Reply to Kjartansdóttir et al.: Chlorovirus ATCV-1 findings not explained by contamination

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We agree with Kjartansdóttir et al. (1) that extreme caution must be used when interpreting high-throughput sequencing results in new hosts. The situation demands thorough investigation and validation beyond the identification of a few sequencing reads. However, we believe it is unlikely that random laboratory contamination explains the results reported in Yolken et al. (2) for the following reasons.

First, the presence of *Acanthocystis turfacea* chlorella virus 1 (ATCV-1) -like DNA sequences in the throat swab samples was confirmed by two methods, sequencing and PCR, and the experiments were performed in different laboratory areas using different procedures. Negative controls and controls containing DNA from other chloroviruses were routinely negative in the PCR reactions, making contamination by laboratory reagents unlikely. Furthermore, procedures used in the human studies involved extraction and amplification of DNA, and hence did not use the DNase or RNA processing reagents listed in Kjartansdóttir et al. (1).

Second, random laboratory contamination cannot explain the association between the detection of viral DNA and variations in the cognitive testing results. Samples were tested in the order they were received, and the persons performing the laboratory tests were unaware of the subject's cognitive results.

Third, the biological plausibility of human colonization with ATCV-1 and association with small alterations in cognitive behavior were supported by the mice gavage experiments. One-time exposure of some mice to ATCV-1 resulted in: (*i*) a specific immune response to viral proteins, (*ii*) alterations in behavior analogous to humans harboring viral DNA, and (*iii*) modulation of gene expression in relevant biological pathways within the hippocampus. These findings strongly support the likelihood that ATCV-1 is capable of biological interactions with humans and other mammals.

Fourth, subsequent experiments used the same PCR methods to test for ATCV-1 DNA in plasma from humans in the same study population. We expected the prevalence of a virus resident in a throat mucosal surface to be lower or absent in blood. None of the plasma samples from the study population tested positive for ATCV-1 DNA ($P < 10^{-8}$ compared with throat swabs, χ^2 analysis). Similar negative results were obtained with blood samples from ATCV-1 exposed and control mice obtained at the time of the antibody measurements. These negative results provide additional evidence that the results from the throat swab samples were not a result of external contamination.

Therefore, the conclusions in our report are based not only on "a few sequence reads" (1), but on confirmatory assays, as well as a highly controlled animal model of oral exposure and subsequent measurement of immune response and behavior. We hope that our work will stimulate further research relating to the possible role of ATCV-1 and other phycodnaviruses in the human virome, and the biological mechanisms of viral interaction with humans and other mammals. These studies would include the measurement of ATCV-1–like sequences at other body sites and the measurement of an immune response to ATCV-1 proteins in humans living in different areas of the world, as well as studies of virus–cell interactions.

1 Kjartansdóttir KR, et al. (2015) Traces of ATCV-1 associated with laboratory component contamination. *Proc Natl Acad Sci USA*, 10.1073/pnas.1423756112.

2 Yolken RH, et al. (2014) Chlorovirus ATCV-1 is part of the human oropharyngeal virome and is associated with changes in cognitive functions in humans and mice. *Proc Natl Acad Sci USA* 111(45): 16106–16111.

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The authors declare no conflict of interest.

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