Association of polymorphisms in genes encoding prothrombotic and cardiovascular risk factors with disease severity in COVID-19 patients: A pilot study

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Abstract
The present study aimed to assess the association of 16 polymorphisms in genes encoding prothrombotic and cardiovascular risk factors with COVID-19 disease severity: FV G1691A, FV H1299R, FII G20210A, MTHFR C677T, MTHFR A1298, factor XIII V34L, PAI-1 4G/5G, EPCR haplotypes (A1/A2/A3), eNOS −786 T > C, eNOS G894T, LTA C804A, ACE I/D, ITGB3 PIA1/A2, ITGA2B Baka/b, ß-Fbg −455 G > A and ApoB R3500Q. The study included 30 patients with severe COVID-19 and 49 non-severe COVID-19 patients. All studied polymorphisms except ITGA2B Baka/b were determined using multilocus genotyping assays CVD StripAssays (ViennaLab Diagnostics), while ITGA2B was genotyped using a real-time PCR method based on TaqMan technology. A higher frequency of carriers of at least one ITGB3 PIA2 allele was found in severe COVID-19 patients (p = 0.009). The distribution of genotypes was significantly different for ß-Fbg −455 G > A (p = 0.042), with only three homozygous AA genotypes found among severe COVID-19 patients. The association with an increased risk for severe COVID-19 was found for ITGB3, with carriers of at least one ITGB3 PIA2 allele having a 3.5-fold greater risk of severe COVID-19 (p = 0.011). Genotype distribution differences were obtained for the combinations of FV H1299R and FXIII V34L (p = 0.026), ITGB3 PIA1/A2 and ITGA2B Baka/b (p = 0.024), and ACE I/D and PAI-1 4G/5G (p = 0.046). ITGB3 polymorphism emerged as an independent risk factor for severe COVID-19 and homozygosity for ß-Fbg −455 G > A mutation could contribute to disease severity. The combined effect of polymorphisms in genes encoding prothrombotic and cardiovascular risk factors could further contribute to disease severity.

KEYWORDS
cardiovascular risk, COVID-19, genetic polymorphisms, thrombosis

1 | INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a novel infection caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), with clinical manifestations ranging from asymptomatic carriage across mild to severe forms that require hospitalization and intensive care accompanied by significant mortality risk. Severe forms of COVID-19 account for up to 15% of all cases and are characterized by the prominent pro-inflammatory response and procoagulant activity, leading to systemic and life-threatening complications that include acute respiratory distress syndrome, acute cardiac, kidney, and liver injury, thromboembolic events, septic shock, and multiorgan failure.
dysfunction or failure. Such exacerbation of COVID-19 is associated with immune system dysregulation accompanied by excessive release of inflammatory cytokines which in turn creates a hyperinflammatory and procoagulant milieu. However, the susceptibility to develop such serious complications is individual and probably a result of a complex interplay that includes several underlying factors such as comorbidities, endothelial dysfunction, oxidative stress but also genetic risk factors. Prothrombotic and cardiovascular complications are the most prevalent adverse events in patients suffering from COVID-19, related to poor prognosis and higher mortality rates. Numerous studies have suggested underlying genetic predisposition as a contributing factor to developing these serious complications. So far, the most studied genes in this context are the ones encoding angiotensin-converting enzymes 1 and 2 (ACE1 and ACE2 genes), since ACE2 is the cellular receptor specifically involved in the binding of SARS-CoV-2 to the nasopharyngeal mucosa and other tissues. The main function of ACE1 is to regulate ACE2 expression through angiotensin II levels, hence regulating blood pressure, systemic vascular resistance, and vasoconstriction. Increased expression of ACE2 receptor caused by ACE1 D allele and a single-nucleotide polymorphism in the ACE2 gene (rs2285666) results in an imbalance of the ACE1/ACE2 arms, consequent disruption of the renin-angiotensin-aldosterone system and has been associated with disease severity and poor outcome in COVID-19. Furthermore, a limited number of studies have investigated the impact of individual prothrombotic risk factors on COVID-19 severity. Among these, the plasminogen activator inhibitor-1 (PAI-1) 4G/5G gene polymorphism was investigated in a single study so far and was found to be associated with post-COVID-19 osteonecrosis mediated by thrombosis. Furthermore, Ponti et al. suggest the involvement of methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism in the incidence and severity of COVID-19. However, the complexity of the clinical course and underlying complications evidenced in severe COVID-19 patients implies that an array of other genetic risk factors might be involved in the pathobiology of COVID-19. Therefore, studies that investigate the genetic background of proteins involved in thrombosis and cardiovascular-associated complications in COVID-19 patients are strongly advocated.

