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# The effect of hereditary thrombotic factors and comorbidities on the severity of COVID-19 disease

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**Abstract. – OBJECTIVE: Coronavirus disease 2019 (COVID-19) has rapidly spread worldwide and presents critical challenges for public health. Due to its chronic and systemic course, COVID-19 is currently accepted as a multi-systemic infectious disease. Here we explore the possible association between disease course and hereditary thrombotic factors and comorbidities.**

**PATIENTS AND METHODS: The patients admitted to the COVID-19 center in the Istanbul Faculty of Medicine were recruited for the study. The patients were classified according to the clinical course, severe *vs.* mild. Five polymorphic loci were analyzed by multiplex PCR: Factor V Leiden (FVL), FII G20210A, Beta-fibrinogen G-455A, and methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C.**

**RESULTS: FII G20210A and Beta-fibrinogen G-455A genotypes were significantly higher in the study group compared to the literature. Wildtype genotype (GG) in Factor V Leiden locus was significantly associated with low D-Dimer levels ($p$ =0.013). The GA genotype increased the D-Dimer levels 2.55-times compared to the GG genotype ($p$ =0.003). Moreover, the Beta-fibrinogen G-455G genotype was significantly higher in the LDH>250 group ($p$ =0.046).**

**CONCLUSIONS: The presence of solid tumors in patients with COVID-19 was related to the severity of the disease course. No evidence of a correlation between the severity of the disease and all five thrombotic mutations was found, whereas the FII G20210A and Beta-fibrinogen G-455A mutations were significantly high compared to previously reported Turkish population data and global carrier rates. This finding will need to be verified by further studies with larger samples since it may reflect a likelihood of having the COVID-19 disease. The high carrier frequency of FVL mutation was more likely present in the D-dimer high group generating an increase in the D-dimer levels 2.55-times compared to the wildtype.**

*Key Words:*
Polymorphism, Hereditary thrombotic factors, D-Dimer, COVID-19, Comorbidity.

## Introduction

The coronavirus epidemic (COVID-19) – caused by serious acute respiratory syndrome coronavirus 2 (SARS-CoV-2) – emerged in late 2019. Although the clinical and imaging findings of the disease are well defined, there are still unknowns for complications and chronic systemic diseases caused by COVID-19. COVID-19 is associated with severe thrombotic complications, such as pulmonary and renal microangiopathy, deep vein thrombosis (DVT), pulmonary embolism (PE), arterial and venous thromboembolism presenting as acute ischemic stroke, and arterial and venous catheter thrombosis or disseminated intravascular coagulation (DIC) syndrome[1]. Acute thrombophilic conditions arise probably due to activation of inflammatory pathways or *via* endothelitis[2]. Moreover, thrombotic complications have been found to correlate with disease severity[3]. Factor V Leiden is the most common mutation related to inherited thrombophilia. Heterozygosity for the Leiden variant occurs in 3-8% of the general US and European populations.

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FII G20210A heterozygosity is the second most common inherited thrombophilia after Factor V Leiden (FVL); heterozygosity for FII 20210G>A occurs in 1.7-3% of the general US and European populations[4].

Polymorphisms of the gene encoding for the methylenetetrahydrofolate reductase (MTHFR) have also been related to an increased susceptibility to develop DVT *via* homocysteine metabolism. Two common MTHFR alleles, C677T and A1298C, lead to mild MTHFR enzyme deficiency. C677T homozygotes and heterozygotes have an average of 30% and 65% of normal activity, respectively, and A1298C homozygotes have 60% of normal enzyme function[5].

In a large study in the Turkish cohort by Sazci et al[6], the percentage of the C677T and A1298C individuals were 47.4% and 46.3%, respectively.

The G-455A polymorphism is located in the promoter region of the beta-fibrinogen gene, -455G→A, which is associated with elevated plasma fibrinogen levels and hyperfibrinogenemia systemic arterial and venous thromboembolism. The overall population's homozygous and heterozygous mutation frequencies are 2.7% and 24.7%, respectively[7].

