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Reply to correspondence by Deora et al. in Human Genomics 18, article no.: 52 (2024): critical insights on "Association of the C allele of rs479200 in the EGLN1 gene with COVID-19 severity in Indian population: a novel finding"

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Abstract
A reply to the correspondence by Deora et al.- Critical insights on "Association of the C allele of rs479200 in the EGLN1 gene with COVID-19 severity in Indian population: a novel finding". The reply contains point-wise rebuttal to the concerns, particularly addressing the epidemiological, statistical, and mathematical issues raised by Deora et al.

We appreciate the critical insights from Deora et al. [1] and carefully considered their points. While we respectfully disagree with some of their critiques, particularly regarding the need to revisit our findings and comments such as "specific epidemiological, statistical, and mathematical issues," we welcome further discussion on these aspects.

Open and constructive dialogue is instrumental in scientific progress. We believe a collaborative exchange of ideas would be beneficial to fully explore this topic. To ensure clarity and avoid any misrepresentation of our views, we would like to take this opportunity to provide clarifications to the concerns on our article.

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Response to concern no. 1

Deora et al. emphasized that T allele of rs479200 has a higher plausibility of being associated with worsening of hypoxia as it happens in COVID-19 as compared to the C allele [1]; *there is no such evidence in the literature, nor generated by Deora et al.*

In fact, in our study it was a novel finding that C allele is significantly more frequent in patients with severe COVID-19. This association was further corroborated by regression analysis, solidifying the link between the C allele and disease severity. Further, we postulated the hypothesis based on the available evidence in the literature [2].

COVID-19 hypoxia has similarities with HAPE, but with subtle differences in their pathophysiology [3, 4]. Moreover, failure of the homeostatic oxygen-sensing has been implicated in severe COVID-19 [4], thus the involvement of the EGLN1 gene and its variants becomes imperative in COVID-19 [5]. This prompted us to investigate the association of C allele of rs479200 of EGLN1 gene in severe COVID-19. Previously, Aggarwal et al. [6] found overexpression EGLN1 in individuals with TT

genotype of rs479200, and indicated insufficient anti-hypoxia responses at high altitudes. Further, EGLN1 overexpression is linked to degradation of HIF-α, and preventing its dimerization with HIF-β, stalling the expression of hypoxia response elements (HRE) and vice-versa [7, 8].

Deora et al. seem to have conflated two distinct phenomena: adaptive responses to hypoxia and enhanced hypoxia responses. The former refers to an individual's ability to cope with hypoxic conditions at high altitudes, characterized by physiological adjustments that facilitate survival and functionality in low-oxygen environments. The latter pertains to the overexpression of hypoxia-responsive elements (HRE), which indicates a heightened cellular response to hypoxia, often involving the upregulation of hundreds of genes to manage reduced oxygen availability.

While Aggarwal et al.'s real-time PCR study on blood samples showed higher EGLN1 expression in the TT genotype, the GTEx portal offers a broader range of tissues but may lack eQTL data for specific tissues of interest.

Our study presented a preliminary analysis, offering a foundation for further investigation. While a larger, homogenous cohort is ideal for a more definitive analysis, this retrospective approach can be pursued in future studies.

We acknowledge the limitations imposed by the short communication format. A more comprehensive analysis would involve collaboration with clinicians, basic scientists, and statisticians. Additionally, the potential influence of SARS-CoV-2 variants, including the Delta variant we studied and its less severe successors, warrants further exploration.

We are open to collaboration and eager to contribute our expertise to a more rigorous validation of these findings.

Response to concern no. 2 & 3

Our study's severity classification was determined by the clinical course during hospitalization, except for asymptomatic patients. SpO_2 is a dynamic physiological parameter, and a single value has limited clinical relevance. The trend of SpO_2 was used to assess which type of oxygen delivery device should be used to maintain SpO_2 levels above 90%. In clinical practice, the step-up approach was adopted during the epidemic to optimize oxygen-delivery devices. The selection of oxygen delivery devices during the hospitalization was decided by the treating physicians to maintain SpO2 levels above 90% (who were not part of this study). The severity of COVID-19 mentioned in reference 14 is based on clinic-radiological features at the time of presentation. We classified the severity based on the type of device required > 24 h. to correct hypoxia during hospitalization. Our manuscript clearly defined the asymptomatic.

