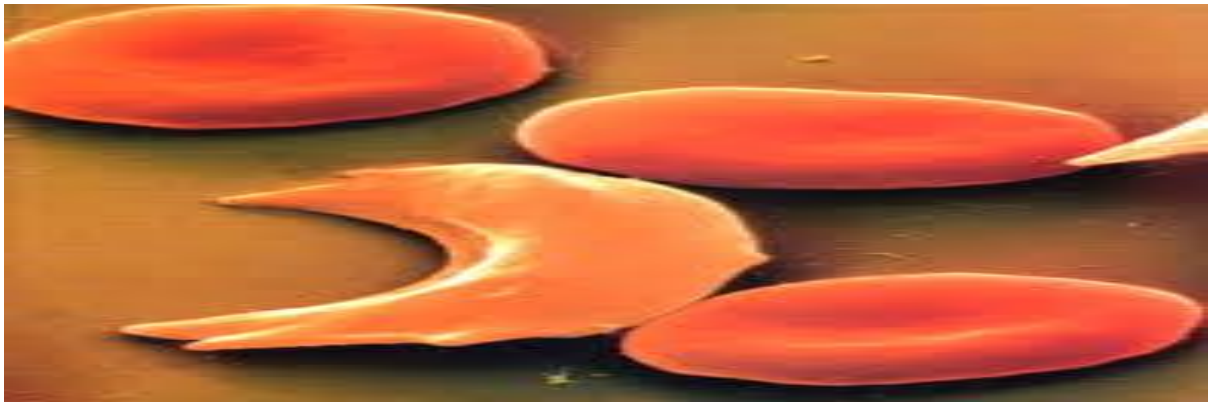
Vol. 1 No. 2
Mar 2014

AfroSickle: Network News

To Promote Science and Improve Patient Care for SCD in Africa



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Network News: Headlines

- Website and blog up and running
- 3 papers published from AfroSickleNet collaborations
- HU trial progressing in Ibadan
- Collaborative Program Development in Kumasi
- Cape Town meeting on ELSI study of SCD
- Pilot studies
- Proposed project on Evolutionary History of S globin locus

Network News: The Stories

1. **Website and Blog**

As most of you already know, the website and blog are gradually coming alive.

Website - http://stritch.luc.edu/sickle_cell/
Blog - http://afrosicklenet.blogspot.com/

Hopefully, you can all access the links from outside. We are going to continue to make it a little more exciting – pictures, pre-prints of papers, copies of funded grants, protocols etc. We could use some "opinion pieces" or "notes from the field". At this point there is no way to know how far we can go toward making it a hub or centerpiece of dialogue on SCD, but we are going to try. I confess to having no experience with Facebook or Twitter but will figure out if they would be useful. As always, suggestions welcome (in fact, necessary . . . .)

2. **Published papers**

There are now 3 papers – listed below – in press, which are a consequence of collaborations through ASN (yes, that's AfroSickleNet . . .)

- *Comparison of Patients from Nigeria and U.S.A. Highlights Modifiable Risk Factors for Sickle Cell Complication*
- *Vitamin D levels in SCD patients in Nigeria and Jamaica*
- *Genetic variants, HbF levels and rate of hospitalization in Cameroon.*

In general I would like to post "pre-prints" – the version accepted for publication. I realize there are still remnants of the "embargo" culture around, but given that the NIH posts pre-prints, and that there is now a site for biology like the famous physics pre-print operation (http://biorxiv.org/about-biorxiv) I think this is the road to the future. The next step will be a mechanism for on-line feed-back and comments, and files with the data. Opinions? What's at stake is a fundamental change in the culture from "work conducted in secret that is published with a big splash in the press" to an open exchange before, during and after publication. The open process would improve the quality and eliminate a lot of posturing and exaggerating.

3. Progress on HU Trial in Ibadan

Victor, Titilola and Bamidele are making good progress toward this important study. Subjects are now being recruited into the Cross-sectional study. The Data Safety and Monitoring Board (DSMB) has been formed for the Clinical Trial phase that will start later. In the light of unavailability of HU placebo in the market, the DSMB agreed with the investigators' proposal to use cross-over design instead of the previously proposed randomized placebo-controlled design. We are also in the process of registering the HU Clinical Trial with the *ClinicalTrials.gov* registry prior to the start of the trial.

