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# Implication of IL-12A, IL-12B, IL-6, and TNF single-nucleotide polymorphisms in severity and susceptibility to COVID-19

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## Abstract

**Background:** The Coronavirus Disease 2019 (COVID-19) pandemic has led to significant global morbidity and mortality. Understanding the genetic factors that influence disease outcomes can provide critical insights into pathogenesis and potential therapeutic targets.
**Objective:** This study aimed to investigate the potential correlation between single nucleotide polymorphisms (SNPs) in *Interleukin 12 Subunit Alpha (IL-12A)*, *Interleukin 12 Subunit Beta (IL-12B)*, *Interleukin 6 (IL-6)*, and *Tumor Necrosis Factor (TNF)* genes and the severity as well as susceptibility to COVID-19 among Moroccan patients.
**Patients and Methods:** Next-Generation sequencing (NGS) was conducted on 325 Moroccan participants, 207 patients with PCR-confirmed Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection and 118 controls. Among these patients, 51% presented moderate to severe symptoms requiring hospitalization, while 49% were asymptomatic or experienced mild symptoms and did not require hospitalization. Statistical analysis was performed using codominant, dominant, and recessive logistic regression models to assess correlations with the severity and susceptibility to COVID-19 infection.
**Results:** No association was found between SNPs of *IL-12A*, *IL-12B*, *IL-6* or *TNF* and COVID-19 severity and susceptibility. However, our results unveiled a noteworthy association with *IL-6* rs2069840, which exhibited a negative correlation (OR = 0.21, 95% CI = 0.07-0.69, $p$ = .006), suggesting a protective effect against SARS-CoV-2 infection.
**Conclusion:** Polymorphisms in *IL-12A*, *IL-12B*, *IL-6*, and *TNF* genes are not correlated to the severity and susceptibility of COVID-19.

## Keywords

COVID-19, host genetics, NGS, IL-12A, IL-12B, IL-6, TNF

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## Introduction

COVID-19 pandemic has presented a global health challenge, causing morbidity and mortality worldwide. Since the beginning of the pandemic, there has been significant research dedicated to understanding the genetic factors influencing the severity and susceptibility to COVID-19.[1] Initially, studies concentrated on identifying the genetic determinants within hosts, especially those related to the severity of the disease and the susceptibility of SARS-Cov-2 infection. In May 2020, the COVID-19 Host Genetics Initiative (HGI) was founded with the aim of uniting the worldwide human genetics community to collect, exchange, and examine data concerning COVID-19 susceptibility, severity, and outcomes.[2]

The severity of SARS-CoV-2 infection exhibits considerable variation among individuals, ranging from asymptomatic cases to severe respiratory distress and multi-organ failure leading to critical outcomes. This variability can be attributed to several factors. Identified risk factors include older age (above 65 yeas), male sex and comorbidities such as diabetes, obesity, cardiovascular disease, hypertension and chronic pulmonary, kidney or liver disease.[3] Among these factors, host genetics has emerged as a crucial determinant of susceptibly and severity of SARS-CoV-2 infection. Large cohort studies, such as Genome-Wide Association Studies (GWAS), have demonstrated that genetic variations within the human genome can significantly influence the response to SARS-CoV-2 infection.[4]

Plasma cytokine levels have been assessed in COVID-19 patients and correlated with disease outcomes, the most severe stage is related to an extrapulmonary systemic hyperinflammation syndrome, where increased cytokine levels exacerbate lung inflammation and contribute to the development of acute respiratory distress syndrome (ARDS), multiple organ failure, and potentially fatal outcomes. IL-6 and TNF- α are two critical components of the cytokine storm.[5] IL-6 plays a critical role in driving hyperinflammation in SARS-CoV-2 infection, with its levels being strongly associated with disease progression, it has been used to monitor the severity of COVID-19. Elevated IL-6 levels have been linked to severe respiratory distress and poorer outcomes in COVID-19 patients. Variability in *IL-6* genes remains important to understand the mechanisms behind IL-6 expression and its impact on disease severity. Moreover, SNPs in the *IL-6* genes vary significantly between different ethnic populations and are associated with disparities in the response to various infectious diseases.[6] TNF-α is also implicated in COVID-19 severity and mortality, as elevated levels have been observed in critical COVID-19 patients, as well as in patients with ARDS and acute kidney injury. The *TNF* SNPs (rs1800629 and rs361525) are considered crucial variants in human infectious diseases susceptibility, potentially influencing cytokine gene transcription. In the context of COVID-19, *TNF* rs1800629 promoter variant has been studied and associated with susceptibility to the disease and a more aggressive disease progression pattern. Studying the *TNF* gene across populations of different ethnicities remains crucial for understanding high levels of TNF-α among COVID-19 patients.[5,7] IL-12 is a key pro-inflammatory cytokine crucial for developing antiviral immune responses, it is considered as a determinant factor bridging innate and acquired immunity. Variability of cytokine synthesis amount is genetically determined by SNPs in *IL-12A* and *IL-12B*, and has been associated with susceptibility and severity to several infectious diseases and response to antiviral therapy.[8,9] Understanding the genetics underpinnings of COVID-19 susceptibility and severity among Moroccan patients is imperative to understand the heterogeneity observed in clinical outcomes and to improve approaches for disease management and prevention.

