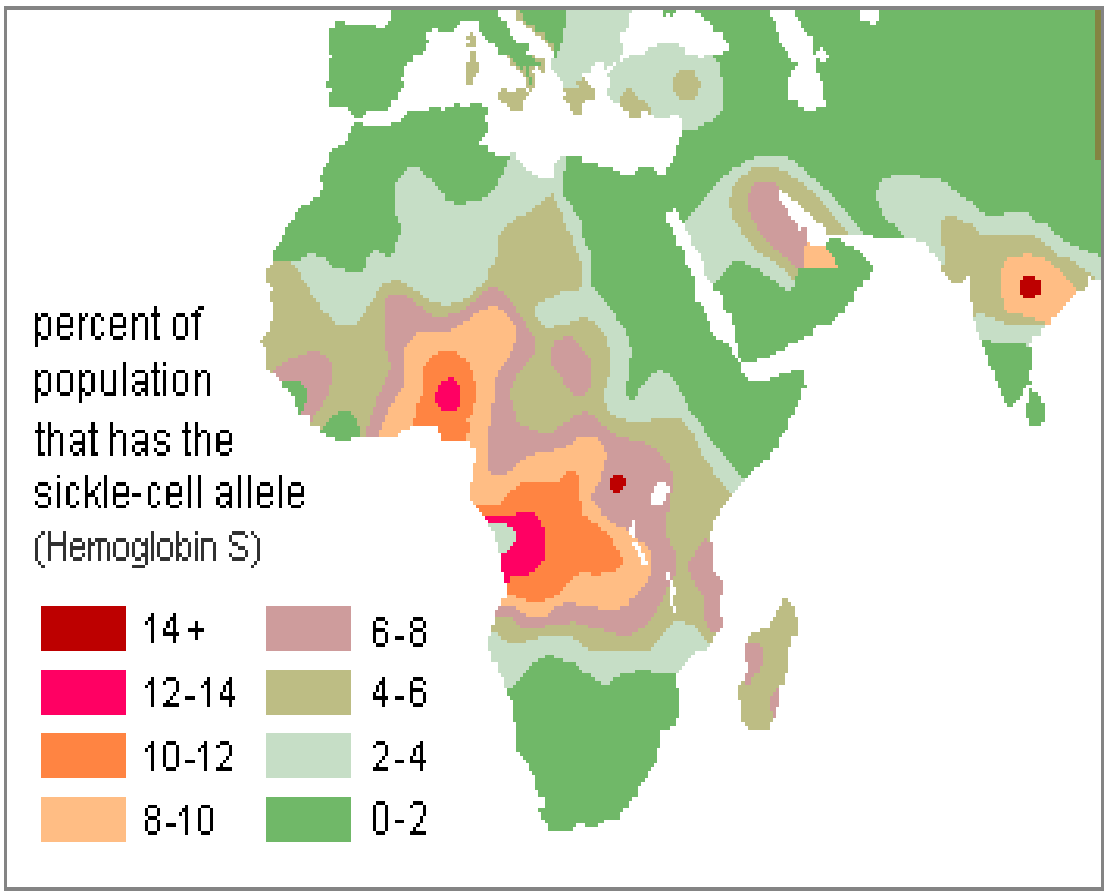
THE RESEARCH AGENDA FOR SICKLE CELL DISEASE IN AFRICA:

UNPRECEDENTED OPPORTUNITIES
UNSOLVED CHALLENGES



INTRODUCTION

Hb disorders (i.e. hemoglobinopathies) are the most common inherited disorder that afflicts our species. It has recently been estimated the 313,000 children are born each year with SCA, 75% of whom live in Africa [1]. Although much remains to be learned about its natural history and its current geographic distribution, in Africa the highest prevalence of the sickle cell mutation occurs between latitudes 15° North and 20° South, and ranges between 10% and 40% of the population; the greatly reduced survival among those with SCD, and the tolerance to malaria among those with HbAS, results in substantial variation in the frequency of these conditions by age. SCD occupies pride of position in human genetics as both the first well-described monogenic disorder and the first such condition in which the molecular basis was understood. However, beyond control of infection and repeated transfusions, relatively little progress has been made in therapy or deeper insights into modifying factors until the last 2 decades. The introduction of hydroxyurea in the 1990's greatly improved the clinical course of SCD for many patients and major advances have now been made in understanding the role of other genes that influence the clinical course of the illness, especially those that regulate fetal hemoglobin (HbF) and the response to hemolysis.