## Patients and Methods

We analyzed FVL, FII G20210A, Beta-fibrinogen G-455A, MTHFR C677T, and A1298C mutations representing well-studied genetic thrombotic contributors throughout the genome in patients with COVID-19.

The patients admitted to the COVID-19 center in Istanbul University, Faculty of Medicine, between April-June 2020 were recruited for the study.

We used the data of previous comprehensive polymorphism studies on the Turkish population to check the accuracy of our data. The institutional clinical research ethics committee approved the protocol (21/05/2020-84539).

### Classification of the Clinical Course, Severe vs. Mild

We performed laboratory confirmation (RT-PCR examination) of SARS-CoV-2 in certified laboratories where RT-PCR assays were carried out following the protocol defined by the World Health Organization[8]. The patients were considered seriously ill when any of the following clinical and laboratory parameters were met: a respiratory rate of ≥30/min, suffering from dyspnea, peripheral oxygen saturation of <90%, getting more than 5 L/min nasal oxygen supply, $PaO_2/FiO_2$ of ≤300, lactate of >2 mmol/L, hypotension (systolic blood pressure 40 mmHg lower than the usual systolic blood pressure) pulse of >100 beats/min, renal, hepatic hematological (thrombocytopenia) or cerebral (confusion) dysfunction findings, presence of sepsis or septic shock, skin findings of capillary return disorder such as *cutis marmorata* and coldness, moderate/severe pneumonia (bilateral infiltration and/or multiple mottling and ground-glass opacity), need for anti-cytokine therapy, and/or broad-spectrum antibacterial therapy.

### DNA Isolation and Genotyping Polymorphisms

DNA was isolated from the collected blood using the Qiagen isolation kit (QIAamp DNA Mini QIAcube Kit, Qiagen, Hilden, Germany). A thrombophilia panel kit was used for amplification. Genetic regions in the Thrombophilia Panel are amplified by two separate multiplex PCRs. Approximately 40-100 ng of genomic DNA was amplified in a 25-µl reaction containing 4.2 µl PCR Master MIX, 15.6 µl $ddH_2O$, 2 µl DMSO, 0.2 µl of Taq DNA polymerase, and 1 µl of each primer. Genomic DNA was denatured for 3 min at 95°C before 15 cycles of denaturation for 30 s at 95°C, annealing for 45 s at 55°C, extension for 60 s at 72°C, and 20 cycles of denaturation for 30 s at 95°C, annealing for 45 s at 58°C, extension for 60 s at 72°C followed by a 10-min 72°C polishing step. The reactions were run on Bio-Rad Thermal Cyclers (Bio-Rad, Hercules, CA, USA). PCR products (8 µl) were purified using Exonuclease I (0.5 µl) and Shrimp Alkaline Phosphatase (SAP, 1 µl).

Purified products were run for 70 min at 37°C and 20 min at 72°C on Bio-Rad Thermal Cyclers. After purification of the PCR products, the Thrombophilia mutation primers in the test system are again taken into two separate multiplex mini-sequencing reactions. Fluorescently labelled dideoxyribonucleoside triphosphates (ddNTPs) in the environment are added to the specific mutation primer, designed separately for each mutation by the polymerase enzyme, and the primer elongation reaction is terminated. In the reaction, 1.5 µl of $ddH_2O$, 0.5 µl of ABI PRISM® SNaPshotTM Mix, 1 µl of Thrombophilia MSQ Primer Mix, and 1 µl of the purified PCR product were placed in separate PCR tubes for each sample. Samples were run 25 cycles of denaturation

for 10 s at 96°C, annealing for 5 s at 50°C, and extension for 30 s at 60°C on Bio-Rad Thermal Cyclers. Mini-Sequencing reaction products were run by adding approximately 4-5 µl of products into 10 µl of HI-DI Formamide to the Applied Biosystems 3500xl capillary electrophoresis device (Waltham, MA, USA). The color of the peaks obtained made it possible to identify single nucleotide polymorphism (SNP) or mutation.

