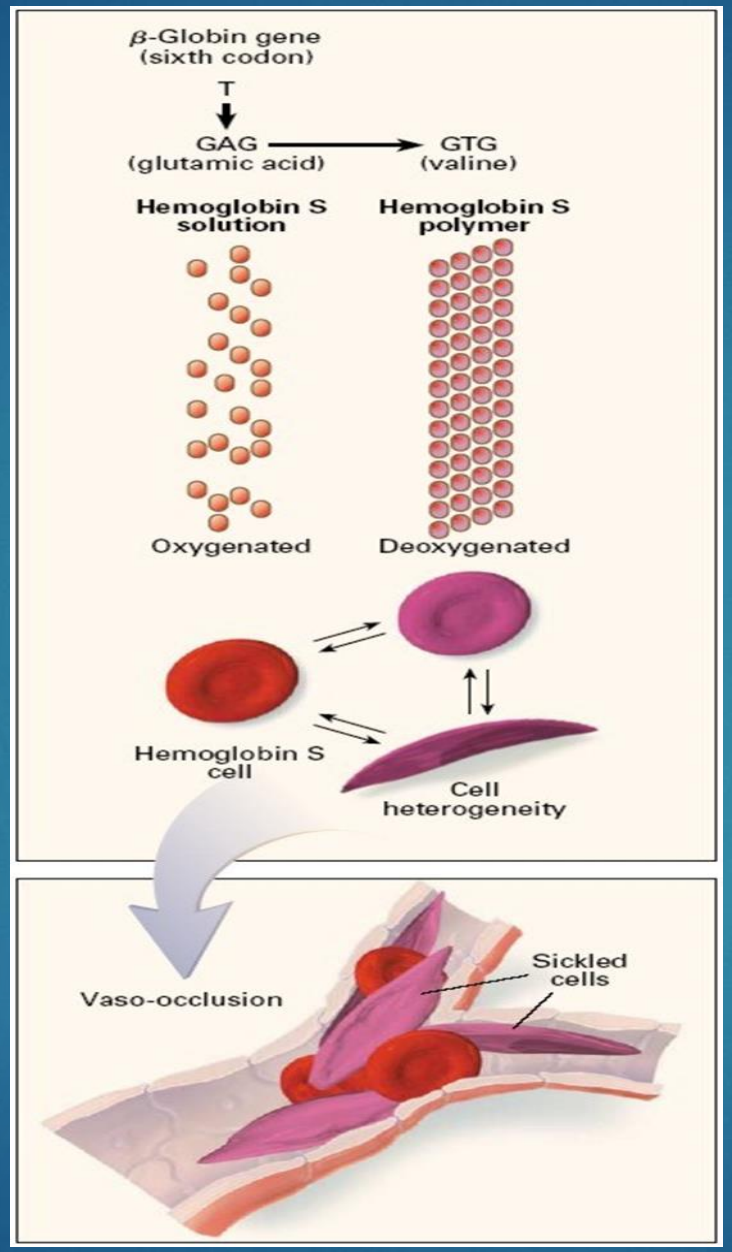
Sickle cell Anemia A Global Health Problem


β-Globin gene
(sixth codon)
T
GAG
(glutamic acid)
GTG
(valine)
Hemoglobin S
solution
Hemoglobin S
polymer
Oxygenated
Deoxygenated
Hemoglobin S
cell
Cell
heterogeneity
Vaso-occlusion
Sickled
cells

The magnitude of the burden of genetic diseases

- 7 million babies are born each year with either a congenital abnormality or a genetic disease
- Five genetic disorders constitute 25% of these births
  - Thalassemia
  - Sickle cell anemia
  - G6PD
  - Oculocutaneous albinism
  - Cystic Fibrosis

History of Sickle Cell Disease-Case 1
thanks to Todd Savitt Ph.D

- September 15, 1904
  - the SS Cearense docked in New York City after an 8 day voyage from Barbados
  - On board was Walter Clement Noel from Grenada who had a painful sore on his ankle that required treatment
  - He then traveled on to Chicago to attend Dental school
- December 1904
  - Around Thanksgiving he developed respiratory problems that finally became so severe he sought treatment the day after Christmas
  - Dr. Ernest Irons an intern performed a history physical and blood work and described pear-shaped and elongated red cells and notified his attending Dr. Herrick

History of Sickle Cell Disease-Case 1 thanks to Todd Savitt Ph.D

- December 31, 1904
  - Ernest Irons did another exam and blood smear and drew a picture of his observations
- For two years Irons and Herrick followed Walter Clement Noel through several bouts of illness but never proved the etiology of his illness
- After graduating from Dental School Walter Clement Noel returned to Grenada to practice dentistry
- 1910
  - Herrick published the findings in the Archives of Internal Medicine

History of Sickle Cell Disease-Case 2
thanks to Todd Savitt Ph.D

- 1885
  - Ellen Anthony was born in rural Campbell county Va
  - There are very few records but she likely worked as a cook and housemaid
- 1907
  - She developed a severe abdominal crisis and presented to a local physician who sent her to UVA to the charity ward
- September 1909
  - During her 4th admission to UVA that lasted 284 days she was seen by medical student B.E. Washburn from Rutherfordton, NC
  - Washburn's Attending, Dr. John S. Davis suggested he submit the case to the Virginia Medical Semi Monthly

STUDIES ON A CASE OF SICKLE-CELL ANÆMIA*

BY JESSIE BOYD SCRIVER, M.D. AND T. R. WAUGH, M.D.,

Montreal

SICKLE-CELL anæmia as a clinical entity is of comparatively recent recognition, being first described by Herrick[1] in 1910. Since then there have appeared in the literature numerous reports of clinical histories, pathological findings, and some laboratory investigations of this condition. Our reason for reporting this case is to place on record our observations on the behaviour of the erythrocytes *"in vivo"*, in this, the first case of sickle-cell anæmia to be reported from our Canadian clinics.

