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Framing the research agenda for sickle cell trait: building on the current understanding of clinical events and their potential implications

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Abstract

Sickle Cell Trait (HbAS), the heterozygous state for the sickle hemoglobin beta globin gene is carried by as many as 100 million individuals including up to 25% of the population in some regions of the world (World Health Organization, Provisional agenda item 4.8, EB117/34 (22 December 2005) or World Health Organization, Provisional agenda item 11.4 (24 April 2006)). Persons with HbAS have some resistance to falciparum malaria infection in early childhood (Piel FB, Patil AP, Howes RE, et al., *Nat Commun* 2010;1104:1–7 and Aidoo M, Terlouw DJ, Kolczak M, et al., *Lancet* 2002;359:1311–1312) and as a result individuals with HbAS living in malarial endemic regions of Africa have a survival advantage over individuals with HbAA. Reports from the US emphasize possible health risks for individuals with HbAS including increased incidence of renal failure and malignancy, thromboembolic disorders, splenic infarction as a high altitude complication, and exercise-related sudden death. The National Heart, Lung, and Blood Institute, National Institutes of Health convened a workshop in Bethesda, Maryland on June 3–4, 2010, *Framing the Research Agenda for Sickle Cell Trait*, to review the clinical manifestations of HbAS, discuss the exercise-related sudden death reports in HbAS, and examine the public health, societal, and ethical implications of policies regarding HbAS. The goal of the workshop was to identify potential research questions to address knowledge gaps.

Background

Global perspectives on the health impacts of HbAS

Studies in the 1950's revealed that the presence of sickle hemoglobin in erythrocytes appeared to protect against malaria, currently responsible for more than 1 million childhood deaths per year in sub-Saharan Africa. In 1,022 children from a birth cohort in Kenya, the

highest mortality in children aged 2 to 16 months occurred in those with HbSS (sickle cell anemia), followed by HbAA (normal hemoglobin) and HbAS (sickle cell trait). The study demonstrated a survival advantage for children with HbAS over those with HbAA, with a 55% reduction in all-cause mortality [3].

More recently, investigators followed 800 children and adults in Kenya for clinical events. While HbAS had no impact on the prevalence of asymptomatic parasitemia, it was 50% protective against mild clinical malaria, 75% protective against hospital admission for malaria, and 90% protective against severe or complicated malaria [4]. In an outpatient clinic in Tanzania, the prevalence of malaria parasitemia was 0.64 for AS, 0.72 for SS, and 2.96 for AA hemoglobin [5]. These studies suggest that children in Africa with HbAS have overall improved survival and lower malaria infection rates, and the difference is largely due to HbAS.

The estimates of prevalence of HbAS in Africa range from 10 to 40% across equatorial Africa and decrease to between 1 and 2% on the North African coast and <1% in South Africa [6]. In all newborns in the U.S., the HbAS prevalence is 1.3% based on a finding of HbAS in 1 out of 12 African Americans [7]. Worldwide, more than 100 million individuals have HbAS.

Potential clinical complications associated with sickle cell trait

A recent review of the medical literature reported that HbAS is not completely benign and provided an evaluation of the strength and specificity of the reported associations [8]. “Definite” associations with HbAS were proposed for exercise-related sudden death, exertional rhabdomyolysis, micro- and macroscopic hematuria, loss of renal concentrating ability, protection against severe falciparum malaria, renal medullary cancer, renal papillary necrosis, and splenic infarction in the setting of low oxygen tension at high altitudes. “Probable” associations with HbAS were suggested for pregnancy-related conditions including fetal loss, low birth weight, preeclampsia, traumatic hyphema, and venous thromboembolic disease.

Acute chest syndrome, asymptomatic bacteriuria in pregnancy, and proliferative retinopathy were reported as “Possible” associations with HbAS. Sufficient evidence was not found to associate avascular necrosis of the femoral head, cholelithiasis, leg ulcers, priapism, or stroke with HbAS. Aspects of this review, including the authors’ conclusions, were addressed by the workshop.

Sudden Death in Sickle Cell Trait: What Is the Evidence?

To review current information, the National Heart, Lung, and Blood Institute, National Institutes of Health convened a workshop to review the clinical manifestations of HbAS, discuss exercise-related sudden death reports in HbAS, and examine the public health, societal, and ethical implications of policies regarding HbAS. The goal of the workshop was to identify potential research questions to address gaps in knowledge. During sessions devoted to discussion of reports of sudden death in individuals with HbAS, three major questions and the potential role for screening were addressed.

