Author's response to reviews

Title: Rheumatic Heart Disease in Uganda: The Association between MHC Class II HLA DRB1 Alleles and Disease: A Case Control Study

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Author's response to reviews: see over
Response to reviewer comments

Dear Editor,

We respectfully re-submit this manuscript following changes made to improve the document as kindly suggested by the reviewers.

We have improved on the language and content of the manuscript and will hopefully meet the standards of your esteemed journal.

Reviewer I

Reviewer: Armando Pucciarelli
Reviewer’s report:
Commentary by Armando Pucciarelli.
This is a very interesting study, however it shows some aspects which need to be better highlighted.
--Abstract,Results.( see also Results in the text of manuscript). ” The mean (SD) and median (IQR) age in years for cases and controls were 29.6 (10.2) and 29(18), and 29.8(9.8) and 31(20), respectively”.
The representation of the data regarding age does not appear to be rational. In fact, in common usage, the data of a given group are represented as mean ± standard deviation if the distribution of data drawn from the study population is normal. Instead, data are usually presented using median plus interquartile range if they are distributed not symmetrically around the mean. For identifying a non-Gaussian distribution of data, the common statistical programs offer the opportunity of exploiting various tests, such as D'Agostino-Pearson method or the Kolmogorov-Smirnov test. Thus, I don't understand why the authors have decided to use both mean and the median for representing the variable "age " in the groups of cases and controls. Please specify the reasons for describing age with mean and median, whose simultaneous use seems redundant to me.
Alternatively, I recommend to execute a test to verify the possible normality of data distribution. In case of normal distribution, please report just the mean ± standard deviation; on the contrary, if at least one of the two groups is characterized by asymmetric distribution around the mean, then please adopt the median plus interquartile range to describe the age in each of the two groups (cases and controls).

- Both in the abstract and in the Results section within the manuscript, the representation of the interquartile range is approximate. In fact, when you use the median to describe a continuous variable, the choice of reporting only the value of the interquartile range it is not sufficiently informative, i.e., the simple difference between the value of the 75th and the one of the 25th percentile doesn't suffice, but rather the numerical values of the 25th and 75th percentiles are required. Thus, please eliminate the value of the difference you reported in round brackets, and replace it by writing the median value followed by (IQR = Q1 to Q3).

1. Response: We appreciate this comment by the reviewer. Data was normally distributed and we agree with the reviewer that we should only present mean plus standard deviation, this change has been made in all sections of the document. (Page 2, line 15 and page 7, line 3)

- Introduction: “Group A streptococcal infection”. Please add the term beta-haemolytic, so as to write: “Group A beta-haemolytic streptococcal infection”. Please always use the adjective “beta-hemolytic” to name this streptococcal bacterial strain throughout the entire text.

2. Response: This change has been made (Page 3, line 13).

- In the Discussion: Rheumatic heart disease could thus depend not only on genetically determined disease susceptibility, but also on the regional and temporal prevalence of a particular streptococcal strain(s). In fact, at least 15 streptococcal strains, each with distinct M proteins, have been associated with outbreaks of rheumatic fever [in this regard see Carlquist JF, et al.( J Am Coll Cardiol. 1995 Aug;26(2):452-7)]. Each different rheumatogenic streptococcal strain might select for a particular DR allele(s) in the pathogenic process, resulting in a high proportion of patients positive for that particular allele during an
outbreak. This could contribute to the explanation of differences between studies which would be otherwise difficult to be justified. For example, there is a strong discrepancy between the data exhibited by the present study, which would show some protective role played by HLA-DR1 against rheumatic heart disease (i.e. rheumatic endocarditis in the large majority of cases), and those reported by Carlquist et al. in their meta-analysis (J Am Coll Cardiol. 1995 Aug;26(2):452-7). According to the latter HLA-DR1 is associated with a greater susceptibility to rheumatic endocarditis in some populations, such as black South Africans and Afroamericans, who are phenotypically quite similar to individuals recruited in the present study. Please comment on this point, i.e., the very evident discrepancy between the protective value of HLA-DR1 against rheumatic endocarditis that you have found in your study and the somewhat conflicting value of risk factor found for this parameter (positivity for HLA-DR1) by Carlquist et al. in similar populations consisting of black South Africans and Afroamericans.

3. Response: We agree that our results conflict with Carlquist et al. and that potential heterogeneity of the causative agent could be a factor. Other explanations include substantial genetic diversity across African populations, low levels of linkage disequilibrium in African populations, small sample sizes in both studies, and different testing methods (serologic testing used in Carlquist et al., and DNA based testing in our study). We have modified the following sentences to our discussion to address these points: “These differences in RHD associations across different populations support the concept of ethnic specific genetic susceptibility for HLA alleles (5). Other explanations for the variability of studies include small sample sizes which may not capture rare HLA alleles, different typing methods (serologic versus DNA based), and heterogeneity of the causative agent. More than fifteen streptococcal strains have been associated with rheumatic fever and each distinct strain may select for a unique DR antigen, resulting in different susceptibility alleles. Africa has one of the most genetically diverse populations, thus differences in HLA alleles is expected between various geographic locations in sub-Saharan Africa.”