This study aims to investigate the correlation of host genetics single nucleotide polymorphisms (SNPs) in immunity genes *IL-12A*, *IL-12B*, *IL-6* and *TNF* with the susceptibility and the severity among Moroccan COVID-19 patients. The data derived from our study is valuable for elucidating the role of host genetics in the pathogenesis of the disease.

## Patients and methods

### Study population

The current study constitutes a case-control association study involving 325 Moroccan participants, consisting of 207 patients COVID-19 positive Polymerase Chain Reaction (PCR) and 118 controls COVID-19 negative PCR. These participants were recruited from three distinct hospital centers participating in a national COVID-19 research consortium. These centers include the Medical Emergency Department at Ibn Sina University Hospital Center in Rabat, the intensive care unit (ICU) at Cheikh Zaid International University Hospital in Rabat, and the Center of Virology, Infectious and Tropical Diseases, Mohamed V Military Teaching Hospital. The study was conducted during a period of the pandemic dominated by the alpha and beta variants.

All participants completed a form detailing socio-demographic information to establish ethnicity, medical history and comorbidities. Patients were categorized as asymptomatic, mild, moderate, and severe. Asymptomatic and mild cases were combined ($N = 101$) and compared to moderate/severe cases ($N = 106$). The control group participants ($N = 118$) tested negative for SARS-CoV-2 PCR; however, they had all been exposed to the virus at least

once, either through family contact or professional exposure during the recruitment year.

### Inclusion criteria

Inclusion criteria encompassed individuals of Moroccan ethnicity, aged 18 to 65 years, who tested positive for SARS-CoV-2 virus through real-time PCR and had not received any vaccinations. Pregnant women were excluded from participation.

### Phenotype definition

COVID-19 disease phenotype was assessed following The National Health Commission and the National Administration of Traditional Chinese Medicine, based on the severity of symptoms and medical needs. The severe cases involve patients exhibiting serious symptoms such as respiratory distress, low blood oxygen levels, or signs of severe pneumonia, requiring immediate medical attention and often hospitalization for intensive care. Moderate cases typically present with fever, cough, and respiratory issues, but do not have severe complications, necessitating medical monitoring and treatment without intensive care. Mild cases entail individuals experiencing mild symptoms like fever, cough, fatigue, and body aches, recovering at home with rest and supportive care without hospitalization. Asymptomatic cases refer to those testing positive for the virus without displaying any symptoms.[10]

To investigate the association of COVID-19 severity, all the positive COVID-19 patients ($N = 207$) were categorized based on hospitalization status: asymptomatic and mild for non-hospitalized patients, as well as moderate and severe for hospitalized patients. Additionally, for susceptibility studies, asymptomatic, mild, moderate, and severe group patients were categorized as Positive COVID-19, while the negative control group consisted of individuals who tested negative for SARS-CoV-2 PCR despite their exposure to the virus in a familial or medical context. All the patients and controls were screened with Next-Generation sequencing.

### Genetic study

For all studied patients, 5 mL EDTA tube were collected and stored at −20°C. DNA was then extracted using the MagPurix® Blood DNA Extraction Kit with a Zinexts MagPurix EVO 24 CE IVD system (Zinexts Life Science Corp., New Taipei City, Taiwan). The quality and quantity of DNA were evaluated using the NanoDrop One and the Qubit dsDNA HS Assay Kit with the Qubit fluorometer (ThermoFisher Scientific). Subsequently, gene-panel next-generation sequencing (NGS) including *IL-12A*, *IL-12B*, *IL-6* and *TNF* was conducted on all participants using Ion Torrent technology (Thermo Fisher Scientific). Library preparation, bead templating (emulsion PCR), and PI Chips v2 loading were carried out using the Ion Chef System, followed by sequencing on the Ion S5 machine. Subsequently, the reads were aligned with the reference genome (hg19) using the Ion Torrent Suite™ server. The generated VCF files were then uploaded to the Ion Reporter Software online server for variant analysis, filtering, and annotations. Variants were categorized based on their quality and functional consequence to select those of interest and compare their frequency across the different sample groups studied.

### Hardy-Weinberg Equilibrium analysis

To assess the genetic equilibrium of the study population, we conducted Hardy-Weinberg Equilibrium (HWE) tests for each locus. An exact test of Hardy-Weinberg Equilibrium was performed using SNPStats software.

