Author's response to reviews

Title: Genetic polymorphisms in MDR1, CYP3A4 and CYP3A5 genes in a Ghanaian population and their relevance on response to ivermectin treatment

Authors:

William Kudzi (wkudzi@yahoo.com)
Alexander N.O Dodoo (alexooo@yahoo.com)
Jeremy J Mills (Jeremy.mills@port.ac.uk)

Version: 2 Date: 19 March 2010

Author's response to reviews:

Dear Editor

Please find attached the revision to the paper MS: 2123107729263686 titled “Genetic polymorphisms in MDR1, CYP3A4, and CYP3A5 genes and its impact on ivermectin therapy in onchocerciasis”. This revision has taken into account the recommendations by the reviewers and corrections have been made to satisfy many of these.

“Keywords” section of the abstract has been removed and “Written informed consents were obtained from all subjects prior to inclusion in the study” inserted into the section on methods

The corrections made in response to each reviewer’s comment are shown below;

Response to reviewer – Anne Slavotinek

Major revisions

Reviewer: The authors need to mention the limitations of their study – an extremely small sample size (21 suboptimal responders only), no exclusion of stratification of the different responder populations, and no replication is provided. In fact, the numbers are so small that it is possible that there are host factors that are important. What power does the study have to detect a significant difference between genotypes with the low numbers?

Response: The authors do agree with the reviewer that the sample numbers were small and this has been stated in the manuscript as a limitation to the study. These were the only documented suboptimal responder samples available for analysis.

Reviewer: How were responders and suboptimal responders defined?

Response: Suboptimal responders were defined as patients observed with high microfilaria loads after many years of treatment with ivermectin and responders as patients who have had their microfilaria cleared after treatment.
Reviewer: You state that the host factors are unlikely to be important in suboptimal response to ivermectin in both the abstract and the discussion, and yet you point out a statistically significant difference between responder and suboptimal responder genotypes: ‘CYP3A5*1/CYP3A5*1 and CYP3A5*1/CYP3A5*3 genotypes were found to be significant different for responders and SORs. Haplotype (*1/*1/*3/*1) was determined to be significant different between responders and SORs’. How do you explain these results if you deem that there no significant host factors?

Response: Although CYP3A5*1/CYP3A5*1 and CYP3A5*1/CYP3A5*3 genotypes were found to be significant different for responders and suboptimal responders, ivermectin is not a substrate for CYP3A5 gene and we are not able to confirm if this will have any significant impact on ivermectin response.

Reviewer: “Some of these variant alleles are being reported for the first time among the Ghanaian population”. – this sentence is too loose – you need to state which variant alleles.

Response: This sentence has been deleted from the manuscript.

Reviewer: For tables 4 onwards, all p values need to be included with the data.

Response: Tables 4 and 5 have been condensed into one and labelled Table 2 in this revised manuscript and the confidence interval (CI) values provided.

Reviewer: Table 1 primers have all been published and should be supplementary data; similarly Table 2 could also be supplementary.

Response: Tables 1, Table 2 and all reference to them have been removed from the manuscript. Brief description of the methods involving these two tables has also been removed from the section on genotyping.

Reviewer: Table 3: Are the genotypes combined for all the study subjects in this table and are those for the responders and SORs listed separately in the Table 4? This should be clarified.

Response: All genotypes investigated in the study population are combined in Table 3 which is now Table 1 in the revised manuscript. Confidence interval has been calculated for the values in this new table. Alleles not detected in the study have been deleted from this new table and a footnote ‘MDR1 (1236C>T), MDR1 (2677G>A), MDR1 (2677G>T), CYP3A4*3 variant alleles were not detected in this study’ provided. Table 2 shows the genotype frequencies for the responders and the SORs.

Genotypes for the responders and SOR were listed separately in Tables 4 and 5. However, these two tables have been condensed into one and labelled Table 2 in this revised manuscript. References to these two tables have been corrected in the manuscript.

Reviewer: At the start of the results section, you should redefine the subject
population. How do your MDR1 and CYP frequencies in the subjects compared to dbSNPs?

Response: Subject population has been redefined at the beginning of the results section with the insertion of the sentence “Although a total of 204 samples were collected for analysis, between 194 and 203 were available for each SNP” and “Twenty one patients documented as having high microfilaria after many rounds of IVM treatment were classified as suboptimal responders”

Minor essential revisions
Reviewer: There are numerous minor points and the manuscript needs further proof reading.
Response: The minor points mentioned by the reviewer have been corrected.

Response to reviewer – Dr Dave Bartley and Dr P Skuce

Major revisions
Reviewers: Objectives of the trials and the work need to be clearly defined, explained and discussed.
Response: The aims of the study have been redefined as;

To genotype pharmacogenetically relevant variants of MDR1, CYP3A4 and CYP3A5 genes in a representative Ghanaian population to address scarcity of data in indigenous African populations.

2) To examine the influence of genetic variations within MDR1, CYP3A4 and CYP3A5 genes on response of patients on ivermectin treatment by examining genotype frequencies in responders and suboptimal responders.

Reviewers: The paper would benefit from the pharmacokinetic data from the various groups to correlate with any impact of genotype on ivermectin therapy.
Response: The group with the pharmacokinetic data is not willing to share the information.

Minor revisions
Reviewers: The title needs to be clear that the genetic polymorphisms occur in the host and not the parasite. Other minor points “their” impact rather than “its” impact, no capital letters require for “ivermectin” or “therapy” and “against” rather than “in” onchocerciasis.
Response: The title of the manuscript has been modified to read “Genetic polymorphisms in MDR1, CYP3A4 and CYP3A5 genes in a Ghanaian population and their relevance on response to ivermectin treatment”

Reviewers: Throughout the document abbreviations such as MDR1, CYP3A4 and CYP3A4*1B are used without ever being defined or explained. It is useful for a global audience to describe these in more detail for example the Cytochrome
P450 monooxygenase 3A4 (CYP3A4).

Response: MDR1 and CYP3A4 have been defined at the beginning of paragraphs 1 and 2 respectively in the background.

Reviewers: The abstract is full of jargon and a huge amount of specific detail, it would help the flow of the abstract if it provided information on the general theme and findings

Response: Specific details such as “CYP3A4*1B was detected at 72%, 60%, and 31%; CYP3A5*3 was detected at 15%, 31%, and 21% and CYP3A5*6 was at 14%, 7%, and 12% for population, responder and SORs respectively” has been removed from the abstract.

Reviewers: More information is required for cytochrome P450 section

Response: The sentence “There are 18 families of CYPs, which are divided further into 44 subfamilies consisting of 57 genes” has been inserted in paragraph 2, page 3.

Reviewers: The paper would benefit from the addition of clearly defined aims within the background section of the manuscript and finish in the discussion with whether they were achieved

Response: The aim of this study has been restructured to read ‘1) To genotype pharmacogenetically relevant variants of MDR1, CYP3A4 and CYP3A5 genes in a representative Ghanaian population to address scarcity of data in indigenous African populations. 2) To examine the influence of genetic variations within MDR1, CYP3A4 and CYP3A5 genes on response of patients on ivermectin treatment by examining genotype frequencies in responders and sub-optimal responders’

Reviewers: Throughout there are a large number of grammatical errors. A large number are listed below but the manuscript would benefit greatly by being thoroughly proof read.

Response: The grammatical errors listed have all been corrected in the manuscript.