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Dear Editor,

We read a recent paper by Li and colleagues with interest, who reported association of genetic polymorphisms in *IFITM3* with COVID-19 susceptibility and severity with implicated conclusions for vaccination or therapeutic goals.[1] In the current study we leveraged the previously discovered association of single-nucleotide polymorphisms (SNPs) for circulating cytokine levels to investigate their causal role in severe COVID-19. To this end, we used the promising methodology of Mendelian randomization (MR) to infer potential causality of risk factor-disease associations. This method uses genetic variants as instrumental variables to test for the effect of exposures on outcomes while minimizing confounding bias. We selected a recently introduced, powerful MR approach, i.e., Generalized Summary-data-based MR (GSMR),[2] to detect any potentially causal links between baseline blood cytokine levels and severe COVID-19 disease. We then confirmed the observed links with MR sensitivity analyses and also validated the findings using replication in three independent datasets: 1) replication in an independent East Asian GWAS for severe COVID-19, 2) internal replication using subsets of the discovery GWAS for severe COVID-19, and 3) replication at transcript level using an independent set of SNPs as instrumental variables. Subsequently, for any cytokine showing a significant effect on severe COVID-19 we performed MR analyses with other cytokine levels as outcomes, to examine their regulatory role.

Our study is based on the GWAS results of 4336 very severe COVID-19 cases and 623,902 controls (Supplementary Table 1), conducted by the Host Genetics Initiative consortium[3] with severe COVID-19 disease (or death due to COVID-19) as the outcome. For the exposures, we used GWAS results of 41 cytokines and growth factors from an independent population of 8293 individuals.[4] For independent replication, we used GWAS results of 65 East Asian severe COVID-19 cases versus 138 ancestry-matched controls[5] and for replication at transcript level, we used expression quantitative trait loci data from blood T cells by Chen et al.[6] Detailed description of the data and methods is given in Supplementary Materials.

We were able to test 13 cytokines having at least 3 independent SNPs for GSMR of which our analyses suggested a causal role for IL-13 in severe COVID-19 with each standard deviation (SD) increase in plasma levels of IL-13 leading to about 20% higher odds of developing severe COVID-19 (OR [CI 95%]=1.23 [1.05–1.44], $P = 0.0087$) (Table 1 and Fig. 1A). The full results of all tested cytokines are represented in Supplementary Table 2.

Leave-one-SNP-out sensitivity analysis showed that none of the SNPs used is driving the association. However, in one case, i.e., when excluding rs9472168, the association becomes stronger (OR [CI 95%] = 1.51 [1.09–2.10]; per standard deviation increase in IL-13 levels) (Fig. 1B). This could be an influential, potentially pleiotropic SNP, the inclusion of which may have resulted in underestimation of the MR effect. Reverse MR analysis of IL-13 did not provide evidence for reverse causation (Beta[se] = −0.05[0.04]; p-value = 0.25). Neither the heterogeneity nor the pleiotropy tests were significant for IL-13 (Q[df] = 1.37[3], Egger intercept[se] = 0.07[0.05]; p-values = 0.71, and 0.23, respectively). Furthermore, except for the MR Egger method all other four MR approaches confirmed the observed causal association (Table 1).

The observed causal role of IL-13 in severe COVID-19 was consistently validated and confirmed by all three validation strategies. First, independent replication in an East Asian GWAS of severe COVID-19 returned a significant effect (OR [CI95%] = 6.17 [1.42–26.84]; $P = 0.01$) (Supplementary Fig. 1). Second, internal replication in the GenOMICC study confirmed the observed significant results (OR [CI 95%] = 1.23 [1.01–1.50], $P = 0.04$). Finally, also replication at transcript level yielded a significant association (OR [CI95%] = 1.27 [1.15–1.41]; $P = 4.09 \times 10^{-6}$) (Supplementary Fig. 2).

We also identified 13 nominally significant causal associations for IL-13 against blood levels of other cytokines, of which six passed a Bonferroni threshold of $P < 0.00125$ i.e., 0.05/40. These include VEGF, IL12p70, IL10, IL7, IL5, and IFNg (Supplementary Table 3).

In summary, we found an OR of about 1.2 for severe COVID-19 per 1 SD increase in IL-13 levels and validated this finding in three independent datasets. IL-13 has previously been studied in asthma with its contribution to IgE production, histamine release and inflammation,[7] as well as barrier damage in the airways.[8] An important observation supporting its role in severe COVID-19 is provided by a recent study reporting that COVID-19 patients receiving Dupilumab, a monoclonal antibody which blocks IL-13/IL-4 sig-

Table 1
Results of IL-13 association with severe COVID-19 from different MR approaches.

