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Clinical impact of single nucleotide polymorphisms within interferon signaling pathway gene in pregnant women with COVID-19

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Abstract

Introduction: This study aimed to investigate if gene expression modifications due to two different single nucleotide polymorphism (SNP) mutations within the *interferon-α/β receptor-2* (*IFNAR2*) gene had an effect on clinical prognosis in pregnant coronavirus disease 2019 (COVID-19) patients.

Methodology: The study included 173 pregnant patients who tested positive for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) using real-time polymerase chain reaction (RT-PCR). Oligonucleotides were designed for the SNPs with nucleotide database codes *rs13050728* and *rs2236757* in the hospital's genetic laboratory, and RT-PCR analyses were performed.

Results: There was a significant difference in upper respiratory tract infection (URTI) symptoms (runny nose, nasal congestion, and fatigue); between *rs2236757* normal–carrier–homozygous mutant pregnant women and the occurrence of symptoms ($p = 0.004$). However, these URTI symptoms were never seen in homozygous mutant pregnant women. Upon analysis of the relationship between *rs13050728* normal–carrier–homozygous mutant pregnant women and the occurrence of symptoms, there was a significant difference between URTI symptoms, and nausea and vomiting ($p = 0.001$, $p = 0.027$, respectively). The URTI symptoms were never seen in homozygous mutant and carrier pregnant women. There was no significant association between *rs13050728* and *rs2236757* normal–carrier–homozygous mutant pregnant women and severity of disease, intensive care unit admission, pregnancy complications, need for oxygen support, and radiologic involvement.

Conclusions: The results of this study will serve as a guide in identifying high-risk individuals, providing treatment, and contributing to the understanding of genetic factors in future viral pandemics.

Key words: COVID-19; pregnant; *IFNAR2*; SNP.

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Introduction

The coronavirus disease 2019 (COVID-19) pandemic, which spread worldwide shortly after it emerged in December 2019, has been associated with various negative outcomes [1]. Following the devastating impact of the pandemic, scientists have initiated numerous studies to investigate the underlying mechanisms of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and identify the risk factors for COVID-19, which can cause symptoms ranging from asymptomatic infection to severe respiratory failure and even death. The risk factors include advanced age; male gender; ethnicity; comorbidities such as cardiovascular disease, hypertension, diabetes, obesity, chronic obstructive pulmonary disease, chronic kidney disease, chronic liver disease, and malignancy; immunosuppressive conditions, and pregnancy [2]. Pregnant women are vulnerable to respiratory pathogens, especially pneumonia, due to physiologic changes including increased oxygen consumption, decreased functional residual capacity and chest compliance, and immunologic changes, e.g., altered T lymphocyte immunity. Therefore, it seems that the prevalence of maternal and fetal morbidity and mortality associated with SARS-CoV-2 virus infections is higher [3,4]. Additionally, host genetic factors were suggested as risk factors for susceptibility to SARS-CoV-2 infection and severe COVID-19 prognosis. Single nucleotide polymorphism (SNP) studies on COVID-19 identified genes associated with different aspects of disease severity, including susceptibility to infection, need for hospitalization, respiratory failure, or death [5–7]. Accordingly, this study aimed to investigate whether gene expression changes due to two SNPs within the *interferon (IFN)-α/β receptor-2* (*IFNAR2*) gene (*rs2236757* and *rs13050728*), which were associated with the severity of disease, had an effect on clinical prognosis in pregnant patients with proven SARS-CoV-2 (based on real-time polymerase chain reaction (RT-PCR) tests) infection. Pregnant patients were considered to be a risk group.

Methodology

Study cohort and data set

The present study included 173 patients who tested positive for SARS-CoV-2 RT-PCR using nasopharyngeal swab samples taken during pregnancy and who met the study criteria. The inclusion criteria were: patients who tested positive for SARS-CoV-2 RT-PCR test during pregnancy, who did not receive the COVID-19 vaccine when they were infected, who did not die, and who were aged ≥ 18 years. The participating patients provided informed consent prior to the onset of the study.