1. Exercise-Related Death
 - a. Is HbAS an independent risk factor for sudden death in young athletes?
 - b. What are the etiologies of sudden death?
 - c. Is sudden death preventable in individuals with HbAS?
2. Can screening for HbAS prevent or mitigate-associated morbidity or mortality?

Sudden death in athletes

From a definition perspective, exercise-related sudden death in all athletes is generally viewed as an irreversible and terminal dependence upon life support implemented within 1 hr of the onset of symptoms. The causes of sudden death have been described as cardiac (myocardial, anatomic, electrical, and combined disease), pulmonary, neurologic (brain stem injury and vagal reactions), metabolic (rhabdomyolysis and malignant hyperthermia), endocrine, hemorrhagic, and thrombotic. For cardiac or idiopathic sudden death, the US National Registry of Sudden Death in Athletes [9] reports 115 cases per year and the 36th Bethesda Conference on Eligibility Recommendations for Competitive Athletes with Cardiovascular Abnormalities estimated a prevalence of 1 in 33,000–50,000 with a male predominance of 5:1 to 9:1 [10]. The number of reported cases increased by about 6% per year with 0.3% of the deaths attributed to HbAS. Of the cardiovascular deaths, 82% were reported as exertional; 2.4% of the deaths were attributed to heat stroke. The most common cardiac etiology was hypertrophic cardiomyopathy (HCM) (36%) followed by coronary artery anomalies (17%). The average age of those affected was 18 ± 5 years and 89% were reported to be male. Hypertrophic cardiomyopathy, inherited as an autosomal dominant trait, has been attributed to mutations in genes that encode for sarcomere proteins [11]. Thirteen genes account for ~50% of HCM cases. HCM is disproportionately present in African American athletes. Of the relevant electrical diseases, the long QT Syndrome is the most important. Inherited in either autosomal or dominant fashion, 13 genes have been described that are involved in the timely execution of the cardiac action potential [12]. The utility of ECG screening for athletes continues to be debated and provides some parallels for the current discussions of screening for HbAS [13]. Issues of cost, test sensitivity and specificity, resource utilization and difficulty of program implementation are central to this discussion [14].

Performance of black athletes

Review of the historical athletic participation of individuals from ancestral populations with higher prevalence of HbAS (e.g. athletes of African ancestry, self identified as black or African American), adds perspective. In the 1936 Olympics, 18 black athletes won 14 medals, 25% of those won by US athletes, and dominated the track and field events. In the 1968 Olympics, nearly 19% of the athletes were black and ~1% (11/1,265) had HbAS. There were no clinically detected adverse events at Mexico City's high altitude (7,350 feet above sea level). Black athletes continue to participate in the National Football League (NFL) (60% of players are black), the National Basketball Association (NBA) (over 80% of players are black), and in college athletics (34% of football players and 45% of male and 33% of female basketball players are black). Many U.S. black athletes compete at high altitude in Denver, Colorado (5,280 feet above sea level), experiencing frequent exposures to reduced oxygen tension, with rare reports of adverse events, although one NFL player with HbAS suffered splenic complications.

Conditions favoring sickling

Acute, intense exercise has multiple effects that could potentially trigger sickling of HbS containing erythrocytes. These effects include a decrease in plasma volume and pH and increases in sympathetic outflow, oxidative stress, heat production, microvascular perfusion, tissue hypoxia, and release of inflammatory mediators. These detrimental effects are balanced by the benefits of chronic physical training which include a reduced resting heart rate and blood pressure, improved autonomic system balance, increased maximal myocardial oxygen uptake, improved endothelial function and vascular compliance, metabolic and neuromuscular modifications, and training-specific muscle adaptations [15].

The potential roles of skeletal muscle function in exercise

Skeletal muscle function is fundamental to exercise and is affected by mechanical, metabolic, hormonal, and neuronal stressors. The consequences of these stressors depend on the degree of loading, the number of contractions, and the thresholds of protective mechanisms for stress-induced damage. The threshold is affected by fiber type, number and oxidative content of mitochondria, energy reserves, speed and strength of fiber contraction, and fiber size. The major types of muscle fibers are Type I (slow twitch), Type IIa (fast twitch), and Type IIb (x) (fast twitch). Slow twitch fibers have higher aerobic capacity, myoglobin content, and fatigue resistance, whereas fast twitch fibers have higher glycolytic capacity, glycogen content, and sarcoplasmic reticulum (SR) development. Contraction speeds are slower for the fast twitch fibers compared with the slow twitch fibers. The three types of fibers are further distinguished by different myosin heavy chains.

