**LETTER TO THE EDITOR**

# Reconciling the debate on deamination on viral RNA

Lai Wei[1]

Dear editor,

A previous study by Di Giorgio et al. (Di Giorgio et al. 2020) reported A-to-I RNA editing events in SARS-CoV-2 transcriptome. Recently, a study in the *Journal of Applied Genetics* written by Zong et al. (Zong et al. 2022) declared that the results produced by Di Giorgio et al. were insufficient to prove authentic A-to-I RNA editing. Then, the Conticello group wrote a commentary to respond to the concerns (Martignano et al. 2022). As far as I can see, the two sides may have some misunderstanding. Here, I would like to reconcile this debate by providing more relevant literatures and data. I hope this would ease both sides.

The core argument of Zong et al. was that the mutation profile shown by Di Giorgio et al. was a typical SNP profile (which is symmetric) rather than A-to-I editing profile (where A > G is dominant) (Zong et al. 2022). However, the conclusion of Zong et al. should be "Not all A > G/T > C sites found by Di Giorgio et al. are A-to-I editing sites" (denoted as "Not All"), instead of "None of the A > G/T > C sites found by Di Giorgio et al. are A-to-I editing sites" (denoted as "None Of"). "Not All" or "None Of"? This should be the key misunderstanding part between the two sides when I read the response by Conticello group (Martignano et al. 2022).

Obviously, Conticello group tried to provide many literatures on the existence of A-to-I RNA editing in order to disprove the "None Of" conclusion of Zong et al. However, Zong et al. was not claiming "None Of" (although they seemed to do so), they were just claiming "Not All". Di Giorgio et al. regarded all the A > G/T > C alterations as A-to-I editing sites, then Zong et al. intended to convey a

message that since the fraction of A > G/T > C was not very high, so the signal-to-noise ratio was not high enough to let one regard all A > G/T > C sites as A-to-I editing. The "Not All" conclusion of Zong et al. was entirely based on the symmetric SNP profile shown by Di Giorgio et al. Again, this is where the two sides misunderstood each other. While Zong et al. concluded "Not All", Conticello group was defending themselves by disproving "None Of". In other words, under this circumstance, both sides are correct. A-to-I editing really exists in SARS-CoV-2, but the A > G/T > C sites in the mutation profile contains not only the A-to-I editing sites but also (probably many) replication errors (termed false positive sites). This statement reconciles the debate between (Zong et al. 2022) and (Di Giorgio et al. 2020)/(Martignano et al. 2022).

Actually, Zong et al. (2022) was not the first paper to question this mutation profile provided by (Di Giorgio et al. 2020) paper. I have read at least four relevant papers (Picardi et al. 2021; Simmonds 2020; Simmonds and Ansari 2021; Song et al. 2022) with similar statement.

Notably, two recent papers (Picardi et al. 2021; Song et al. 2022) held strikingly similar views with the Zong et al. paper. I will discuss these two papers in detail. As I know, the corresponding authors of these two papers (Picardi et al. 2021; Song et al. 2022), Ernesto Picardi and Rui Zhang, are two prestigious experts in the field of A-to-I RNA editing (Picardi et al. 2017a, b; Picardi and Pesole 2013; Ramaswami et al. 2013; Zhang et al. 2017). The two groups (Picardi et al. 2021; Song et al. 2022) also made critical comments on the Di Giorgio et al. results (Di Giorgio et al. 2020), particularly the mutation profile.

(1) Picardi et al. gently pointed out that "Recently, it has been shown that A-to-G changes … (Di Giorgio et al. 2020) …, but strong evidence of A-to-I RNA editing in the SARS-COV-2 genome has not been provided" (Picardi et al. 2021). The statement "strong evidence has not been provided" almost means the same with the title "poor evidence for …" by (Zong et al. 2022). That's why I think their views are strik-

**One sentence summary** While A-to-I RNA editing in SARS-CoV-2 indeed exists, the identification requires meticulous methodology.

✉ Lai Wei
  wl@bnu.edu.cn

[1] College of Life Sciences, Beijing Normal University, Beijing, China

ingly similar. However, no one has denied the existence of A-to-I editing in SARS-CoV-2 transcriptome.