The condition which Herrick described occurred in a negro, 20 years old, who presented a cardiac enlargement, albuminuria, general adenopathy, icterus, and a secondary anæmia which was not remarkable for the great reduction in red corpuscles or hæmoglobin, but was strikingly atypical in the large number of nucleated red corpuscles of the normoblastic type and in the tendency of the erythrocytes to assume a slender sickle-like shape. The shape of the red cells was very irregular and there was a large number of thin elongated sickle-shaped and crescentic forms which were seen both in fresh specimens and in specimens fixed by heat, alcohol, and ether, and stained with the Ehrlich triacid stain, as well as with the control stain. The second case was reported by Washburn[2] in 1911, and in 1915 Cook and Meyer[3] reported the third case and considered the condition of familial incidence. Emmel,[4] who studied this third case and reported his findings in 1917, presented the important observation that the number of sickle-shaped cells increased in sealed wet blood preparations, and that during periods of remission, when no sickle cells were found in the patient's blood, the typical forms would appear in sealed wet smears on standing. This cultural characteristic he considered specific of the condition, and demonstrated its presence in an apparently healthy member of the patient's family. He suggested that the phenomenon, in part at least, was due to accentuation or abnormal activity of the same factors which in normal hæmatogenesis are involved in the transformation of the original spherical erythrocyte into a biconcave disc-shaped form.

Sydenstricker,[5] in 1923, who was the first to report sickle-cell anæmia in children, considered the condition a familial and hereditary disease, showing no sex preference and probably confined to the negro race. He suggested that it might be a hereditary defect of the spleen and blood-forming organs with the resulting change in the erythrocytes which predisposes to hæmolysis and phagocytosis. He drew attention to the following symptoms in these cases; dyspnœa, palpitation, and weakness referable to the anæmia; muscle and joint pains of considerable severity at frequent intervals; frequent attacks of nausea and vomiting, associated with epigastric and left hypochondriac pain; low fever; frequent night sweats; a greenish yellow discolouration of the scleræ, which varies in intensity and is most marked during the abdominal crises; pale mucous membranes, general lymphadenoid hyperplasia; heart changes associated with anæmia; enlarged liver; peculiar leg ulcers in some cases, or scars of these ulcers (these are rare in children); urobilinuria.

The blood picture in these cases is characteristic, showing a secondary anæmia with smears presenting the peculiar sickle-shaped erythrocytes in varying numbers, and the tendency of the erythrocytes when prepared in sealed wet preparations to exhibit an increase in the number of sickles on standing or incubating, as has already been mentioned.

Huck,[6] in 1923, showed that in sealed wet preparations there was "sickling" of practically all cells at the end of 24 hours but

* From the Department of Medicine, McGill University Clinic, Royal Victoria Hospital, and the Department of Pathology, McGill University, Montreal.

Read in abbreviated form before the Canadian Medical Association, Montreal, June, 1929.

History of Sickle Cell Disease

- In 1945 Linus Pauling sat in the audience of a lecture by Dr. William Castle about a sickle cell patient
- In that lecture, Dr. Castle noted that the sickling phenomenon was different in arterial blood samples and venous blood samples
- No sickling in arterial blood but sickle forms present in venous blood

November 6, 1946

Dr. William B. Castle
Boston City Hospital
Boston
Massachusetts

Dear Bill:

I now have a graduate student (Harvey Itano, M.D.) beginning work on the problem of the relation between the nature of the hemoglobin in sickle cell anemia and the phenomenon of sickling.

He has not found any references in the literature to the work that you were telling me about, which, if I remember correctly, indicated that the dividing line between sickling and non-sickling came at 50 percent oxygenation of the hemoglobin or 50 percent combination with carbon monoxide. Could you tell me whether you and your collaborators have published any of this work, send me reprints if it has been published, and send me a brief statement about the results if it has not been published.

Last summer Dr. Burch told me that he felt sure that the phenomenon was due to a large amount of carbon dioxide. I have read his papers, and it seems to me that all of his results can be explained by assuming that the carbon dioxide treatment removes oxygen.

We are hoping to get some interesting results by studying other compounds of hemoglobin.

With best regards, I am

Sincerely yours,

LP:gv

Linus Pauling

W. B. CASTLE, M.D.
BOSTON CITY HOSPITAL
BOSTON 18

APPOINTMENTS
TELEPHONE KENMORE 8600

November 25, 1946

Dr. Linus Pauling
Gates and Crellin Laboratories of Chemistry
California Institute of Technology
Pasadena 4, California

Dear Linus:

How nice it is to have a word from you and probably to learn something more as a result of your work about that most interesting condition, sickle cell anemia. With regard to the facts about sickling, it is well established that oxygen and carbon monoxide prevent sickling, and that exposure to other gases produce sickling by the removal of one or the other of these. Naturally, there is some effect from carbon dioxide in so far as it alters the saturation curve for oxygen of the hemoglobin by a change in the pH of the system. I would agree with you that Doctor Burch's papers can all be interpreted in terms of removal of oxygen and, indeed, I wrote a critique of one of them for the Year Book of Medicine in which I simply interpolated the abstract of his communication in terms of this explanation.

I think that the literature that would be most useful for you, both for its content and for the references given, is the following:

Scriver, J. B., and Waugh, T. R. Canad. Med. Assn. J. 1930, 23, 375-380.

Murphy, R. C., and Shapiro, S. Arch. Int. Med., 1944, 74, 28. (Cf. Bibliography)

Same authors. Annals Int. Med. 1945, 23, 576. (Cf. Bibliography)

Our own observations here confirm those of Scriver and Waugh that sickling begins at about 35 to 40 millimeters oxygen tension. I determine the sickling by the effect on the "viscosity", that is, the timed flow of blood through a viscosimeter with appropriate arrangements to maintain the blood in equilibrium with various tensions of oxygen. The viscosity of the blood begins to increase at about 40 millimeters oxygen tension, and rises

November 25, 1949
Sickle cell Disease is recognized as the first Molecular disease

Reprinted from SCIENCE, November 25, 1949, Vol. 110, No. 2865, pages 543–548.

Sickle Cell Anemia, a Molecular Disease[1]

Linus Pauling, Harvey A. Itano,[2] S. J. Singer,[2] and Ibert C. Wells[3]

Gates and Crellin Laboratories of Chemistry,
California Institute of Technology, Pasadena, California[4]

THE ERYTHROCYTES of certain individuals possess the capacity to undergo reversible changes in shape in response to changes in the partial pressure of oxygen. When the oxygen pressure is lowered, these cells change their forms from the normal biconcave disk to crescent, holly wreath, and other forms. This process is known as sickling. About 8 percent of American Negroes possess this characteristic; usually they exhibit no pathological consequences ascribable to it. These people are said to have sicklemia, or sickle cell trait. However, about 1 in 40 (*4*) of these individuals whose cells are capable of sickling suffer from a severe chronic anemia resulting from excessive destruction of their erythrocytes; the term sickle cell anemia is applied to their condition.