Data collection

Collection of patients' data

Patients’ data was screened using the hospital's information system. The hospitalization decision for pregnant women who presented to the hospital was based on the Turkish Ministry of Health's guideline, namely "Follow-up of pregnant women during the period when COVID-19 respiratory system diseases are prevalent" [8]. In this study, the severity of disease in pregnant women with COVID-19 at initial presentation to the hospital and during follow-up were classified as asymptomatic infection, mild disease, moderate disease, serious/severe disease, and critical disease; based on the criteria set by the US National Institute of Health. In this study, gestational weeks 1–13 were defined as the first trimester, weeks 14–26 as the second trimester, and weeks 27–41 as the third trimester.

SNP analysis based on patients' blood samples

The following procedures were performed on the waste blood samples obtained during the follow-up of patients who had COVID-19 during their pregnancies and recovered. Oligonucleotides were designed for the SNP with nucleotide database code *rs13050728*, and RT-PCR analysis was performed within the scope of the project (Supplementary Table 1). The primer3plus program was used to design primers (https://www.primer3plus.com/primer3plusAbout.html). The lyophilized primers that were synthesized for the project were dissolved with nuclease-free dH_2O to a concentration of 100 mM. SensiFAST™ Probe No-ROX Kit (Bioline, London, UK) was used for RT-PCR reactions. A 20 µL reaction mix containing 2X qPCR MasterMix, 0.4 mM forward primer (10 mM) and 0.4 mM reverse primer (10 mM), 0.1 mM probe (10 mM), and 5 µL DNA were used. The reaction was performed on a Bio-Rad CFX96 Touch RT-PCR (Bio-Rad, Hercules, CA, USA) device using 8 strip tubes, with initial denaturation at 95 °C for 5 minutes; followed by 40 cycles at 95 °C for 10 seconds, and 66 °C for 30 seconds (reading). Base changes were shown based on allele-specific probe data obtained from an RT-PCR test. A point mutation (*rs2236757* A > G) from the *IFNAR2* gene (Supplementary Table 2) was examined within the scope of the project. These regions were amplified with specific primers (Oligomer, Ankara, TURKEY) and then verified based on melting peaks. Different primers were used for different alleles (Supplementary Table 3). The mix used for PCR included 1× SNP PCR buffer, 3 mM $MgCl_2$, 0.2 mM of each dNTP, 0.8 pmol P1 and P2, 1.2% dimethyl sulfoxide, 0.5 µL EvaGreen® Dye (Biotium, Fremont, CA, USA), and 1 unit HotStart *Taq* DNA polymerase (NanoHelix, Daejeon, South Korea). PCR grade water was added to the mix to a total volume of 25 µL. The PCR programs required for amplifying the gene region and melting analysis are provided in Supplementary Table 4 and Supplementary Table 5. Bio-Rad CFX96 Brand model Real Time Device (Bio-Rad, Hercules, CA, USA) was used for the analyses.

Ethical approval

Approval for the study was obtained from the Selcuk University Ethics Committee (29.03.2022-2022/163) and Selcuk University Scientific Research Projects Coordination Office (09.06.2022-22122011). This study was conducted pursuant to the principles of the World Medical Association Declaration of Helsinki—Ethical Principles for Medical Research Involving Human Subjects (2000).

Statistical analyses

Kolmogorov–Smirnov and Shapiro–Wilk tests were used to test the normal distribution hypothesis for numerical data. Frequency and percentage were used to describe categorical data, and median (min–max) was used to describe numerical data. When analyzing numerical data, either the independent groups t test or Mann–Whitney U test was used for data with and without normal distribution, respectively. When comparing numerical variables in three or more groups, the one-way analysis of variance (ANOVA) test, post hoc LSD and Tamhane analysis, Kruskal–Wallis analysis of variance, and post hoc Mann–Whitney U test with Bonferroni correction were used for variables with normal distribution. Friedman test was used to compare baseline, day 3–5, and day 7–10 laboratory data; and a mixed ANOVA test was used to evaluate the factors affecting repeated measures. A logistic regression test was used to analyze multivariate factors affecting intensive care unit (ICU) hospitalization and the severity of the disease. A *p* value of < 0.05 was considered statistically significant.

Results

The mean age of the pregnant women was 29.75 ± 5.37 years, and their demographics and clinical characteristics are summarized in Supplementary Table 6. The most prevalent symptoms were cough (57.8%), dyspnea (34.7%), and muscle and joint pain (32.9%). The most prevalent radiologic finding was ground glass infiltration (64.2%). The median values of vital signs and laboratory parameters of pregnant women at admission are listed in Supplementary Table 7.