### Statistical Analysis

Descriptive statistics were given as median (minimum-maximum) and frequencies with percentages. Analyses were performed with the Mann-Whitney U test, Chi-Square test, and Fisher's exact test. We performed multivariable binary logistic regression analysis with entering and backward LR methods to determine the risk factors for D-dimer levels and the severity of COVID-19. The level of significance was taken at $\alpha = 0.05$. SPSS Version 25.0, (IBM Corp., Armonk, NY, USA) software was used for statistical analysis.

## Results

A total of 189 PCR-positive cases were enrolled in the study. The median age of the patients was 48 years (range, 19-62 years). We outlined the clinical features of COVID-19 patients, such as gender, comorbidity, symptoms, and severity (Table I).

The most common comorbidities were hypertension 72 (25.5%) and diabetes mellitus 36 (12.8%). The severe disease was observed in 80 (30.3%) patients. In the follow-up, mortality occurred in 9 (6.0%) patients, and 14 (9.5%) patients needed intensive unit care.

All five genotype frequencies and comorbidities in severe and mild disease groups are listed in Table II.

There was no statistically significant difference between mild and severe patient groups for either of the five genotypes ($p$>0.05). The severe disease course was more likely to be present in elder people ($p$<0.001).

The only comorbidity that seemed to be associated with the severity was a solid malignancy. Patients not carrying a solid malignancy had a significantly milder disease course ($p$=0.004) (Table II).

Age and solid malignancy were the risk factors for the severity of COVID-19. The patients presenting with a tumor were 2.02-times more likely to have severe disease than patients without a solid tumor ($p$=0.041) (Table III). For older people, the COVID-19 disease seemed to be more severe ($p$<0.001). Older patients experience 1.06-times more severe disease courses ($p$<0.001).

To check the genotype frequency results, they were compared with the population frequency based on the Turkish literature with the biggest cohorts (Table IV).

The wildtype FII genotype was significantly higher in the patient group compared to the control group reported by Gundogdu et al[9] ($p$=0.013). The Beta-fibrinogen G-455A genotype was significantly higher in the total patient group compared to the control group reported by Yilmaz et al[10] ($p$=0.048). To avoid errors due to small-size controls, the genotype frequency in the patient group was later compared with the genotype frequencies in the Topmed database and revealed a similar significant association ($p$<0.001)[7].

A1298C frequencies were similar to other Turkish healthy population data[6,10,11] reported previously in the literature ($p$<0.001). MTHFR C677T and FVL did not differ significantly between the literature[6,11] and patient group in our

**Table I.** Clinical characteristics of COVID-19 patients.

| | n (%) |
|---|---|
| Male | 155 (54.8) |
| Female | 128 (45.2) |
| ICU (n = 148) | 14 (9.5) |
| Ex (n = 144) | 9 (6.0) |
| **Comorbidity** | |
| Hypertension | 72 (25.5) |
| DM | 36 (12.8) |
| COPD/asthma | 30 (10.6) |
| Solid malignancy | 26 (9.2) |
| Coronary Artery Disease | 17 (6.0) |
| Hematologic malignancy | 7 (2.5) |
| Congestive Heart Failure | 5 (1.8) |
| **Severity** | |
| Mild | 184 (69.7) |
| Severe | 80 (30.3) |
| **Symptoms** | |
| Fever | 152 (53.9) |
| Coughing | 168 (59.6) |
| Sputum | 1 (0.4) |
| Dyspnea | 94 (33.3) |
| Fatigue/myalgia | 143 (50.9) |
| Nausea | 28 (10.0) |
| Diarrhea | 21 (7.4) |
| Anosmia | 12 (4.3) |

DM: Diabetes Mellitus Type2, COPD: Chronic Obstructive Pulmonary Disease, ICU: Intensive Care Unit, ex: Exitus.