The main observable difference between the erythrocytes of sickle cell trait and sickle cell anemia has been that a considerably greater reduction in the partial pressure of oxygen is required for a major fraction of the trait cells to sickle than for the anemia cells (*11*). Tests *in vivo* have demonstrated that between 30 and 60 percent of the erythrocytes in the venous circulation of sickle cell anemic individuals, but less than 1 percent of those in the venous circulation of sicklemic individuals, are normally sickled. Experiments *in vitro* indicate that under sufficiently low oxygen pressure, however, all the cells of both types assume the sickled form.

The evidence available at the time that our investigation was begun indicated that the process of sickling might be intimately associated with the state and the nature of the hemoglobin within the erythrocyte. Sickle cell erythrocytes in which the hemoglobin is combined with oxygen or carbon monoxide have the biconcave disk contour and are indistinguishable in that form from normal erythrocytes. In this condition they are termed promeniscocytes. The hemoglobin appears to be uniformly distributed and randomly oriented within normal cells and promeniscocytes, and no birefringence is observed. Both types of cells are very flexible. If the oxygen or carbon monoxide is removed, however, transforming the hemoglobin to the uncombined state, the promeniscocytes undergo sickling. The hemoglobin within the sickled cells appears to aggregate into one or more foci, and the cell membranes collapse. The cells become birefringent (*11*) and quite rigid. The addition of oxygen or carbon monoxide to these cells reverses these phenomena. Thus the physical effects just described depend on the state of combination of the hemoglobin, and only secondarily, if at all, on the cell membrane. This conclusion is supported by the observation that sickled cells when lysed with water produce discoidal, rather than sickle-shaped, ghosts (*10*).

It was decided, therefore, to examine the physical and chemical properties of the hemoglobins of individuals with sicklemia and sickle cell anemia, and to compare them with the hemoglobin of normal individuals to determine whether any significant differences might be observed.

EXPERIMENTAL METHODS

The experimental work reported in this paper deals largely with an electrophoretic study of these hemoglobins. In the first phase of the investigation, which concerned the comparison of normal and sickle cell anemia hemoglobins, three types of experiments were performed: 1) with carbonmonoxyhemoglobins; 2) with uncombined ferrohemoglobins in the presence of dithionite ion, to prevent oxidation to methemoglobins; and 3) with carbonmonoxyhemoglobins in the presence of dithionite ion. The experiments of type 3 were performed and compared with those of type 1 in order to ascertain whether the dithionite ion itself causes any specific electrophoretic effect.

Samples of blood were obtained from sickle cell anemic individuals who had not been transfused within three months prior to the time of sampling. Stroma-free concentrated solutions of human adult hemoglobin were prepared by the method used by Drabkin (*3*). These solutions were diluted just before use with the

[1] This research was carried out with the aid of a grant from the United States Public Health Service. The authors are grateful to Professor Ray D. Owen, of the Biology Division of this Institute, for his helpful suggestions. We are indebted to Dr. Edward R. Evans, of Pasadena, Dr. Travis Winsor, of Los Angeles, and Dr. G. E. Burch, of the Tulane University School of Medicine, New Orleans, for their aid in obtaining the blood used in these experiments.

[2] U. S. Public Health Service postdoctoral fellow of the National Institutes of Health.

[3] Postdoctoral fellow of the Division of Medical Sciences of the National Research Council.

[4] Contribution No. 1333.

October 13, 1956
The difference between normal and sickle globin genes is recognized

(Reprinted from Nature, Vol. 178, pp. 792–794, October 13, 1956)

A SPECIFIC CHEMICAL DIFFERENCE BETWEEN THE GLOBINS OF NORMAL HUMAN AND SICKLE-CELL ANÆMIA HÆMOGLOBIN

By Dr. V. M. INGRAM

Medical Research Council Unit for the Study of the Molecular Structure of Biological Systems, Cavendish Laboratory, University of Cambridge

A NEW and rapid technique of characterizing the chemical properties of a protein in considerable detail has been devised ; by its application a specific difference is found in the sequence of amino-acid residues of normal and sickle-cell hæmoglobin. This difference appears to be confined to one small section of one of the polypeptide chains.

Of all the abnormal human hæmoglobins, the one that has been most intensively studied is hæmoglobin *S* from patients with sickle-cell anæmia. In 1949 Pauling and his collaborators[1] demonstrated by electrophoretic experiments that at neutral *p*H the hæmoglobin *S* molecule has a net charge which is more positive by three units compared with the normal molecule, hæmoglobin *A*. It has since been suggested[2] that this difference is really due to hæmoglobin *S* having fewer free carboxyl groups than does hæmoglobin *A*. It is also known that in the reduced state the abnormal protein has a much lower solubility[3]. However, careful determinations of the amino-acid composition of the two proteins[4,5] did not show any significant differences between them within the accuracy of the methods employed. Comparison of the N-terminal[6] and C-terminal[7] amino-acids and of the sulphydryl groups[8] was equally disappointing. On this evidence alone, it is not possible to decide whether the difference between the proteins, which is in any event small, lies in the amino-acid sequences of the polypeptide chains or whether it lies in the folding of these chains leading to a masking of some amino-acid side-chains.