Black, African men had significantly more Type IIa fibers (49 vs. 42%) and fewer Type I fibers (33 vs. 41%) than Caucasian men [16]. The muscle activities of glycolytic pathway enzymes were 40 to 76% higher in black than Caucasian men and the average creatine kinase nearly twice as high. Obese black women have been reported to have a higher percentage of Type IIb fibers and a lower percentage of Type I fibers than Caucasian women [17].

A study of young, military recruits with Exertional Heat Illness (EHI) in Taiwan found that 24 of 37 with EHI developed rhabdomyolysis and that the percentage of Type II fibers was 66% in controls, 75.5% in individuals with EHI alone, and 81% in those with EHI and rhabdomyolysis [18].

Other genetic factors influencing muscle function

Rapid rises in cytoplasmic calcium are required for skeletal muscle contraction. The increases in calcium are the result of calcium release through type I ryanodine receptors (*RYR1*). Analysis of *RYR1* is used as a screening test for susceptibility to Malignant Hyperthermia. A possible relation between malignant hyperthermia, EHI, and rhabdomyolysis has been proposed. Small clinical series have suggested a relationship between *RYR1* variants and Malignant Hyperthermia and coexistent HbAS [19]. Other potential genetic factors contributing to sudden death in African Americans include polymorphisms in the cardiac sodium channel gene, *SCN5A* Y1102, associated with increased risk of sudden death and ventricular arrhythmias in black adults [20].

Lessons from the military experience

Individuals with HbAS could be at increased risk for EHI due to the polymerization of sickle hemoglobin in an exercise induced hypoxic, dehydrated, acidotic, hyperthermic environment. Agonal hypoxia causes extensive sickling in these individuals and may not have a causal role. There is no evidence that antemortem sickling can be differentiated from postmortem sickling in HbAS carriers who die suddenly. To study this on a population basis, all natural deaths among ~2 million Armed Forces Recruits were reviewed from 1977 to 1981. The prevalence of HbAS was estimated based on the number of African American recruits (8%) plus 0.08% of the recruits from other races. Fewer than 10% of the sudden death cases were tested for hyperthermia or rhabdomyolysis. The calculated number of African American HbAS recruits had a 30-fold increased relative risk of exercise-related death compared with those without hemoglobin S ($P < 10^{-6}$).

Excess mortality related to exercise has been investigated in military recruits and may help evaluate interventions to reduce the risk for participation in college level athletics [21–23]. Half of the cases of military recruit deaths had fatal EHI with exertional rhabdomyolysis,

heat stroke, and/or acute renal failure although requirements for life support were delayed. The other half suffered idiopathic sudden death in cardio-pulmonary arrest. About 1 out of 15 to 1 out of 30 of these cases were found to have acute EHI as the cause of sudden death; however, only 3% were screened for EHI. The spectrum of clinical presentations described in military recruits included collapse, rapid progression of muscle pain, slowly developing painful muscle(s), and hyperthermia.

In response to these findings, an interventional program was devised when the Wet Bulb Temperature Index increased. The program included limiting exercise intensity, reducing work/rest ratios, ordering observed water consumption and use of light clothing in hot weather conditions if possible. Both Test and Control groups used prompt evaluation and triage of any recruit with early signs of EHI including failure to keep up with group activities, determination of field rectal temperature, cooling, rehydration, and rapid transport to medical facilities. The intervention reduced deaths in both the HbAS cohort (relative risk for death = 0.06 [$P < 0.0001$]) and the HbAA groups compared with those not receiving the intervention. Both idiopathic and EHI deaths were prevented by the intervention [22].

In a small cohort of military recruits, presumptive alpha-thalassemia was an additional factor reducing risk for exercise-related death. The lower HbS concentration in the cells of those with HbAS-alpha thalassemia may theoretically have reduced HbS polymerization.

As of the date of the 2010 workshop, in collegiate football, 18 deaths related to sickling were reported in the lay press since 1974. Of the 16 cases reported during the last decade, 10 of the individuals had HbAS [24]. Individuals with HbAS represent 3 to 4% of National Collegiate Athletic Association (NCAA) Division I football players. The NCAA attributes the deaths to an “Intensity Syndrome,” not Exertional Heat Illness. The deaths were seen as metabolic complications and are not usually sudden (within 1 hr). For each reported death, there are a total of 3–5 nonfatal events.

Studies in maximally exercising normal individuals demonstrate that hypoxemia and lactic acidosis occur within 5 min [25]. In military recruits with HbAS, the percentage of sickled cells in venous blood increased with exercise and to a greater extent with increased altitude [26]. However, performance was not affected. In contrast, sickling was virtually absent in arterial blood suggesting that sickling may have been reversed by exposure to oxygen tensions in the pulmonary microcirculation.

