Genetic factors associated with COVID-19 severity and mortality: TYK2 and NOTCH4

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Received September 26, 2025; Accepted July 31, 2026

DOI: 10.3892/br.2026.2198

Abstract. The clinical manifestations and outcomes of coronavirus disease (COVID-19) vary among patients. Emerging evidence indicates that host genetic factors may influence disease severity. Genome-wide association studies (GWAS) have identified several genetic loci, including *TYK2* and *NOTCH4*, as contributors to COVID-19 pathogenesis. The present study examined the association between genetic variants of *TYK2* (rs74956615) and *NOTCH4* (rs3131294) and the severity and mortality of COVID-19 in a Jordanian cohort. The present study included 362 patients with COVID-19 who were admitted to a hospital in Amman, Jordan. Clinical severity was categorized according to the WHO guidelines (non-severe, severe and critical), and outcomes were classified as survivors or non-survivors. Blood samples were collected, and DNA was extracted. Genotyping of *TYK2* and *NOTCH4* single-nucleotide polymorphisms was performed using PCR and Sanger sequencing. The frequencies of heterozygous and variant alleles of *TYK2* and *NOTCH4* were significantly higher in patients with critical disease than in non-survivors (P<0.001). Logistic regression analysis revealed that individuals with heterozygous or variant alleles of *NOTCH4* had 15.3 times higher odds of developing severe/critical conditions (P=0.010), while those with variant alleles of *TYK2* and *NOTCH4* had approximately eight times higher odds of mortality (P<0.001 for both). *TYK2* and *NOTCH4* variants are markedly associated with increased COVID-19 severity and mortality, indicating their potential as genetic biomarkers for risk stratification. These findings support the importance of host genetics in disease progression and may guide future personalized treatment strategies for this condition.

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Key words: COVID-19, *TYK2*, *NOTCH4*, genetic variants, severity, mortality, Jordan

Introduction

The coronavirus disease-2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was first detected in Wuhan, China, in 2019, followed by a widespread trajectory throughout the world (1), making COVID-19 one of the 21st-century pandemics owing to its high morbidity and mortality rates worldwide (2). Historically, several viruses from the same coronavirus family have emerged, including the famous SARS-CoV and MERS-CoV. However, the characteristics of the strain that caused the COVID-19 pandemic made the resulting infection more severe and the spread of the disease faster (1). SARS-CoV-2 has affected over 759 million individuals worldwide and caused over 6.8 million mortalities.

The severity and clinical outcomes of COVID-19 vary markedly among individuals, with some remaining asymptomatic, whereas others develop severe respiratory illnesses, organ damage, or even death. Identifying the factors that predispose individuals to COVID-19 susceptibility and poor outcomes has been the subject of intense research. One such area of investigation is the role of genetics in COVID-19 susceptibility and its exacerbation. Genetic factors have been proposed to contribute to the susceptibility to and severity of COVID-19.

Genome-wide association studies (GWAS) have been conducted to investigate the genetic factors that may contribute to the susceptibility and exacerbation of COVID-19 (3,4). These studies identified genetic variations associated with an increased risk of developing COVID-19 and severe clinical outcomes. These genetic variations affect various aspects of the immune response, including cytokine regulation, immune cell responses, and susceptibility to viral infections (4). One GWAS investigated different genetic mechanisms associated with COVID-19 in a large cohort of individuals. The study highlighted several single-nucleotide polymorphisms (SNPs) in several genes, such as interleukin-6 (IL6), tumor necrosis factor (TNF) and genes involved in angiotensin-converting

enzyme 2 (ACE2) regulation, which have been proven to be involved in the immune response. Other significant SNPs were identified in the *NOTCH4* (rs3131294) and *TYK2* (rs74956615) genes, which have been proven to be associated with critical illness in COVID-19 patients and an exacerbating effect in these patients (3).