(2) Song et al. directly claimed that "an initial attempt to identify A-to-I editing sites in SARS-CoV-2 (Di Giorgio et al. 2020) … inconsistent with the common view of ADAR-mediated RNA editing, suggesting that it identifies a large number of false-positive sites" (Song et al. 2022). Of course, readers with some knowledge on A-to-I editing would know that the nucleotide downstream an editing sites should favor G, but (Di Giorgio et al. 2020) failed to identify this pattern. Then, the judgement by Song et al. (2022) is intuitive and reasonable. However, again, "many false positive sites" does not contradict with "several true positive sites". Logically, Di Giorgio et al. (2020) could still claim that there are several true editing sites contained in the variation sites they have found.

Meanwhile, there are also differences between (Zong et al. 2022) paper and (Picardi et al. 2021; Song et al. 2022) papers. Picardi/Song et al. additionally carried out more stringent criteria to remove potential SNPs (replication errors) and artefacts (Picardi et al. 2021; Song et al. 2022), and also resorted to the hyper-editing methodology (Porath et al. 2014) to retrieve more clustered RNA editing sites. This methodology considers that RNA editing events take place in clusters (due to the nature of editing enzymes) while SNPs are essentially replication errors introduced by polymerases so that SNPs should be randomly distributed/scattered. The key improvement of the hyper-editing pipeline is to transform the reference sequence with a particular type of mutation and see whether a read (if heavily edited) could be aligned to the transformed genome (Porath et al. 2014). Strikingly, a sharp peak at $A > G$ appeared after the meticulous pipeline (Picardi et al. 2021), representing the genuine A-to-I editing sites. Moreover, the ADAR motif has been optimized by the multiple-step strategy, suggesting higher confidence of the RNA editing sites (Song et al. 2022). The evolutionary relevance of the host-dependent RNA editing in SARS-CoV-2 has been discussed by Song et al. by analyzing a few missense mutations in spike protein (Song et al. 2022). Notably, their idea that "SARS-CoV-2 hijacks hosts' ADARs to fuel its own evolution" is absolutely true but has already been proposed by earlier literatures (Zhang et al. 2021).

Apart from the critical comments by Ernesto Picardi and Rui Zhang groups (Picardi et al. 2021; Song et al. 2022), another similar paper (Simmonds, 2020) wrote that "Intrahost diversity in this study (Di Giorgio et al. 2020) was, however, dominated by minor populations generated from $G > A$ and $U > C$ substitutions; their symmetry and lack of 5' or 3' context led the authors to propose the editing effects of ADAR in viral dsRNA". Simmonds directly questioned why Di Giorgio et al. (2020) found many $T > C$

substitutions which were not supposed to be the dominant type. In addition, Simmonds and Ansari (2021) wrote that "A tendency to misincorporate a U instead of a C would therefore be reflected in a parallel number of $G > A$ mutations where it occurred on the minus strand. However, as observed previously for SARS-CoV-2 [citations including (Di Giorgio et al. 2020)], the frequency of $G > A$ mutations was substantially lower than $C > U$ changes". Indeed, although (Simmonds and Ansari, 2021) found this mutation profile very strange, they finally gave an explanation for this unexpected mutation profile.

All these aforementioned literatures questioned the mutation profile found by (Di Giorgio et al. 2020), supporting the "Not All" conclusion. However, these concerns did not deny the existence of A-to-I RNA editing, which is to say, no literatures said "None Of". I believe that Zong et al. also intended to claim the "Not All" conclusion instead of "None Of". The only problem for Zong et al. (2022) is that they did not provide an alternative (better) method to exclude the false positive sites as other papers did (Picardi et al. 2021; Song et al. 2022).

To summarize, the debate on RNA editing in SARS-CoV-2 could be reconciled by the fact that the typical "asymmetric profile" of editing signature could be produced by utilizing more stringent pipeline to run the variant calling and filtering processes (Picardi et al. 2021; Song et al. 2022). RNA editing in SARS-CoV-2 exists and has contributed to the fast evolution of the virus (Li et al. 2020a, b; Simmonds 2020; Yu et al. 2021; Zhang et al. 2021). While Di Giorgio et al. might have partially misinterpreted the bioinformatic pipeline of editing detection, it did not preclude the existence of RNA editing in SARS-CoV-2. The only trick is that when detecting RNA editing sites in the transcriptome of SARS-CoV-2, meticulous methodology and rational interpretation should be applied rather than automatically regarding all the $A > G/T > C$ variations as A-to-I RNA editing events.