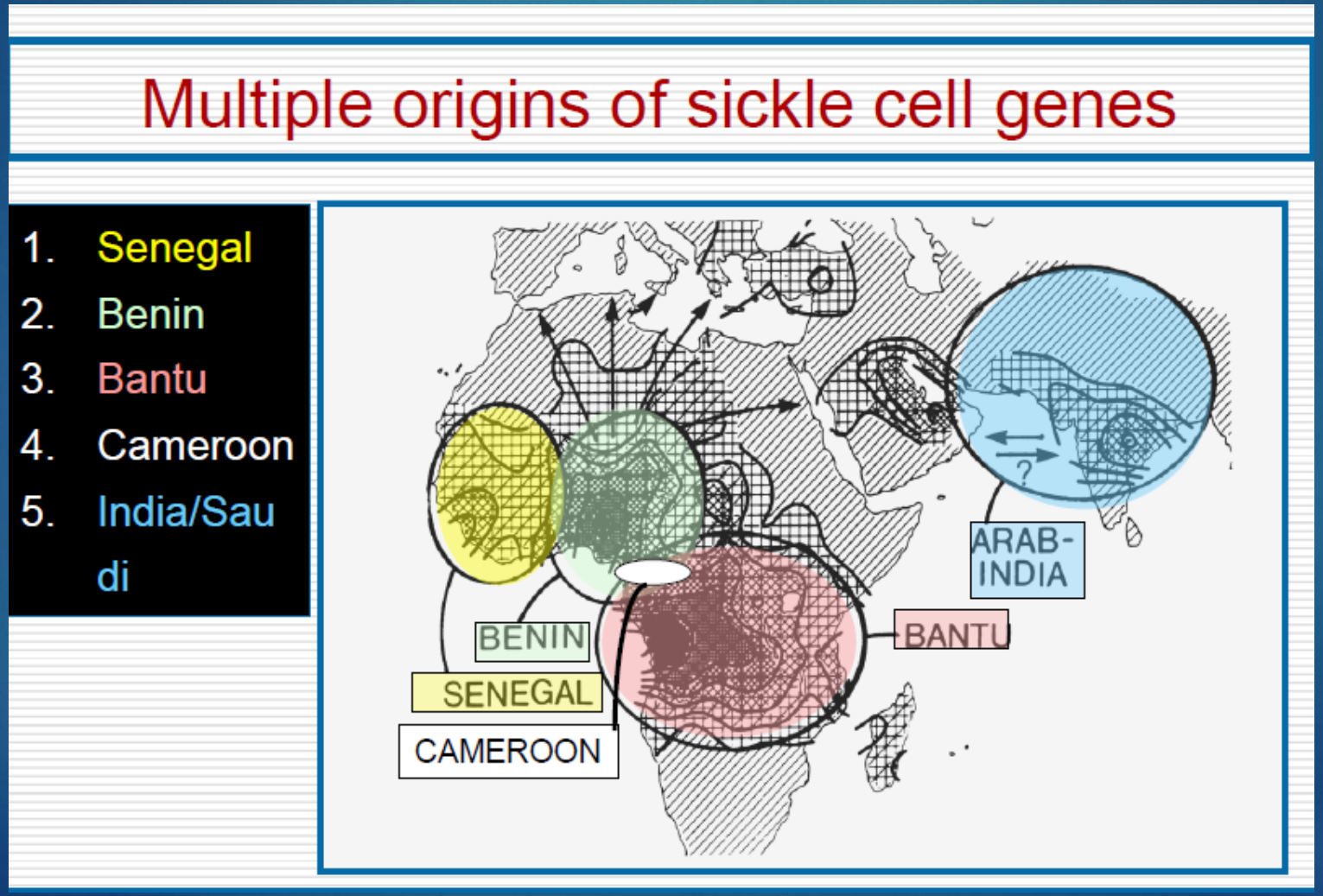
Hæmoglobin is still too large a molecule for detailed analysis of amino-acid sequence. However, it was thought that if a rapid method could be found of characterizing the chemical properties of the peptides in a tryptic digest, then perhaps a replacement of even a single residue for another might be detected without elaborate analysis.

History of Sickle cell Anemia

- 1954 Allison et al. published in the British Journal of Medicine and article proving the protective effect of Sickle cell trait (AS) against malarial disease explaining the distribution of Sickle cell disease
- However despite this advantage Sickle trait incidence in populations is typically less than 25% due to the severe disadvantage in survival of the homozygous (SS) state
- The distribution of Sickle cell anemia was not completely explained by the protection against Malarial diseases

- *If all cases of Sickle cell anemia had the same origin all patients would be cousins and their disease should all be very similar.*
- *This led researchers to look into the origins of Sickle cell anemia to see if there was one place of origin or multiple regions of origin*

There are multiple origins of the Sickle cell Gene.



Data based on associated restriction site polymorphisms that exist with the Sickle Cell Gene