TYK2 encodes a tyrosine kinase involved in cytokine signaling pathways, including those of type I and III interferons (IFN) (5). *TYK2* is also associated with the IFNAR1 subunit of type 1 IFN receptors (6). Interferons play a critical role in the antiviral immune response by inducing the expression of genes that inhibit viral replication and promote the destruction of infected cells. Several studies have identified *TYK2* variants that are associated with an increased risk of severe COVID-19 symptoms. For example, Pairo-Castineira *et al* (3,4) reported that a missense variant in *TYK2* (p.Arg203His) is associated with a higher risk of respiratory failure in COVID-19 patients (3,4). Another study reported that *TYK2* haploinsufficiency is associated with an increased risk of severe COVID-19 in a cohort of patients with innate immunity defects. A GWAS conducted by the COVID-19 Host Genetics Initiative found that genetic variations in *TYK2* were associated with an increased risk of severe COVID-19. Specifically, a study found that a rare variant of *TYK2* is associated with a 3.5-fold increased risk of severe COVID-19 (7). Another study identified that the rs2304257C>T polymorphism in *TYK2* is associated with increased susceptibility to severe COVID-19 in the Chinese population (8). These findings highlight the importance of *TYK2* in the immune response to SARS-CoV-2 infection, and suggest that *TYK2* variants may serve as biomarkers for predicting COVID-19 outcomes.

Another genetic factor implicated in COVID-19 susceptibility and exacerbation is *NOTCH4*, which encodes a transmembrane receptor involved in immune system regulation. NOTCH signaling plays a critical role in the differentiation and function of immune cells, including T, B, and myeloid cells. *NOTCH4* gene expression is increased in patients with COVID-19 (9). This increased expression results in the disruption of the repair mechanism in Regulatory T cells, leading to the exacerbation of respiratory infections in COVID-19 patients (10). This also indicates that *NOTCH4* signaling is an obstacle to tissue repair and a promoter of more severe respiratory infections (10,11). Several studies have reported an association between *NOTCH4* variants and the risk of developing severe COVID-19 (3,10,11). These findings suggest that *NOTCH4* plays a critical role in the immune response to SARS-CoV-2 infection and may serve as a potential therapeutic target for COVID-19.

The present study aimed to investigate the association between genetic variants of *TYK2* (rs74956615) and *NOTCH4* (rs3131294) and the severity and mortality of COVID-19 in a Jordanian cohort.

Materials and methods

Study design and population. The present study included individuals diagnosed with COVID-19 between March 2020 and February 2021 who were hospitalized at the Prince Hamzah Hospital in Amman, Jordan. Cases were defined as individuals with laboratory-confirmed COVID-19 infection as determined by a positive PCR test. All participants in the COVID-19 study were Jordanian. The clinical characteristics of the included patients are given in Table I.

The present study conducted a post-hoc power analysis to assess the adequacy of the sample size and robustness of the observed associations. Using the effect sizes derived from the logistic regression models and the proportion of variant carriers in the cohort, the analysis demonstrated that the study had sufficient statistical power (>80% for the primary outcomes) to detect moderate-to-large effect sizes for both *TYK2* and *NOTCH4* variants in the cohort.

The power calculation was based on the number of cases with complete genotype and outcome data included in the regression models (complete-case analysis), excluding individuals with missing genotypic data.

Sample collection. A total of 410 peripheral blood samples were collected in ethylenediaminetetraacetic acid (EDTA)-treated tubes and labeled with the same medical record ID number for each patient to facilitate access to the patient's medical file. The medical records and samples were accessed during the period February 2021 to December 2022. The collected blood samples were transferred on ice to the -80˚C freezers at Applied Science University, Amman, Jordan. DNA extraction and PCR amplification were conducted in the Laboratory for Molecular and Microbial Ecology (LaMME) at the University of Jordan, Amman, Jordan. Sanger sequencing was conducted through the service provider Macrogen, Inc. Biometric data were collected for each patient, such as the severity index of the case, concentrations of O_2 and CO_2, the need to be placed on oxygen ventilation, and other disease variables that would help establish a correlation between genomic information and disease status. All these data were collected during the study period (between March 2020 and February 2021). Ethical approval was obtained from the ethics committee of Prince Hamza Hospital (approval no. 6-11-2021-129), and the samples were collected respectively. The requirement for written informed consent was formally waived by the Institutional Review Board. All procedures were conducted in accordance with institutional ethical standards and the Declaration of Helsinki.