Unfortunately, even the most basic interventions - such as neonatal screening and protection against infection - have not been widely implemented in Africa. It is universally recognized that the recent advances in clinical and research capacity must urgently be made available to patients, medical practitioners and researchers in Africa, and numerous conferences and meetings have been held to discuss this question. In spite of these attempts progress on the ground thus far has been slow and many questions about the course of the illness, the risk:benefit ratio of available therapies, and the impact of both known and unknown genetic modifiers on the course of SCD in Africa remain unanswered. The SCD research agenda for Africa therefore involves a broad spectrum of topics, including genetics, pathophysiology, drug trials, and implementation of known therapeutic modalities.

In the following sections we provide a brief description of some of the key clinical aspects of SCD and recent scientific advances in understanding the disease process and treatment modalities and offer proposals on the agenda to move forward and improve control of intercurrent illness.

Basic genetic modifiers of SCD

As noted, SCD is not only SS, or the homozygous state. The other variants and their range of clinical severity teach lessons about the immense importance of the content of the red cell in determining the clinical manifestations of SCD. The four common variants of SCD can be arranged in the following decreasing order of clinical severity:

1- SS; 2 - S beta-zero thalassemia; 3 – SC; 4 - S beta-plus thalassemia

The most common laboratory and clinical feature of SCD is anemia (ie, chronic hemolytic anemia); this represents the fundamental determinant of severity. While the range of "steady-state" Hb levels within each genotype is relatively narrow and may overlap at the borders, it is extremely rare to find a patient with SCD with a normal Hb level and a normal reticulocyte count – a measure of the rate of hemolysis. At the other end of the scale, it is equally rare to see a patient with S beta-plus thalassemia with severe anemia and a high reticulocyte count.

Beyond anemia, the range and severity of the complications of SCD fall into the following genotypic rules: - Early overwhelming pneumococcal bacterial infection (a consequence of splenic dysfunction), frequent severe pain, and stroke are more common in SS than in SC, or S beta-plus thalassemia.

As noted, there is substantial inter-individual variation in the clinical course of SCD. Much of this variation has been ascribed to variation in the levels of HbF that persist after the age of 2. In the last several years, genetic variants at the BCL11A, HBS1L-MYB and beta-globin loci have been identified and shown to explain about 45% of the variance in HbF. Genome editing techniques have been used to verify the mechanism of action of non-coding SNPs within a BCL11A intron and inactivation of BCL11A in the erythroid lineage eliminated the SCD phenotype in a transgenic SS murine model (2). Other variants have also been identified which appear to influence

vascular complications and acute chest syndrome [3]. The impact of variation at *BCL11A* has been demonstrated in 2 African cohorts, however much more remains to be learned about allelic variation at the loci influencing HbF as well as other potential modifying factors [3,4].

B. The role of Hb F

Fetal hemoglobin (HbF) is produced during in utero development and remains the dominant form of circulating Hb until birth. A developmental switch turns off the production of HbF and results in predominance of HbA through the remaining life cycle. HbF is studied in SCD mostly from the viewpoint of its percentage of total Hb (in hemolysate) but rarely from the viewpoint of F cells (RBC's containing HbF). The latter is more difficult to measure however, in SCD, a cellular disease; F cells may carry more clinical meaning. The best natural experiment on the effects of HbF and F cells on SCD is in healthy individuals (not SCD patients) who have inherited the rare condition pancellular HPFH (hereditary persistence of fetal Hb) from one parent and S allele from the other parent. Even though HbS is the predominant Hb in their red cells, 30-40% of the total Hb is F, which is present in all their red cells, (100% F cells, or pancellular distribution). This leads to normal Hb/reticulocyte levels and disappearance of clinical manifestations of SCD. On the other hand, infants with SS begin to infarct their spleens at the time when their HbF levels are still very high (20-30% in infants 6-12m of age), making the interval from 6 to 12 months the period of highest incidence of pneumococcal bacteremia - the leading cause of death in SCD prior to penicillin prophylaxis and anti-pneumococcal vaccination. Why is HbF percent not protective against splenic dysfunction in these infants? As infants switch to make more beta-globin predominant cells, their F cell percentage drops to low levels, leaving most of their cells as S cells (with little or no F). Making 100% HbF in only 30% of the red cells, is not the same as having F present in every red cell (as in S-HPFH); both situations yield HbF of 30%.

In the early studies of the efficacy of hydroxyurea for SCD, it was shown that the response to hydroxyurea was more dramatic in terms of F cell production (10-15% baseline to >75% on hydroxyurea) than in HbF% (5-10% baseline to 15-20% on

hydroxyurea). As far as can be determined, hydroxyurea has not been shown to increase gamma-globin gene expression. Its main effect on HbF in SCD is likely due to its effect on the cell cycle, causing the production of more F cells in response to the additional stress hydroxyurea places on erythropoiesis. The F cell production locus (if there is such a locus . .) was estimated to be responsible for 40% of the variation in HbF levels in SCD ([5]. Almost all SCD patients, especially children, have much higher HbF levels than those with normal RBC's. This is not due to obvious Mendelian inheritance as the parents with AS have normal HbF levels.

