit, an average 9.2 years after baseline, 4,836 participants with baseline known AF status and genotyping had an ECG. There was a similar association with incident AF and SCT as seen in prevalent AF with the OR in model 3 being 1.25 but the 95% confidence intervals were wider and crossed 1 (0.77, 2.03) (Table 3).

Table 3: Odds Ratios (ORs) and 95% Confidence Intervals (CIs) of incident AF over 9.2 years by sickle cell trait

	N	N with incident AF	OR (95% CI)
Model 1	4,836	208	1.25 (0.77, 2.03)
Model 2	4,795	206	1.27 (0.78, 2.07)
Model 3	4,764	206	1.28 (0.78, 2.08)
Model 1: adjustment for age, sex, income and education. Model 2: Model 1+ adjustment for body mass index, cardiovascular disease history, diabetes. Model 3: Model 2 + adjustment for chronic kidney disease and hypertension.			

In a sensitivity analysis classifying prevalent AF as AF present at either REGARDS exam, the association of SCT with AF was stronger than in analysis from the

baseline visit, with an OR of 1.39 (1.01, 1.92) for model 3 (Table 4). In this analysis, the

total prevalence of ever having reported a history of AF or ever having ECG evidence of

AF in the participants available for a 2nd in-home visit was slightly higher than the main

cohort, at 10.1%.

Table 4 Odds Ratios (ORs) and 95% Confidence Intervals (CIs) of cross-sectional analysis of all prevalent AF at 2nd in-home visit by sickle cell trait

	N	N with prevalent AF	OR (95% CI)
Model 1	4,836	490	1.43 (1.05, 1.97)
Model 2	4,795	483	1.41 (1.03, 1.94)
Model 3	4,764	482	1.39 (1.01, 1.92)
Model 1: adjustment for age, sex, income and education. Model 2: Model 1+ adjustment for body mass index, cardiovascular disease history, diabetes. Model 3: Model 2 + adjustment for chronic kidney disease and hypertension			

In an additional sensitivity analysis of the odds of prevalence of ECG evidence of

AF, the association of SCT with AF was greater, with ORs of 1.7-1.8 in all three models,

while the association was weaker for AF defined by history alone (OR of 1.26 in all three

models), but in both instances the 95% CIs included 1.0. When we restricted the analysis

to individuals with PCs of genetic ancestry available, there was no association of SCT

with AF in this subset (OR 1.01; 95% CI 0.71, 1.44), as such we did not further pursue

adjusting for PC of genetic ancestry in additional models.

2.4. Discussion

The main findings of this study were a 32% higher odds of prevalent AF in

African Americans with SCT after adjusting for age, gender, education, income, and

cardiovascular disease. Additional adjustment for CKD or hypertension did not attenuate

this association.   There was also a 26% higher odds of incident AF, though this finding was not statistically significant.

To our knowledge, the association of SCT with AF has not been reported before. We had hypothesized that SCT could be associated with AF by acting through the intermediaries of CKD (Watanabe et al., 2009) which in turn may be associated with hypertension (Kannel, Abbott, Savage, & McNamara, 1982). Arguing against CKD and hypertension mediating the association of SCT with AF is that after adjusting for CKD, hypertension, and known cardiovascular disease, the association of SCT with AF did not meaningfully change. These data are confusing but consistent with the surprising but robust recent epidemiological research demonstrating that SCT was not independently associated with risk of ischemic stroke among African Americans in the United States (H. I. Hyacinth et al., 2018), even though ischemic stroke is associated with CKD and SCT is associated with risk of CKD.

While we have previously shown the substantial accuracy of self-reported AF(Soliman et al., 2011), a strength of this study includes the ability to capture AF data by both medical history and ECG. Medical history reported by REGARDS participants has also been validated by comparing it with Medicare claims data in other cardiovascular diseases (Colantonio et al., 2017) and it provides a better capture of paroxysmal or persistent AF. These are conditions which are still associated with thromboembolism and that require anticoagulation (Ganesan et al., 2016) as well as

rhythm-controlled AF, and reported history of AF has been found to be a significant risk factor in developing ischemic stroke (Soliman et al., 2011). Furthermore, when a more strict definition of AF by ECG criteria alone was used, the direction of the association was similar with a greater magnitude. Additionally, the fact that there was a similar prevalent and incident association is helpful in that is provides an internal replication of results.

This study has several limitations. While data on prevalent AF at the time of entry into the study is robust, the data on incident AF was less so: medical histories and a second in-home ECG were available from only about half of the participants, with approximately 12% dying between visits, 21% withdrawing from the study, and 21% not assessed for other reasons. The number of participants with incident AF was relatively small so our power was limited. Finally, when we looked at the subset of participants with PC of genetic ancestry available there was no association between SCT and AF. PC data was available in only a subset of the cohort, and as there were significantly more participants with AF in the subset that did not have PC data available, it appeared to be non-random, rather than a simple loss of power. In theory, SCT could be a marker of another nearby mutation and adjusting for population substructure may have attenuated this association. While possible, this would be unlikely due to the known pathologic implications of SCT and SCD as well as lack of attenuation of SCT with adverse outcomes after adjusting for PC of genetic ancestry for other diseases in other populations (H. I. Hyacinth et al., 2018; R. P. Naik et al., 2014). Finally, as genotyping

for SCT was limited to African American participants in REGARDS, these findings may not be generalizable to people of other races or living in other regions.

2.5. Conclusion

SCT was associated with AF independent of risk factors for AF. The mechanism does not appear to act through CKD and hypertension, known risk factors for AF and conclusions regarding a proposed mechanism cannot be drawn from the data. Given the association of hypertension and CKD with AF, these data support the need to better understand the clinical consequences of SCT as well as other polymorphisms which may impact the association of SCT with cardiovascular diseases. These data add AF to the potential diseases associated with SCT and demonstrate the keen need to understand the health consequences of SCT as it seems less and less to be an asymptomatic carrier state of a common genetic disease.

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