Author’s response to reviews

Title: Structural Variation of Centromeric Endogenous Retroviruses in Human Populations and their Impact on Cutaneous T-cell Lymphoma, Sézary Syndrome, and HIV infection

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Response to the Reviewers’ comments:

Reviewer reports:

Jonas Blomberg, M.P., Ph.D. (Reviewer 1): Critique of the Kaplan et al paper on HERV-K111 (K111) integrations in pericentromeric regions.

The paper uses PCR and sequencing to study differences in copy number of K111 in a large number of healthy and diseased individuals. It spans over wide swathes, from retrovirology to genomics to the clinic. It is generally well written, and represents the state of knowledge on HERV-K(hml2) adequately. Methodology seems sound, but some conclusions unduly far reaching.

Response: We thank very much the reviewer’s comments and believe that the paper has improved substantially with the help of the reviewer.

I have some general remarks:

1. The definition of "-/ K111" genotype rests on a PCR where the left primer binds to CER repeats. Absence of signal could in principle be due to changes or absence in the CER repeat as well as in K111. Please clarify the nature of this genotype. Long amplimer sequencing could reveal the true nature of this genotype, but I am not sure it covers all possible explanations.

Response: We agree with the reviewers that this could be the case. However, we performed the reaction with other set of primers that bind to other areas of the 5’ flanking region of K111 still did not produce amplification products confirming the -/ K111 genotype. In the revised manuscript we have included this information and now it says “We previously showed that using 4 additional primers that bind to other areas of the 5’ flanking region of K111 still did not produce amplification products in -/ K111 cells. This confirmed that the -/ K111 genotype observed with the primers P1 and P4 is not the result of mutations in the 5’ flanking area preventing the binding of the P1 primer during PCR, but is due to absence of K111 provirus (26)”, page 19, lines 443-447. In addition, the inability to detect K111 with the LNA primer set in the gag region of -/ K111 individuals also indicates that the failure to detect K111 is not due to some mutation in the CER elements flanking the virus, but is due to the absence of K111, page 25, lines 611-613.

2. The correlation of K111 to CTCL and HIV progression looks convincing, even if the mechanistic interpretation is vague. In principle, K111 should provide similar sequences,
RNA and protein as the other hml2 proviruses. Np9 is mentioned, but may not be unique to K111. Please remark on possible unique contributions that K111 could provide.

Response: We agreed with the reviewer and have further explained the possible mechanisms K111 Np9 variants may have on HIV infection and T-cell development. In the revised manuscript we now say “Finally, K111s are type 1 proviruses that have the ability to make functional Np9 proteins, similar to other HK2 Type 1 proviruses. Recent evidence has shown that K111 can also produce alternative spliced variants of np9 (32). The function of Np9 has begun to be elucidated, but the function of Np9 variants is not yet known. Therefore, K111 encoded Np9 protein variants may play some important role in T-cell biology and HIV infection. In the case of CTCL, the absence of K111 np9 variants may contribute to failure of cells to die and thus to become neoplastic, while in HIV this may provide protection against T-cell lysis from HIV and result in better preservation of T-cells as seen in HIV LTNPs. Clearly, modern humans have hundreds to thousands of copies of K111, each one with the potential of generating an Np9 protein or variants of Np9. Greater understanding of these proteins may be very important for delineating the function of the T-cell in CTCL and/or in HIV infection, leading perhaps to better therapies for T-cell disorders”, page 34, lines 890-901.

3. The term "pericentromeric instability" is mentioned in several places. How is that condition defined and studied?

Response: As genome instability refers to mutation that can change the genome structure, having K111 deletions will create genomic instability in the pericentromeres. We defined this as having pericentromeric instability. This is mentioned in the abstract, page 4, lines 78-79, and in the introduction section, page 10, lines 203-206. For consistency, we have changed the term pericentric to pericentromeric.

4. The patient and disease characterization is sometimes diffuse. For example, see Sèzary syndrome is a kind of CTCL. Please revise how Sz is mentioned with respect to CTCL. This information is included in the revised manuscript methods section, page 12

Response: We agreed with this observation and have explained how the diagnosis of Sézary Syndrome, a worse case of CTCL, is determined. This information is described in the revised manuscript methods section, pages 12-13, lines 248-270.

Specific:

P9 L134 retrovirus
Response: We have changed the word “retrovirus” to “provirus”, page 8, line 164.

