



Peer Community In Infections

Understanding the in vitro evolution of Cyprinid herpesvirus 3 (CyHV-3), a story of structural variations that can lead to the design of attenuated virus vaccines

Jorge Amich  based on peer reviews by **Lucie Cappuccio** and **Veronique Hourdel** ?

Nurul Novelia Fuandila, Anne-Sophie Gosselin-Grenet, Marie-Ka Tilak, Sven M Bergmann, Jean-Michel Escoubas, Sandro Klafack, Angela Mariana Lusiastuti, Munti Yuhana, Anna-Sophie Fiston-Lavier, Jean-Christophe Avarre, Emira Cherif (2022) Structural variation turnovers and defective genomes: key drivers for the in vitro evolution of the large double-stranded DNA koi herpesvirus (KHV). Missing preprint_server, ver. Missing article_version, peer-reviewed and recommended by Peer Community in Infections. <https://doi.org/10.1101/2022.03.10.483410>

Submitted: 11 March 2022, Recommended: 21 July 2022

Cite this recommendation as:

Amich, J. (2022) Understanding the in vitro evolution of Cyprinid herpesvirus 3 (CyHV-3), a story of structural variations that can lead to the design of attenuated virus vaccines. *Peer Community in Infections*, 100001. <https://doi.org/10.24072/pci.infections.100001>

Published: 21 July 2022

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Structural variations (SVs) play a key role in viral evolution, and therefore they are also important for infection dynamics. However, the contribution of structural variations to the evolution of double-stranded viruses is limited. This knowledge can help to understand the population dynamics and might be crucial for the future development of viral attenuated vaccines.

In this study, Fuandila et al (1) use the Cyprinid herpesvirus 3 (CyHV-3), commonly known as koi herpesvirus (KHV), to investigate the variability and contribution of structural variations (SV) for viral evolution after 99 passages in vitro. This virus, with the largest genome among herpesviruses, causes a lethal infection in common carp and koi associated with mortalities up to 95% (2). Interestingly, KHV infections are caused by haplotype mixtures, which possibly are a source of genome diversification, but make genomic comparisons more difficult.

The authors have used ultra-deep long-read sequencing of two passages, P78 and P99, which were previously described to have differences in virulence. They have found a surprisingly high and wide distribution of SVs along the genome, which were enriched in inversion and deletion events and that often led to defective viral

genomes. Although it is known that these defective viral genomes negatively impact viral replication, their implications for virus persistence are still unclear.

Subsequently, the authors concentrated on the virulence-relevant region ORF150, which was found to be different in P78 (deletion in 100% of the reads) and P99 (reference-like haplotype). To understand this loss and gain of full ORF150, they searched for SV turn-over in 10 intermediate passages. This analysis revealed that by passage 10 deleted and inverted (attenuated) haplotypes had already appeared, steadily increased frequency until P78, and then completely disappeared between P78 and P99. This is a striking result that raises new questions as to how this clearance occurs, which is really important as these reversions may result in undesirable increases in virulence of live-attenuated vaccines.

We recommend this preprint because its use of ultra-deep long-read sequencing has permitted to better understand the role of SV diversity and dynamics in viral evolution. This study shows an unexpectedly high number of structural variations, revealing a novel source of virus diversification and confirming the different mixtures of haplotypes in different passages, including the gain of function. This research provides basic knowledge for the future design of live-attenuated vaccines, to prevent the reversion to virulent viruses.

References:

- (1) Fuandila NN, Gosselin-Grenet A-S, Tilak M-K, Bergmann SM, Escoubas J-M, Klafack S, Lusiastuti AM, Yuhana M, Fiston-Lavier A-S, Avarre J-C, Cherif E (2022) Structural variation turnovers and defective genomes: key drivers for the in vitro evolution of the large double-stranded DNA koi herpesvirus (KHV). bioRxiv, 2022.03.10.483410, ver. 4 peer-reviewed and recommended by Peer Community in Infections. <https://doi.org/10.1101/2022.03.10.483410>
- (2) Sunarto A, McColl KA, Crane MStj, Sumiati T, Hyatt AD, Barnes AC, Walker PJ. Isolation and characterization of koi herpesvirus (KHV) from Indonesia: identification of a new genetic lineage. Journal of Fish Diseases, 34, 87-101. <https://doi.org/10.1111/j.1365-2761.2010.01216.x>

Reviews

Evaluation round #2

Reviewed by [Lucie Cappuccio](#), 28 June 2022

Dears authors,
Thank you for corrections, and congratulations for your work.

Evaluation round #1

DOI or URL of the preprint: <https://doi.org/10.1101/2022.03.10.483410>
Version of the preprint: 2

Authors' reply, 28 June 2022

Dear Dr. Amich,
We have now provided a thorough point-by-point response to the reviewers' comments. We would also like to thank you and the reviewers for their time and effort in giving constructive feedback on our article.

Please find below the responses to the general comments of Lucie Cappuccio. The remaining comments in the review file have been directly addressed (changes+responses) in the marked documents (Fuandila_etal_trackchanges.docx).

Reviews

Reviewed by Lucie Cappuccio, 22 Apr 2022 11:12

-> It is an interesting article, well presented and discussed with promising future work.

Abstract: present simply and clearly the objective.

Introduction: well structured to understand the state of art and the aim of this work.

Material and Methods:

ligne 213: "inconsistent/supplemental "

#Response: The correction has been made.

Figure 5: the information is clear, however, if it is possible, it could be more complete and visual by adding the percentage of SVs cited in the text on the figure (like in the supplementary Table 3)

#Response: The percentages have been added to Figure 5.

Result: Did you keep the supernatant of the different passages to test the effect of the SVs on TCID50 or PFU if cytopathic effects are observable with KHV? Same question about the CCB cells: is it possible to test the impact of infection on cell viability?

#Response: Though in very weak quantities, we still have some supernatant of the different passages that could be used to test the effect of SVs on the TCID50. However, we do not expect specific differences because we did neither observe any variations in the cytopathic effects nor in the kinetics of infection along cell culture passages. Therefore, the influence of SVs should be tested in vivo, but this is more difficult regarding ethics and animal welfare.

Discussion: very interesting discussion, maybe more details/literature about the RING family and the corroboration with ORF150

#Response: More details and literature have been added in the Discussion section, Lines 411-416.

The supplementary_Information has also been modified according to the comments.

[Download tracked changes file](#)

Decision by [Jorge Amich](#) , posted 27 May 2022

Minor revisions

Dear Dr Cherif and co-authors,

I am glad to inform you that the review process for your manuscript "Structural variation turnovers and defective genomes: key drivers for the in vitro evolution of the large double-stranded DNA koi herpesvirus (KHV)", submitted to PCI Infections, has now been completed. Apologies for the time it has taken to reach this point.

Two independent reviewers have evaluated your manuscript. Both agree that the work is interesting and sound. Nevertheless, they raise a few comments and questions that would need to be addressed before a final decision can be made.

Please, respond to all the reviewers' comments through the PCI infections website and upload an accordingly edited version of the manuscript in the Preprint server.

Thank you very much for believing in Peer Community In.

Dr Jorge Amich

Recommender and co-Administrator for PCI Infections

Reviewed by [Lucie Cappuccio](#), 22 April 2022

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Abstract: present simply and clearly the objective.

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Discussion:very interesting discussion ,maybe more details/litterature about the RING family and the corroboration with ORF150

[Download the review](#)

Reviewed by Veronique Hourdel, 25 May 2022

[Download the review](#)