The aim of the present study was to perform a comprehensive analysis covering a selection of polymorphisms in genes encoding prothrombotic and atherosclerotic risk factors in COVID-19 patients and assess their possible association with disease severity.

2 | METHODS

2.1 | Setting, study design, and participants

This cross-sectional study was conducted at the University Hospital Center Zagreb and included 30 patients with a severe form of COVID-19 hospitalized from January to May 2021 at the COVID-19-dedicated intensive care unit (ICU), as well as a control group consisting of 49 patients with non-severe COVID-19. The diagnosis of COVID-19 was confirmed by reverse transcription real-time polymerase chain reaction (PCR) from nasopharyngeal and oropharyngeal swabs. All patients admitted to the ICU presented with bilateral pneumonia and fulfilled at least one of the following criteria for severe COVID-19: oxygen saturation <94%, a ratio of arterial partial pressure of oxygen to fraction of inspired oxygen <300 mmHg, respiratory frequency >30 breaths/min, or lung infiltrates >50%, thus requiring respiratory support.

The following 16 polymorphisms in 12 candidate genes encoding proteins associated with prothrombotic and cardiovascular risk were studied: factor V (FV) G1691A (Leiden), FV H1299R (R2), factor II G20210A, MTHFR C677T, MTHFR A1298, factor XIII (FXIII) V34L, PAI-1 4G/5G, endothelial protein C receptor (EPCR) haplotypes (A1/A2/A3), endothelial nitric oxide synthase (eNOS) G894T, lymphotoxin-alpha (LTα) C804A, ACE 1/D, integrin beta-3 (ITGB3) PIA1/A2, integrin alpha-IIIb (ITGA2B) Baka/b, β-fibrinogen −455 G > A, and Apolipoprotein B R3500Q.

All analyses performed for the purposes of the study were conducted from leftover samples, otherwise destined for discarding, without any need for additional blood sampling. Laboratory analyses were performed at the Department of Laboratory Diagnostics, University Hospital Center Zagreb, Croatia.

The study was conducted according to the principles of the Declaration of Helsinki and with the consent of each participant, and was approved by the University Hospital Center Zagreb Ethics Committee (8.1-21/41-2, 02/21-JG).

2.2 | Laboratory analyses

Genomic deoxyribonucleic acid (DNA) was isolated from ethylenediamine-tetraacetic acid whole blood samples drawn into 3-ml tubes (Greiner Bio-One), using the salting-out method, while its quality and quantity were determined spectrophotometrically at 260 and 280 nm using NanoDrop Lite (Thermo Fischer Scientific). DNA extracts were amplified in two parallel multiplex PCRs using biotinylated primers. The size of the amplification products was analyzed by agarose gel electrophoresis.

All studied polymorphisms except ITGA2B Baka/b were determined using commercial multilocus genotyping assays named CVD StripAssays (ViennaLab Diagnostics). The principle of the assays is based on the hybridization of DNA products to a test strip containing allele-specific oligonucleotide probes. Bound biotinylated sequences are detected with streptavidin-alkaline phosphatase and color substrates. The results were read independently by two laboratory professionals. Assay kits were used strictly adhering to the manufacturer’s defined protocol. ITGA2B was genotyped using a real-time PCR method based on TaqMan technology on a 7500 Real-time PCR system (Applied Biosystems).

2.3 | Statistical analysis

Data distribution normality was assessed using the Shapiro-Wilk test. Categorical data were reported as absolute numbers and proportions, while numerical data as means and standard deviations.
The distribution of genotype frequencies among the two groups of patients was compared using the X² test or Fisher's exact test, where appropriate. Univariate logistic regression with the calculation of odds ratios and corresponding 95% confidence intervals was used to test the impact of the assessed polymorphisms between severe and non-severe COVID-19 patients, \( p < 0.05 \) was considered statistically significant. The dominant model was used, meaning that homozygous and heterozygous variants were compared to the homozygous wild type. For analysis of EPCR haplotypes, the genotypes were grouped according to the presence of at least one A3 allele. Statistical analysis was performed in MedCalc, v.19.5.2 (MedCalc).

3 | RESULTS

The study included 30 severe COVID-19 patients (mean age: 61 years, from 33 to 86; 60% males) treated at the ICU and 49 non-severe COVID-19 patients (mean age: 57, from 26 to 88; 51% males). Complications that were developed in severe COVID-19 patients, as well as their comorbidities, are listed in Table 1.