P10 L145 number of K111 copies in modern man. In the germ line or in somatic cells?
Response: We have indicated that is in the germline, page 9, Line 178.

P13 L227 The paragraph on CTCL characteristics is vague, and written like a review. Please be more specific regarding which property was actually observed in the patients studied.
Response: In the revised manuscript we described The CTCL and Sz characteristics as informed by the diagnosis team in the clinic, page 12-13, lines 248-270.

P17 L303 "gaited slowly"??
Response: the terms “gaited slowly” were changed to “run”, page 16, line 382.

P21 L375 Vague. See comment on P13 L227.
Response: We found this statement to be accurately written and of interest for clinicians.

P21 L369 Healthy controls from the same ethnic group?
Response: In the revised manuscript we have indicated that the healthy controls were of the same ethnicity, page 20, line 459.

P21 L386 "unusual"?
Response: We have changed the word, unusual to uncommon, page 21, line 478.

P25 L475 A very long primer!
Response: We thank the reviewer for catching this typo. In the revised manuscript we say: Using the primer that binds to 3460 of the pro region of both K111 and K222, a low molecular weight
band is barely visible in the -/-K111 patients while a strong band at 4240 bp is seen in +/-K111 normal patients, page 25, lines 594.

P26 L489 Sz is a form of CTCL, see remark 5.
Response: We thank the reviewer for this observation. In the revised manuscript we say “Patients with CTCL and, the severe form Sz, had statistically significantly fewer copy numbers of K111 than did control subjects”, page 25, lines 607-609.

P26 L494 How much of this copy variation is somatic, how much in germ line?
Response: In the revised manuscript we indicated that is the number of copies in somatic cell, page 26, line 622.

P27 L500 Can one be sure that -/- K111 individuals have no K111 whatsoever?
Response: As explained in the comment No. 1 (see above), a line of evidences suggest that -/- K111 individuals have not K111 proviruses.

P28 L522 predominantly
Response: We change the word “predominately” to “mostly found”, page 27, line 662.

P36 L704 Please revise language in this section.
Response: We have revised the language of this section. In the revised manuscript we say “Our data indicate that HK2 proviruses in the pericentromeres of several chromosomes, or at least the 5’ portion of their genomes, are deleted in a subset of modern humans. Considering that ~1000 of these retroelements exist in the pericentromeres, having a deletion in the 5’ portion of these retroelements indicates that 3400 Kb of pericentromeric sequence is missing. Further, it appears that pericentromeric instability is associated with survival of a subset of CD4+ T-cells, which together contribute to the development of neoplastic transformation with concomitant severe CTCL. In another unknown way, pericentromeric instability allows a different subset of CD4+ T-cells to survive better in HIV infection resulting in long term survival”, page 35, lines 912-920.
Reviewer 2 (Reviewer 2): PEER REVIEWER ASSESSMENTS:

OBJECTIVE - Full research articles: is there a clear objective that addresses a testable research question(s) (brief or other article types: is there a clear objective)?

Yes - there is a clear objective

DESIGN - Is the current approach (including controls and analysis protocols) appropriate for the objective?

Yes - the approach is appropriate

EXECUTION - Are the experiments and analyses performed with technical rigor to allow confidence in the results?

Yes - experiments and analyses were performed appropriately

STATISTICS - Is the use of statistics in the manuscript appropriate?

Yes - appropriate statistical analyses have been used in the study

INTERPRETATION - Is the current interpretation/discussion of the results reasonable and not overstated?

Yes - the author's interpretation is reasonable

OVERALL MANUSCRIPT POTENTIAL - Is the current version of this work technically sound? If not, can revisions be made to make the work technically sound?

Yes - current version is technically sound
PEER REVIEWER COMMENTS:

GENERAL COMMENTS: I found this article sound. Experiments were well designed and performed. Authors discovered only indirect proof for the role of K111 elements in cancer and HIV development. Further studies needed to find direct evidence. I conclude that this is a correlation study. Nevertheless, it was done well. This study meets best practice. I recommend the manuscript for publication.

Response: We thank the reviewers for his positive comments.

ADDITIONAL REQUESTS/SUGGESTIONS:

I found typos all over the manuscript. Presently, the quality of Figures are poor. I recommend to replace the Figures with high resolution pictures.

Response: We checked for typos along the manuscript and have made changes throughout the manuscript. We have submitted the figures with higher resolution. In addition, we made sure there are no typos and the language was written proficiently.