PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	ORGAN DAMAGE IN SICKLE CELL DISEASE STUDY (ORDISS): PROTOCOL FOR A LONGITUDINAL COHORT STUDY BASED IN GHANA
AUTHORS	Anie, Kofi; Paintsil, Vivian; Owusu-Dabo, Ellis; Ansong, Daniel; Osei-Akoto, Alex; Ohene-Frempong, Kwaku; Aikins Amissah, Kofi; Addofoh, Nicholas; Bonwin Ackah, Ezekiel; Owusu-Ansah, Amma; Ofori-Acquah, Solomon

VERSION 1 - REVIEW

REVIEWER	Dr. Richard K.D. Ephraim Department of Medical Laboratory Science School of Allied Health Sciences University of Cape Coast Ghana
REVIEW RETURNED	23-Mar-2017

GENERAL COMMENTS	1. Who will do the examinations mentioned? 2. What time will the urine be collected and what type of urine collection will the authors use for this study? 3. A reference should be provided for the manual counting of reticulocytes. 4. How will the school performance be assessed?

REVIEWER	Trisha Wong Oregon Health and Science University, Portland, OR. USA
REVIEW RETURNED	29-Mar-2017

GENERAL COMMENTS	This is a well-written manuscript of a prospective, longitudinal study protocol proposing to study sickle cell disease and subsequent organ damage in children seen at one institution in Ghana. It is a collaboration amongst KATH and KCCR in Ghana and University of Pittsburgh (I assume University of Pittsburgh Medical Center, UPMC) in the USA. The objectives and study protocol are well delineated and I look forward to seeing the end results. However, I have a few suggestions that could strengthen this protocol manuscript. Specific suggestions: Title page: this is not an epidemiological cohort study so please remove that from the title (or if it is, that needs to be explained better in your analysis) Intro: Please tell us a little more about K-CSCD? What professionals

	and services are provided in the clinic (MD? RN? Pharmacy? Radiology?). How many patients are seen annually? You say in the eligibility criteria that all genotypes of children registered at K-CSCD will be included, but how/why are patients registered at K-CSCD? For example, have they all had Hgb electrophoresis to confirm SCD or is there a certain constellation of symptoms that you look for? Intro: Your intro should also reference the data/publications that establish the utility of at least some of the biomarkers listed in Table 1 Page 6: Enrollment is missing an L Page 7: I believe that Complete Blood Count (CBC) is the more established term, rather than FBC...unless they are actually different tests. Page 9: Regarding the sentence "shipment...to VMI will take place once every year," why is it in the future tense? The rest of the article is written in present tense and if this protocol has been active since May 2015, the first shipment should have already happened. Page 9: is the VMI institute the same as the Center for Translational and International Hematology mentioned at the top of page 6? If so, keep the name consistent. If not, please delineate which each UPMC center is doing for the study. Page 10: under Ethical and Safety, please comment on whether data sent to VMI will be de-identified or completely anonymous? Do members of UPMC come to Ghana to work in clinic? Also, please comment on Material Transfer approval for sending data and biologic samples, including DNA, internationally? Page 10: The conclusion needs to somehow specify that this study only pertains to children with sickle cell. Page 11: please be more specific about your funding. Is it funded from a UPMC private foundation or endowment? Or did UPMC apply and win funding from somewhere else, such as a federal or private agency? Page 12: your most recent reference is 2013. There are more recent articles that review major organ failure in SCD than 1990! Page 13: please include what genetic assays you plan on doing with the DNA in Table 1 Page 14: Although I appreciate the organization of Table 2, I'm not sure exactly what it is. it doesn't seem precise enough to be a protocol for defining organ failure but I'm guessing that is what it is. A lot of tests (blood, imaging, etc.) are mentioned, so who decides when/how those tests will be ordered. For example, "ECHO is the most commonly used technique used to measure cardiac function"--does every enrolled subject get an ECHO? If so, how often? If not, what criteria will be used to decide who needs and ECHO and who doesn't? General suggestions: - Would consider moving paragraphs and sections around to follow more intuitively. For example, include the IRB approvals under the Ethics section and move it earlier in the paper, while keeping Dissemination at the end. - Please reference your tables within the text. Currently, they are free standing and it is hard to interpret why and in what context they were included. - Please include a section on your proposed analysis. Such as what is your estimated/goal accrual. How long do you think it will take to accrue your goal?...or are you just accruing for a set time (ie: a sample of convenience?) Will you publish after the last-enrolled subject is followed for 3 years? - I'm sure the assays listed in Table 1 were well discussed but why

	not INR, PT, Fibrinogen, ESR, CRP, ferritin, adhesion molecules (p or e selectin). Which cytokines? Again, your discussion in the intro with references to publications regarding the utility (and inutility) of some assays can be used to to justify your list of assays. Thank you for doing this important work and I look forward to reviewing your resubmission.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

1. Who will do the examinations mentioned?

Statement inserted – Page 7, Paragraph 3.

2. What time will the urine be collected and what type of urine collection will the authors use for this study?

Statement inserted – Page 8, Paragraph 1.

3. A reference should be provided for the manual counting of reticulocytes.

A reference has been included – Page 8, Paragraph 1, and Reference list (Page 14).

4. How will the school performance be assessed?

Statement inserted – Page 9, Paragraph 1.