Distributions of genotype frequencies between severe and non-severe COVID-19 patients for all assessed polymorphisms are presented in Table 2. A statistically significant higher frequency of carriers of at least one ITGB3 PIA2 allele was found within the group of severe COVID-19 patients (\( p = 0.009 \)). The distribution of genotypes among the two compared groups was found to be significantly different for \( \beta \)-fibrinogen \( -455 \) G \( > \) A (\( p = 0.042 \)), with a higher frequency of GA genotypes in non-severe COVID-19 patients, while the only three homozygous AA genotypes were found among severe COVID-19 patients.

The association with an increased risk for severe COVID-19 was not found for the majority of polymorphisms (Table 3). The only exception was ITGB3 for which it emerged that carriers of at least one PIA2 allele have a 3.5-fold greater risk of severe COVID-19 (\( p = 0.011 \)).

When frequencies of combined polymorphisms were compared, a statistically significant difference in genotype distribution among the two groups of patients was obtained for the combinations of FV H1299R and FXIII V34L, ITGB3 PIA1/A2 and ITGA2B Baka/b, as well as ACE I/D and PAI-1 4G/5G, as shown in Table 4.

4 | DISCUSSION

The present study represents the first comprehensive analysis involving the association of genetic polymorphisms related to prothrombotic and cardiovascular risk factors with COVID-19 severity. Among the wide array of genetic polymorphisms studied, we evidenced that ITGB3 PIA2 is independently associated with increased risk for severe COVID-19, while the single-nucleotide polymorphism in the \( \beta \)-Fbg gene was found in the homozygous mutated form only among severe COVID-19 patients.

Specifically, the obtained results unequivocally suggest a 3.5-fold increased risk for severe COVID-19 in carriers of at least one ITGB3 PIA2 allele. ITGB3 and ITGA2B genes encode the subunits of the platelet integrin glycoprotein IIb/IIIa (GPIIb/IIIa) complex which is responsible for platelet adhesion and activation. ITGB3 PIA1/A2 polymorphism results from a single amino acid substitution (leucine \( \rightarrow \) proline) at residue 33 in the gene of the \( \beta \)-chain of GPIIb/IIIa, resulting in PIA1 or PIA2 isoforms. This alters the structural conformation of its \( \beta \)-subunit, transforming it into a fully active state which exhibits enhanced platelet reactivity and adhesion capacity, resulting in increased thrombogenicity.\(^{17,18}\) The association of ITGB3 PIA1/A2 polymorphism with the occurrence and extent of different thrombotic and cardiovascular events, including coronary artery disease, ischemic stroke, and deep venous thrombosis,\(^{19-21}\) has already been reported, indicating its involvement in the pathogenesis of prothrombotic-associated complications. Based on the presence of these adverse events in our cohort of severe COVID-19 patients and the higher frequency of the ITGB3 PIA2 allele which was strikingly more present in patients with thrombotic complications, it can be speculated that this genetic predisposition has a role in the already complex and deleterious pathophysiology of COVID-19, especially hemostasis derangement.

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**Table 1** Complications and comorbidities in severe COVID-19 patients treated at the intensive care unit.

| Complications in severe COVID-19 patients (\( N = 30 \)) | \( N \) (proportion) |
|--------------------------------------------------------|----------------------|
| Multimorgan failure                                     | 8 (0.27)             |
| Sepsis                                                 | 7 (0.23)             |
| Acute cardiovascular event                              | 6 (0.20)             |
| Pulmonary embolism                                      | 5 (0.17)             |
| Cardiorespiratory arrest                                | 5 (0.17)             |
| **Comorbidities in severe COVID-19 patients (\( N = 30 \))** |                     |
| Diabetes mellitus                                       | 10 (0.33)            |
| Hypertension                                            | 9 (0.30)             |
| Prior cardiovascular event                              | 5 (0.17)             |
| Chronic kidney disease                                  | 4 (0.13)             |
| Hematological disease                                   | 4 (0.13)             |
| Atrial fibrillation                                     | 3 (0.10)             |
| Other                                                   |                      |
| Acute liver injury                                      | 3 (0.10)             |
| Disseminated intravascular coagulation                  | 2 (0.07)             |
| Thrombosis                                              | 1 (0.03)             |
| **Other**                                               |                      |
| Asthma                                                  | 2 (0.07)             |
| Autoimmune hepatitis                                    | 1 (0.03)             |
| Crohn's disease                                         | 1 (0.03)             |
| Postpartum period                                       | 1 (0.03)             |

Abbreviation: COVID-19, coronavirus disease 2019.
Thus contributing to disease severity. Although ITGA2B Baka/b polymorphism was not found to be associated with disease severity in this study, the frequency of the combination of heterozygosity in both ITGB3 and ITGA2B genes was found to be higher in severe COVID-19 patients, further supporting the role of platelet antigens in COVID-19 pathogenesis.