Response to concern no. 4

While we appreciate the author's attention to detail in finding typos, our statistical analysis remains accurate. A few minor printing errors were identified (detailed in Supplementary Table 1) but have no impact on the data or conclusions. *We kindly clarify that the manuscript's statistical integrity is sound.*

The sample size mentioned in the manuscript indicates the number of samples included in the association analysis (Table 1). Variation between the sample numbers in the supplementary table and the main table arose due to instances, where samples lack discernible gender ($N=9$) and clinical category ($N=4$) details, thus necessitating their exclusion from the association analysis. In summation, our dataset comprised gender details for 162 samples and clinical category details for 167 samples individually. However, upon amalgamating all variables for association analysis, only 158 samples manifested both gender and clinical category data presented concurrently.

However, the typing mistakes in the total row of the supplementary Table 1 can be read as follows:

We also acknowledge the printing errors in the abstract and in the main text "adjusted and unadjusted," which should be correctly read as "unadjusted and adjusted, respectively". Thank you for bringing this to our attention.

The methodology employed by the authors of the commentary for odds ratio calculation remains undisclosed, rendering the analysis unreliable. Moreover, the authors have overlooked the specified total sample size of 158, which diverges from the total sample size provided in the supplementary table. The raw data, as indicated in the manuscript's availability of data and materials section, is accessible upon request from the authors. Regrettably, the authors failed to acknowledge this provision

Supplementary Table 1 Clinical and demograhic profiles of COVID-19 patients from India included in the current study

		Asymptomatic		Mild		Severe		Total	
		N	**%**	***N***	**%**	***N***	**%**	***N***	**%**
Age (Mean ± SD)		49.7 ± 17.9		54.3 ± 15.7		34.9 ± 15.6		45.9 ± 18.3	
Gender	**Female**	9	23.70%	11	17.70%	18	31.00%	38	24.05%
	Male	29	76.30%	51	82.30%	40	69.00%	120	75.95%
	Total	**38**	**24.05%**	**62**	**39.24%**	**58**	**36.71%**	**158**	**100.00%**

and instead arrived at erroneous and unsubstantiated conclusions.

Odd ratio (OR) calculations (as per supplementary Table 2):

	Diseased (Severe)	No-case (asymptomatic)
Exposed (CC)	29	8
Unexposed (TT)	7	12

OR = AD/BC
= 348/56
= 6.214

When adjusted with age and Gender the result is = 9.421 (N = 158).

We maintain the validity of our odds ratio data, derived from the stated sample size in the manuscript, and refute any allegations of misinterpreting the strength of associations between the C allele of rs479200 and the severe category of COVID-19.

Our study employed rigorous statistical analysis to ensure the validity of the findings. We acknowledge the data collection challenges presented by the COVID wave, which may have resulted in missing information. To maintain data integrity, we excluded this incomplete data. We firmly believe our analysis is sound and reject any accusations of flawed methodology or distorted results. Questioning the integrity of our work is unsubstantiated.

Response to concern no. 5

We did observe the frequency of C allele in severe COVID-19 group to be 0.66, contrary to the C allele frequency reported in 1000 genome project in South-east Asian population. Indeed, this was a novel finding and its implications for severe COVID-19 are worth investigating. Further, the Indigenome samples were taken from healthy samples, whereas our sampling was totally based on the clinical population having hypoxia or related symptoms from the Indian cohort. The data in our study also showed T allele frequency (0.45) and C allele (0.55) in total studied clinical population. In the study by Aggarwal et al., it was reported that the C allele frequency was 0.66 in Kapha prakriti constitution individuals, which is a segregated population, concurring with our findings of C allele frequency (0.66) in severe COVID-19. Although, this inference from these two studies should be investigated in more detail. Similarly, in our asymptomatic COVID-19 patients showed a high T allele frequency (0.55) and a comparable trend in the frequency of C allele (0.45) with the Indigenome database.

We strongly refute the assertions regarding the inclusion of selective samples in COVID-19 categories. The sample inclusion and collection are explicitly described in the methods section.

We understand that the exact allele frequencies may vary based on the ethnicity of the studied population. However, the observations in our study were rigorously inferred from PCR-RFLP data and thorough statistical analyses. As stated previously, a larger cohort of COVID-19 categorised patients may be recruited, to understand the association analysis of C allele in severe COVID-19, which can have implications in the investigation of EGLN1 gene in COVID-19 hypoxia.

Further, the HWE status in a small clinical population may vary from a large general population.

We believe this response conclusively addresses the concerns raised by Deora et al. and provides a valuable balanced perspective for the readers.

Author contributions

PKS, RH, SD, KCP and KV conceived, wrote and reviewed the manuscript in response to the correspondence by Deora et al.

Data availability

No datasets were generated or analysed during the current study.

Declarations

Competing interests

The authors declare no competing interests.

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