DNA extraction, quantification, amplification and DNA sequencing. Genomic DNA was extracted from blood samples using the Wizard Genomic DNA Purification Kit (Promega Corporation), following the manufacturer's protocol. DNA yield and quality were measured using NanoDrop to ensure that the amount of DNA in each sample was sufficient for PCR and to ensure the efficiency of the extraction kit and protocol.

PCR amplification of *NOTCH4* and *TYK2* was performed using the following primers: *NOTCH4* Forward 5'-CCATCT CTGGGCTGAGAATC-3', *NOTCH4* Reverse 5'-GCCTCA AGTGAGGACAAGTG-3', *TYK2* Forward 5'-GGGCCTTGA AGGAATCAGAG-3' and *TYK2* Reverse 5'-CCCTGCAGC CTTAAGAGAGA-3'. Thermocycling was initiated with a denaturation step at 95˚C for 3-5 min, followed by 30 cycles consisting of denaturation at 95˚C for 30 sec, annealing at 56˚C for 30 sec and elongation at 72˚C for 30-45 sec, with a final extension completed at 72˚C for 10 min. Subsequently, the amplified products were resolved on a 1.5% agarose gel

Table I. Clinical characteristics of the included patients.

Parameter	Frequency	Percentage	Mean ± standard deviation
Total patients	365	100.0	
Sex			
Male	206	56.6	
Female	157	43.4	
Age			54.28 (±16.000)
COVID-19			
Non-severe	141	39.0	
Severe	117	32.3	
Critical	104	28.7	
Clinical outcome			
Non-survivor	82	22.7	
Survivor	280	77.3	
ICAM5-*TYK2*_variant			
TT (reference genotype)	104	28.7	
TA (heterozygous genotype)	13	3.6	
AA (homozygous variant genotype)	2	0.6	
Missing	243	67.1	
*NOTCH4*_variant			
CC (reference genotype)	252	69.6	
CT (heterozygous genotype)	28	7.7	
TT (homozygous variant genotype)	3	0.8	
Missing	79	21.8	

and visualized using ethidium bromide. The amplified DNA was subjected to Sanger sequencing (Macrogen, Inc.) (12,13).

Bioinformatics analysis. After obtaining the sequenced products, full-sequence analysis of the genes was performed for each sample. The resulting genomic data were then compared with different patient variables collected previously to assess the significance and correlation of polymorphisms in the genes with the disease severity.

Data collection occurred during early 2020-2021; therefore, the dominant variant circulating in Jordan was the ancestral strain and before the widespread emergence of variants of concern (VOCs). During this period, the dominant circulating strain in Jordan corresponded to early SARS-CoV-2 lineages closely related to the Wuhan-Hu-1 reference strain (lineage B).

Methodology for stratifying the severity and clinical outcomes of COVID-19. The present study extracted all patient data from the medical records. The data included age, sex, length of hospital stay, comorbidities, treatment, laboratory results, and clinical outcomes.

Patients were divided into two classes. The first was discharge status: Survivor or non-survivor. Disease severity (non-severe, severe, or critical) was used to determine the second classification. The WHO established this classification using the same cohort as that in a recent study (14).

Non-severe cases were defined as individuals with peripheral capillary oxygen saturation (SpO_2) ≥94% on room air, no signs of respiratory distress, and normal or mild radiographic findings. Severe disease was classified by the presence of SpO_2 <94%, respiratory rate ≥30 breaths/min, or radiological evidence of pneumonia involving >50% of the lung fields within 24-48 h. Critical cases were defined as patients presenting with respiratory failure requiring mechanical ventilation, shock, or multi-organ dysfunction.

Statistical analysis. Data analysis was conducted using SPSS version 26 (IBM Corp.). When applicable, continuous variables were reported as the mean with standard deviation or the median with interquartile range. Categorical variables are presented as frequencies and percentages. The χ^2 test and Fisher's exact test were used to compare various categorical variables, specifically between survivor and non-survivor categories, as well as between different categorical variables and the severity classification of COVID-19 patients. Logistic regression models were used to assess the association between genetic variations in *TYK2* and *NOTCH4* and COVID-19 severity.

Binomial logistic regression models were constructed to evaluate the association between *TYK2* and *NOTCH4* variants and COVID-19 severity and mortality. All models were modified for significant clinical covariates, including age and sex, due to their recognized influence as predictors of COVID-19 outcomes. The adjusted odds ratios (ORs) with 95% confidence intervals (CIs) were reported.