Another interesting finding of this study pertains to the significantly higher frequency of the single-nucleotide polymorphism in the promoter region of the fibrinogen gene (β-Fbg -455G>A) found in patients with the non-severe COVID-19 form of the disease. However, homozygous mutant genotype was found in three cases only among the severe COVID-19 patients. As this polymorphism is associated with elevated plasma levels of the acute phase protein fibrinogen and is considered to be an independent predictor of coronary heart disease,22 this finding might further support its role in enhancing susceptibility to hyperinflammatory state development, which is one of the hallmarks of severe COVID-19.

**Table 2** Distribution of genotype frequencies of all assessed polymorphisms in COVID-19 severe compared to non-severe patients.

| Polymorphism | Genotype | Severe COVID-19 patients (N = 30), N (proportion) | Non-severe COVID-19 patients (N = 49), N (proportion) | p |
|--------------|----------|-------------------------------------------------|-------------------------------------------------|---|
| FV Leiden (G1691A) | GG | 29 (0.97) | 48 (0.98) | 1.000 |
| | GA | 1 (0.03) | 1 (0.02) | 0.000 |
| | AA | 0 (0) | 0 | 0.595 |
| FV R2 (H1299R) | HH | 21 (0.70) | 38 (0.78) | 0.284 |
| | HR | 9 (0.30) | 11 (0.22) | 0.042 |
| | RR | 0 (0) | 0 (0) | 0.042 |
| FII G20210A | GG | 30 (1.00) | 46 (0.94) | 0.229 |
| | GA | 0 (0) | 3 (0.06) | 0.009 |
| | AA | 0 (0) | 0 (0) | 0 (0) |
| MTHFR C677T | CC | 11 (0.37) | 27 (0.55) | 0.688 |
| | CT | 14 (0.47) | 18 (0.37) | 0.127 |
| | TT | 5 (0.16) | 4 (0.08) | 0.042 |
| MTHFR A1298C | AA | 12 (0.40) | 15 (0.31) | 0.566 |
| | AC | 14 (0.47) | 27 (0.55) | 0.566 |
| | CC | 4 (0.13) | 7 (0.14) | 0.566 |
| FXIII V34L | VV | 16 (0.53) | 32 (0.65) | 0.566 |
| | VL | 13 (0.43) | 16 (0.33) | 0.566 |
| | LL | 1 (0.04) | 1 (0.02) | 0.566 |
| PAI-1 4G/5G | 4G/4G | 12 (0.40) | 10 (0.20) | 0.688 |
| | 4G/5G | 13 (0.43) | 24 (0.49) | 0.688 |
| | 5G/5G | 5 (0.17) | 15 (0.31) | 0.688 |
| EPCR haplotype (A1/A2/A3) | A1/A1 | 7 (0.23) | 17 (0.35) | 0.308 |
| | A1/A2 | 5 (0.17) | 7 (0.14) | 0.308 |
| | A2/A2 | 7 (0.23) | 5 (0.10) | 0.308 |
| | A1/A3 | 6 (0.20) | 6 (0.12) | 0.308 |
| | A2/A3 | 5 (0.17) | 14 (0.29) | 0.308 |
| | A3/A3 | 0 (0) | 0 (0) | 0 (0) |
| eNOS −786 T > C | TT | 8 (0.27) | 20 (0.41) | 0.643 |
| | TC | 13 (0.43) | 21 (0.43) | 0.643 |
| | CC | 9 (0.30) | 8 (0.16) | 0.643 |
| eNOS G894T | GG | 16 (0.53) | 22 (0.45) | 0.566 |
| | GT | 12 (0.40) | 21 (0.43) | 0.566 |
| | TT | 2 (0.07) | 6 (0.12) | 0.566 |
| LTA C804A | CC | 16 (0.53) | 29 (0.59) | 0.643 |
| | CA | 13 (0.43) | 18 (0.37) | 0.643 |
| | AA | 1 (0.04) | 2 (0.04) | 0.643 |

Note: *p* < 0.05 is considered statistically significant.