**Table II.** Comparison of genetic polymorphisms and comorbidities with the severity of the disease.

| | Severity | | |
|---|---|---|---|
| | **Mild (*n* = 145)** | **Severe (*n* = 44)** | ***p*-value** |
| MTHFR 1298 AC | | | 0.367 |
| CC | 17 (11.7%) | 2 (4.5%) | |
| AC | 70 (48.3%) | 24 (54.5%) | |
| AA | 58 (40.0%) | 18 (40.9%) | |
| FIIGA | | | 0.736 |
| GG | 135 (93.1%) | 42 (95.5%) | |
| GA | 10 (6.9%) | 2 (4.5%) | |
| MTHFR 677CT | | | 0.598 |
| TT | 12 (8.3%) | 2 (4.5%) | |
| CT | 67 (46.2%) | 19 (43.2%) | |
| CC | 66 (45.5%) | 23 (52.3%) | |
| FVGA | | | 0.767 |
| GG | 132 (91.0%) | 41 (93.2%) | |
| GA | 13 (9.0%) | 3 (6.8%) | |
| BfibGA | | | 0.659 |
| GG | 78 (54.5%) | 26 (60.5%) | |
| GA | 54 (37.8%) | 13 (30.2%) | |
| AA | 11 (7.7%) | 4 (9.3%) | |
| **Age** | **40.0 (19.0-84.0)** | **52.0 (31.0-89.0)** | **<0.001** |
| Gender | | | 0.142 |
| Male | 74 (51.0%) | 28 (63.6%) | |
| Female | 71 (49.0%) | 16 (36.4%) | |
| Hypertension | | | 0.736 |
| No | 134 (92.4%) | 42 (95.5%) | |
| Yes | 11 (7.6%) | 2 (4.5%) | |
| DM | | | 0.688 |
| No | 137 (94.5%) | 43 (97.7%) | |
| Yes | 8 (5.5%) | 1 (2.3%) | |
| COPD/asthma | | | 0.077 |
| No | 135 (93.1%) | 37 (84.1%) | |
| Yes | 10 (6.9%) | 7 (15.9%) | |
| Coronary Artery Disease | | | 0.136 |
| No | 144 (99.3%) | 42 (95.5%) | |
| Yes | 1 (0.7%) | 2 (4.5%) | |
| Congestive Heart Failure | | | 0.412 |
| No | 144 (99.3%) | 43 (97.7%) | |
| Ye | 1 (0.7%) | 1 (2.3%) | |
| **Solid malignancy** | | | **0.004** |
| **No** | **141 (97.2%)** | **37 (84.1%)** | |
| **Yes** | **4 (2.8%)** | **7 (15.9%)** | |
| Hematologic malignancy | | | 0.551 |
| No | 143 (98.6%) | 43 (97.7%) | |
| Yes | 2 (1.4%) | 1 (2.3%) | |

Data were presented with median (min-max) and n (%). COPD: Chronic Obstructive Pulmonary Disease, MTHFR: methylenetetrahydrofolate reductase, DM: Diabetes Mellitus Type2, Bfib: Beta fibrinogen, FV: factor V, DNA molecule: adenine (A), cytosine (C), guanine (G), and thymine (T).

study, respectively ($p$=0.428, $p$=0.845). The FV Leiden GA genotype was found in 8% of the total patients, similar to the population frequency of 7.37% in the healthy Turkish population[11].

The association between the five polymorphisms and D-Dimer levels is presented in Table V. A significant relationship was observed between FVL and D-dimer levels.