4. Collaborative Program Development in Kumasi

Solomon spent the month of Feb in Ghana setting up his research operations in Kumasi. In collaboration with Drs. Ellis Owusu-Dabo (Director of KCCR), and Ohene-Frempong (Emeritus Professor at Penn, and President, Sickle Cell Foundation of Ghana), they are setting up a research hematology laboratory in KCCR to support various research projects focused on sickle cell disease, which will include the proposed international collaboration AfroSickleNet. For additional information on KCCR (i.e. Kumasi Collaborative Center for Research in Tropical Medicine), visit http://kccr-ghana.org/kccr/. Currently, this lab has the capacity to store samples at -80 degrees on site, and perform DNA extractions using an automated analyzer. There are plans to hire a full-time hematology technician to work in this lab, pending a contract agreement between KCCR and the University of Pittsburgh. A memorandum of understanding (MOU) between Pitt and the parent institution of KCCR, the Kwame Nkrumah University of Science and Technology (KNUST), which will support these activities has been signed by the Ghanaian party including the KNUST Vice-Chancellor, and is now awaiting the Pitt signatures.

In addition to the laboratory-based activities, he made progress also with the clinical team in Kumasi, in the Komfo Anokey Teaching Hospital (KATH). There were several meetings with the pediatricians involved with this project. Together they have drafted a clinical research protocol for a longitudinal observation study (ORDISS), which is now been finalized for submission for ethical review by the KNUST Committee on Human Research Publication and Ethics. They anticipate that this study will be running in the next 3 months. Solomon is planning a follow-up visit to Kumasi in July.

5. Cape Town Short Course on Genomics, Bioethics and SCD (24th February to 2nd March 2014)

H3Africa provides an unprecedented opportunity to study genetic and genomic technologies into research, diagnosis, intervention, and treatment for sickle cell disease (SCD) in Africa. Convened by A. Wonkam (Cameroon and South Africa), the course aimed to bring together all the investigators and trainees involved in the first ELSI project of H3 Africa devoted to the study of the perspective of public health intervention in Sickle cell Disease. For each of the three study sites (Cameroon, Tanzania, Ghana), we invited 6 trainees to attend the short course. The short course was advertised within the H3Africa community and the number of attendees exceeded our expectation: 20 from 6 African countries, as many other attendees found the course relevant to their endeavour.

Course Description

Genomics is expected to have a major impact on the prevention, diagnosis and
Our NIH funded project focuses on two critical components of SCD research in Africa: 1) challenges of implementing genomic research and 2) identification of psychosocial factors associated with the burden of SCD in order to improve the health of people with SCD across Africa. The work proposed in this project will lay the groundwork for more extensive societal implications of genomics research in Africa in the future.
The course content was developed around four main themes: 1) Genomics, 2) bioethics and public policy issues; 3) Sickle cell disease and public health intervention in Africa, 4) Qualitative Research Methodologies and its application to SCD.

Pedagogic Approach

The course was taught in 20 theoretical, practical or interactive sessions, each session lasting one to two hours, (36 hours in total). The course was designed to encourage active involvement by participants. 6 international faculties (4 from the USA, including K Ohene-Frempong and S. Ofori-Aquah) was involved in teaching.
From participants' evaluation and instructors comments, this first experience was an immense success that deserved to be repeated, maybe in different African settings.

6. Pilot studies

We are still in process of pulling together protocols, IRB approvals etc. for a couple of pilot studies. The first on the list is an examination of markers of hemolysis in SS patients and AA participants from the general population. At the moment the delay is my fault (ie, RC) – I am traveling and haven't moved this forward.

7. Proposed project on Evolutionary History of S globin locus

Neil Hanchard has prepared an short protocol for a study of the population/evolutionary history of the S locus – see below. He is also developing a collaboration with the MalariaGen program at Oxford/Sanger to explore joint analyses of their extensive data base. He plans to visit Oxford in April.

R. Cooper

NB: We have posted all of this material in the website and the blog, in a format open to anyone - if you have reservations about that approach, for some specific pieces of information, let us know.