These findings raise a number of important research questions. Is the erythropoietic response to stress similar in all patients? Is it mediated by other less obvious genetic factors that would not be detectable in unstressed parents/siblings? Is the effect similar to the effect of the Xmn 1 (-158 Gg) site in the Senegal or Saudi-Indian S-haplotype? Is the erythropoietic stress response factor the same as the "F cell production locus"? Patients who show less myelotoxicity in response to hydroxyurea therapy show higher Hb F/F reticulocyte response; is that evidence of better "erythropoietic stress management"?

C. SCD and malaria

The environmental conditions that lead to emergence of the S mutation insure – at least in equatorial Africa - that the highest prevalence of SCD is found in regions where the malaria parasite is still endemic. Malaria is a major erythropoietic stress factor and profoundly alters the clinical expression of SCD in Sub-Saharan Africa. While the AS state is protective against severe malaria, patients with SS are highly vulnerable to serious complications or death from infection with malaria. Hemolytic crisis from acute malaria competes with pneumococcal sepsis for the status as the leading cause of death in children with SCD (SS) in Sub-Saharan Africa. At the same time, the combination of malaria and SCD, two major hemolytic stressors, presents unique research opportunities to explore the clinical course of SCD in Africa.

A further question that requires an urgent answer is whether there are genetic factors that enable better management of this pair of stressors. Plasmodium falciparum does not grow well in HbS or HbF containing red cells and probably less well in S cells

with high F. The question thus arises, in SCD, are the patients with better F cell response to hemolytic stress of malaria less likely to be overwhelmed by acute malaria infection? Malaria, like hydroxyurea, can also suppress erythropoiesis, again calling for delicate management of the stress.

D. Alpha thalassemia and SCD

The clinical picture observed with the complex Hb abnormalities is highly varied. Alpha thalassemia protects patients from some features of SCD, eg, slightly less anemia, reduced risk of stroke. On the other hand, it worsens some features, such as pain, acute chest syndrome, and avascular necrosis, among others. A few reports on the higher frequencies of α+ thalassaemia amongst SCD patients as compared to unaffected controls seem to indicate a positive selective effect on survival among SCD patients; testing this hypothesis on a birth cohort could offer important new evidence. In addition, alpha thalassemia is associated with altitude, age of individuals and endemicity of malaria. Therefore negative epistasis between α+ thalassaemia and sickle cell trait can modulates the inter-population variations. The concurrent effects of the various genomic loci that affect the level HbF, alpha-thalassemia and infections (especially bacteraemia and malaria), on in various setting deserve further investigation.

E. Research on SCD must begin at birth

In Africa the highest mortality of SCD is in the first 3 years in areas where newborn screening and early preventive management is not available. The clinical phenotype observed in older children and adults in Africa is influenced by a huge survivor bias and misses the true impact of devastating complications such as infection and stroke. Stroke and related silent brain infarction, more common in children than adults, carry a high index of suspicion for the role of other genetic factors. Stroke in SCD does not correlate with pain frequency, the most commonly used clinical measure of SCD severity. However stroke, silent brain infarcts, and neurocognitive dysfunction in SCD all correlate with severity of anemia. Chronic organ damage is also very important in the "survivors" of childhood SCD; even in the US, close to 80% of SCD

mortality occurs in the setting of a short illness in subjects not being managed for any chronic end-organ damage.

REFERENCES

1. Piel FB, Patil AP, Howes RE, Nyangiri OA, Gething PW, et al. (2013) Global epidemiology of sickle haemoglobin in neonates: a contemporary geostatistical model-based map and population estimates. Lancet 381: 142–151.

2. Bauer, D.E., Kamran, S.C., Lessard, S., Xu, J., Fujiwara, Y., Lin, C., Shao, Z., Canver, M.C., Smith, E.C., Pinello, L., et al. 2013. An erythroid enhancer of BCL11A subject to genetic variation determines fetal hemoglobin level. *Science* 342:253-257.

3. Ghosh, S., Adisa, O.A., Chappa, P., Tan, F., Jackson, K.A., Archer, D.R., and Ofori-Acquah, S.F. 2013. Extracellular hemin crisis triggers acute chest syndrome in sickle mice. *J Clin Invest* 123:4809-4820.

4. Miller, M.L., Gao, G., Pestina, T., Persons, D., and Tuomanen, E. 2007. Hypersusceptibility to invasive pneumococcal infection in experimental sickle cell disease involves platelet-activating factor receptor. *The Journal of infectious diseases* 195:581-584.

External Links

WHO. "Sickle-cell anaemia - Report by the Secretariat" (PDF).

"Sickle Cell Disease: Data & Statistics". Centers for Disease Control and Prevention. 16 September 2011.