In the Discussion, “Identification of individuals with susceptibility gene to RHD could potentially provide an opportunity to screen anyone with streptococcal pharyngitis and place them on primary penicillin prophylaxis. This will make it more cost-effective to provide primary prophylaxis.” It seems to me that the Authors conceive a possible scenario of periodic prophylactical use of benzathine-
penicillin or other antibiotic agents to be implemented only for subjects with ascertained genetic predisposition to the rheumatic endocarditis. However, the project should be better clarified. Please provide more information about this project.

- In my opinion, the Authors should better specify the candidate individuals to be proposed for periodical administration of antibiotics active against” Group A beta-hemolytic streptococcus”. In particular the Authors should declare whether this prophylaxis, that is likely to have to be maintained for long period( several years), should be denied to children and adolescents who are negative for HLA DR 11 even though they present with a clinical history of recurrent throat infections by group A beta-hemolytic streptococcus. Please discuss this issue.

4. Response: We had suggested using HLA to screen susceptible individuals for ARF/RHD but now realize that it is still controversial given the conflicting reports from different studies, we have therefore withdrawn this statement from the document.

Level of interest: An article of importance in its field
Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:
I declare that I have no competing interests

Reviewer 2

Title: Rheumatic Heart Disease in Uganda: The Association between MHC Class II HLA DRB1 Alleles and Disease: A Case Control Study
Version: 1 Date: 3 February 2014
Reviewer: Giuseppina Di Biase
Reviewer's report:
Commentary by Giuseppina Di Biase
In this research paper the Authors have broached a very interesting issue, i.e the point that several alleles of the HLA class II genes appear to be the dominant contributors to the development of rheumatic fever and rheumatic heart disease. There are several aspects which need to be better elucidated. They are briefly
Results
- “HLA-DR1 (OR=0.42, CI 0.21-0.85, P=0.01, Pc=0.09) showed a positive trend towards association with not having RHD”. In my opinion, the term you used here is improper and would need to be corrected. In fact, the word “trend” denotes a non significant relationship, so it is characterized by a nonsignificant p-value, although rather close to statistical significance (e.g. p=0.076). By contrast, in this sentence a significant association (p= 0.01) rather than a true, properly called "trend" is noticeable. Thus the sentence should be amended as follows: "HLA-DR1 was demonstrated to be associated with a decreased risk of RHD (OR=0.42, CI 0.21-0.85, P=0.01 ...)". -“HLA-DR1 (OR=0.42, CI 0.21-0.85, P=0.01, Pc=0.09) showed a positive trend...” Moreover I don't understand the meaning of the acronym Pc( Pcorrected?). As a rule, full term should be provided before introducing in the text any acronym. Please clarify accordingly. If the interpretation “P corrected “ is right, please specify the sense of this "P correction"

5-Response: We have made this change as kindly suggested by the reviewer. Pc means corrected P value and this change has been made in the document. (Page 2, line 17 and page 7, line 9)

Discussion
-“We found .. a positive trend towards association of HLA-DR1 with healthy controls” The improper use of the word “trend” is re-proposed here. Please correct again, by underscoring the existence of a significant association ( not a trend) between HLA-DR1 and the freedom from RHD.

6- Response: This change has been made (See response 5 above)

-According to Guilherme et al.( Ann Pediatr Cardiol. 2011 Jan-Jun; 4(1): 13–21) “The HLA-DR7 allele that was found in Brazilian, Turkish, Egyptian, and Latvian patients could be considered the HLA class II gene that is most consistently associated with rheumatic fever and rheumatic heart disease". Nevertheless, in the present study the association evidenced by other Authors between this allele and rheumatic disease is lacking. Please illustrate more thoroughly the possible reasons that have led to very different results, when searching for candidate
alleles within the chromosoma 6, depending on the ethnic composition of the analyzed population samples or as a consequence of different genotypical profiles of the involved microbial strains in a given country and time period.

7- Response: We agree that a more thorough discussion is warranted given the difference in DR association across studies, including pointing out the heterogeneity of the causative agent. “Other explanations include substantial genetic diversity across African populations, low levels of linkage disequilibrium in African populations, small sample sizes in both studies, and different testing methods (serologic testing versus DNA based testing in our study). We have modified the following sentences to our discussion to address these points: “These differences in RHD associations across different populations support the concept of ethnic specific genetic susceptibility for HLA alleles. Other explanations for the variability of studies include small sample sizes which may not capture rare HLA alleles, different typing methods (serologic versus DNA based), and heterogeneity of the causative agent. More than fifteen streptococcal strains have been associated with rheumatic fever and each distinct strain may select for a unique DR antigen, resulting in different susceptibility alleles. Africa has one of the most genetically diverse populations, thus differences in HLA alleles is expected between various geographic locations in sub-Saharan Africa.” (Page 8, lines 1-8)

Discussion- “Identification of individuals with susceptibility genes to RHD could potentially provide an opportunity to screen patients with borderline RHD for secondary prophylaxis, and to place such people in a more routine follow up for streptococcal surveillance”. In the opinion of some, secondary prophylaxis with benzathine penicillin, once a week in the first month and subsequently once a month for a few years, should be systematically executed in patients with a history of one or more episodes of rheumatic fever, especially in areas where it is endemic, without relying on the tests of genetic susceptibility. In fact they could be misleading, since a complete agreement has not been reached yet for them among the researchers. In the Discussion please also illustrate this controversial point and related conflicting outlooks concerning possible therapeutic strategies.

8- Response; We appreciate the controversial nature of this suggestion and we have decided to withdraw it. (See response 4 above)
Level of interest: An article of importance in its field
Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests: I declare that I have no competing interests