Global Burden of Sickle Cell Disease

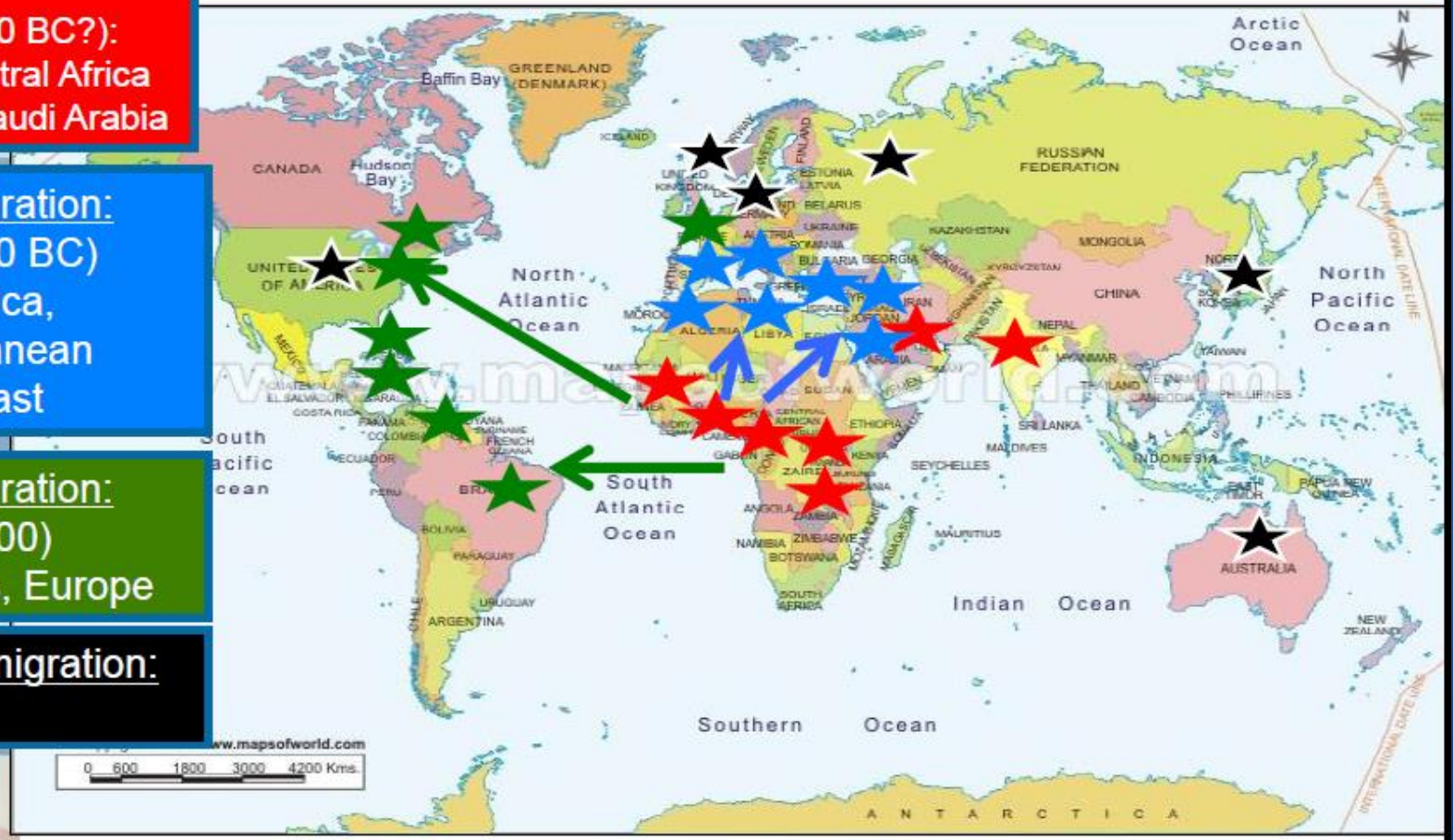
Spread of Sickle Genes

Origins
(2000-1000 BC?):
West, Central Africa
India/E. Saudi Arabia

Early migration:
(1000-200 BC)
North Africa,
Mediterranean
Middle East

Later migration:
(1500-1900)
Americas, Europe

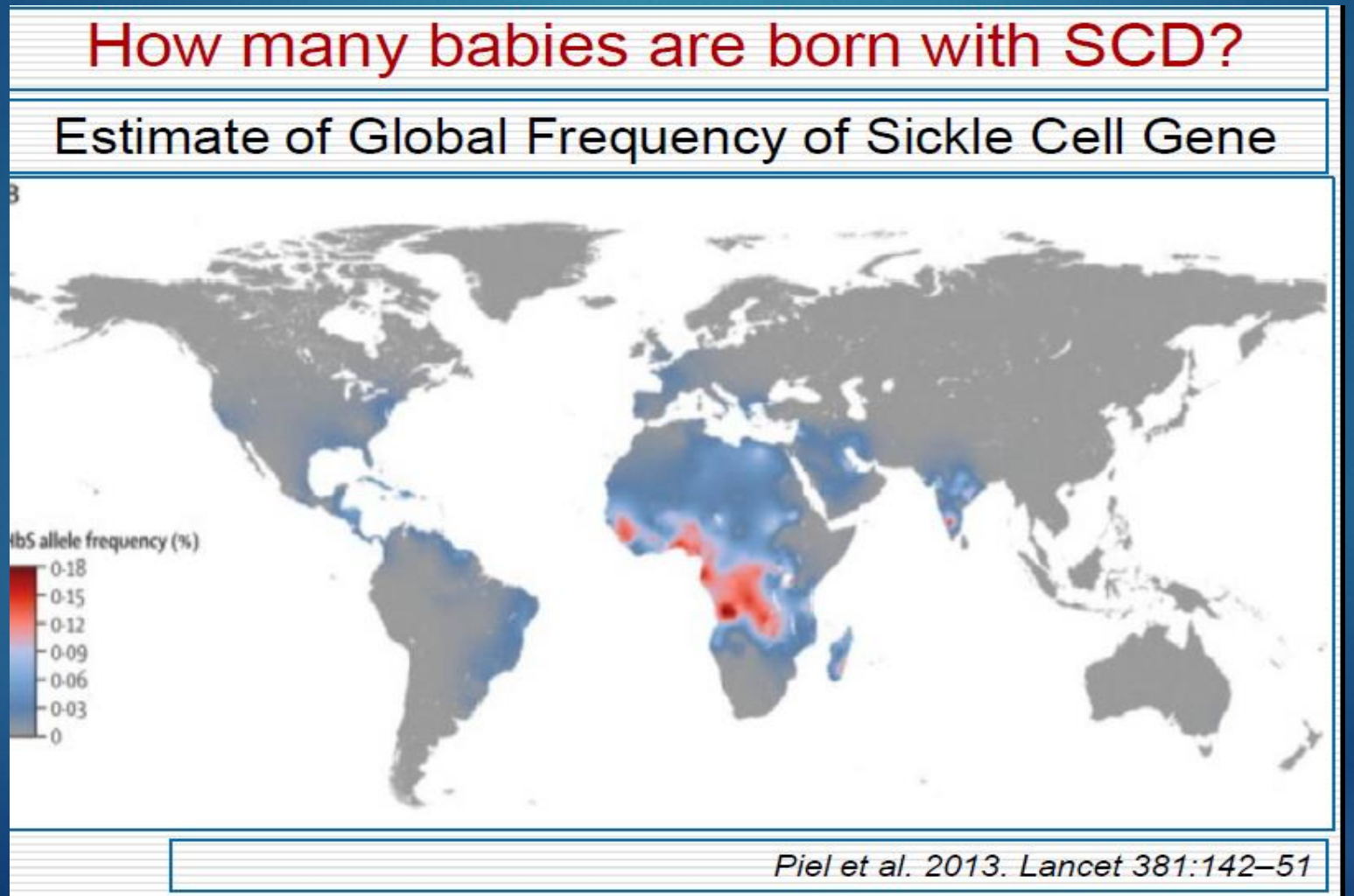
Modern migration:
Global



SCDAA
"Break The Sickle Cycle"

Sickle cell disease, a truly global health problem

Where is Sickle cell Anemia the greatest Healthcare burden?



Estimates of number of Cases of Sickle trait and Sickle cell anemia in 2010

How many people are born with SCD?

