Reviewer's report

Title: Genetic susceptibility to Chagas disease cardiomyopathy: involvement of several genes of the innate immunity and chemokine-dependent migration pathways.

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Reviewer: Daniel Hoft

Reviewer's report:

This is an interesting manuscript presenting data in support of the hypothesis that there is a genetic component to Chagas disease progression. The authors focus on three immune genes (CCR5, CCL2, TIRAP) and find an association between polymorphisms in these genes and disease susceptibility or protection. While this study focuses on previously identified genes, the strengths of this report include: 1) it is the largest study done to date, 2) the authors use asymptomatic seropositive individuals instead of seronegative ones as controls, and 3) the Tag SNP approach is used to expand the areas of the genes studied. Through these techniques, they were able to identify new polymorphisms in the CCR5, CCL2, and TIRAP genes. This could potentially have prognostic or therapeutic value in the future and therefore should be published. However, there are limitations to this study that are not clearly mentioned in the manuscript. First, this is a cross-sectional study which compares T. cruzi-infected individuals with no disease symptoms (ASY) to those with either moderate or severe chronic Chagas cardiomyopathy (CCC). With no knowledge of when these individuals were infected, we do not know whether or not the ASY patients will develop disease manifestations in the future or whether the CCC group has simply been infected for a longer period of time. A longitudinal study would be more ideal; however this would be very difficult, especially with this large and mostly rural population. Second, this report involves a “test” population, and the results require validation in additional populations. Third, the study identified polymorphisms but does not correlate these polymorphisms with levels of gene expression. Without knowing how the SNP affects expression or function, it is hard to deduce the importance of each SNP. Overall, this study yields exciting results by analyzing polymorphisms from a large number of CCC patients, but it is important for the authors to recognize and clearly state the limitations.

Specific comments:

1. In the background section, a few statements are overstated. For example, the authors say that Chagas disease occurs exclusively in the Americas, but I think they meant to say that it is endemic to Latin America and vector-based transmission happens exclusively in the Americas. Another example is stating that CCC is “by far the most important clinical consequence” since this is more of an opinion than a fact. Perhaps revise this to the most common consequence or one of the most important consequences?
2. Also in the background section, the authors mention previous data with CXCL9 and then jump to a statement that this is consistent with an accumulation of CCR5+ Th1 T cells in the CCC heart tissue. Perhaps they could have another sentence directly linking CXCL9 to their decision to study CCR5?

3. In the methods section, they mention that data for LVEF were missing for 10 patients with CCC, however fail to mention whether or not they used data from these 10 individuals for any of the analysis completed. If so, the authors should state their group assignments (moderate to severe CCC).

4. In the methods section, it would be nice if the authors could clarify the SNP selection process in more detail. Were the 15 tag SNPs in Table 1 the only 15 for those 3 genes with a minor allele frequency over 20%? And what is the rationale for using the 20% MAF cutoff?

5. In the results section, for the CCR5 rs11575815 A/T polymorphism, the authors combine individuals expressing the more frequent homozygous genotype (AA) and those with the heterozygous genotype (AT) for comparison with the rare homozygous genotype (TT). However, in all the other examples, they compare the more frequent homozygous expressing group to combinations of the heterozygous and rare homozygous genotypes. Is there a reason why the analysis was done differently for the CCR5 rs1157815 SNP? If so, the rationale should be explained?

6. In the results section text, the authors present results of the multivariate analyses for all the studied genes. However, all tables included with the manuscript present only univariate analyses. Inclusion of a table with an overview of the multivariate analyses would be helpful.

7. In the discussion section, there should be more information on how the disease associated SNPs might affect gene expression and then how the altered gene expression patterns could influence disease progression. There is some mention of previous studies that looked at gene expression with different SNPs in these three genes, but it is not clear what the authors’ predictions are for these particular SNPs.

8. In the discussion section, they mention a few previously identified SNPs in the CCR5, CCL2, and TIRAP genes. Could the authors speculate on why they did not identify these same SNPs? Were these polymorphisms present but at minor allele frequencies lower than 20% and thus excluded?

9. In the conclusion, the authors comment on the multigenic character of CCC, with “each polymorphism imparting a small contribution.” Have they analyzed whether or not the expression of multiple SNPs associated with CCC patients correlates with increased disease severity?

In the conclusion, it also would be informative if the authors could speculate on exactly how to proceed with these data and what are the next steps for translating this information into prognostic or therapeutic value.

**Level of interest:** An article of importance in its field
Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:

I declare that I have no competing interests.