Using Genetics to Inform The Role of Hemostatic Factors in COVID-19: A Mendelian Randomization Study

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Abstract

Background: Severe corona virus disease 19 (COVID-19) is challenging to treat as it presents with thrombotic as well as inflammatory features. Specifically, endothelial cells are thought to be damaged in COVID-19. Here we assessed whether key druggable targets related to endothelial function, i.e., nitric oxide and platelets, are affected specifically in severe COVID-19, using a Mendelian randomization study.

Methods: We compared genetically predicted circulating nitric oxide, proxied by three relevant *NOS3* functional variants (rs2070744, rs1799983 and rs3918226), and genetically predicted platelets, proxied by a functional variant related to platelet reactivity from *GRK5* (rs10886430) and by platelet count (based on 243 variants), because they share a phenotype, in people with severe COVID-19 with the general population (cases=536, non-cases=329,391) largely based on studies from Northern Europe. We made a similar comparison for any COVID-19.

Results: Nitric oxide was inversely associated with severe COVID-19 (odds ratio (OR) 0.84 per raising allele, 95% confidence interval (CI) 0.75 to 0.95), but was not associated with any COVID-19. Associations of both platelet reactivity and platelet count with severe COVID-19 were in a harmful direction (OR 1.20, 95% CI 0.95 to 1.50 and OR 1.23, 95% CI 0.97 to 1.56 respectively) with combined OR 1.21, 95% CI 1.03 to 1.42, but associations with any COVID-19 were null.

Conclusions: Genetic validation of the role of nitric oxide and possibly of platelets in specifically severe COVID-19 suggests they could be targets of intervention for which well-established treatments exist.

Background

Treatment of severe corona virus disease 19 (COVID-19) has improved substantially over the course of the pandemic, informed by clinical insights and findings from trials. Nevertheless, COVID-19 presents a complex challenge as a respiratory disease with thrombotic features, whose origins are not entirely clear, but may involve a range of factors including the endothelium.[1] COVID-19 damages endothelial cells;[2] a key part of the hemostatic system. Specifically, endothelial cells regulate arterial tone by generating contracting factors such as thromboxane A2 and relaxing factors, such as nitric oxide.[3, 4] Nitric oxide, formerly known as endothelium-derived relaxing factor, can act as a vasodilator and anti-thrombotic agent.[3, 5] Several trials are currently investigating the role of inhaled nitric oxide as a treatment for COVID-19, https://clinicaltrials.gov/ct2/results/details?term=nitric+oxide+COVID, but . systemic nitric oxide has had less attention https://racetoacure.stanford.edu/clinical-trials/991, although it is the target of long-standing cardiovascular drugs, such as nitroglycerin.

Endothelial cells also modulate platelet aggregation,[6, 7] possibly via thrombin,[8] which is the key coagulation factor associated with ischemic heart disease.[9] Thrombin induced platelet reactivity and aggregation has recently been shown to be relevant to a range of cardiovascular diseases,[10] consistent with the well-established role of platelets in thrombosis.[11] Platelet reactivity and aggregation is also the

target of long-standing cardiovascular drugs, such as aspirin. The relevance of platelets to COVID-19, particularly severe COVID-19, is unclear, although more platelet aggregation has been seen in more severe cases.[12] However, observational studies in selected samples, such as patients, can be difficult to interpret, given potential selection bias, confounding, and typically small sample sizes.

As COVID-19 may impact endothelial function, investigating factors relevant to haemostatic processes in severe COVID-19 may provide additional insight. In this study, we used mendelian randomization, a design robust to confounding, to assess whether nitric oxide or platelets contributed to very severe COVID-19. We used any COVID-19 as a control outcome as we would not expect factors involved in the response to COVID-19 to necessarily to be the same as the factors that result in COVID-19 infection, particularly as severe COVID-19 is only experienced by a minority of those infected. We included hospitalized COVID-19 for completeness.

Methods

This is a two-sample mendelian randomization study. We obtained genetic instruments for nitric oxide and platelets from the largest publicly available published studies. Preferentially we selected genetic instruments on functional grounds, because functional variants known physiologically to correspond to a specific phenotype are most likely to capture it both comprehensively and exclusively. Otherwise, we selected genetic variants statistically (as reaching genome wide significance. We applied these genetic predictors of nitric oxide and platelets to the largest publicly available genome wide association study (GWAS) of COVID-19 to determine if people with genetically different levels of nitric oxide or platelets also differed in their vulnerability to very severe COVID-19, and obtained mendelian randomization estimates. We similarly considered any and hospitalized COVID-19.