**Table III.** Examining risk factors for severity of COVID-19.

| | Wald | OR | 95% CI for OR | *p*-value |
|---|---|---|---|---|
| Solid malignancy | | | | |
| (Reference) No | – | – | – | – |
| Yes | 4.17 | 2.02 | 1.03-3.98 | 0.041 |
| Age | 17.02 | 1.06 | 1.03-1.08 | <0.001 |
| Constant | 17.37 | 0.41 | | <0.001 |
| Nagelkerke $R^2$ | | | | 0.206 |
| Hosmer and Lemeshow test | | | | 0.506 |

Binary logistic regression model ($p<0.001$). Variable (s) entered on step I: age and solid malignancy. OR: odds ratio, CI: confidence interval. Formula for Calculating Nagelkerke R-squared in Logistic Regression Analysis = [1-(L0/L1)^(2/N)]/[1-L0^(2/N)].

The wildtype Factor V GG genotype was significantly associated with low D-Dimer levels ($p=0.013$). We found no significant relationship between the other four polymorphisms and D-Dimer levels ($p>0.05$). Other risk factors for high D-dimer levels are listed in Table VI.

According to binary logistic regression analysis, the FVL, age, and severity were the risk factors for high D-dimer levels. The FV GA genotype increases the D-dimer levels 2.55-times compared to the GG genotype ($p=0.003$). Older age increases the D-dimer levels 1.04-times ($p=0.005$). The severity of the disease increases the D-Dimer levels by 2.16-times ($p<0.001$).

## Discussion

In this study, we found that age was significantly related to the severity of the disease ($p<0.001$). Other studies[12] and meta-analyses previously confirmed the age-severity relation. The most common comorbidity in the cohort was hypertension (n=72, 25.5%). A meta-analysis by Fang et al[12] found that hypertension was associated with a higher rate of disease severity among other comorbidities. However, we did not find a relationship between the severity of the disease and hypertension. The only comorbidity related to the severity was the presence of a solid tumor ($p=0.004$) (Table III). This

**Table IV.** Comparison of genetic polymorphisms between the literature data and this study.

| | Patient n (%) | Control n (%) | *p*-value |
|---|---|---|---|
| **FII G20210A** | | **Gundogdu et al[9]** | **0.013** |
| **GG** | **177 (93.7%)** | **130 (99.2%)** | |
| **GA** | **12 (6.3%)** | **1 (0.8%)** | |
| **Beta fibrinogen** | | **Yilmaz et al[10]** | **0.048** |
| **GG** | **104 (83.9%)** | **20 (16.1%)** | |
| **GA** | **67 (90.5%)** | **7 (9.5%)** | |
| **AA** | **15 (100.0%)** | **0 (0.0%)** | |
| MTHFR A1298C | | Sazci et al[6] | 0.632 |
| AA | 76 (40.2%) | 736 (43.7%) | |
| AC | 94 (49.7%) | 779 (46.3%) | |
| CC | 19 (10.1%) | 69 (10.0%) | |
| MTHFR C677T | | Sazci et al[6] | 0.428 |
| TT | 14 (8.0%) | 162 (92.0%) | |
| CT | 86 (9.7%) | 799 (90.3%) | |
| CC | 89 (11.0%) | 723 (89.0%) | |
| Factor V Leiden | | Ozbek[11] | 0.845 |
| GG | 173 (4.2%) | 3931 (95.8%) | |
| GA | 16 (4.4%) | 345 (95.6%) | |

MTHFR: methylenetetrahydrofolate reductase. DNA molecule: adenine (A), cytosine (C), guanine (G), and thymine (T).