Bonferroni correction was used on the set of statistical tests that had already been chosen to take into account multiple comparisons. In particular, there were eight comparisons,

Table II. Genotypic distribution, allelic frequencies, Hardy-Weinberg equilibrium and association analysis of *TYK2* and *NOTCH4* variants.

A, *TYK2* (rs74956615)

Allele Frequencies	Allele Count (n)	Frequency (%)
T	221	92.9%
A	17	7.1%

Model	Outcome	Adjusted OR (95% CI)	P-value
Dominant (TA+AA vs. TT)	Severity	2.55 (0.83-7.84)	0.104
Dominant (TA+AA vs. TT)	Mortality	8.29 (2.53-27.13)	<0.001

B, *NOTCH4* (rs3131294)

Category	CC (Ref)	CT	TT	Total (n)	HWE χ^2 (P-value)
Genotype n (%)	252 (89.0%)	28 (9.9%)	3 (1.1%)	283	4.33 (0.037)

Allele Frequencies	Allele Count (n)	Frequency (%)
C	532	94.0%
T	34	6.0%

Model	Outcome	Adjusted OR (95% CI)	P-value
Dominant (CT+TT vs. CC)	Severity	15.29 (1.95-120.02)	0.010
Dominant (CT+TT vs. CC)	Mortality	8.14 (2.35-28.25)	0.001

Frequencies calculated excluding missing genotypes. ORs derived from logistic regression models adjusted for age and sex. Dominant model used due to low frequency of homozygous variant genotypes. HWE, Hardy-Weinberg equilibrium assessed using χ^2 test on complete-case genotypic data; OR, odds ratios; CI, confidence interval.

and the level of statistical significance was changed to match (α=0.05/8 = 0.00625). All reported P-values were analyzed in relation to this adjusted significance level. P<0.05 was considered to indicate a statistically significant difference.

Results

A total of 362 individuals diagnosed with COVID-19, comprising diverse populations from different geographic regions, were included in the analysis. Participants were categorized into three groups based on disease severity: Non-severe, severe and critical, and into two groups based on clinical outcomes: non-survivors and survivors. The clinical characteristics of the study participants were presented in Table I.

Genotypic and allelic distributions of *TYK2* (rs74956615) and *NOTCH4* (rs3131294), along with Hardy-Weinberg equilibrium testing and association measures, were summarized in Table II. The distribution of genotypes showed a predominance of the wild-type alleles for both SNPs, with relatively low frequencies of homozygous variant genotypes. HWE analysis based on complete-case data indicated no major deviation for *TYK2* (P>0.05), whereas a slight deviation was observed for *NOTCH4*, which may reflect small genotype counts or missing data rather than genotyping error.

TYK2 gene variants and COVID-19 severity. Analysis of *TYK2* variants revealed that 94.6% of the participants in the non-severe category had the homozygous reference genotype, 3.6% had the heterozygous genotype, and 1.8% had the homozygous variant genotype. The severe category showed 94.6% homozygous reference and 5.4% heterozygous genotypes. The critical group comprised 61.5, 34.6 and 3.8% of patients with homozygous reference, heterozygous, and homozygous variant genotypes, respectively. These findings indicate that the critical group included the highest heterozygous genotype compared to the other genotypes, followed by the homozygous variant genotype (Pearson's chi-square test, P<0.001; Table III).

NOTCH4 variants and COVID-19 severity. Analysis of *NOTCH4* gene variants revealed that 99% of the participants in the non-severe category were homozygous for the reference genotype and 0.8% were heterozygous. The severe category showed 94.0% homozygous reference and 6.0% heterozygous genotypes. The critical group was distributed

Table III. Distribution of the *NOTCH4* and *TYK2* gene variants according to COVID-19 severity and mortality.