IFNAR2 rs2236757 and *rs13050728* normal, carrier, and homozygous mutant distribution of pregnant women are summarized in Table 1, and the allele frequencies of the pregnant women are summarized in Table 2. The association of *IFNAR2* SNP variations with disease severity was investigated using four genetic models (codominant, dominant, recessive, and overdominant). Disease severity was divided into two groups: mild (asymptomatic + mild) and severe (moderate + severe + critical). Based on this analysis, the *rs2236757A* and *rs13050728T* risk alleles were not associated with the severity of COVID-19. The findings are summarized in Tables 3 and 4. There was a significant difference between *rs2236757* normal–carrier–homozygous mutant pregnant women and the occurrence of symptoms related to upper

Table 1. Gene analysis of pregnant women with COVID-19.

	Normal n (%)	**Carrier n (%)**	**Homozygous mutant, n (%)**
rs2236757	(AA) 27 (15.6%)	(AG/GA) 70 (40.5%)	(GG) 76 (43.9%)
rs13050728	(TT) 36 (20.8%)	(TC/CT) 80 (46.2%)	(CC) 57 (32.9%)

COVID-19: coronavirus disease 2019.

Table 2. Allele frequencies of pregnant women with COVID-19.

GEN	**Risk allele n (%)**	**Other allele n (%)**
rs2236757	124 (35.8%) (A)	222 (64.2%) (G)
rs13050728	152 (43.9%) (T)	194 (55.1%) (C)

COVID-19: coronavirus disease 2019.

Table 3. Association of *IFNAR2 rs2236757* with severity of disease.

SNP	**Genetic model**	**Mild disease, n (%)**	**Serious disease, n (%)**	***p* value**
IFNAR2 rs2236757(A>G)	**Codominant**			
	AA	13 (14.9%)	14 (16.3%)	0.861
	AG/GA	34 (39.1%)	36 (41.9%)	
	GG	40 (46.0%)	36 (41.9%)	
	Dominant			
	AA	13 (14.9%)	14 (16.3%)	0.837
	AG/GA + GG	74 (85.1%)	72 (83.7%)	
	Recessive			
	AA + AG/GA	47 (54%)	50 (58.1%)	0.647
	GG	40 (46%)	36 (41.9%)	
	Over-dominant			
	AA + GG	53 (60.9%)	50 (58.11%)	0.758
	AG/GA	34 (39.1%)	36 (41.9%)	

SNP: single nucleotide polymorphism; *IFNAR2*: interferon-α/β receptor-2.

Table 4. Association of *IFNAR2 rs13050728* with severity of disease.

SNP	**Genetic model**	**Mild disease, n (%)**	**Serious disease, n (%)**	***p* value**
IFNAR2 rs13050728 (T>C)	Codominant			
	TT	16 (18.4%)	20 (23.3%)	0.724
	TC/CT	41 (47.1%)	39 (45.3%)	
	CC	30 (34.5%)	27 (31.4%)	
	Dominant			
	TT	16 (18.4%)	20 (23.3%)	0.459
	TC/CT + CC	71 (81.6%)	66 (76.7%)	
	Recessive			
	TT + TC/CT	57 (65.5%)	59 (68.6%)	0.747
	CC	30 (34.5%)	27 (31.4%)	
	Over-dominant			
	TT + CC	46 (52.9%)	47 (54.7%)	0.879
	TC/CT	41 (47.1%)	39 (45.3%)	

SNP: single nucleotide polymorphism; *IFNAR2*: interferon-α/β receptor-2.

respiratory tract infection (URTI), including runny nose, nasal congestion, and malaise ($p = 0.004$). These URTI symptoms were never seen in homozygous mutant pregnant women. There was no difference in other symptoms (Table 5). Upon analysis of the relationship between *rs13050728* normal–carrier–homozygous mutant pregnant women and the occurrence of symptoms, there was a significant difference between URTI symptoms, and nausea and vomiting ($p = 0.001$, $p = 0.027$, respectively). These URTI symptoms were never seen in homozygous mutant and carrier pregnant women. All pregnant women with nausea and vomiting were carrier pregnant women. There was no difference in other symptoms (Table 6). There was no significant association between *rs13050728* and *rs2236757* normal–carrier–

Table 5. Association of symptoms with *rs2236757* normal–carrier–homozygous mutant.