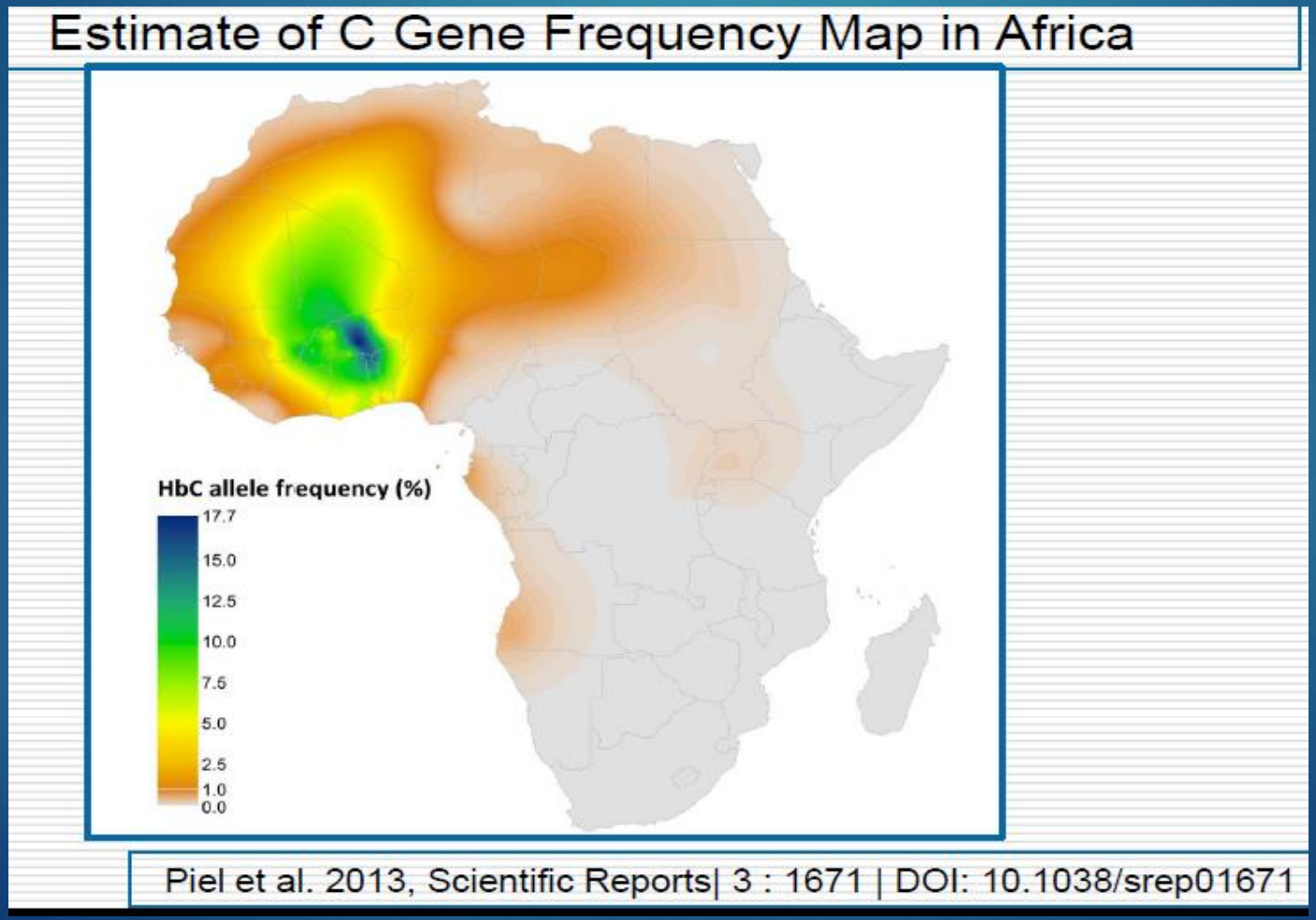
Estimated Newborns with AS and SCD-SS

2010

Region	AS	SS	%
Global	**5,476,407**	**312,302**	**100**
Americas	386,430	12,802	4.6
Arab-India	1,147,477	46,826	16.9
Eurasia	256,163	7,493	3.0
Southeast Asia	2,535	21	0.0
Sub-Saharan Africa	3,580,207	235,681	75.5

Piel et al. 2013. Lancet 381:142–51

Other Hemoglobinopathies such as Hemoglobin C can affect the rate of Sickle disorders.



How many Children are born with C trait or Homozygous Hemoglobin C

Estimated Newborns with AC and CC Disease in Africa

2013		
Region	AC	CC
AFRO	**672,117**	**28,703**
1. Nigeria	148,423	3,099
2. Burkina Faso	131,454	9,592
3. Ghana	98,153	4,707
4. Mali	79,506	4,354

Piel et al. 2013, Scientific Reports| 3 : 1671 | DOI: 10.1038/srep01671

What are the predictions for what will happen to Sickle Cell Anemia in the future?

2010-2050 Estimated Newborns with SCD-SS

Country	2010		2050		2010-2050
	SS	%	SS	%	% change
Global	**305,773**	**100**	**404,190**	**100**	**+32.2**
1. Nigeria	91,011	29.8	140,837	34.8	+54.7
2. India	44,425	14.5	33,890	8.4	-23.7
3. Congo DR	39,743	13.0	44,663	11.1	+12.4
21. USA	2,842	0.93	3,379	0.83	+18.9
14. Ghana	5,815	1.90	6,855	1.70	+17.9