Reports from the NCAA and responses

The reported deaths in NCAA Division I athletes all occurred during conditioning, not during game play, either in sprinting or speed drills and in one case with weight lifting. Reports of the clinical presentation of events attributed to a proposed “sickling syndrome” suggest that affected individuals experience weakness but do not exhibit slowly evolving, painful muscle cramping, and do not lose consciousness. A syndrome of rhabdomyolysis is apparent in these individuals with typical laboratory findings of hyperkalemia, acidosis, elevated creatine phosphokinase, and renal failure secondary to myoglobinemia. The hypothesis that exercise-induced sickling triggers vaso-occlusion in muscle, which in turn precipitates rhabdomyolysis, has not been demonstrated empirically. Alternative explanations were proposed, such as rhabdomyolysis triggering a reinforcing cascade of events in athletes with HbAS that intensified the rhabdomyolysis syndrome, making a fatal outcome more likely. These discussions highlighted the need for further research in this area.

Altitude with concomitant hypoxia and the potential adverse influence of dietary supplements in the setting of strenuous exercise may also play a role in poor outcomes in

individuals with HbAS. Anecdotal reports of college athletes suggest that sickling precedes rhabdomyolysis. A muscle biopsy study in nonathletes revealed remodeling of the microvasculature in muscles in individuals with HbAS characterized by lower capillary density, lower capillary tortuosity, and enlarged microvessels when compared with controls [27]. In a small series, individuals with HbAS were found to have higher mean blood viscosity at rest compared with healthy controls that was normalized by hydration during exercise [28].

Interventions to mitigate this symptom complex worthy of further study include taking longer breaks during practice and shortening intensity and length of practice sessions held in hot weather or high altitude. A known genetic predisposition to malignant hyperthermia might pose a genetic risk factor in conjunction with HbAS. HbAS phenotypes may be the result of interactions between conditioning status and genetic influences such as muscle fiber types and regulation of cellular calcium and sodium flux.

Interest in the potential risks of participation at college level athletics for individuals with HbAS was brought into focus in 2007 by the National Athletic Trainers' Association (NATA) consensus statement, "HbAS and The Athlete" [29]. NATA recommended that athletes with HbAS can participate in all sports; however, screening and simple precautions should be implemented to help prevent deaths. Although not an evidence-based review, the case for screening was determined to be "strong" by NATA [30]. Coaches and athletes should receive education to reduce risks and to understand the response to serious complications. The Sickle Cell Disease Association of America (SCDAA) did not support the consensus statement. The NCAA recommended that member institutions test student athletes for HbAS in 2009. In April 2010, the NCAA adopted legislation that required screening of athletes. This action required that incoming Division I athletes must be tested for HbAS, show proof of a prior test, or sign a waiver releasing an institution from liability.

Potential role of pathology methods

Pathological evaluation of several individuals with delayed death, survival long enough for medical intervention, determined that all had exerted effort beyond their conditioning level and all presented with metabolic acidosis soon followed by rhabdomyolysis, renal, failure, and DIC [31]. Individuals with sudden or rapid death who experience cardiac arrest before medical intervention should be evaluated using toxicology, pathology, and electrocardiography when feasible. Hemoglobin determination by high pressure liquid chromatography (HPLC) is the method most commonly used to diagnose HbAS at autopsy although it may be confounded by inadequate sampling or sample degradation. Histologic diagnosis has been used to identify HbAS cases with a very high degree of sensitivity and specificity even years after the time of death [31]. Establishment of the diagnosis postmortem does not specifically address whether sickling occurred antemortem or postmortem.

Research Opportunities Related to Sudden Death in Athletes

- i. Conduct a large epidemiological study to address some of the most pressing public health issues of HbAS. Prospective studies could collect information about other morbid states and determine methods to establish the causes of death in athletes and the occurrence of lesser, but important, clinical events. The study should:
 - a. Incorporate information from the NCAA database of college athletes including outcomes data for athletic-related sports
 - b. Assess preventive strategies such as the effects of training and hydration methods in college athletics and/or the military

- ii. Evaluate the use and impact of a standardized content educational message about the risks of injury and death in athletics for people with HbAS.

Ethical, Legal, and Social Implications of Sickle Cell Trait Screening Programs

The identified impediments to research included under-representation of the African–American community in preclinical and translational research projects and limited study in health disparities research.

Ethical considerations in screening athletes for sickle cell trait

A potential framework was proposed for a public health ethics analysis to identify data needs and ethical implications of programs, policies, and interventions following the implementation of NCAA policy on HbAS screening of athletes [32].