Exposures

Nitric Oxide

We used three established functional genetic variants relevant to endothelial nitric oxide synthase (eNOS),[13] i.e., rs2070744 (*NOS3*), rs1799983 (*NOS3*) and rs3918226 (*NOS3*).[14] We did not include the 4b/4a VNTR variant (rs61722009 (*NOS3*)), because it is seldom included in GWAS, and is not in the 1000 Genomes reference panel.

Platelets

We used both platelet reactivity in response to thrombin and platelet count, because few genetic predictors of platelet reactivity are available, and platelet count may be phenotypically similar to platelet reactivity.[10] One genetic variant (rs10886430 (*GRK5*)) functionally and statistically relevant to thrombin induced platelet reactivity is available.[10] We extracted genetic variants for platelet count from a published GWAS giving independent genome-wide significant genetic predictors.[15] We presented platelet reactivity

estimates in terms of a square root transform, which was used to normalize the distribution,[10] and platelet count in effect sizes.[15]

Outcome

We obtained associations with COVID-19 from the latest publicly available GWAS summary statistics. (https://www.covid19hg.org/results/) (accessed 2nd July 2020) comparing genetic make-up for different severities of COVID-19 with the population, i.e., very severe COVID-19 (cases=536, non-cases=329,391), hospitalized COVID-19 (n=3199, non-cases=897,488), and any COVID-19 (cases=6,696, non-cases=1,073,072). Case status for very severe COVID-19 was laboratory confirmed COVID-19 hospitalized with respiratory support or death. Case status for hospitalized COVID-19 was hospitalized with laboratory confirmed infection, hospitalization due to COVID-19-related symptoms or self-reported hospitalized COVID-19 positive. Case status for any COVID-19 was laboratory confirmed infection, doctor diagnosis or self-report. The COVID-19 GWAS is mainly based on people of European descent from existing cohort studies and was adjusted for study covariates, principal components, age, sex, age^2 and sex*age, as appropriate. https://docs.google.com/document/d/1Pcq1jttF8W7ifEUXA6-a1WVMsUyEoAybS6IqvuP-Uv8/edit?ts=5e964dc2#.

Statistical Analysis

We aligned genetic variants for the exposures (eNOS or platelets) and genetic associations with COVID-19 on the same effect allele. There were no palindromic SNPs for eNOS or platelet reactivity. For the genetic variants predicting platelet count we did not drop palindromic SNPs because the GWAS for platelet count and COVID-19 both used the same strand direction. For genetic variants not in the COVID-19 GWAS, proxies (r^2>0.8) were identified. We meta-analyzed genetic variant specific Wald estimates (genetic variant on COVID-19 divided by genetic variant on exposure) with the standard error obtained from the first term of Fieller's theorem.[16] We used inverse variance weighting, with multiplicative random effects for three or more genetic variants. We also used the weighted median and MR-Egger estimates as sensitivity analysis,[17] where possible (i.e., when three or more independent genetic predictors of each exposure were available). Since variants for nitric oxide are correlated, we obtained their correlations using LdLink (https://ldlink.nci.nih.gov/), and included them in the analysis. We obtained mendelian randomization estimates using the MendenlianRandomization R package. All analyses were performed using R Version 3.6.1 (R Development Core Team, Vienna, Austria).

Ethics approval

This study only used publicly available summary statistics and hence no ethics approval is required. This study complies with the Declaration of Helsinki.

Results

Nitric Oxide

Each of the three eNOS genetic variants (rs2070744 (*NOS3*), rs1799983 (*NOS3*) and rs3918226 (*NOS3*)) associated with greater eNOS activity was also associated with a lower risk of very severe COVID-19 (Supplementary Table 1). Taken together, accounting for correlations, genetically predicted nitric oxide was inversely associated with very severe COVID-19 but was not associated with hospitalized or any COVID-19 (Figure 1 and supplementary Table 2).

