**Author's response to reviews**

**Title:** PXR and CAR single nucleotide polymorphisms influence plasma efavirenz levels in South African HIV/AIDS patients

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**Author's response to reviews:** see over
The Editor,
BMC Medical Genetics
Biomedical Genetics

Re: Submission of revised manuscript “1739677433807398: PXR and CAR single nucleotide polymorphisms influence plasma efavirenz levels in HIV/AIDS patients’ for consideration for publication; Swart et al ).

Thank you for giving us the opportunity to improve our manuscript by responding to reviewers comments. Please find at the end of this letter, detailed responses to all the questions raised by the reviewers.

Our research focusses on investigating the human genetic variation that is associated with differential response to treatment. Although such research is being done in other parts of the world, South Africa and Africa in general presents with a unique human genetic diversity that affects pharmacokinetic and pharmacodynamics profiles of drugs. In addition, the scourge of HIV and subsequent introduction of highly active antiretroviral therapy (HAART) has allowed prolonged survival of HIV-infected individuals, thus, introducing other unforeseen problems such as drug adverse events (ADEs). As part of our contribution in understanding the genetic factors associated with response to efavirenz-based HAART regimens, we report on the role of genetic variation in PXR and CAR genes. The objective of the article is to show that genetic variation in nuclear receptors such as PXR and CAR should be taken into consideration in pharmacogenomics studies as the current emphasis on drug metabolising enzyme genes in personalised medicine is likely to miss some of the variation due to these nuclear receptors.

We hope you find our revised manuscript suitable for publication in your prestigious journal.

Sincerely,
Response to reviewer’s comments

Reviewer 1
Reviewer comment:
Use the term “healthy subjects” instead of “healthy controls”. The study is not a case-control study.

Response:
We agree with the suggestion by the reviewer and the wording “healthy controls” was replaced by “healthy subjects” throughout the manuscript.

Reviewer 2:

Major Compulsory Revisions
Comment 1:
It is well known that efavirenz is metabolized by CYP2B6 and CYP2B6 SNPs are significantly associated with its disposition. Authors should genotype for CYP2B6 SNPs and add the observed results to that to get more complete picture of the genetic analysis. It is possible that after analyzing for CYP2B6 the association with NR1I2 SNPs might be different than reported.

Response 1:
The reviewer’s comment has been taken into consideration. The participants were further genotyped for the CYP2B6 SNPs and other SNPs which are not part of this manuscript. An analysis for the major CYP2B6 SNP, 516G>T was done. The NR1I3 rs3003596 SNP was stratified by CYP2B6 516G>T genotypes and a new Figure, Figure 2 (A-C) has been added in the manuscripts. We still observed decreased EFV levels in association with the NR1I3 rs3003596 C/C genotype among the CYP2B6 T/T genotype carriers (who lack CYP2B6 activity and are expected to present with higher levels of EFV compared to the CYP2B6 516 G/G and G/T carriers)

Comment 2: Authors have not included information on any association of gender differences; this should be acknowledged given the fact that there are gender differences in drug disposition.

Response 2:
We did not observe gender differences EFV disposition in this particular group although gender is known to affect drug disposition in some cases. We have added the following sentence to the text “Although gender differences are known to result in differences in drug disposition, no significant association of gender with plasma efavirenz levels were observed in this study with average plasma efavirenz levels of 5.25 µg/mL and 4.43 µg/mL in males and females, respectively (P=0.307)".
Comment 3:
Table 3 is not clear, it should be indicated that these are result of resequencing n number of subjects.

Response 3:
The title of Table 3 was changed to include the number of individuals that were used in the sequencing as follows: **Table 3: NR1I2 and NR1I3 genetic variants in 32 HIV/AIDS patients following targeted sequencing of the NR1I2 and NR1I3 DNA binding domains.**

Comment 4:
Although authors have performed haplotype analysis they have not clearly indicated association of haplotype analysis with the clinical data. Also please include SNPs in the haplotype in the manuscript text.

Response 4:
We would like to thank the reviewer for this observation. The association of haplotype analysis with plasma efavirenz levels was included in the manuscript and a revised figure, Figure 3 (A-B) has been included. In addition, the following sentence on page 12 was corrected to include the SNPs in the haplotype in the manuscript text: “By observation, the NR1I2 T-G-G haplotype (with respect to rs2472677-rs3732356-rs6785049), which occurs in about 34% of the patients, was associated with above therapeutic range efavirenz levels and this may influence treatment regimen change (Figure 3A-B”).

Minor Essential comments:
Comment 5:
Table 7 is not required in the manuscript and should be moved to supplementary tables.

Response 7:
Table 7 has been changed to Supplementary Table S1

Comment 8:
Figure 1 please include the exact p-value instead of p<0.05 for TT vs. TC

Figure 1 has been modified as suggested

“Our Mission is to be an outstanding teaching and research university, educating for life and addressing the challenges facing our society.”
Comment 9:
Multiple spelling mistakes should be corrected

Response
Thank you for this observation, we have carefully checked the document now and corrected mistakes