Reviewer: 2

This is a well-written manuscript of a prospective, longitudinal study protocol proposing to study sickle cell disease and subsequent organ damage in children seen at one institution in Ghana. It is a collaboration amongst KATH and KCCR in Ghana and University of Pittsburgh (I assume University of Pittsburgh Medical Center, UPMC) in the USA. The objectives and study protocol are well delineated and I look forward to seeing the end results. However, I have a few suggestions that could strengthen this protocol manuscript.

Specific suggestions:

Title page: this is not an epidemiological cohort study so please remove that from the title (or if it is, that needs to be explained better in your analysis)

This is a longitudinal cohort study. We have not stated ‘epidemiological’ in the title.

Intro: Please tell us a little more about K-CSCD? What professionals and services are provided in the clinic (MD? RN? Pharmacy? Radiology?). How many patients are seen annually? You say in the eligibility criteria that all genotypes of children registered at K-CSCD will be included, but how/why are patients registered at K-CSCD? For example, have they all had Hgb electrophoresis to confirm SCD or is there a certain constellation of symptoms that you look for?

We have already stated that patients registered at (K-CSCD) are identified through newborn screening (Page 4, Last Paragraph). Nonetheless, we have included further information –

Page 5, Paragraph 2.

Intro: Your intro should also reference the data/publications that establish the utility of at least some of the biomarkers listed in Table 1

References for Table 1 have been included – Pages 18-19.

Page 6: Enrollment is missing an L

British English is spelt ‘Enrolment.’

Page 7: I believe that Complete Blood Count (CBC) is the more established term, rather than FBC...unless they are actually different tests.

‘Full Blood Count (FBC)’ is a British established term also used in Ghana.

Page 9: Regarding the sentence "shipment...to VMI will take place once every year," why is it in the future tense? The rest of the article is written in present tense and if this protocol has been active since May 2015, the first shipment should have already happened.
Amendment has been made – Page 10, Paragraph 1.
Page 9: is the VMI institute the same as the Center for Translational and International Hematology mentioned at the top of page 6? If so, keep the name consistent. If not, please delineate which each UPMC center is doing for the study.

This has been clarified – Page 6, Paragraph 2.

Page 10: under Ethical and Safety, please comment on whether data sent to VMI will be de-identified or completely anonymous? Do members of UPMC come to Ghana to work in clinic? Also, please comment on Material Transfer approval for sending data and biologic samples, including DNA, internationally?

This has been clarified; we have included a statement – Page 11, Paragraph 1.
There are no members of UPMC that go to Ghana to work in the clinic.

Page 10: The conclusion needs to somehow specify that this study only pertains to children with sickle cell.

The word ‘children’ has been inserted.
Page 11: please be more specific about your funding. Is it funded from a UPMC private foundation or endowment? Or did UPMC apply and win funding from somewhere else, such as a federal or private agency?

This has been clarified.

Page 12: your most recent reference is 2013. There are more recent articles that review major organ failure in SCD than 1990!

Powars (1990) was a landmark study; there have been no studies of the same kind in organ damage of patients with sickle cell disease without interventions since then. This highlights the importance of our current research. However, we have included another relevant publication to emphasise the issue – see Reference Page.

Page 13: please include what genetic assays you plan on doing with the DNA in Table 1
These have been included.

Page 14: Although I appreciate the organization of Table 2, I'm not sure exactly what it is. it doesn't seem precise enough to be a protocol for defining organ failure but I'm guessing that is what it is. A lot of tests (blood, imaging, etc.) are mentioned, so who decides when/how those tests will be ordered. For example, "ECHO is the most commonly used technique used to measure cardiac function"--does every enrolled subject get an ECHO? If so, how often? If not, what criteria will be used to decide who needs and ECHO and who doesn't?

Table 2 provides information about consensus guidelines and clinical definitions for the management of sickle cell disease. These will be utilised as appropriate and not routinely for every patient.

General suggestions:

Would consider moving paragraphs and sections around to follow more intuitively. For example, include the IRB approvals under the Ethics section and move it earlier in the paper, while keeping Dissemination at the end.

The sections correspond to the BMJ Open format for protocol manuscripts. However, we have moved the IRB approvals statement to ‘Ethical and safety considerations’ section – Page 10, Last Paragraph.

Please reference your tables within the text. Currently, they are free standing and it is hard to interpret why and in what context they were included.

Tables are already referred to in the text – Page 10, Paragraph 2.

Please include a section on your proposed analysis. Such as what is your estimated/goal accrual. How long do you think it will take to accrue your goal?...or are you just accruing for a set time (ie: a sample of convenience?) Will you publish after the last-enrolled subject is followed for 3 years?

We have included a statement about convenience sampling under ‘Participants and Recruitment’– Page 6, Paragraph 3.

We have included ‘Statistical Analysis’ under ‘Methods and Analyses’ – Page 10, Paragraph 3. We intend to publish after the last-enrolled subject is followed for 3 years?

I'm sure the assays listed in Table 1 were well discussed but why not INR, PT, Fibrinogen, ESR, CRP, ferritin, adhesion molecules (p or e selectin). Which cytokines? Again, your discussion in the intro with references to publications regarding the utility (and inutility) of some assays can be used to to justify your list of assays.

Assays are standard for our work. References have included – Pages 20-21.

VERSION 2 – REVIEW

REVIEWER	Trisha Wong OHSU, USA
REVIEW RETURNED	23-Jun-2017

GENERAL COMMENTS	The authors addressed the reviewer concerns sufficiently. Thank you.