1. What are the public health goals for the proposed intervention, policy or program? These include prevention or reduction in the risk of exercise-related morbidity/mortality associated with HbAS.
2. How effective is the intervention, policy, or program in achieving its stated goals? The more burdensome the program, the stronger the evidence that is needed.
3. What are the known or potential burdens of the program? These may include unwarranted loss of opportunities, risks to privacy and confidentiality, a potential to stigmatize the HbAS athlete, a potential to induce anxiety or fear in the athlete or other burdens.
4. How can the burdens be minimized? One method would be a universal program to reduce heat/exertional illness among athletes.
5. Is the program implemented fairly? Inequalities and programs targeted to “high-risk” groups must have strong justification.
6. How can the public health benefits and the accompanying burdens be balanced? The benefits of the program must outweigh the identified or anticipated burdens.

Bioethics, behavioral, and social sciences research

The diagnosis of the carrier state for a genetic disease may be associated with serious health problems. This has been highlighted by a study of carriers of glucocerebrosidase mutations that identified a 5-fold increased risk for Parkinson’s disease [33]. The potential link between HbAS and exercise-related illness could represent another such association.

The passage of the National Sickle Cell Anemia Control Act in 1972 (Public Law 92–294) emphasized screening and counseling programs, development and dissemination of information and educational materials, and research in the “...diagnosis, treatment, and control of sickle cell anemia.” Problems were identified in the subsequent surge in interest in sickle cell disease [34]. These problems included a potential overemphasis on prevention of births of infants with sickle cell disease as the goal of genetic counseling, the portrayal of HbAS as a personal health hazard, and determination of the harms and risks of a testing program. These risks included psychological impact, stigmatization, and the potential for discrimination [35].

Ethical and social considerations in sickle cell counseling

HbAS/sickle cell disease counseling takes place in four contexts: premarital, prenatal, neonatal, and community based. The predictive uncertainty of genetics and genomics is

illustrated by difficulties in predicting the clinical phenotype of individuals affected by sickle cell disease when the genotype is well described [36].

Although newborn sickle cell screening is mandated in all 50 states, information about HbAS status may be poorly communicated to affected individuals [37]. Counseling and education are uncommonly conveyed outside of the family resulting in perpetuation of misinformation. Imparting genetic information affects the entire family including misattributed parenthood. Confidentiality of results and biospecimens must be considered. The prevalence of HbAS varies widely in racial-ethnic groups and affects values about health and wellness and self image of affected individuals.

Sickle carrier detection—Lessons learned over 75 years

The diagnosis of HbAS was initially made by microscopic examination of blood [38]. More than 8% of specimens from at risk individuals had preparations compatible with HbAS. In 1949, the solubility test and use of electrophoretic mobility were introduced to determine the presence of HbS and a diagnosis of HbAS [39].

The lack of counseling, use of poor methodologies, and poorly trained personnel reduced enthusiasm for mass screening following passage of the National Sickle Cell Anemia Control Act [40]. In the 1980's, genetic counseling had limited impact on reproductive decision making [41]. Newborn screening for sickle cell disease received a boost when the results of the Penicillin Prophylaxis in Sickle Cell Disease (PROPS) trial were published [42]. This randomized trial concluded that children should be screened in the newborn period for SCD and that those with sickle cell anemia should receive prophylactic therapy with oral penicillin by 4 months of age to reduce pneumococcal-associated morbidity and mortality. Individuals with HbAS are also detected by newborn screening [43]. In 2005, of 4,190,705 US births, 72,111 were identified as hemoglobin variant carriers including HbS (53,532), HbC (14,814), or HbE (3,695). Carrier followup should include notification of the carrier's parents and provision of both immediate education and counseling and when the carrier reaches reproductive age. Inadvertent side effects of carrier testing for HbAS including decreased self-image, parental overprotection, and discrimination with respect to marriage, employment, or insurance have made the process more difficult [44]. Misdiagnosis of carriers as individuals with SCD may contribute to oversight of other serious medical problems.

Informing the public health agenda with data

Efforts to bring sickle cell screening to the local community in the early 1970's encountered difficulties [45]. The public health risks associated with SCD were seen as low compared with hypertension and environmental diseases. The community had concerns about discrimination and counseling messages that were insufficiently conveyed or understood. Similarly, a pilot education and counseling program did not succeed due to inadequate staffing, poor followup, and insufficient counselor knowledge.