		IFNAR2 rs2236757			***p***
		Normal, n (%)	**Carrier, n (%)**	**Homozygous mutant, n (%)**	
COVID-19 compliant symptom	No	2 (7.41%)	7 (10%)	7 (9.21%)	0.922
	Yes	25 (92.59%)	63 (90%)	69 (90.79%)	
Fire	No	20 (74.07%)	56 (80%)	62 (81.58%)	0.715
	Yes	7 (25.93%)	14 (20%)	14 (18.42%)	
Cough	No	13 (48.15%)	31 (44.29%)	29 (38.16%)	0.599
	Yes	14 (51.85%)	39 (55.71%)	47 (61.84%)	
Shortness of breath	No	18 (66.67%)	43 (61.43%)	52 (68.42%)	0.667
	Yes	9 (33.33%)	27 (38.57%)	24 (31.58%)	
Headache	No	25 (92.59%)	63 (90%)	70 (92.11%)	0.876
	Yes	2 (7.41%)	7 (10%)	6 (7.89%)	
Sore throat	No	26 (96.3%)	59 (84.29%)	64 (84.21%)	0.167
	Yes	1 (3.7%)	11 (15.71%)	12 (15.79%)	
Muscle joint pain	No	20 (74.07%)	46 (65.71%)	50 (65.79%)	0.691
	Yes	7 (25.93%)	24 (34.29%)	26 (34.21%)	
Loss of taste and smell	No	27 (100%)	65 (92.86%)	72 (94.74%)	0.186
	Yes	0 (0%)	5 (7.14%)	4 (5.26%)	
Diarrhea	No	27 (100%)	69 (98.57%)	76 (100%)	0.403
	Yes	0 (0%)	1 (1.43%)	0 (0%)	
Other symptoms of URTI (runny nose, nasal congestion, weakness)	No	23 (85.19%)	67 (95.71%)	76 (100%)	0.004
	Yes	4 (14.81%)	3 (4.29%)	0 (0%)	
Nausea and vomiting	No	27 (100%)	65 (92.86%)	75 (98.68%)	0.065
	Yes	0 (0%)	5 (7.14%)	1 (1.32%)	
Non-COVID-19 symptom	No	24 (88.89%)	59 (84.29%)	65 (85.53%)	0.840
	Yes	3 (11.11%)	11 (15.71%)	11 (14.47%)	

IFNAR2: interferon-α/β receptor-2; COVID-19: coronavirus disease 2019; URTI: upper respiratory tract infection.

Table 6. Association of symptoms with *rs13050728* normal–carrier–homozygous mutant.

		IFNAR2 rs13050728			***p***
		Normal, n (%)	**Carrier, n (%)**	**Homozygote mutant, n (%)**	
COVID-19 compliant symptom	No	2 (5.56%)	7 (8.75%)	7 (12.28%)	0.530
	Yes	34 (94.44%)	73 (91.25%)	50 (87.72%)	
Fever	No	26 (72.22%)	69 (86.25%)	43 (75.44%)	0.130
	Yes	10 (27.78%)	11 (13.75%)	14 (24.56%)	
Cough	No	16 (44.44%)	37 (46.25%)	20 (35.09%)	0.404
	Yes	20 (55.56%)	43 (53.75%)	37 (64.91%)	
Shortness of breath	No	23 (63.89%)	52 (65%)	38 (66.67%)	0.960
	Yes	13 (36.11%)	28 (35%)	19 (33.33%)	
Headache	No	33 (91.67%)	72 (90%)	53 (92.98%)	0.825
	Yes	3 (8.33%)	8 (10%)	4 (7.02%)	
Sore throat	No	32 (88.89%)	69 (86.25%)	48 (84.21%)	0.813
	Yes	4 (11.11%)	11 (13.75%)	9 (15.79%)	
Muscle joint pain	No	28 (77.78%)	52 (65%)	36 (63.16%)	0.280
	Yes	8 (22.22%)	28 (35%)	21 (36.84%)	
Loss of taste and smell	No	34 (94.44%)	75 (93.75%)	55 (96.49%)	0.760
	Yes	2 (5.56%)	5 (6.25%)	2 (3.51%)	
Diarrhea	No	36 (100%)	79 (98.75%)	57 (100%)	0.461
	Yes	0 (0%)	1 (1.25%)	0 (0%)	
Other symptoms of URTI (runny nose, nasal congestion, weakness)	No	29 (80.56%)	80 (100%)	57 (100%)	< 0.001
	Yes	7 (19.44%)	0 (0%)	0 (0%)	
Nausea and vomiting	No	36 (100%)	74 (92.5%)	57 (100%)	0.027
	Yes	0 (0%)	6 (7.5%)	0 (0%)	
Non-COVID-19 symptom	No	34 (94.44%)	65 (81.25%)	49 (85.96%)	0.134
	Yes	2 (5.56%)	15 (18.75%)	8 (14.04%)	

