Author's response to reviews

Title: Genetic Risk Factors for Cerebrovascular Disease in Children with Sickle Cell Disease: Design of a Case-Control Association Study and Genomewide Screen

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Dear Ms. Veitch,

Please find below a point-by-point response to the reviewer's comments of our article, ID-2060759621461142:

1. Some of the terms for stroke are nonstandard. For example, in line 4 of the abstract, the term 'infarctive stroke' should be replaced by 'ischemic stroke.'
   This change was made.

2. Patients were randomized in the Stroke Prevention Trial in Sickle Cell Anemia and not 'to' the trial (see p. 2, line 10).
   This change was made.

3. Acronyms should be defined the first time they appear in the manuscript. In the abstract, many acronyms are undefined (TNF alpha, MALDI-TOF, VCAM-1, etc.). In fact, there are so many acronyms, that a table of abbreviations might be the best way to define them.
   We have added a List of Abbreviations.

4. There is far too much generic background about sickle cell disease. Material that can be found in any general medical text should be avoided (e.g. the first paragraph on page 4). It just dilutes the message.
   This paragraph was shortened according to the suggestions by the reviewer.

5. In line 17, page 4, it is said that in sickle cell patients under age 20 years, stroke is predominantly 'thrombotic.' Are we really sure that the ischemic stroke cases are related to thrombosis? Could it not also be that the ischemic stroke cases are mostly due to non-thrombotic vascular occlusion from red cell clumping?
   "Thrombotic" was replaced with "ischemic."

6. The statement "...only a few genetic risk factors are known to influence stroke risk;" (page 4, line 20) should be supported with at least one reference.
   Reference 7 was added here.

7. The normal cerebral blood flow velocity for sickle cell children is reported as "140 cm/sec" (p. 5,
line 6), but should there not be a range of normal values? As a point of reference, it might be helpful to also state what the age related normal range is for non-sickle children. A normal range has been given for TCD values.

8. The ages should come with units. Examples: "2-16" (line 9, page 5 and line 21, page 5) should read "2-5 years." It is important not to be ambiguous. This change was made throughout the manuscript.

9. The term "abnormals" (page 6, line 11) needs to be clarified. '(i.e., TCD >200 cm/sec)' was added to clarify "abnormals"

10. It is stated that "TCD remains the only proven indicator of cerebrovascular disease and stroke risk in Hb SS [p. 7, lines 24-25]" However, there is some evidence that silent stroke as seen on brain magnetic resonance imaging is also an indicator of stroke risk. While there is new evidence that silent infarcts on MRI may be an indicator of stroke risk, this has not yet been proven and validated in controlled clinical trials and in a large number of patients, as is the case with TCD.

11. The statement "...results obtained in smaller studies [p. 8 line 2]" should be supported by one or more references. Reference 5 was added here.

12. Line 5 on page 8 states "Currently, 18 of the 130..." It would be better to give a date rather than use the vague statement "Currently". The date of the study termination has been clarified.

13. The demographics of the patient population should be described in more detail. How many centers were involved in STOP and how many centers are now involved in STOP II? Where are these centers located (e.g. US, US and Canada, etc.)? Information on the number and location of STOP and STOP II centers has been provided.

14. I suggest that the Human Studies material be placed in its own subsection at the end of the methods section (paragraph 3 on page 9). 'Human Biological Materials' was added as a heading for this section.

15. The matching variables are not precisely defined prospectively, but it appears that the authors are writing in some 'wiggle room' into the protocol (see paragraph 2, page 9). The matching variables should be clearly stated. This sentence has been rewritten and now reads: 'Cases and controls will be matched for age, sex and weight, which are the most important covariates.'

16. Information about the company and company location (city, state) should be provided parenthetically for every key piece of equipment, reagent, or software. For example, instead of saying "Sequenom's MassARRAY system [p.10, line 7]" one could say "MassARRAY system (Sequenome; city, state)." This is common practice in journals like the Journal of Biochemistry, and it fosters independent confirmation of published findings. This change was made.

17. "NTP [page 10, line 16]" is not defined. NTPs are nucleotide triphosphates, which is now written in full.

18. The statement "more accurate compared to hybridization-based methods of SNP detection [p. 11, line 13]" should be supported by one or more references. References 17 and 18 were added here.
19. There should be some discussion of the clinical implications of finding risk modifier genes of modest effect. How sensitive and specific is TCD for identifying high-risk individuals? How would 'positive' results of the proposed study be integrated into a rational screening program for preventing Hb SS associated cerebrovascular disease? How effective are genetic screening programs for diseases with well-established genetic risk factors like breast cancer? Would the same level of effectiveness be anticipated in the sickle cell population?
A paragraph addressing these issues has been added to the discussion section.

20. It would appear that the right half of table 2 is supposed to be an extension of the bottom part of the left half of the table. In its current form, the table is confusing.
We agree and have reformatted the Table.