Gene variant	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
ICAM5-*TYK2*_variant	TT (reference genotype)		TA (heterozygous genotype)		AA (homozygous variant genotype)	
non-severe	53	95%	2	4%	1	1%
severe	35	95%	2	5%	0	0
critical	16	62%	9	34%	1	4%
*NOTCH4*_variant	CC (reference genotype)		CT (heterozygous genotype)		TT (homozygous variant genotype)	
non-severe	129	99%	1	1%	0	0
severe	79	94%	5	6%	0	0
critical	44	64%	22	32%	3	4%
ICAM5-*TYK2*_variant	TT		TA		AA	
Non-survivor	10	56%	7	39%	1	5%
Survivor	94	93%	6	6%	1	1%
*NOTCH4*_variant	CC		CT		TT	
Non-survivor	38	67%	16	28%	3	5%
Survivor	214	95%	12	5%	0	0

at 64, 32 and 34% for the homozygous reference, heterozygous, and homozygous variant genotypes, respectively. These findings indicated that the critical group included the highest proportion of heterozygous genotypes compared to other genotypes, followed by the homozygous reference genotype (Pearson's χ^2 test, P<0.001; Table III).

TYK2 gene variants and clinical outcome. In terms of clinical outcomes, the presence of certain *TYK2* variants was markedly associated with COVID-19 mortality. The TT variant was found in 93% of survivors and 56% of non-survivors. The TA variant was detected in 39 and 6% of the non-survivors and survivors, respectively. Moreover, the AA homozygous variant genotype was detected in 5.6% of the non-survivor group and 1.0% of the survivor group (P<0.001; Table III).

NOTCH4 gene variants and clinical outcome. In terms of clinical outcomes, the presence of specific *NOTCH4* variants was markedly associated with COVID-19 mortality. The TT variant was found in 94.7% of survivors and 66.7% of non-survivors. The TA variant was detected in 28.1 and 5.3% of the non-survivors and survivors, respectively. Moreover, the AA homozygous variant genotype was detected in 5.3% of the non-survivor group and 0.0% of the survivor group (P<0.001; Table III).

Binomial logistic regression was performed to ascertain the effects of the variants of the two tested genes on the likelihood of severe or critical status and mortality. The linearity of continuous variables with respect to the logit of the dependent variable was assessed using Tidwell's method (15). The Bonferroni correction was applied using all eight terms in the model, resulting in statistical significance being accepted when P<00625 (16). Based on this assessment, all continuous independent variables are linearly related to the logit of the dependent variable. The logistic regression model was statistically significant, $\chi^2(2)=17.188$, P<0.001 and $\chi^2(2)=24.476$, P<0.001 for the severity and mortality statistics, respectively.

The heterozygous or variant *NOTCH4* genotypes were 15. The odds of exhibiting a severe or critical state of COVID-19 were 285 times higher than those of the reference genotype (P=0.010). Furthermore, heterozygous and variant genotypes of both genes (*NOTCH4* and ICAM5-*TYK2*) had approximately eight-fold higher odds of mortality than the reference genotype (P=0.001 and <0.001, respectively; Table IV).

Discussion

The present study addressed the association between genetic variants of *TYK2* and *NOTCH4* and the severity and clinical outcomes of COVID-19. The analysis encompassed a diverse population of 362 individuals from different geographic regions, who were categorized based on disease severity and clinical outcomes. The findings revealed significant associations between specific gene variants and the severity and mortality of COVID-19. The present study found that variant alleles of *TYK2* and *NOTCH4* were increased in individuals with COVID-19 as a function of disease severity and were associated with heightened mortality, demonstrating that this mechanism is effective in SARS-CoV-2 infection and may be critically involved in the pathogenesis of acute respiratory failure.

TYK2 produces tyrosine kinase, which is essential for cytokine signaling, particularly in the type I and III interferon pathways (5). *TYK2* plays an essential role in the stable expression of IFNAR1 on the cell surface (17,18). Thus, appropriate *TYK2* activity is a key step in the initiation of the type I IFN response (17,18). IFNs are important antiviral cytokines that induce the expression of genes that inhibit viral replication during the early stages of viral

Table IV. Binomial logistic regression results.

