Reviewer’s report

Title: KIR3DS1/L1 and HLA-Bw4-80I affect HIV disease progression among HIV typical progressors and long-term nonprogressors

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Reviewer: Brian Long

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In this study, Jiang et al. report on natural killer cell immunoglobulin receptor (KIR) and HLA genotype and relate KIR gene frequency and expression levels with HIV viral load, CD4+ T cell count and disease progression in a cohort of 132 HIV seropositive ethnic Han subjects. The authors first determine the KIR and HLA genotype of the infected subjects, and analyze the HLA-B locus for the presence of isoleucine at position 80 (Bw4-80I) that has been previously associated with delayed HIV disease progression in the presence of particular KIR ligands, namely KIR3DS1. This information is then related back to categorized disease status parameters within the study group. Next, the mRNA expression levels of 2 pertinent KIR genes, KIR3DS1 and KIR3DL1, which segregate as alleles, were determined by quantitative PCR analysis. Gene expression level was then related back to disease parameters to reinforce the notion that particular KIR receptors are associated with improved outcomes in HIV disease. The results of this study are in agreement with a multitude of previously published reports on the effect of the KIR3DS1 and HLA-Bw4-80I compound genotype on HIV disease progression initiated by Martin et al. Nature Genetics 31 (2002). The novelty of this study is restricted to the fact the study subjects are all Chinese and ethnic Han, but as such represents a valuable contribution to the literature on this subject.

Major Compulsory Revisions

1. The KIR gene frequency and HLA genotyping appear to be well done and provide for compelling data. However, there are some serious concerns regarding the methodology for the quantitative PCR. Most importantly, it appears the data are not normalized for the number of input cells, as RNA was isolated from an unspecified number of PBMC (Materials and Methods, pg. 9-10). This is problematic in that some subjects may have more or less PBMC per unit volume of whole blood. More importantly, irrespective of the PBMC number, the frequency of NK cells within the PBMC population can be highly variable, especially in HIV infected subjects. For example, you will find a larger quantity of KIR mRNA in a sample that has twice the number of NK cells per unit volume of whole blood relative to a subject that has half the number. The data in this case needs to be normalized to the number of NK cells that the RNA was isolated from, or at a minimum to the number of PBMCs. Perhaps the authors have some
CBC data from the time of collection they could refer back to.

2. The authors list several primers in the materials and methods used to amplify the KIR3DS1 and KIR3DL1 gene products. However, upon performing a BLAST search of the sequences (http://www.ncbi.nlm.nih.gov/tools/primer-blast/), it appears each is specific for several different KIR genes. For example, the KIR3DS1 primer set listed will also amplify KIR3DL1 and KIR3DL2. The authors will need to provide evidence that the primers they’ve designed have specificity for the intended gene products.

3. There is no mention in the methods as to whether the isolated RNA was assayed for quality and quantitation. Were the starting concentrations of RNA normalized? I would assume this to be the case, but it needs to be described.

4. Ideally, qPCR should be normalized to more than one housekeeping gene (GAPDH in this case) as it has been demonstrated that a single housekeeping gene can have varying levels of expression in different cell types. I would suggest the authors refer to J. Huggett et.al, Genes and Immunity (2005) 6, 279-284 for clarification. It is now standard procedure for qPCR to normalize to at least 3 to 6 internal control genes with an algorithmic determination of the most suitable candidate (i.e. GeNorm, http://medgen.ugent.be/~jvdesomp/genorm/).

Minor Essential Revisions

1. As the paper is currently configured, Table 1 is supplementary data, and the manuscript begins with Table 2 (i.e., there is no Table 1 in the manuscript itself). Table 2 should be relabeled as Table 1, and Table 3 should be relabeled as Table 2.

2. The table formatting needs revision. The column headings especially are difficult to discern, and the tables themselves spread over more than one page.

3. Under Methods for HIV viral load measurement, the authors misstate the detection limit as between 20 and 10^7 copies per ml, however the manufacturer states the detection limit is from 50 to 7.5 x 10^5 copies per ml (http://molecular.roche.com/assays/Pages/COBASAMPLICORHIV-1MONITORTestv15.aspx). The authors should further specify that they are measuring RNA copies.

4. It would be interesting to know the viral loads of the long term nonprogressors relative to the typical progressors. Do any of their subjects meet the definition of viral controllers? (i.e. viral loads below the limit of detection in the absence of therapy and normal CD4 counts). If so, are there correlations with KIR and HLA?

Discretionary Revisions

1. On page 12, second paragraph, first sentence. Should add the word ‘maintain’ in front of CD4+ T cell counts.

2. Page 5, reference 8 refers to Rhesus macaques. Is there similar data or references specific to human KIR and HLA?

3. On page 16, last sentence. Remove the word DNA following KIR3DS1/3DL1. Should read “KIR3DS1/3DL1 gene is associated”. That a gene is DNA is a given.
4. On Page 17, second paragraph, last sentence. I wouldn’t say mRNA levels of KIR3DL1 promote disease progression. There’s no evidence for that. I would say it is associated, but promoting implies a more active participation than has been demonstrated.

5. Page 17 to page 18. Remove the word ‘might’. The data does demonstrate this, not that it might demonstrate this.

6. Top of page 21, the word autologous seems to have a capital ‘F’ placed in the middle of it. Also, the next sentence should be ‘At the DNA level’, not ‘On the DNA level’.

7. Please review the figure legends for typos. For instance, in Figure legend 1, there is a sentence that starts with ‘And’.

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

**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