Method	N_SNPs	Effect estimate	SE	P	OR	CI 95%
Primary MR analysis						
GSMR	5	0.21	0.08	0.008752	1.23	1.08–1.39
MR sensitivity analysis						
Inverse variance weighted	5	0.21	0.08	0.008422	1.23	1.08–1.39
Weighted median	5	0.17	0.08	0.042155	1.19	1.03–1.34
MR Egger#	5	−0.02	0.17	0.921923	0.98	0.65–1.31
MR-PRESSO*	5	0.21	0.07	0.049244	1.23	1.10–1.37

As the directional pleiotropy test is not significant, the MR Egger result is not informative.
* P-value of global test (pleiotropy hypothesis) = 0.5. P-values of heterogeneity and pleiotropy tests for IL-13 are 0.71 and 0.23, respectively.
SE: Standard error of estimate; N_SNPs: the number of genome-wide significant SNPs for IL-13 that are used as instrumental variable in GSMR analysis.
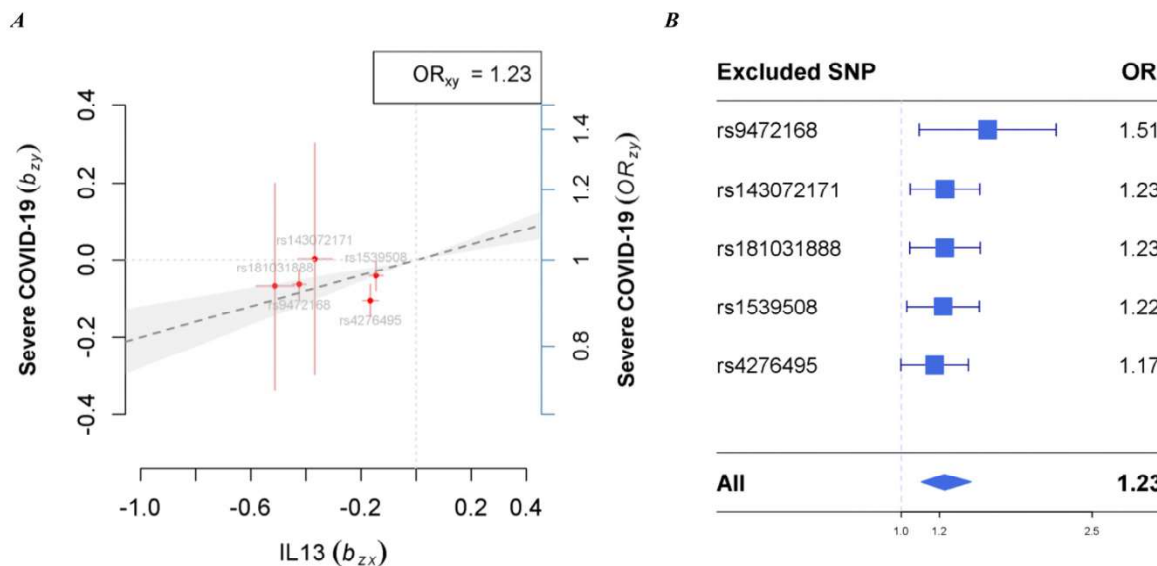
Fig. 1. (A) The independent ($r^2 < 0.05$) SNPs associated with IL-13 (p-value $< 5 \times 10^{-8}$) and their associations with severe COVID-19. (B) MR leave-one-out sensitivity analysis of IL-13 levels on severe COVID-19, using the five significant, independent IL-13 SNPs ($r^2 < 0.05$, p-value $< 5 \times 10^{-8}$). b_{zx} indicates the effect of SNP (z) on IL-13 level as exposure (x); OR_{zy} and b_{zy} indicate the effect of SNP (z) on severe COVID-19 as outcome (y), in odds ratio and logodds scale, respectively. Red lines indicate standard error of effect estimates. These five SNPs explain ~9% of IL-13 variance.[4] The dashed line shows the overall estimated effect of IL-13 levels on severe COVID-19 based on all SNPs through a generalized least square approach (GSMR). GSMR effect size (se): 0.2 (0.08), OR (CI95%) = 1.23 (1.05–1.44), $P_{GSMR} = 0.0087$; i.e. one SD increase in IL-13 levels will lead to a 23% higher chance of developing severe COVID-19 symptoms.

naling, had less severe disease.[9] Warranting further investigations, a study in SARS-CoV-2-infected mice has shown that IL-13 inhibition leads to reduced hyaluronan expression, a polysaccharide which deposits in lungs of severe COVID-19 patients, as well as reduced mortality and disease severity.[9] Our observation of a positive causal effect of IL-13 on a number of other cytokine levels is in line with a previously described key regulatory role of IL-13 in the cytokine storm of COVID-19 patients, where IL-13 recruits inflammatory cells (such as neutrophils, macrophages, eosinophils, and lymphocytes) to the lung mucosae, leading to hyper-production of various pro-inflammatory cytokines.[10]

Despite successful identification (and consistent independent validation) of a causal relationship for IL-13 on severe COVID-19, our study also suffers from a number of limitations: First, the causal effect of IL-13 on severe COVID-19 did not survive Bonferroni correction ($P < 0.0038$ i.e., 0.05/13; with 13 as the number of tested cytokines). This can be due to the limited power of a modest number of suitable genetic instruments available for MR. Second, cytokines without sufficiently strong genetic instruments were not included in our MR analyses and therefore, our results do not reject potential causal associations for these cytokines. Third, the majority of study populations for our analyses are of European descent, which limits the generalizability of the results to other ancestries. While we independently replicated our findings using GWAS results of severe COVID-19 in an East Asian population ($n = 203$), we didn't have IL-13 GWAS in the same ancestry. Larger efforts using exposure and outcome GWASs from the same/similar populations are needed to unravel trans-ancestry portability of this finding.

To conclude, our study provides evidence for a causal effect of IL-13 on severe COVID-19. As such, further investigation is warranted exploring IL-13 as a potential therapeutic target for patients with severe COVID-19 or for those that are at risk of developing severe symptoms.

Declaration of Competing Interest

The authors declare that they have no competing interests.

Acknowledgement

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2022.05.024.

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