Abbreviations: ACE, angiotensin-converting enzyme; ApoB, apolipoprotein B; COVID-19, coronavirus disease 2019; eNOS, endothelial nitric oxide synthase; EPCR, endothelial protein C receptor; FII, coagulation factor II; FV, coagulation factor V; FXIII, coagulation factor XIII; ITGA2B, integrin alpha IIb; ITGB3, integrin beta-3; LTA, lymphotixin-alpha; MTHFR, methylenetetrahydrofolate reductase; PAI-1, plasminogen activator inhibitor-1.
TABLE 3 | Distribution of genotype frequencies in severe and non-severe COVID-19 patients, with calculated odds ratios (ORs) and corresponding 95% confidence intervals (CIs).

| Polymorphism | p     | OR (95% CI) |
|--------------|-------|-------------|
| FV R2 (H1299R) | 0.457 | 1.48 (0.53–4.14) |
| FII 20210A    | 0.087 | N/A         |
| MTHFR C677T   | 0.110 | 2.12 (0.83–5.38) |
| MTHFR A1298C  | 0.395 | 0.66 (0.26–1.71) |
| FXIII V34L    | 0.292 | 1.65 (0.65–4.16) |
| PAI-1 4G/5G   | 0.158 | 2.21 (0.71–6.87) |
| EPCR haplotype (A1/A2/A3) | 0.149 | 2.06 (0.77–5.47) |
| eNOS -786 T > C | 0.197 | 1.90 (0.71–5.10) |
| eNOS G894T    | 0.466 | 0.71 (0.29–1.77) |
| LTA C804A     | 0.611 | 1.27 (0.51–3.17) |
| ACE I/D       | 0.610 | 1.31 (0.46–3.75) |
| ITGB3 A1298C  | 0.011 | 3.52 (1.34–9.28) |
| ITGA2B Baka/b | 0.297 | 1.67 (0.64–4.34) |
| β-fibrinogen 455 G > A | 0.586 | 0.77 (0.30–1.96) |
| ApoB R3500Q   | N/A   | N/A         |

Note: p < 0.05 is considered as statistically significant.
Abbreviations: ACE, angiotensin-converting enzyme; ApoB, apolipoprotein B; eNOS, endothelial nitric oxide synthase; EPCR, endothelial protein C receptor; FII, coagulation factor II; FXIII, coagulation factor XIII; ITGB3, integrin alpha-IIb; ITGA2B, integrin alpha-IIIb; LTA, lymphotoxin-alpha; MTHFR, methylenetetrahydrofolate reductase; N/A, not applicable; PAI-1, plasminogen activator inhibitor-1.

The role of other assessed polymorphisms was not found to be significant in the progression towards the severe form of COVID-19 in our study population. Contrary to previously published studies,11,12,23 the ACE1 I/D polymorphism was not identified as a contributor to disease severity in our patients. However, for a more profound insight into the involvement of the renin-angiotensin system in modulating the course of COVID-19, this investigation should be complemented with the analysis of ACE2 gene polymorphisms due to the direct involvement of ACE2 receptor in the ingestion of the SARS-CoV-2 from the cell surface. Large-scale population studies and in silico analyses report the existence of multiple ACE2-altering variants which affect virus-host interaction differently, either by increasing susceptibility or having a protective effect.24,25 Furthermore, the role of MTHFR was assessed through the analysis of two most frequent polymorphisms (C677T and A1298C), equally not yielding a clear association with COVID-19 severity. Similarly, polymorphisms of LTA and eNOS genes involved in oxidative stress and endothelial dysfunction were not found to directly contribute to the development of the severe form of COVID-19 in our study.

Given the lack of empirical studies in the field, the assumption about the possible involvement of PAI-1 4G/5G in the pathophysiology of COVID-19 was based solely on its well-known role in coagulopathy and inflammation where upregulation of PAI-1 leads to thrombosis formation but also induces the secretion of pro-inflammatory cytokines and chemokines by binding to macrophages.26 In our study, the contribution of PAI-1 4G/5G to COVID-19 severity was not confirmed. Although not reaching statistical significance, it is worthwhile to notice that we evidenced a twofold higher frequency of the 4G/4G genotype in severe COVID-19 patients as well as an approximately twofold higher risk of severe COVID-19 in carriers of the 4G allele. Given that PAI-1 4G/4G genotype is associated with