Outcome	Gene	B	SE	Wald	df	Sig.	OR	95% CI EXP(B)	
Severity (non-severe vs. severe/critical								Lower	Upper
	*NOTCH4*_variant (heterozygous or homozygous variant genotype)	2.727	1.051	6.726	1	0.010	15.285	1.947	120.016
	ICAM5-*TYK2*_variant (heterozygous or homozygous variant genotype)	0.934	0.574	2.648	1	0.104	2.545	0.826	7.842
Mortality (survivor vs. non-survivor)									
	*NOTCH4*_variant (heterozygous or homozygous variant genotype)	2.097	.635	10.921	1	0.001	8.143	2.348	28.247
	ICAM5-*TYK2*_variant (heterozygous or homozygous variant genotype)	2.114	.605	12.205	1	<0.001	8.285	2.530	27.132

B, regression coefficient; SE, standard error; df, degrees of freedom; OR, odds ratio; CI, confidence interval; EXP(B), exponentiated regression coefficient (odds ratio); ICAM5, intercellular adhesion molecule 5; TYK2, tyrosine kinase 2; NOTCH4, Notch receptor 4.

infection (6,17,18). Studies have shown that COVID-19 infection does not elicit a competent IFN response, leading to decreased disease severity (19,20). The analysis of *TYK2* variants revealed a strong association between them and COVID-19 severity. This association is congruent with that reported by Pairo-Castineira *et al* (3), who reported that rs74956615 increases the severity of COVID-19 by 1.6 and has a very strong P-value (OR=1.6, discovery $P=2.3x10^{-8}$). Located within the chromosome 19 genomic sequence (NCBI Accession: NC_000019.10), this regulatory eQTL locus (at position 10,317,045) is highly critical because its risk allele correlates with upregulated *TYK2* expression, functioning as an inflammatory driver that promotes the hyper-activation of downstream JAK-STAT cytokine signaling during severe infection (3). The genotype distribution varied markedly among the non-severe, severe and critical groups, with the critical group exhibiting a higher proportion of heterozygous genotypes than those in the other groups. This finding suggests a potential role for *TYK2* variants in influencing the progression of critical illness (7). The observed association aligns with the existing literature implicating *TYK2* in immune response regulation, supporting the idea that genetic variations in this gene may contribute to diverse clinical outcomes in COVID-19 patients (7). These findings may contribute to an improved understanding of the mechanisms underlying the disease progression.

Several studies have identified *TYK2* variants that are associated with an increased risk of severe COVID-19 symptoms. For example, Pairo-Castineira *et al* (3) reported that a missense variant in *TYK2* (p.Arg203His) is associated with a higher risk of respiratory failure in COVID-19 patients (4,17). Another study reported that *TYK2* haploinsufficiency is associated with an increased risk of severe COVID-19 in patients with inborn errors of immunity (5). Notably, a study found that a rare variant of *TYK2* is associated with a 3.5-fold increased risk of severe COVID-19 disease (7). Another study identified that the rs2304257C>T polymorphism in *TYK2* is associated with increased susceptibility to severe COVID-19 in the Chinese population (8). These findings highlight the importance of *TYK2* in the immune response to SARS-CoV-2 infection, and suggest that *TYK2* variants may serve as biomarkers for predicting COVID-19 outcomes. As aforementioned, *TYK2* prevents the accumulation of IFNAR1 in the intracellular compartment and increases its stabilization at the cell surface (17,18). However, another route of IFNAR1 stabilization at the cell surface is *TYK2* independent. For example, the RNA-binding protein RBM47 stabilizes IFNAR1 transcripts (21).

NOTCH4 is a critical regulator of disease severity in various respiratory viral infections. *NOTCH4* encodes a transmembrane receptor crucial for immune system regulation. NOTCH signaling is vital for the differentiation and function of immune cells, such as T, B and myeloid cells. Patients with COVID-19 exhibit elevated *NOTCH4* gene expression levels, indicating its potential involvement in the disease (9).

The findings of the current study revealed that *NOTCH4* variants were substantially associated with the severity of COVID-19. The critical group displayed a higher prevalence of heterozygous genotypes, indicating a potential correlation between *NOTCH4* variants and critical illness development. This finding was consistent with the role of *NOTCH4* in immune regulation and suggested that genetic variations in this gene may contribute to the variability in COVID-19 severity. Studies have reported that *NOTCH4* expression increases in tissue Treg cells early in lung inflammation and promotes the innate immune response when adaptive

immunity has not yet been effectively mobilized (10,11). In the context of severe virus-mediated damage, *NOTCH4* promotes the development of an intrinsic protective response driven by lung epithelium-derived danger signals in the host. This physiological function of *NOTCH4* is lost in severe respiratory viral diseases, leading to uncontrolled and excessive activation of innate immunity, which harms lung tissue (10). Moreover, several studies have reported an association between *NOTCH4* variants and the risk of severe COVID-19 (3,22,23). These findings suggest that *NOTCH4* plays a critical role in the immune response to SARS-CoV-2 infection and may serve as a potential therapeutic target for COVID-19. These findings could aid in the development of targeted therapeutic approaches for managing COVID-19 based on genetic profiles.