**Table V.** The association between the polymorphisms and D-Dimer levels.

| Genotypes | D-dimer ≤ 550 µg/L (n = 107) n (%) | D-dimer > 550 µg/L (n = 80) n (%) | *p*-value |
|---|---|---|---|
| MTHFR1298 | | | 0.936 |
| CC | 11 (10.3%) | 8 (10.0%) | |
| AC | 52 (48.6%) | 41 (51.2%) | |
| AA | 44 (41.1%) | 31 (38.8%) | |
| FIIGA | | | 0.601 |
| GG | 101 (94.4%) | 74 (92.5%) | |
| GA | 6 (5.6%) | 6 (7.5%) | |
| MTHFRCT | | | 0.077 |
| TT | 9 (8.4%) | 5 (6.3%) | |
| CT | 41 (38.3%) | 44 (55.0%) | |
| CC | 57 (53.3%) | 31 (38.8%) | |
| **FVGA** | | | **0.013** |
| **GG** | **103 (96.3%)** | **69 (86.3%)** | |
| **GA** | 4 (3.7%) | 11 (13.8%) | |
| BfibGA | | | |
| GG | (n = 105) | (n = 79) | 0.706 |
| GA | 56 (53.3%) | 47 (59.5%) | |
| AA | 40 (38.1%) | 26 (32.9%) | |
| | 9 (8.6%) | 6 (7.6%) | |

MTHFR: methylenetetrahydrofolate reductase, Bfib: Beta fibrinogen, FV: factor V, DNA molecule: adenine (A), cytosine (C), guanine (G), and thymine (T).

**Table VI.** Other risk factors for high D-dimer levels.

| | Wald | OR | 95% CI for OR | *p*-value |
|---|---|---|---|---|
| FVGA | | | | |
| GG | | – | – | – |
| GA | 8.53 | 2.55 | 1.36-4.77 | 0.003 |
| Age | 7.87 | 1.04 | 1.01-1.06 | 0.005 |
| Severity | | | | |
| Mild | | – | – | – |
| Severe | 13.82 | 2.16 | 1.44-3.25 | <0.001 |
| Constant | 1.19 | 0.49 | | 0.275 |
| Hosmer and Lemeshow test | | | | 0.401 |
| Nagelkerke $R^2$ | | | | 0.26 |

Binary logistic regression model ($p<0.001$). Variable(s) entered on step 1: FVGA, age, severity, Hypertension history, Diabetes History, Coronary Artery Disease, Solid malignancy. OR: odds ratio, CI: confidence interval, FV: factor V, DNA molecule: adenine (A), cytosine (C), guanine (G), and thymine (T).

association was initially stated by Liang et al[13] and weakly proven by Fang et al[12].

Studies[9] with the biggest Turkish cohorts were picked from the literature to compare the mutation frequencies with the healthy population. A discrepancy with FII G20210A mutation between our patient cohort and healthy population data is driven by the study conducted by Gundogdu et al[9], who reported the FII G20210A mutation rate as 0.8% in 131 control patients, and this rate was 6.3%, where 12 out of 189 were carrying FII G20210A mutation in the present study. According to this data, there was a significant difference between the study by Gundogdu et al[9] and our cohort ($p<0.001$) (Table IV). Moreover, in one of the most comprehensive studies about the carrier frequency of a healthy

population with a total of 5,527 individuals, Rosendaal et al[14] found that 111 heterozygous carriers of the FII G20210A mutation were found (2%). This prevalence was as high as 3.0% in southern Europe (95% CI: 2.3-3.7%), where the mutation is most frequently seen worldwide[14]. Therefore, one can clearly say that the carrier frequency in our patient cohort was found to be more than twice that when compared to the most frequently seen population. Studies with bigger cohorts in a healthy Turkish population are needed before claiming a possible relation with COVID-19 disease.

In the total study cohort, the beta fibrinogen G-455A genotype was significantly high when compared to the population cohort ($p$=0.048) (Table IV). It is obvious that, with this data, it is not proper to claim that thrombotic events or increased beta fibrinogen activity may contribute to COVID-19 infection; however, previous data[15] prove the link between beta fibrinogen and immune system response is consistent with what we have found. An example of viral and beta fibrinogen association was reported by Ait-Goughoulte et al[15] showing that the Hepatitis C virus core protein interacts with beta fibrinogen and attenuates cytokine-stimulated acute phase response.