Platelets

The one genetic variant (rs10886430) functionally and statistically relevant to thrombin induced platelet reactivity[10] was available for each COVID-19 case definition. Of 287 published variants predicting platelet count,[10] 230 were available for very severe COVID-19, based on position or rsid, and a further 13 had proxies (r^2>0.8), giving 243 genetic predictors in total for platelet count, more variants were available for hospitalized (n=263) and severe COVID-19 (n=268). Both platelet reactivity and platelet count had associations in the direction of higher risk of severe COVID-19 (Figure 1 and Supplementary Table 3). Estimates for platelet count were similar in sensitivity analysis (Supplementary Table 3). MR-Egger gave no indication of pleiotropy (Supplementary Table 3). Taken together as a platelet phenotype, platelets were positively associated with severe COVID-19 (odds ratio 1.21, 95% confidence interval 1.03 to 1.42). Platelets were unrelated to any COVID-19 or hospitalized COVID-19 (Figure 1 and Supplementary Table 3).

Discussion

Consistent with the role of endothelial cells in severe COVID-19,[2] we present genetic validation of a protective role of a druggable factor, nitric oxide, that regulates endothelial function,[3, 4] in specifically severe COVID-19. In addition, we show that a platelet phenotype might also be relevant specifically to severe COVID-19.

Nitric oxide and platelets are the targets of some of the oldest treatments for cardiovascular disease. Nitric oxide forms the basis of a very long-standing treatment for angina and acute myocardial infarction, i.e., nitroglycerin,[18] while aspirin is one of the most effective anti-platelet agents in secondary prevention.[19] Another, very well-established treatment for cardiovascular disease, statins, may improve endothelial function,[20] and increase eNOS[21] as well as reducing platelet count.[15] These findings also have some consistency with men being more vulnerable to severe COVID-19 than women.[22] Typically men have lower nitric oxide than women,[23] and lower endothelial function,[24] but also lower platelet counts with little difference in platelet reactivity.[25] Whether any common underlying factor, such as hormones, is driving associations of haemostatic and coagulation factors with COVID-19, might bear consideration.

Despite providing some genetic validation for the role of key druggable haemostatic factors in severe COVID-19, this study has limitations. First, valid genetic instruments need to fulfil three assumptions, i.e., relate strongly to the exposure, not be associated with potential confounders of the effects of the exposure on the outcome and only affect COVID-19 via the relevant exposure. The genetic variants for

nitric oxide are well-established functionally relevant variants from a protein coding gene (*NOS3*).[14] The variant for platelet reactivity was partly selected on functional grounds.[10] The sensitivity analysis for platelet count did not give any indication of pleiotropy (Supplementary Table 3). Confounding by population stratification could create bias, although genetic associations for both the exposures (nitric oxide and platelets)[10, 13, 15] and the outcomes, COVID-19, largely concern people of European ancestry (https://www.covid19hg.org/results/). Mendelian randomization studies can be open to selection bias, particularly if the genetic variants affect survival and other causes of survival and the outcome, here COVID-19, exist.[26] However, whether nitric oxide or platelets affect survival has not been clearly established. The underlying GWAS for COVID-19 obtained the non-cases from the same population as the cases (https://www.covid19hg.org/results/) which also reduces any possible selection bias, as does comparing with the general population to avoid index event bias.[27] Buffering of genetic effects could occur during development, i.e., canalization, however prior adaption to COVID-19 is unlikely. Findings, largely in Europeans, may not apply to other populations. Causes would be expected to act consistently, although may be less relevant in different settings.[28] We were not able to assess vulnerability to severe COVID-19 by sex, because no sex-specific GWAS exists. Exploration of differences by sex could be important, given response to viral infections, including COVID-19, differs by sex.[29]

Conclusions

This study suggests that the haemostatic factors, nitric oxide and a platelet phenotype, may underlie some of the thrombotic features of severe COVID-19. Investigation of the role in COVID-19 of well-established, widely available essential medicines targeting nitric oxide and platelets, such as nitrogylcerin, aspirin and statins, might be worthwhile.

Abbreviations

COVID: Coronavirus disease

CI: confidence interval

eNOS: endothelial nitric oxide synthase

GWAS: genome wide association study

OR: odds ratio

Declarations

Ethics approval and consent to participate

This study only uses publicly available summary statistics

Consent for publication

None needed

Availability of data and materials

The data is publicly available, and the code is available on request

Competing interests

The authors declare that they have no competing interests

Funding

None

Authors' contributions

CMS conceptualized the study, did the initial analysis and wrote the first draft. JVZ and SLAY contributed to the conceptualization. JVZ checked the analysis. All authors approved the final version.

