Review 1: "Host genome analysis of structural variations by Optical Genome Mapping provides clinically valuable insights into genes implicated in critical immune, viral infection, and viral replication pathways in patients with severe COVID-19"

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RR:C19 Evidence Scale rating by reviewer:

- **Potentially informative.** The main claims made are not strongly justified by the methods and data, but may yield some insight. The results and conclusions of the study may resemble those from the hypothetical ideal study, but there is substantial room for doubt. Decision-makers should consider this evidence only with a thorough understanding of its weaknesses, alongside other evidence and theory. Decision-makers should not consider this actionable unless the weaknesses are clearly understood and there are other theory and evidence to further support it.

Strength of Evidence Rating Reasoning:*Potentially informative*

This paper demonstrates the first speculation about the role of structural variants (SVs) in susceptibility to severe COVID-19 in 37 patients. However, gene expression studies should confirm the effect of SVs on gene expression level and be compared with asymptomatic or mild COVID-19 patients. Additionally, in vitro studies are needed using cells derived from patients, cell cultures, or animal models to study knock out and knock-in of those genes and study the effect on viral replication, interferon and cytokine levels, and severity of manifestations.

Review:

The overall conclusion of the investigators is that optical genome mapping can be utilized to discover large structural genome variants (> 500 bp) that impact COVID-19 severity and survival. While genome structural variants (SVs) are involved in neurodegenerative or cancer. This is the first time to study large structural variants as a cause of severe COVID-19. Next-generation sequencing can discover single nucleotide variants (SNVs) or small structural variants but won't be able to discover large structural variants or repetitive elements variation in the human genome.

However, there are many limitations to this study. Investigators used a single arm to study SVs involved in severe COVID-19 without the inclusion of a control arm of mild or asymptomatic COVID-19 patients. The control arm in the study was a dataset of Bionano genomics but they did not tell us about the demographic characteristics of their control arm and whether they are healthy individuals or patients and if their number (267 controls) is sufficient to call the SVs unique. Hence, the inclusion of a control arm of mild or asymptomatic COVID-19 patients and studying structural variants in them is essential and this control arm should be of sufficient size to make statistically significant conclusions. Moreover, the number of patients is limited to 37 patients and the rare variants are found in only one

or two patients. Family studies of SVs can be useful here especially in related family members with mild or asymptomatic COVID-19. To report a rare variant in a single patient to be the cause of a disease, you need rigorous lab validation to confirm the effect of this variant in susceptibility to severe viral infection. Validation of the effect of SVs was done only for a single gene (STK26) which proved that this gene is differentially expressed in severe COVID-19 patients. STK26 inhibits TRAF6 (a gene essential for TLR3 and TLR7 signaling to produce inflammatory cytokines and interferon). Differential gene expression of STK26 was studied in a sample of 12 severe COVID-19 patients and a control of 11 asymptomatic or mild COVID-19 patients. Investigators did not provide demographics of those patients and if the 12 severe COVID-19 patients were drawn from the original sample of 37 patients from the main study.

Additionally, gene expression does not provide a causative implication. In vitro studies are needed to prove its implication in severe COVID-19 using cell culture or animal models knocking in and out this gene using different techniques as CRISPR or siRNA. The higher expression of STK26 might be a consequence not a cause of severe COVID-19. Besides, for the common four SVs in candidate loci previously associated with severe COVID-19, they used a cutoff of 20%. There was no clear explanation for this cutoff. Besides, I expected a cutoff of 5% because critical (ICU admission) COVID-19 occurs in around 5% of the population hence 5% cutoff seems more reasonable. Finally, patients admitted to the study have different comorbidities that predispose to severe COVID-19 including diabetes mellitus (16 patients), hypertension (22 patients), chronic kidney disease (9 patients), and asthma (2 patients). Ideally, case report studies of monogenic causes of inborn errors of immunity recruit healthy patients because comorbidities are confounding factors for severe viral diseases.

Confirmation of the role of genes reported in this paper in susceptibility to severe COVID-19 is needed through in vitro studies using cells derived from patients, cell cultures, and animal studies using CRISPR, siRNA, or RNAseq technologies.