Examining the association between gene variants and clinical outcomes, the present study found that specific *TYK2* variants were markedly associated with COVID-19 mortality. TA and AA variants were more prevalent in the non-survivor group, whereas the TT variant was predominant in the survivor group. This emphasized the potential prognostic value of *TYK2* variants in predicting mortality outcomes in COVID-19 patients. Similarly, specific *NOTCH4* variants were found to be markedly associated with COVID-19 mortality. The non-survivor group exhibited a higher prevalence of TA and AA variants, indicating the potential role of *NOTCH4* gene variants as predictors of adverse clinical outcomes in COVID-19 patients.

GWAS examining genetic factors in COVID-19 susceptibility and severity have identified variations associated with an increased risk of contracting the virus and experiencing severe clinical outcomes (3,4,8). These genetic variations affect diverse aspects of the immune response, including cytokine regulation, immune cell responses, and susceptibility to viral infections (4). Notably, specific SNPs in genes such as *NOTCH4* (rs3131294) and *TYK2* (rs74956615) have been linked to critical illnesses in COVID-19 patients, underscoring the genetic basis of disease exacerbation (3,7).

The methodology for accurately stratifying the exacerbation level of COVID-19 patients can be based on a combination of clinical prediction scores, radiographic imaging and biomarkers. Studies have shown that combining these methodologies can lead to a more accurate and holistic assessment of a patient's condition (24-26). Binomial logistic regression analysis confirmed the significant effects of *TYK2* and *NOTCH4* variants on COVID-19 severity and mortality. The model explained a considerable proportion of the variance in both the severity and mortality, highlighting the potential predictive power of these genetic variants. Notably, individuals with heterozygous or variant alleles of *NOTCH4* have markedly higher odds of exhibiting severe or critical disease, emphasizing the clinical relevance of this gene in determining disease severity. Additionally, heterozygous or variant alleles of both *NOTCH4* and *TYK2* were associated with increased odds of mortality, highlighting the potential utility of genetic information in identifying patients at a higher risk of adverse outcomes.

It is important to note that the methods mentioned in the present study have been utilized in several studies; however, it is recommended that further validation studies be conducted on diverse populations worldwide, including the population in which the present study was conducted. Additionally, these methods should not be used in isolation, but as part of an integrated approach that considers other risk factors, such as environmental and lifestyle factors.

Collapsing the heterozygous and mutant genotypes into a single 'variant-carrier' category is justified and consistent with the biological rationale and statistical characteristics of the present study dataset. First, the homozygous mutant genotype was extremely rare in the cohort, accounting for <1% of patients. Separate estimation of the effects of the mutant genotype would yield unstable odds ratios with wide confidence intervals and limited interpretability. Combining heterozygous and mutant individuals into a single 'variant-present' category is an accepted approach for rare alleles in genetic epidemiology and preserves the statistical power without introducing artificial comparisons. Second, previous GWAS and functional studies have demonstrated that both heterozygous and homozygous non-reference forms of *TYK2* and *NOTCH4* produce a similar directional biological effect on interferon signaling and immune dysregulation. Therefore, expanding the analysis to include additive, recessive and codominant models is unlikely to provide additional meaningful insights and may instead introduce unstable estimates due to sparse cell counts. Thus, the current modelling strategy remains justified.