IFNAR2: interferon-α/β receptor-2; COVID-19: coronavirus disease 2019; URTI: upper respiratory tract infection.

homozygous mutant pregnant women with regard to ICU admission, pregnancy complications, need for oxygen support, and radiologic involvement.

Discussion

IFNs are a family of cytokines that increase resistance to viral infection, induce cell-intrinsic programs, and act by binding to the IFNAR1 and IFNAR2 receptor complex [9]. It was reported that SARS-CoV-2 had a higher susceptibility to type I IFN than other coronaviruses, and genetic variations in the type I IFN pathway were implicated in severe COVID-19 [7,10]. Previous studies reported an association between the *IFNAR2* gene *rs2236757* and *rs13050728* SNP variations on chromosome 21q22.1, and critical COVID-19 disease and the need for hospitalization [5,6]. Nevertheless, no study has been conducted in Turkey that investigated the association between the clinical prognosis of COVID-19 and the *IFNAR2 rs2236757* and *rs13050728* SNP variations.

It was suggested that increased expression of *IFNAR2* reduced the risk of critical disease, consistent with the type 1 IFN pathway in antiviral defense [6,11]. A Mendelian randomization study by Gaziano *et al*., reported that the *rs13050728* C allele was associated with higher *IFNAR2* expression in all tissues (except skeletal muscle) and lower risk of hospitalization, indicating that drug trials targeting *IFNAR2* may be prioritized for early treatment of COVID-19 [11]. A study on the role of host genetics and innate immune cells in the severity of COVID-19 reported that the *rs13050728 T* allele might contribute to severe COVID-19 by increasing *IFNAR2* expression in classical monocytes [12]. It was also reported that the *IFNAR2 rs2236757 G* allele reduced the likelihood of severe COVID-19 by increasing *IFNAR2* expression [6]. Pairo-Castineira *et al.* reported that the *IFNAR2 rs2236757 A* allele increased the severity of COVID-19 and that the *rs2236757* variant was copied, especially in the Caucasian population [6]. Other findings suggest that the origin of the population contributes to the occurrence of severe COVID-19. Raza and Abbasi reported that *rs2236757* was associated with severe COVID-19, and the *rs2236757 A* allele recently showed positive selection in the African population [13]. A Brazilian study by Dieter *et al.* on non-white patients reported that *rs2236757/IFNAR2 A/A* genotype was associated with the risk of ICU admission and mortality [14]. Fricke-Galindo *et al.* conducted a validation on a Mexican population, and investigated the importance of *IFNAR2* in the clinical outcomes of patients with severe COVID-19. They reported that the dominant allele modeling according to *rs2236757* (*AG* or *GG* genotype), *rs3153*, *rs1051393*, and *rs2834158* variants were associated with mortality [15]. Abdelhafez *et al.* investigated the association of *IFNAR2 rs2236757* and *oligoadenylate synthetase 3 rs10735079* gene polymorphisms with the risk and severity of COVID-19 infection among Palestinians [16]. In this study, there was a significant association between *IFNAR2 rs2236757A* risk allele and critical COVID-19 disease ($p < 0.025$). In addition, it was also reported that patients who were *rs2236757GA* carriers were more likely to experience sore throat (odds ratio [OR], 2.52; 95% confidence interval [CI] 1.02–6.24; $p = 0.011$); and occurrence of the *rs2236757A* risk allele was associated with an increased risk of breathlessness (OR, 4.70; 95% CI 1.80–12.27); $p < 0.001$) [16]. This is consistent with previous studies that associated the symptom of shortness of breath with severe disease [17]. Previous studies reported that different symptoms were associated with different clinical outcomes [18,19]. In the present study, the *IFNAR2 rs2236757GG* homozygous mutant pregnant women did not have certain URTI symptoms, including runny nose, nasal congestion, and malaise. At the same time, pregnant women with *IFNAR2 rs13050728 CC* homozygous mutants and *rs13050728 CT* carriers never had these URTI symptoms. This was consistent with studies reporting that the frequency of nasal congestion and fatigue increased in severe disease and that the *rs2236757 G* allele and *rs13050728 C* allele were protective in severe disease [20,21]. In the present study, there was no significant difference between *IFNAR2 rs2236757* and *IFNAR2 rs13050728* normal–carrier–homozygous mutant pregnant women based on disease severity, ICU admission, need for oxygen support (single-double), and need for non-invasive mechanical ventilation and intubation. Although the results of the present study were not consistent with a number of studies, which reported that variations were associated with COVID-19 severity, they were consistent with a study on Moroccan patients, which indicated that the *IFNAR2 rs2236757* variation had no effect on the severity of COVID-19 [22]. This may be explained by the varying clinical impact of genetic polymorphisms in different populations [13]. Nevertheless, this was associated with the lower frequency of *IFNAR2 rs2236757 A* allele, which has been associated with COVID-19 severity, in the patients in this study, and also with the fact that mortal patients for both SNPs could not have been included in the present study. In addition, it was suggested that changing pregnant immunology may cause differences in the host immune response to COVID-19, and the immunological response to infections during pregnancy is the result of a combination of signals originating from the maternal immune system and the fetal-placental unit [23]. Type I IFN expression in the placenta is characteristic in several species, including humans. It was suggested that placental type I IFN was critical in the control of viral replication, and uncontrolled viral replication in its absence could harm the fetus and the mother [24]. Racicot *et al*. conducted a study on mice and reported that viremia increased in *IFNAR−/−* mice, but placental and fetal IFN signaling could protect the mother even in the absence of IFNAR in the mother, reducing viremia and mortality [25]. Nevertheless, a study on pregnant cattle reported that *IFNAR* expression increased in placenta and cartilage tissue [26]. Accordingly, it was considered that the altered immunology of pregnant women contributed to the lack of association between *IFNAR2* SNP mutations and the prognosis of the disease.