Piel et al. (2013) PLoS Med 10(7): e1001484. doi:10.1371/journal.pmed.1001484

So Now, a little more

History

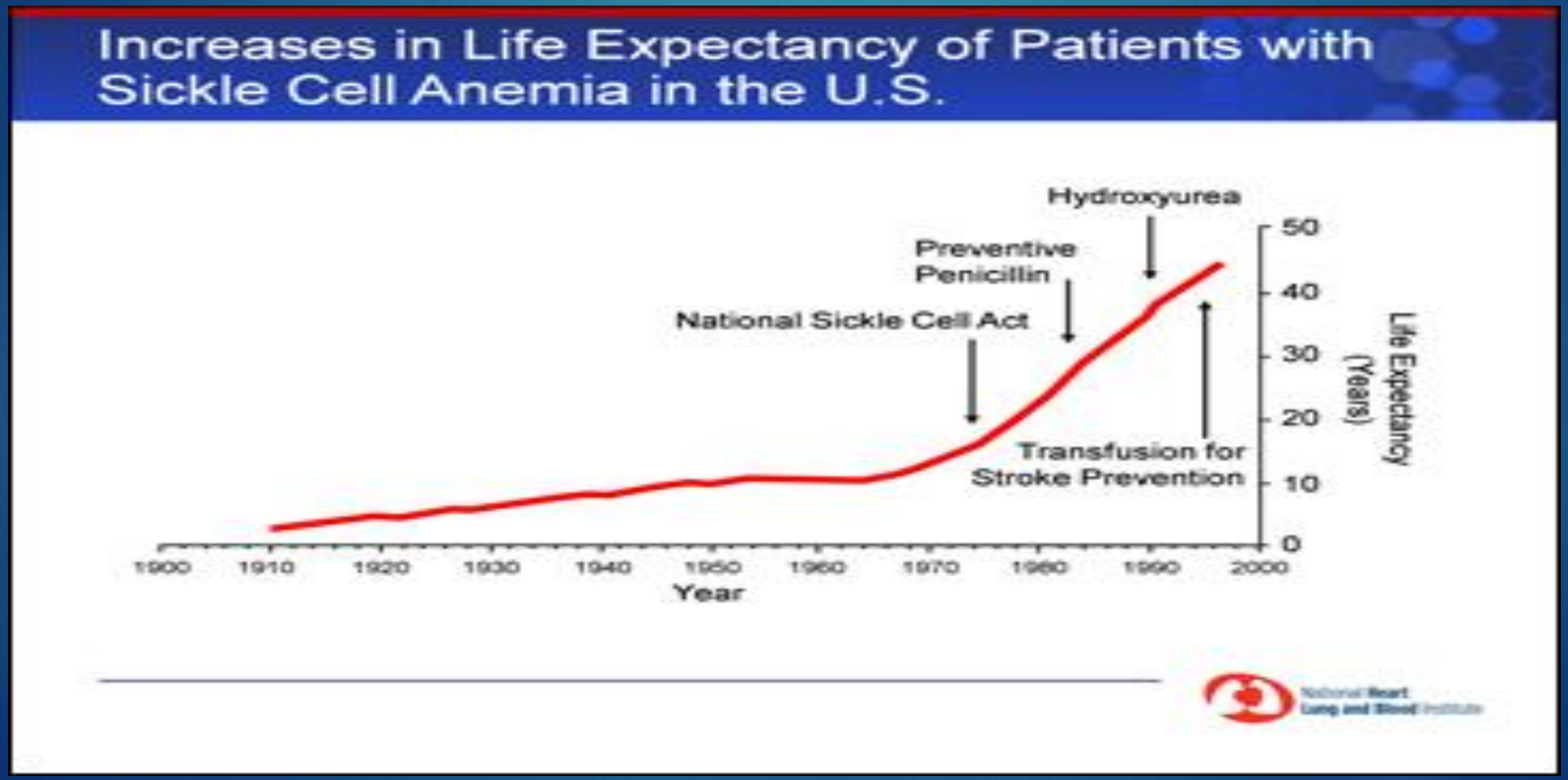
History of Sickle cell Disease

- 1977 the NIH funded the Cooperative Study of Sickle cell Disease with the following objectives
  - To study the effect of SCD on growth and development from birth through adolescence
  - To study "painful crisis" including the manifestations and therapies being used
  - To determine the nature, duration and outcomes of the major complications of SCD
  - To determine the nature, prevalence, and age related incidence of organ damage due to SCD
  - To determine the economic, educational and vocational levels in patients with SCD

What are some of the complications which were understood due to this study?

- Early mortality from infections during childhood due to splenic hypofunction
- Painful crisis
- Acute chest syndrome crisis
- Pulmonary arterial hypertension
- Thrombotic stroke and silent strokes in children and adolescents
- Bone infarcts and avascular necrosis
- Kidney and eye changes
- Early mortality before many reach adulthood or during early adulthood.

What have we done here in the Unitesd States to improve life for Sickle cell patients?