There are currently two Health Resources and Services Administration (HRSA) data collection efforts. These are the SCD Newborn Screening Initiative (SCD-NBS) which includes a national coordinating center and 17 grantees participating in a minimum dataset project and the SCD Treatment Demonstration Program (SCDTDP). Both programs depend on telephone interviews, requests for written information, clinic and home visits, and encounters with community-based organizations (CBOs). The expected outcomes of the data collection are increased SCD knowledge for families including affected children and carriers of HbAS and to improve NBS and carrier followup activities. Recommendations from the initiative include the use of multicultural-multilingual educational media outreach,

collaboration with CBOs to increase culturally appropriate educational materials, and targeting diverse and emerging populations through the use of culturally diverse staff members and support groups. Unmet needs include the creation of a standardized newborn screening followup procedure in all states, and standardization of referral and treatment protocols for both subspecialists and primary care providers.

The Secretary's Advisory Committee on Heritable Disorders in Newborns and Children (SACHDNC) was charged with evaluating research and findings on HbAS status and health outcomes; the impact of the NCAA recommendations on the affected population, community service providers, and public health; current guidelines on HbAS status screening; and recommendations on HbAS screening.

In a letter to the Secretary, HHS, on June 14, 2010, the Committee made several recommendations [46].

- All individuals should know their risk for carrier status for various inherited genetic conditions such as sickle cell disease.
- Genetic testing or screening should not be a prerequisite for participation in athletic endeavors.
- Evaluation and screening for sickle cell disease and other genetic conditions should take place within the individual's medical home. That evaluation should include an assurance of the privacy of genetic information.
- All potential athletes should receive annual education on safe practices for prevention of exercise- and heat-related illnesses.
- Research is needed to determine if some athletes with sickle cell trait are at increased risk of exercise-related sudden death.

Societal and public health implications for sickle cell trait and community-based participatory research programs

Community-Based Participatory Research may enable the study of changing social, political, and environmental conditions that affect health outcomes [47,48]. The study of HbAS falls within this approach that includes participation by the affected community as defined by a sense of identity and purpose, not geography. Successful education and outreach efforts in HbAS will engage the public, the athletic community, agencies and programs to effect change in the current approach. In this type of methodology, the location of power is in the research process.

Important public health concerns regarding HbAS that can be addressed include as follows:

- Protection, Nondiscrimination, and Stigmatization of the carrier
- Practical recommendations communicated to communities, individuals, professionals (medical and athletic), and the general public
- Knowledge, Education, and Counseling
- Public, Community, and Stakeholder Roles and Level of Engagement
- Harmonizing Science, Law, Policy, Values, Ethics, Clinical Utility, and Service
- Monitoring and actions for new information including mortality reports and new policies

Research Opportunities Related to Implications of HbAS Screening Programs

- Assess the risks and benefits of the NCAA HbAS screening program as an example of “occupational” genetic testing.
- Assess the risks including stigmatization, ineffective communication, and benefits of carrier state detection for athletes and in general.
- Evaluate implications for societal, community, health care provider and individual responses to carrier detection and knowledge bases

Research Opportunities Related to Behavioral, Social, and Ethical Research

- Evaluate the impact of disease construction—The impact on individuals and health care of establishing HbAS as a disease.

Medical Knowledge Base for Sickle Cell Trait

Background Information

It is known that HbAS-containing RBC can be induced to sickle in vitro within minutes [49,50]. Hypoxia is required and the sickling process is promoted by acidic pH, hypertonicity, and altitude. Exercise produces conditions conducive to sickling. For example at an altitude of ~1,300 m, venous pO₂ falls to the mid-30 mmHg range and pH also decreases and is associated with an increased percentage (1 to 2%) of sickling red cells in the blood [51]. The effect is more marked at a height of 4,000 m. Fluid restriction during lengthy (40 min) treadmill exercise and thermal stress is also associated with peripheral venous blood sickling [52]. Whether or not these effects are relevant in vivo during the one second or less circulatory transit time in the systemic capillaries is not known [53,54]. In addition to changes in hemoglobin polymerization, HbAS carriers have higher rigidity of red cells at rest and after a brief supramaximal exercise period [55]. Exercise-induced plasma soluble vascular cell adhesion molecule-1 (sVCAM-1) increase is higher and more prolonged in athletes with HbAS [56]. This may signal microcirculatory disturbances with endothelial activation and increased potential for adhesive events. In studied subjects, alpha-thalassemia reduced the observed effects, suggesting a possible link to HbS-polymerization.

Is sickle cell trait a risk factor for venous thrombosis?