The present study has certain limitations, including the lack of new SARS-CoV-2 variants, the lack of a control patient group, and the fact that mortal patients could not be included because the study was conducted with patients who returned for follow-up after recovery from COVID-19. Nevertheless, this is the first study of its kind to evaluate the genetic factors of COVID-19 in pregnant women. Additionally, this study is the first in Turkey and one of the few studies worldwide investigating the role of human genetic factors on signs and symptoms of COVID-19.

Conclusions

Although the initial devastating impact of the COVID-19 pandemic is over, it continues to cause serious morbidity and mortality. It is also predicted that other viral pandemics may occur in the future as a result of mutations, which may occur due to the nature of viruses. Pregnant women are one of the patient groups that need attention due to their physiologic and immunologic changes during pregnancy. This study will serve as a guide in a possible future viral pandemic in terms of identifying those who are at risk, and providing treatment and prevention by contributing to the elucidation of genetic factors.

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Conflict of interest

No conflict of interest is declared.

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Annex – Supplementary Items

Supplementary Table 1. Oligonucleotides designed for *rs13050728* SNP.

Rank	Oligo Name	5' mark	5-Series -3	3' mark	Tm
1	rs13050728_SNP_F1		GGCATAGTTTCACTCTGTTG		66 °C
2	rs13050728_SNP_R1		GCCTGTAATCCCGTCTAC		
3	rs13050728_SNP_P1_1	FAM	TGGGAGGCTAAGGCAGGAGA	BHQ1	
4	rs13050728_SNP_P1_2	HEX	TGGGAGGCTGAGGCAGGAGA	BHQ1	

SNP: single nucleotide polymorphism.

Supplementary Table 2. *rs2236757* GeneBank information.

Gene region	Series
rs2236757	NC_000021.9:g.33252612A>G

Supplementary Table 3. *rs2236757* primer sequences.

rs2236757AP1	GACAAATCCCAAAAGAGATTAAGGCCAA
rs2236757GP1	ACAAATCCCAAAAGAGATTAAGGCCAG
rs2236757OP2	CAATGATATAGGTGAAATTTCCACTCATG

Supplementary Table 4. Optimized polymerase chain reaction (PCR) program for *IFNAR2 rs2236757* and *rs13050728* single nucleotide polymorphism (SNP) region amplification. This PCR protocol was used to amplify target genomic regions encompassing the *IFNAR2 rs2236757* and *rs13050728* SNPs prior to genotyping.