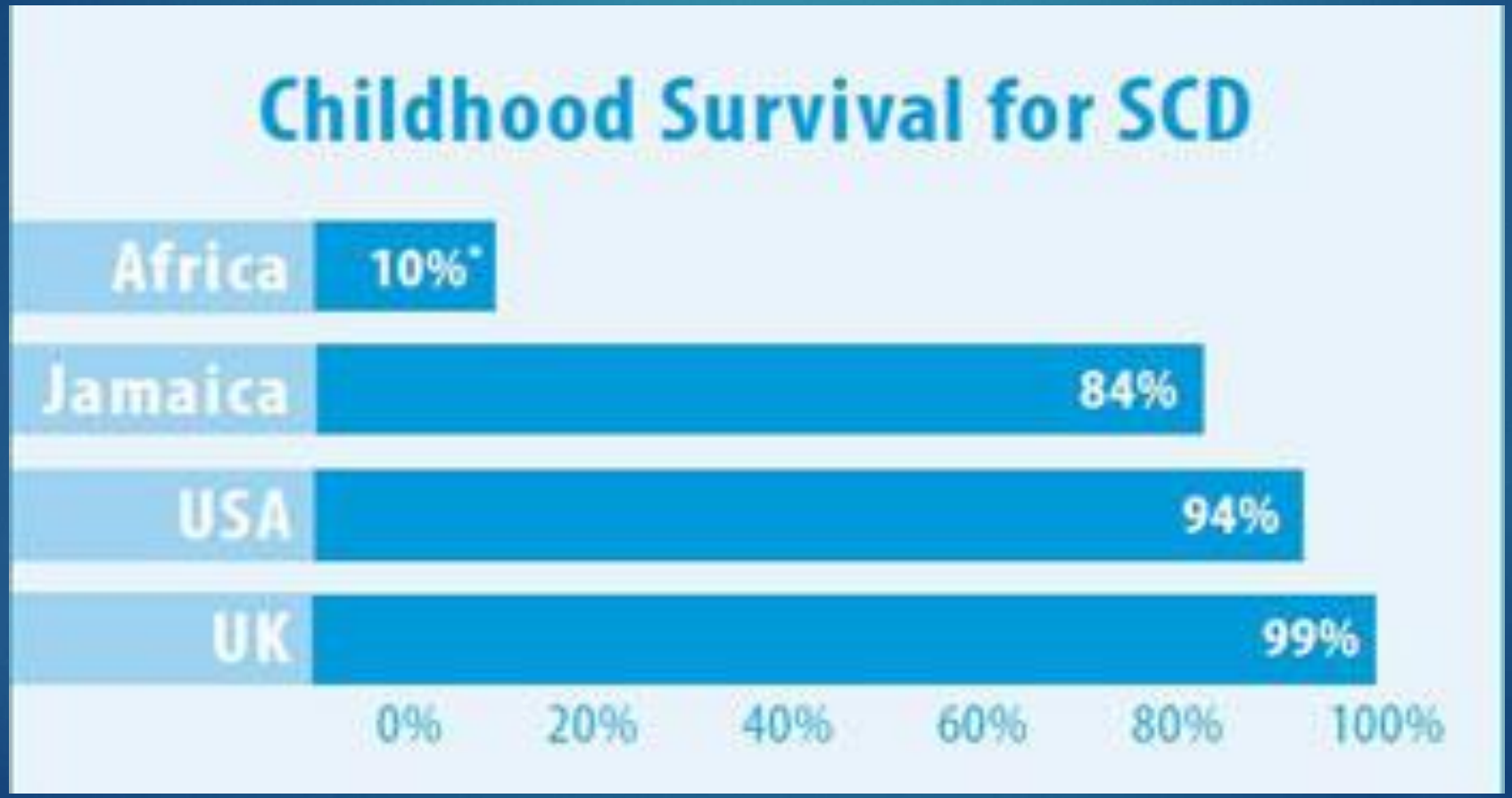


https://www.nhlbi.nih.gov/news/spotlight/success/reducing-burden-sickle-cell-disease

How have we addressed these Sickle cell complications in the United States?

- Diagnosis unknown – Universal New Born Screening, genetic counseling
- Early mortality – Penicillin prophylaxis for children
- Acute pain crisis/Acute Chest syndrome – Hydroxyurea
- Strokes and silent strokes – transcranial dopplers, RBC exchange, hydrea
- Cure for sickle cell anemia –Stem Cell Transplantation

How big is the Healthcare gap between Sickle cell patients in the US and in Africa?



http://www.fic.nih.gov/News/GlobalHealthMatters/november-december-2014/Pages/sickle-cell-disease.aspx

Just a reminder of the burden of Sickle cell disease

Burden of Sickle Cell Disease	
COUNTRY	SICKLE CELL BIRTHS/YEAR
Nigeria	91,011
Dem. Rep. Congo	39,743
Tanzania	11,877
Uganda	10,877
Angola	9,017
Cameroon	7,172
Zambia	6,039
Ghana	5,815
Guinea	5,402
Niger	5,310
Sub-Saharan Africa Total	242,187
Worldwide Total	305,773

Sickle Cell Disease in Africa

A Neglected Cause of Early Childhood Mortality

Scott D. Grosse, PhD, Isaac Odame, MB, ChB, MRCP, Hani K. Atrash, MD, MPH, Djesika D. Amendah, PhD, Frédéric B. Piel, PhD, Thomas N. Williams, PhD

…..although current data are inadequate to support definitive statements, they are consistent with an early-life mortality of 50%–90% among children born in Africa with SS disease."

WORLD HEALTH ORGANIZATION

FIFTY-NINTH WORLD HEALTH ASSEMBLY **A59/9**
Provisional agenda item 11.4 **24 April 2006**

Sickle-cell anaemia

Report by the Secretariat

… sickle-cell anemia contributes the equivalent of 5% of under 5 deaths on the African continent, more than 9% of such deaths in West Africa, and up to 16% of under-5 deaths in individual West African countries.

How various levels of Public Health infrastructure can impact Sickle cell mortality

Table 2. Summary of the level of public health infrastructure and excess mortality considered per income class and for each of the four scenarios tested.

Scenario	Low-/Middle-Income Countries ($GNI_{pc} \leq US\$12,275$)		High-Income Countries ($GNI_{pc} > US\$12,275$)	
	General Level of Public Health Infrastructures for Under-Five Children with SCA	Excess Mortality in Under-Five Children with SCA	General Level of Public Health Infrastructures for Under-Five Children with SCA	Excess Mortality in Under-Five Children with SCA
Scenario 1	Poor access to public health infrastructures	90%	Good access to public health infrastructures	10%
Scenario 2	Good access to public health infrastructures	50%	Specific interventions for children with SCA (e.g., diagnosis, treatment)	5%
Scenario 3	Specific interventions for children with SCA (e.g., diagnosis, treatment)	10%	Universal screening programme (optimum)	0%
Scenario 4	Universal screening programme	5%	Universal screening programme (optimum)	0%

doi:10.1371/journal.pmed.1001484.t002

Health-altering Interventions

Intervention	Access in Africa
1. Newborn screening; penicillin prophylaxis; anti-pneumococcal vaccination	Affordable / increasing availability
2. Comprehensive care coordination	Limited / Affordable
3. Better pain management	Affordable / very limited availability
4. Family-patient education	Possible / affordable
5. Hydroxyurea	Affordable / increasing availability
6. Chronic RBC transfusion	Affordable / increasing availability
7. Hematopoietic stem cell transplantation	Very limited availability

Newborns with SCD Increasing Globally

2010-2050 Estimated Newborns with SCD-SS

Impact of Public Health Interventions:

1. Implementation in 2015 of
   prenatal diagnosis,
   penicillin prophylaxis, and
   "vaccination" for children with SCD-SS,
   can reduce mortality among children under-5 with SCD-SS,
   prolong the lives of 5,302,900 SCD-SS newborns with by 2050.

2. Large-scale universal screening
   could save the lives of up to 9,806,000 newborns with SCD-SS globally, 85% of whom will be born in sub-Saharan Africa

Piel et al. (2013) PLoS Med 10(7): e1001484. doi:10.1371/journal.pmed.1001484

Thank you for your attention

- http://www.nhlbi.nih.gov/health-pro/guidelines/sickle-cell-disease-guidelines