In 1979, of more than 65,000 consecutively admitted black males at 13 Veterans Administration hospitals 7.8% had HbAS [57]. No age-dependent differences in frequency of HbAS were found. HbAS did not have an effect on average age at hospitalization or death, overall mortality, or length of hospitalization. The only diagnoses in which there was a difference in frequency were “essential” hematuria and clinically diagnosed pulmonary embolism (1.5% in individuals with HbAA compared with 2.2% in those with HbAS).

In individuals with HbAS compared with HbAA controls laboratory parameters are consistent with activation of blood coagulation. D-dimer, a reliable indicator of future risk of venous thrombosis, is higher [58]. Similarly, thrombin antithrombin complexes (TAT) and prothrombin fragment 1.2 are elevated in individuals with HbAS compared with controls. The studies were sex matched but females represented 83% of the subject population.

A case-control study [59] identified HbAS as a risk factor for venous thromboembolism (VTE). The risk of VTE was approximately twice as high in blacks with HbAS compared with controls. HbAS also appeared to confer a 4-fold increased risk of pulmonary embolism.

The study concluded that, "...HbAS is a more important cause of VTE among blacks than is the prothrombin [G20210A] mutation among whites." The risk was even higher for a small sample of African American women taking oral contraceptives [60].

A potential coagulation role for microparticles, phospholipid microvesicles of 0.05 to 1.5 μm in size [61], derived from red cells, platelets, monocytes, and endothelial cells, has also been suggested.

Sickle cell trait and pregnancy

The available medical literature is largely based on retrospective cohorts from single institutions. Screening for hemoglobinopathies in study populations has not been consistently thorough and complications of pregnancy had variable or incomplete definitions. Control groups were not incorporated into the studies and confounding variables such as coexistent alpha-thalassemia or iron deficiency were not evaluated.

Single case reports during pregnancy describe HbAS as the cause of pulmonary embolism, sudden death [62], death associated with cardiomyopathy [63], death associated with aortocaval compression [64], hypopituitarism [65], and proliferative retinopathy in association with gestational diabetes and hypertension [66].

Asymptomatic bacteriuria in women with HbAS during pregnancy has been studied in retrospective cohorts. A 2-fold increase [67], 1.4-fold increase [68], and no increase [69] have been reported. The occurrence of pyelonephritis in the series is higher in individuals with HbAS but is associated with risk factors including prior episodes of pyelonephritis. Lower HbS concentrations are associated with a lower risk of urinary tract infection in pregnancy [70]. Screening for bacteriuria at first prenatal visit has been recommended.

In one prospective cohort study, preeclampsia occurred twice as frequently in women with HbAS although women with HbAS had a 2-fold increased history of preeclampsia in a prior pregnancy [71]. Other studies failed to confirm the report [72–74]. Placental abnormalities including amniotic fluid infection and meconium histiocytosis have been noted to be 3- to 9-fold more common in pregnant women with HbAS and associated with fetal loss after the first trimester [75,76]. Other studies have not shown an increase in preterm delivery rate [70,72].

Renal perspectives including malignancy

Hemoglobin S is known to polymerize at low oxygen tension, high hydrogen ion concentration, higher intracellular HbS concentration, and under conditions of high osmolality. These conditions all exist in the renal medulla and have been shown to be associated with fewer vessels and a greater degree of disruption of the vasa recta in the kidney [77].

Hematuria is possibly the most common renal manifestation of HbAS [57]. Hospitalized African American veterans with HbAS have an ~2-fold increased risk of hematuria (2.5%). In addition, there are numerous case reports that describe episodes of papillary necrosis.

The community prevalence of Chronic Kidney Disease (CKD) is 7–8%. In diabetic populations, the prevalence ranges from 18 to over 30% [78–80]. A contributing role for HbAS in this context has been sought in a small series of patients.

A recent epidemiological study reported that HbAS prevalence is twice as frequent in African American patients with End Stage Renal Disease (ESRD) ($P < 0.001$) and HbAC is ~2.5 times more prevalent ($P = 0.01$) than African American control subjects [81]. Although

these relationships might reflect an effect caused by the variant hemoglobin, alternatively HbAS and HbAC might be surrogate markers for other risk factors that are associated with African ethnicity. HbAS occurred more frequently in a black population with autosomal dominant polycystic kidney disease and was associated with an earlier onset of ESRD ($P < 0.003$) [82].

Renal medullary carcinoma is a rare tumor that arises centrally in the renal medulla and grows rapidly in an infiltrative pattern to invade renal sinuses. Patients with HbAS account for nearly 90% of ~130 reported cases [83,84]. The prognosis is generally poor with diagnosis made at the time of metastatic disease and poor responses to treatments [85,86].