PCR Program		
95 °C	3 minutes	1 loop
95 °C	15 seconds	35 cycles
60 °C	1 minute	

Supplementary Table 5. Melting curve analysis program for SNP-specific PCR product validation. This melting program was applied after PCR to assess the specificity of the amplification and detect potential nonspecific products or primer-dimer formations in *rs2236757* and *rs13050728* genotyping.

Melting program	
95 °C	1 minute
65 °C	0.05 seconds
95 °C	5 seconds

SNP: single nucleotide polymorphism; PCR: polymerase chain reaction.

Supplementary Table 6. Demographics and clinical characteristics of pregnant women with COVID-19.

	n	%
Age range (years)		
< 25	38	22
25.1–30	59	34.1
30.1–35	51	29.5
35.1–40	20	11.6
> 40	5	2.9
Oxygen saturation value (%)		
95–100 (normal)	123	71.1
91–94 (mild hypoxia)	24	13.9
86–90 (moderate hypoxia)	17	9.8
≤ 85 (severe hypoxia)	9	5.2
Trimester distribution		
1st trimester	25	14.5
2nd trimester	50	28.9
3rd trimester	98	56.6
Comorbidities		
No	132	76.3
Yes	41	23.7
Severity of disease		
Asymptomatic	15	8.7
Mild	72	41.6
Moderate	36	20.8
Serious/severe	33	19.1
Critical	17	9.9
Hospitalization status		
Outpatient follow-up	32	18.5
Hospitalized	141	81.5
ICU admission		
No	155	89.6
Yes	18	10.4
Oxygen needed	88	50.9
No	85	49.1
Yes	54	31.2
Nasal O_2 (2–4 L/min)	31	17.9
Nasal + Mask O_2 (8–10 L/min)	14	8.1
High flow	9	5.2
CPAP	8	4.6
Intubation	88	50.9

COVID-19: coronavirus disease 2019; ICU: intensive care unit; O_2: oxygen; CPAP: continuous positive airway pressure.

Supplementary Table 7. Median values of vital signs and laboratory parameters of pregnant women at admission.

	Median (min–max)
Median values of vital signs	
Body temperature (°C)	36.7
Pulse rate (per minute)	95
Systolic blood pressure (mmHg)	110
Diastolic blood pressure (mmHg)	70
Respiratory rate (per minute)	14
Leukocyte (3.5–10.5 K/uL)	7300 (3300–29710)
Hemoglobin (12–15.5 g/dL)	12 (7.6–15.5)
Neutrophil (1.7–7 K/mL)	5800 (2000–25380)
Lymphocyte (0.9–2.9 K/mL)	1000 (200–3510)
Platelets (150–450 K/mL)	202 (83–527)
Creatinine (0.5–0.9 mg/dL)	0.5 (0.3–3.0)
ALT (0–35 U/L)	18.5 (4.0–2320)
AST (0–35 U/L)	28.0 (10–2456)
LDH (126–222 U/L)	220 (127–3414)
CK (30–145 U/L)	47.5 (10–2231)
Glucose (72–106 mg/dL)	96 (57–348)
Albumin (3.5–5.2 g/dL)	3.3 (2.0–4.7)
Ferritin (13–150 ng/mL)	36.4 (5.4–2253)
Procalcitonin (0–0.5 ug/L)	0.13 (0.02–1.95)
Troponin (0–14 ng/L)	3 (0.10–114.0)
IL-6 (pg/mL 0.5–6.4)	19.65 (0.90–145.8)
D-dimer (0–500 ng/mL)	762 (120–10968)
INR (0.8–1.2)	0.90 (0.70–1.08)
PT (9–15)	10.5 (8.1–12.7)
aPTT (25–40)	28.4 (20.5–40.8)
Fibrinogen (200–500 mg/dL)	466 (192–780)
CRP (0–8 mg/L)	18.4 (1.2–254)

Min: minimum; max: maximum; ALT: alanine aminotransferase; AST: aspartate aminotransferase; LDH: lactate dehydrogenase; CK: creatine kinase; IL-6: interleukin-6; INR: international normalized ratio; PT: prothrombin time; aPTT: activated partial thromboplastin time; CRP: C-reactive protein.