Reviewer’s report

Title: Association of non-synonymous mutations in the Plasmodium falciparum kelch13 gene (PF3D7_1343700) with parasite clearance rates after artemisinin-based treatments - a WWARN individual patient data meta-analysis

Version: 0 Date: 02 Jul 2018

Reviewer: Khalid Beshir

Reviewer's report:

This manuscript is clearly presented and the text is well written. The significance of the manuscript lies on the largest evaluation of genotype-phenotype association of artemisinin resistance as well as the identification of additional mutations at pfk13 associated with slow clearance rate. The manuscript adds great value to the ongoing effort to understand the emergence and spread of artemisinin resistance. The authors carried out a meta-analysis of a correlation between parasite clearance (as measured by half-life) and different mutations in pfk13 gene. They conducted a systematic literature review to find studies that were published between 2000 and 2017. Four databases were searched to find studies that include frequent parasite count and pfk13 mutation analysis and 18 studies met the criteria. Using multivariable regression models, the authors assessed the correlation between log transformed parasite clearance half-life and different mutations at pfk13. The authors reported that 22 mutations (mutations in the propeller region of pfk13 as well as allele 252Q) were significantly associated with slower clearance rate compared to the wild type isolates (1.5-2.7 fold difference). They observed no difference in Africa. Discretionary revisions are below.

Minor comments:

Page 6, Line 1: The authors assumed a wild type if no mutation is reported. Please mention if the whole pfk13 was genotyped by sequencing and the whole DNA sequence was analysed rather than reported candidate point mutations. In Supplementary material 1, there was one study (No. 8) reported to have moderate risk of bias due to genotyping method. Was the analysis (Supp Fig S2) also done by excluding this study?

Page 6, Line 4: The authors excluded mixed genotypes and the authors did not provide rational for exclusion and possibly provide evidence that mixed genotypes does not correlate with clearance rate.

Page 6, Line 20-25: Are the cut-off values random or based on previous findings? Please clarify.
When PC1/2 was compared to microscopic positive on day 3 false positive (when parasitaemia is very high) and false negative (when the initial parasitaemia is low) was observed. Was there qPCR data in any of the studies? It would be interesting to see whether qPCR, either Parasite Reduction Ratio at 48 hours (PRR48) after treatment or days 3 qPCR positivity, can reduce the false positive and the false negative respectively.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Not applicable

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

Yes

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

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