Reviewer’s report

Title: In vitro characterization of novel and functional regulatory SNPs in the promoter region of IL2 and IL2R alpha in a Gabonese population

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Reviewer: Katie A Smith

Reviewer’s report:

This paper describes a number of polymorphisms in the IL2 and IL-2R locus that occur in a population of male Gabonese individuals exposed to malaria. These variants were then validated for their allelic gene expression by transient transfection in vitro. The possibility that these variants may contribute to immunity to parasite infection is discussed, although there have no attempts to perform regulatory T cell functional assays from these individuals, or attempt to associate these particular polymorphisms with parasite susceptibility or disease state. Although this paper may add to our current knowledge of gene polymorphisms associated with parasite infection, some major compulsory revisions would need to be performed before a decision could be made on whether this paper could be accepted for publication.

Major Compulsory Revisions

1) In Figure 1, I struggle to understand how the authors can describe the data shown as “induction” of each construct, given there is no significant increase in relative light units by luciferase assay of PMA and ConA stimulated cells in comparison to non-stimulated cells. Please clarify this figure, including which Jurkat cell line was used, or provide some other example of “stimulation” perhaps using anti-CD3 to demonstrate “induction” (if they are Jurkat T leukemia cells). I also do not understand why individual genetic variants reported in Table 1 have been combined for the transfection analysis, especially as the rs2067006 AT variant is not present in this population of patients. It would be helpful if the authors took a more reductionist approach, using the IL2 major allele (I assume this is the sequence of the promoter region of native IL2?) in combination with each individual genetic variant, before combining them to assess which individual variant, or combination of variants, might alter luciferase activity. Also, it would be useful to have come idea of background luciferase activity in un-transfected controls, or include the positive and negative controls mentioned in the materials and methods section of the paper.

2) In Figure 2, there is more convincing data that a novel variant of IL2R alpha might affect leuciferase activity, but again I would ask for the positive and negative controls, as well as transfection of single variants before the transfection of multiple variants.

3) Please clarify in the methods, whether genomic DNA has been isolated from
whole blood or from a purified T cell population, as this impacts greatly on your discussion (Paragraph 2, penultimate sentence).

Minor Essential Revisions

1) The sentence structure and grammar needs to be very carefully re-written throughout. Please obtain advice on the appropriate adjectives to use to describe your work (e.g. Background, paragraph 2, do you mean transcribe instead of produced? Results, paragraph 1, do you mean stimulation, rather than induction?). Please try to clarify your sentences, especially within Background, paragraph 2 and Discussion, paragraph 2, as well as avoid making unsupported statements (e.g. Background, paragraph 1 and 2). Please carefully re-visit the literature to include references, which might better support your arguments (e.g. Background, paragraph 1, sentence 3 would be better supported by the publications of Belkaid Y et al., Sher A et al., or Maizels RM et al.).

2) In the Discussion (Paragraph 1) the statement “Foxp3 is described as a master regulatory of natural Tregs development and function and ablate the efficiency of thymocytes to differentiate into mature T cells” is incorrect. The publication of Lin et al., instead demonstrates that mature regulatory T cells expressing a non-functional fusion protein of Foxp3 lack suppressor function and fail to develop from CD4+EGFP- cells upon transfer to a lymphopenic host. The sentence should be re-written to correctly summarise this publication.

3) What does the phrase “but this may be extraneous in the in situ state” in the Discussion (paragraph 2) mean? Do you mean that this hypothesis would need to be confirmed in T cells isolated directly ex-vivo from the patients?

4) Please could you also clarify the infectious status of the individuals used in this study. Are they symptomatic? Can malarial life-stages be detected in blood smear? Have they been treated with anti-malarial drugs? I assume all have P.falciparum infection? Also, please could you include the ethics committee reference number, if there is one, associated with this study in the materials and methods section.

5) As a list of abbreviations has not been given, please make sure to fully describe them in the text the first time they are mentioned, especially terms such as ETS and LD. Related to this, please clarify what NA stands for in Table 1 and 2 either in the text or associated figure legend. When referring to transcribed gene products (e.g. Background, paragraph 2), please use the correct nomenclature (these should not be in italics).

6) In the description of statistical analysis, please clarify if you have pooled all of your data, normalised your data, or adjusted the data to take into account it's distribution.

Discretionary Revisions.

1) Transfection of these variants into other cell lines and in particular, primary T cells, may strengthen your paper and in particular, enhance your discussion.
(paragraph 2).

2) It would be very interesting to analyse Foxp3 expression in the individuals who donated samples to this study as mentioned in the Discussion (paragraph 1) and to determine if regulatory T cells from these individuals are altered in their suppressive function. Variants in IL2 and IL-2Ralpha could then be associated more specifically with Treg function and parasite infectious status.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Not suitable for publication unless extensively edited

**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