Population genetics and selection of HbS beta-globin haplotypes

Background

The sickle allele (HbS) of the beta-globin gene (*HBB*), which in the homozygous state gives rise to sickle cell disease (SCD), occurs commonly in populations of African ancestry as a result of its protective effect against severe malaria [1-4]. HbS is classically thought to have arisen multiple times around the same time in recent human history (~5,000 years ago), largely driven by observations of distinct haplotype backgrounds across sub-Saharan Africa, Arabia and India [5-7]. These classical βs haplotypes are named according to their putative geographical origins – Benin, Bantu (Central African), Cameroon, Senegal and Arab. In general, in regions where the βs allele is found at substantial frequency, a specific βs haplotype usually predominates, although other haplotype backgrounds – both classical and 'atypical' - are also observed. For example, although the Senegal βs haplotype is the most commonly observed in Senegal, the Benin haplotype is not uncommonly seen [8,9]; similarly, we have observed a commonality of Benin haplotypes among βs haplotypes sampled in Yaoundé, Cameroon, as oppose to the expected 'Cameroon-type' (unpublished data). Combined with observations of broad diversity among even geographically isolated βs haplotypes [8,10,11], there remains some uncertainty surrounding the origins and interpretation of classical βs haplotypes, particularly the contribution of complex genomic rearrangements and ancestral population events [12-15].

Much of the conventional wisdom concerning the origin of βs haplotypes was concluded from the patterns of restriction fragment length polymorphisms observed in the early 1980's [5,6,16]. The authors observed a single major ancestral haplotype in each population as well as several less-common, but more diverse, ancestral haplotype backgrounds. It was postulated that the observed data reflected either a single (most-common) mutation followed by complex genomic rearrangements *or* three to four independent mutations occurring independently. The relative simplicity of the latter explanation subsequently gained traction, bolstered by subsequent population surveys [17] and more detailed haplotype inference [18]. Since those original descriptions, however, there have been significant technological advances that have informed our understanding of the genomic architecture of the beta-globin region and potentially have implications for the accepted dogma of βs haplotypes. In addition to the aforementioned increase in DNA sequence diversity on classical RFLP haplotypes, a propensity for gene conversion/structural chromosomal events in the beta-globin locus has since been described [19-21], and new signatures of selection, punctuated by long-

range ancestral haplotypes, have been described in association with the HbS allele [9,22-24]. The dynamics of how these more recent observations interplay with one-another remains uncertain [25], but taken together, they suggest that our understanding of the genetic context and recurrent nature of the HbS allele may be substantially more complex than hitherto thought, and not fully captured by the original βs haplotype descriptions. Given reported associations between βs haplotypes and variability in SCD severity [26-28], and the central contribution of HbS to our understanding of population genetics [29], a updated characterization of βs haplotypes could have profound implications for our understanding of human history and population migration; as well as for the first molecularly understood genetic disease - SCD.

Purpose and Objectives

The primary purpose of this study is the technological re-evaluation and characterization of βs haplotypes. The specific aims are:

1. Describe the distribution of βs haplotypes across multiple populations of African Ancestry

We will provide a robust overview of African βs haplotypes by evaluating the frequency and diversity of Beta-S haplotypes in a cohort of SCD patients from across Africa.

2. Evaluate the genomic evidence for multiple, simultaneous origins for HbS using βs haplotypes

2a. Define traditional beta-globin haplotypes utilizing next-generation sequencing technologies

We will combine next-generation sequencing technology with traditional RFLP genotyping to characterize and define classical βs haplotypes in the context of common sequence variation.

2b. Compare long-range- and classical- βs haplotypes

We will compare extended ancestral βs chromosomes derived from long-range sequencing with ancestral chromosomes inferred from classical βs haplotypes (characterized as in 2a). This data will be evaluated against haplotype expectations consistent with the multi-origin hypothesis for HbS.

Protocol Risks/Subjects/ Design/Procedure

We propose to obtain 500 DNA samples from SCD patients in Africa. For two-hundred of these patients, DNA will also be obtained from the patients' mother and father. Relevant demographic information – age, gender, grandparents' ethnic affiliation – will also be recorded. Samples will be genotyped using RFLP to determine classical beta-S haplotypes. The samples will be sequenced over a 400 kilobase region encompassing beta-globin.

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