TABLE 4 | Distribution of genotype frequencies in severe and non-severe COVID-19 patients for the combination of polymorphisms for which statistically significant difference was observed.

| Combination of polymorphisms | Genotype | Severe COVID-19 patients (N = 30), N (proportion) | Non-severe COVID-19 patients (N = 49), N (proportion) | p     |
|-----------------------------|----------|-----------------------------------------------|--------------------------------------------------|-------|
| FV H1299R/FXIII V34L        | HH/VV    | 12 (0.4)                                      | 23 (0.47)                                        | 0.026 |
|                             | HR/VL    | 5 (0.17)                                      | 1 (0.02)                                         |       |
|                             | RR/LL    | 0 (0)                                         | 0 (0)                                            |       |
| ITGB3 A1298C/ITGA2B Baka/b  | PIA1/A1/Baka/a | 9 (0.3)                                      | 25 (0.51)                                        | 0.024 |
|                             | PIA1/A2/Baka/b | 5 (0.17)                                      | 2 (0.04)                                         |       |
|                             | PIA2/A2/Bakb/b | 0 (0)                                         | 0 (0)                                            |       |
| ACE I/D/PAI-1 4G/5G         | II/5G/5G | 1 (0.03)                                      | 3 (0.06)                                         | 0.046 |
|                             | ID/4G/5G | 5 (0.17)                                      | 8 (0.16)                                         |       |
|                             | DD/4G/4G | 7 (0.23)                                      | 1 (0.02)                                         |       |

Note: p < 0.05 is considered statistically significant.
Abbreviations: ACE, angiotensin-converting enzyme; COVID-19, coronavirus disease 2019; FV, coagulation factor V; FXIII, coagulation factor XIII; ITGA2B, integrin alpha-IIIb; ITGB3, integrin beta-3; PAI-1, plasminogen activator inhibitor-1.
higher PAI-1 levels and consequently increased prothrombotic risk, this finding implies that PAI-1 4G/4G genotype might have a role in the development of severe COVID-19. Importantly, our study showed that combined homozygosity of ACE DD and PAI-1 4G/4G genotypes was strikingly more present in severe COVID-19 patients. In addition, we have shown that a higher number of patients with the severe course of the disease have underlying combined heterozygosity of FV H1299R and FXIII V34L polymorphisms. However, to clearly understand the roles of any genetic prothrombotic risk factors in the course of COVID-19, large-scale population studies should be conducted. Only by including a representative large sample, their true impact could be elucidated, and this should be highlighted as the main limitation of our study. However, to the best of our knowledge, this is the first study that aimed to simultaneously evaluate the impact of a large number of the most common prothrombotic and cardiovascular genetic risk factors in COVID-19 patients. Since studied genes are involved in different pathophysiological mechanisms, the present study provides valuable insight in the possible role of genetic predisposition in the development of severe complications associated with COVID-19 and represents the basis for future investigations in the field.

In conclusion, the results obtained herein demonstrate that ITGB3 polymorphism is an independent risk factor for severe COVID-19. Furthermore, our results indicate that homozygosity for β-Fbg −455 G>A mutation could contribute to disease severity. The combined effect of polymorphisms in two genes encoding prothrombotic and cardiovascular risk factors, as in the case of concomitant ACE DD and PAI-1 4G/4G genotype, could further contribute to disease severity. Our study clearly shows that the underlying complications leading to severe COVID-19 cannot be unambiguously explained by studied genetic risk factors only and once again confirms the complexity of COVID-19 pathophysiology.

AUTHOR CONTRIBUTIONS
Ivana Lapić conceived and designed the study, performed laboratory analyses, analyzed and interpreted the data, and wrote the manuscript. Margareta Radić Antolcic conceived and designed the study, performed laboratory analyses, analyzed and interpreted the data, and critically revised the manuscript. Ivana Horvat performed laboratory analyses and analyzed and interpreted the data. Vedran Premužić recruited patients, collected samples, and analyzed and interpreted the data. Jozefina Palić performed laboratory analyses and analyzed the data. Dunja Rogić conceived and designed the study, analyzed and interpreted the data, and co-wrote the manuscript. Renata Zadro conceived the study, analyzed and interpreted the data, and critically revised the manuscript. All authors approved the final version of the article for submission.

CONFLICTS OF INTEREST
The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT
All data from this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT
The study was approved by the University Hospital Center Zagreb Ethics Committee (Protocol number: 8.1-21/41-2, 02/21-JG).

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