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Figures

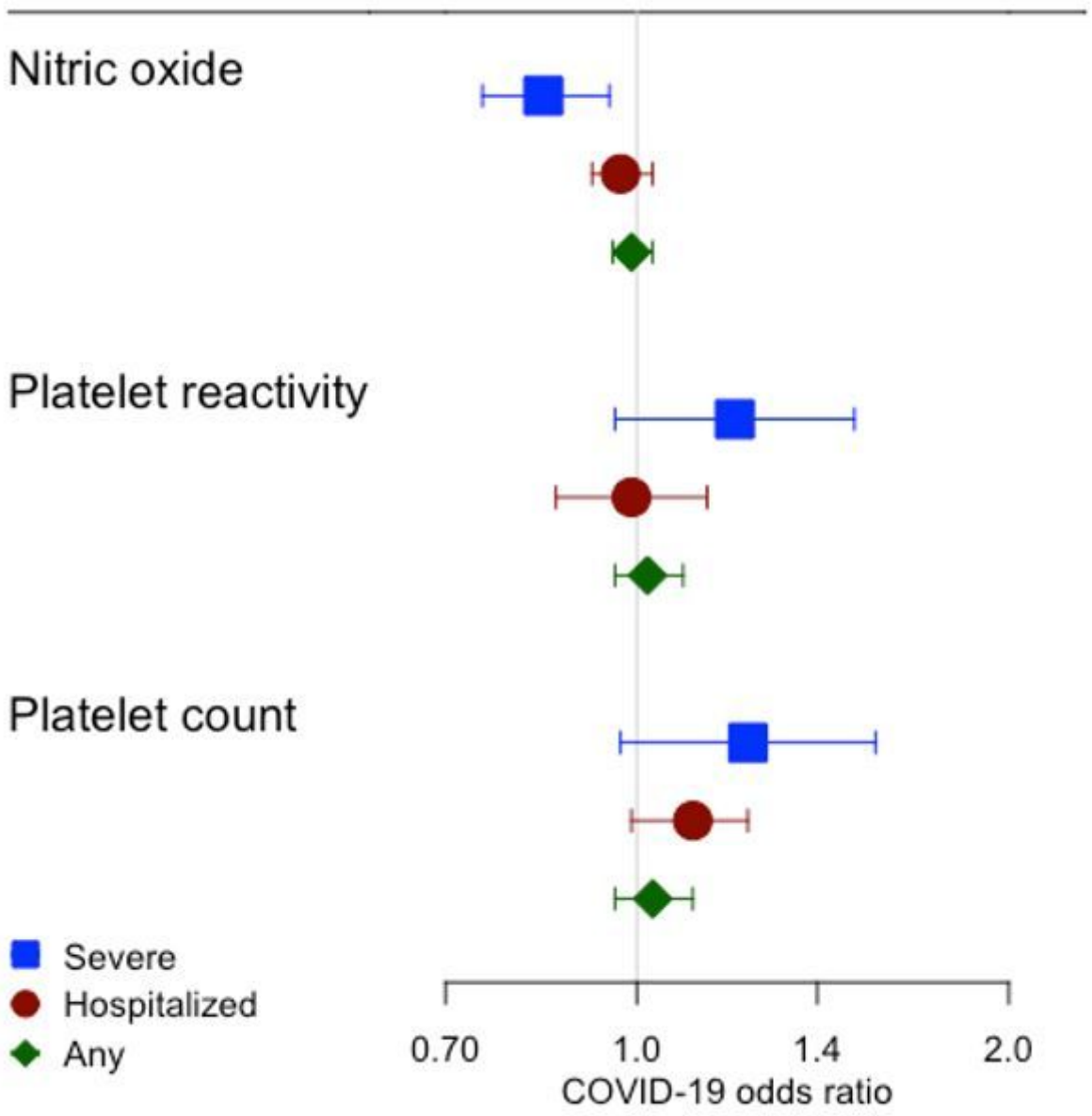

Figure 1

Mendelian randomization estimates of nitric oxide (per allele higher*) based on rs2070744, rs1799983 and rs3918226, platelet reactivity (per square root transform) based on rs10886430, and platelet count (per effect size) based on 243+ variants on severe, hospitalized and any COVID-19 (*For rs1799983 carriers of the nitric oxide lowering allele have nitric oxide lower by -0.27 □mol/L 30).

Supplementary Files

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