Other potential manifestations of sickle cell trait

Most information is in the form of case reports. The impact of HbAS on severe malaria in endemic areas also confounds analyses [87].

Since the initial description of splenic infarct in HbAS in the 1940's, over 80 cases have been reported [88,89]. The event may occur 1 to 2 days after exposure to high altitude and also may occur during unpressurized air travel. From 1986–2006, 25 cases were identified at the Colorado Sickle Cell Center [90]. All were male, ages ranged from 4 to 40 years. Thirty-six percent were African-American, 36% non-Hispanic white, 16% Hispanic, and 12% unknown. The infarcts occurred at greater than 7,500 feet elevation for non-Denver residents and greater than 9,000 feet for Denver residents. Treatments have included return to lower altitudes and splenectomy.

More recently, there has been a report of an increased rate of invasive pneumococcal disease in children with HbAS and HbC trait [91]. Children born from 1996 to 2003 in Tennessee were followed through 2005 or to their fifth birthday, to evaluate the onset of a pneumococcal event or death. The introduction of pneumococcal conjugate vaccine (PCV7) led to reduced rates of invasive pneumococcal disease in all children and an 82% reduction in children with HbAS, but the rates in HbAS and HbC trait remained elevated (adjusted RR of ~1.8) [92]. The differences were identified after adjusting for known high-risk conditions: age, asthma, and race.

The role of HbAS as a risk factor for stroke has been debated [93,94]. A small study in Guadeloupe found that the risk of ischemic stroke in HbAS was less frequent (4%) than in controls (8.5%) [95]. Potential cofactors for stroke risk include hypoxemia, acidosis, dehydration, and viscosity.

Following blood bank storage, red cells from HbAS donors survive normally in normoxemic recipients [96]. However, use of these cells for infants or patients with sickle cell disease undergoing exchange transfusion is not recommended. Removal of white blood cells by filtration is commonly practiced to reduce the risks of alloimmunization, febrile reactions, CMV infections, and potentially immune suppression. In the case of HbAS donors, leukodepletion filtration often leads to line occlusion and loss of the unit. HbS polymerization due to collection into an acidic citrate anticoagulant environment and low oxygen tensions appear to explain filter failures [97]. The addition of air to storage bags to increase oxygen levels greatly improves the chances of filtration success [98].

Research Opportunities to Improve the Medical Knowledge Base

- Are there potential genetic modifiers of phenotypic expression of HbAS? What is the impact of comorbid conditions such as asthma or diabetes mellitus on clinical outcomes in individuals with HbAS?

- Evaluate the development of animal models for hypothesis testing.
- Determine the potential interaction of HbAS with the development of renal disease, including the significance of HbC as a risk factor for renal disease.
- Quantify the impact of known modifiers of disease severity for SCD including HbF level, alpha-thalassemia and HbC.
- What risk factors combined with HbAS result in thrombosis? Are there genetic factors? Is there a role of microparticles in HbAS?
- Viewing HbAS as a genetic polymorphism, how might it interact with other genetic polymorphisms to influence the development of clinical disease?
- Improve understanding of the protective effect of HbAS against malaria.
- Improve understanding of the potential pathophysiology of HbAS containing red blood cells, including red cell membrane abnormalities, vascular wall interactions, and impact on immune function and infectious disease.
- What is the possible impact of cofactors such as intense athletic exercise, high altitude exposure, and pre-eclampsia.

Summary and Conclusions

The purpose of the workshop was to identify research topics about potential health risks of HbAS. Various mechanisms of sudden death in young athletes were discussed and a genetic basis for some of the outcomes including the risk of rhabdomyolysis was proposed. Case reports on sudden deaths include potentially important information for consideration. However, these anecdotal reports lack uniform definitions and add uncertainty. A more methodologically sophisticated approach is needed. However, from a practical perspective, implementation of preventive measures for EHI for all athletes seems to be warranted. Other potential complications and aspects of HbAS should be investigated. These include renal and coagulation complications and access to genetic counseling. Ethical, social, and behavioral research should be conducted in concert with investigations to improve understanding of the basic biology and the clinical implications of HbAS. This condition also provides an opportunity to conduct multidisciplinary research. NIH, through its Clinical and Translational Science Award Program, in collaboration with CDC and HRSA plans to support research on the clinical implications for individuals with Sickle Cell Trait. Research in HbAS is also in alignment with the NHLBI Strategic Plan, specifically, Goal 2; “to improve understanding of the clinical mechanisms of disease and thereby enable better prevention, diagnosis, and treatment” [99].

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