## Declarations

**Ethics approval and consent to participate** Not applicable.

**Consent for publication** Not applicable.

**Competing interests** The authors declare no competing interests.

# References

Di Giorgio S, Martignano F, Torcia MG, Mattiuz G, and Conticello SG. (2020). Evidence for host-dependent RNA editing in the transcriptome of SARS-CoV-2. Sci Adv 6, eabb5813.

Li Y, Yang X, Wang N, Wang H, Yin B, Yang X, Jiang W (2020) GC usage of SARS-CoV-2 genes might adapt to the environment of human lung expressed genes. Mol Genet Genomics 295:1537–1546

Li Y, Yang XN, Wang N, Wang HY, Yin B, Yang XP, Jiang WQ (2020) The divergence between SARS-CoV-2 and RaTG13 might be overestimated due to the extensive RNA modification. Future Virol 15:341–347

Martignano F, Di Giorgio S, Mattiuz G, Conticello SG (2022) Commentary on "Poor evidence for host-dependent regular RNA editing in the transcriptome of SARS-CoV-2." J Appl Genet 63:423–428

Picardi E, D'Erchia AM, Lo Giudice C, Pesole G (2017) REDIportal: a comprehensive database of A-to-I RNA editing events in humans. Nucleic Acids Res 45:D750–D757

Picardi E, Horner DS, Pesole G (2017) Single-cell transcriptomics reveals specific RNA editing signatures in the human brain. RNA 23:860–865

Picardi E, Mansi L, Pesole G (2021) Detection of A-to-I RNA Editing in SARS-COV-2. Genes (Basel) 13:41. https://doi.org/10.3390/genes13010041

Picardi E, Pesole G (2013) REDItools: high-throughput RNA editing detection made easy. Bioinformatics 29:1813–1814

Porath HT, Carmi S, Levanon EY (2014) A genome-wide map of hyper-edited RNA reveals numerous new sites. Nat Commun 5:4726

Ramaswami G, Zhang R, Piskol R, Keegan LP, Deng P, O'Connell MA, Li JB (2013) Identifying RNA editing sites using RNA sequencing data alone. Nat Methods 10:128–132

Simmonds P. (2020). Rampant C-->U Hypermutation in the Genomes of SARS-CoV-2 and Other Coronaviruses: Causes and Consequences for Their Short- and Long-Term Evolutionary Trajectories. mSphere 5:e00408- 20. https://doi.org/10.1128/mSphere.00408-20

Simmonds P, Ansari MA (2021) Extensive C->U transition biases in the genomes of a wide range of mammalian RNA viruses; potential associations with transcriptional mutations, damage- or host-mediated editing of viral RNA. PLoS Pathog 17:e1009596

Song Y, He X, Yang W, Wu Y, Cui J, Tang T, Zhang R (2022) Virus-specific editing identification approach reveals the landscape of A-to-I editing and its impacts on SARS-CoV-2 characteristics and evolution. Nucleic Acids Res 50:2509–2521

Yu YY, Li Y, Dong Y, Wang XK, Li CX, Jiang WQ (2021) Natural selection on synonymous mutations in SARS-CoV-2 and the impact on estimating divergence time. Future Virol 16:447–450

Zhang R, Deng P, Jacobson D, Li JB (2017) Evolutionary analysis reveals regulatory and functional landscape of coding and noncoding RNA editing. PLoS Genet 13:e1006563

Zhang YP, Jiang W, Li Y, Jin XJ, Yang XP, Zhang PR, Jiang WQ, Yin B (2021) Fast evolution of SARS-CoV-2 driven by deamination systems in hosts. Future Virol 16:587–590

Zong J, Zhang Y, Guo F, Wang C, Li H, Lin G, Jiang W, Song X, Zhang X, Huang F et al (2022) Poor evidence for host-dependent regular RNA editing in the transcriptome of SARS-CoV-2. J Appl Genet 63:413–421