Furthermore, the multiple-testing strategy employed in the present study must be understood within the framework of a hypothesis-driven design. The analysis concentrated on two predetermined SNPs (*TYK2* rs74956615 and *NOTCH4* rs3131294) derived from previous GWAS findings, rather than employing an exploratory genome-wide or multi-locus screening methodology. The Bonferroni correction for the small number of planned comparisons (eight tests) is therefore considered appropriate and sufficiently conservative. Extending the correction to encompass all potential genetic inheritance models (additive, recessive, and codominant) or implementing false discovery rate (FDR) adjustments would not conform to the established analytical framework and could introduce superfluous statistical penalties, especially in the context of sparse genotype frequencies.

The present study had some limitations. The sample size of the present study was relatively small. Future studies with larger sample sizes could provide more definitive insights into the roles of *TYK2* and *NOTCH4* in COVID-19 susceptibility and exacerbation. Additionally, the genetic factors examined accounted for only a small proportion of the COVID-19 susceptibility and severity. Other genes, environmental and clinical factors (such as treatment strategies) may also serve a role in COVID-19 severity and mortality; however, these factors were not analyzed in the present study, which is a limitation. Finally, data were collected during early 2020-2021; therefore, the dominant variant circulating in Jordan was the ancestral strain. Another limitation of the present study is the use of a dominant genetic model, whereby heterozygous and homozygous variant genotypes were combined due to the very low frequency of the mutant genotype; although this approach preserved statistical power, it precluded evaluation of additive or

recessive effects, which could be explored in larger cohorts with sufficient genotype representation.

Another limitation pertains to the multiple-testing correction strategy. The present study applied the Bonferroni correction to the predefined comparisons, but did not apply it to all possible genetic models (additive, recessive and codominant) or adjust for FDR. This approach aligns with the study's hypothesis-driven design; however, it may increase the risk of residual type I error when interpreting the reported associations.

A further constraint is the absence of functional validation for the identified genetic associations within the study cohort. While rs74956615 has established eQTL regulatory roles (3), no independent mechanistic analyses (such as eQTL or sQTL evaluations) were conducted here to ascertain their specific biological impacts in the sample population. As a result, while these associations align with known expression pathways, direct causality within the cohort has yet to be determined.

In conclusion, the present study provided valuable insights into the roles of *TYK2* and *NOTCH4* variants in the severity and clinical outcomes of COVID-19. Understanding the genetic determinants of disease progression may contribute to the development of personalized and effective management strategies for COVID-19 patients. Further research and validation studies are warranted to confirm these associations and to explore the mechanisms underlying the interplay between host genetics and COVID-19 outcomes. Accurately stratifying the exacerbation level of COVID-19 patients is crucial for identifying high-risk patients who require intensive care and for guiding treatment and management strategies. A scientific methodology based on clinical data, including clinical prediction scores, radiographic imaging and biomarkers, combined with a multidisciplinary approach, can be used to accurately stratify COVID-19 patients based on the severity of their illness. Further research is needed to validate these methods and determine the most effective approach for stratifying COVID-19 patients based on disease severity. The present study provided evidence that genetic factors, specifically *TYK2* and *NOTCH4* variants, may contribute to COVID-19 susceptibility and severity. These findings have important implications for understanding the underlying mechanisms of COVID-19 and developing more targeted prevention and treatment strategies.

Acknowledgements

The present study benefited from the Prince Hamzah Hospital, which provided support in collecting COVID-19 samples for this research. The authors also relied on the data and assistance provided by the hospital staff, which were essential to the completion of the present study.

Funding

The present study was supported by the Deanship of Scientific Research at the University of Jordan through a sabbatical leave for Dr. Mamoon Al-Rshaidat during the academic year 2024-2025.

Availability of data and materials

The data generated in the present study are included in the figures and/or tables of this article.

Authors' contributions

Conceptualization was by MA, AA, HA and MZ. Formal analysis was by MA, AA, OA, AI and MZ. Project administration was by MA, AA and MZ. Writing the original draft was by MA, AA, OA and MZ. Writing, review and editing was by MA, AA, OA, AI, HA and MZ. MA, AA and MZ confirm the authenticity of all the raw data. All authors reviewed and approved the final manuscript.

Ethics approval and consent to participate

Ethical approval was obtained from the ethics committee of Prince Hamza Hospital (approval no. 6-11-2021-129) and the samples were collected respectively. The requirement for written informed consent was formally waived by the Institutional Review Board. All procedures were conducted in accordance with institutional ethical standards and the Declaration of Helsinki.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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