D-dimer is a specific fibrin degradation product considered a global marker of coagulation activation and fibrinolysis. Concerning hereditary factors, it is well established that inherited resistance to activated protein C (APC-resistance) is caused primarily by a point mutation in the factor V gene leading to the replacement of arginine on the 506$^{th}$ position with a glutamine (factor V R506Q or FV Leiden mutation, factor V Leiden) is associated with functional impairment of the protein C anticoagulant system and is an established risk factor for thrombosis in the general population. In the present study, statistical analysis indicated that the FV Leiden genotype was significantly more common in the high D-Dimer group when compared to the normal D-Dimer group. Chaireti et al[8] investigated the association between D-dimer and Factor V Leiden mutation in a patient cohort who had venous thromboembolism and found a statistically significant difference in the D-dimer levels between the controls and the heterozygotes as well as controls and the homozygotes. However, the difference between the hetero and homozygotes was not statistically significant. They stated that using multi-variant analysis, the difference in D-dimer levels between the patients carrying FVL and controls was due to the greater number of patients with confirmed thrombosis in the Leiden positive group and concluded that neither D-dimer concentration nor thrombin generation did depend on FV Leiden. Our study observed a statistically significant relationship between high D-dimer levels and Factor V Leiden mutation (Table V). Since there were no homozygous patients in the cohort, the impact of homozygosity could not be observed. None of the patients had a symptomatic finding related to coagulopathy which can be attributed to early anticoagulant use. All patients had received anticoagulant therapy empirically. Therefore, we could not also use international normalized ratio (INR) levels as a parameter. Knowing that the mortality in COVID-19 is related to high D-dimer levels, we also compared the thrombotic mutations and D-dimer levels with mortality; however, the death rate was quite low to make an assertion at the time of follow-up.

Patients with severe disease or comorbidities (including hypertension, diabetes mellitus, solid malignancy, and coronary artery disease) were more likely to present in the D-dimer high group, and the median age of the D-dimer high group was significantly higher than D-dimer low group, as expected. Besides, logistic regression analysis revealed that the FV Leiden GA genotype increases the D-dimer levels 2.55-times compared to the GG genotype (Table VI). According to binary logistic regression results, the FVL, age, and severity of the disease were the risk factors for high D-dimer levels. We did not find any significant association between comorbidity parameters and genetic polymorphisms.

## Conclusions

This study investigated the effect of hereditary thrombotic factors as well as comorbidities on the severity of the disease and the relation between specific biochemical parameters of COVID-19. There was no evidence for a direct correlation between the severity of the disease and FVL, FII G20210A, Beta-fibrinogen G-455A, MTHFR C677T, and A1298C mutations.

The only comorbidity that affects the severity of COVID-19, was the presence of solid tumors. Besides, significantly higher frequency of FVL mutation in the D-dimer high group showed an explicit relation between FVL and D-dimer levels in COVID-19 disease. We therefore suggest, evaluating thrombotic events in COVID-19, not solely with D-dimer levels but also with the presence of FVL mutation.

The carrier frequency of FII G20210A and Beta-fibrinogen G-455A mutations was significantly higher than previously reported Turkish population data and global carrier rates. The study's limitations regarding these two mutations were the absence of our control data and minimal population studies regarding the five thrombotic mutations. Therefore, further studies with larger samples need to verify these findings since they may reflect a likelihood of having the COVID-19 disease.

### Ethics Approval

The study protocol was approved by the Istanbul Medical School Ethics Committee, Istanbul University (21/05/2020-84539). The study abided by the ethical standards laid down in the Declaration of Helsinki of 1964 and its later amendments.

### Informed Consent

All participants provided written informed consent.

### Availability of Data and Materials

Data and materials are available and can be sent up on request.

### Conflict of Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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### Authors' Contributions

G.Y.S., N.S. and H.K.: conceptualization, methodology, software.
Y.O. and A.E.: data curation, writing, original draft preparation.
S.P., A.E. and M.K.: visualization, investigation.
M.K. and G.Y.S.: supervision.
N.S., A.E.: software, validation.
G.Y.S. and M.K.: writing, reviewing, editing.

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