### Statistical analysis

The normality of the distribution of continuous variables was assessed using the Kolmogorov-Smirnov test. Continuous variables are presented as median with interquartile range (IQR). Analysis of continuous variables between groups were performed using Mann-Whitney U test. Categorical variables are presented as frequencies and percentages. The Pearson's Chi-squared test and Fisher's exact test were used for comparison of categorical variables between groups. The association of SNPS with COVID-19 severity and susceptibility under codominant, dominant and recessive models, were evaluated using logistic regression analysis via SNPstats software. All analyses, including graphical representations, were performed using RStudio (version R 4.2.3). We considered a $p$ value <.05 to be statistically significant.

### Ethics committee

This study was approved by the ethics committee of Cheikh Zaid Hospital of Rabat (ID: CEFCZ/AB/PR_RFG). Written informed consent was diligently obtained from all participating patients, ensuring adherence to ethical standards.

## Results

Demographic and phenotypic data are presented in Table 1, the mean age of all patients was 45.9 ± 12.7 years, with a minimum age of 20 and a maximum of 68. Our findings reveal a statistically significant association between advanced age and disease severity when comparing between groups asymptomatic/mild and moderate/severe patients ($p < .001$). Additionally, the median ages were 41 and 54 respectively. As anticipated, the presence of

comorbidities in patients also showed a significant difference in COVID-19 phenotypes ($p$ = .007). However, there was no difference observed between genders ($p$ > .05).

Our analysis revealed several relatively rare variants, a total of 105 SNPs were detected; 22 in *IL-12A*, 45 in *IL-12B*, 17 in *IL-6*, and 21 in *TNF*. The SNPs subjected to association investigation had a MAF greater than 10%, as shown in Table 2. Among them, rs2243136 was located in the Intron, and rs35990253 was located in the exon (p.Val211Met) of the *IL-12A* gene. In *IL-12B*, rs11574790 and rs919766 were located in the intron, and rs3212227 was located in the 3′UTR region. *IL-6* and *TNF*, in turn, revealed one variant each rs2069840 and rs3093662, respectively, in the intron region. Additionally, one novel mutation was identified, namely c.-1 + 34T>G in intron region of *IL-12B*. COVID-19 is a multifactorial disease. According to the common disease/common variant hypothesis, only common variants with MAF >10% were considered. Furthermore, all these variants were in Hardy-Weinberg Equilibrium ($p$ > .05).

In terms of susceptibility to COVID-19 infection, comparative analysis between positive and negative groups unveiled polymorphisms across four loci, with no discernible significant disparity noted within the *IL-12A*, *IL-12B*, and *TNF* loci (Table 3). However, at the *IL-6* locus, rs2069840 G demonstrated a negative correlation (OR = 0.21 95% CI = 0.07-0.69 $p$ = .006), indicating a protective effect against susceptibility of COVID-19. Furthermore, no deviation from Hardy-Weinberg Equilibrium was observed for rs2069840 (HWE $p$ > .05).

To assess the association of the exhibited SNP with the severity of COVID-19, a comparative analysis was conducted between two groups: hospitalized and non-hospitalized participants (Table 4). Investigation of these SNPs, whether in codominant, dominant or recessive models, revealed no significant association with the severity of the disease ($p$ > .05).

## Discussion

In this study, we aimed to examine the association between several genetic variants within *IL-12A*, *IL-12B*, *IL-6*, *TNF* and disease severity among COVID-19 Moroccan patients, as well as their susceptibility to SARS-CoV-2 infection. Our results revealed several significant observations worthy of discussion.

As anticipated, Our demographic data showed a statistically significant association between advanced age of patients and disease severity. This is consistent with numerous prior studies identifying age as a major risk factor for severe forms of COVID-19. Our findings support this observation, with a higher median age among patients exhibiting severe forms of the disease. Additionally, the presence of comorbidities was also associated with

**Table 1.** Patients demographic by clinical phenotype.

| Characteristic | *N* | Overall, *N* = 207 | Asymptomatic/Mild *N* = 101 | Moderate/Severe *N* = 106 | *p*-value[a] |
|---|---|---|---|---|---|
| Age, median (IQR) | 207 | 48 (35, 57) | 41 (29, 54) | 54 (41, 59) | **<0.001** |
| Sex, *n* (%) | 207 | | | | 0.5 |
| F | | 93 (45%) | 43 (43%) | 50 (47%) | |
| M | | 114 (55%) | 58 (57%) | 56 (53%) | |
| Hospitalization, *n* (%) | 207 | | | | **<0.001** |
| Hospitalized | | 106 (51%) | 0 (0%) | 106 (100%) | |
| Not hospitalized | | 101 (49%) | 101 (100%) | 0 (0%) | |
| Comorbidity, *n* (%) | 207 | 52 (25%) | 17 (17%) | 35 (33%) | **0.007** |
| Mortality, *n* (%) | 207 | 7 (3.4%) | 0 (0%) | 7 (6.6%) | **0.014** |

[a]Mann-Whitney U test; Pearson's Chi-squared test; Fisher's exact test.

**Table 2.** Variants detected with Minor Allele Frequency (MAF).

| Gene | Locus | rs ID | Variant | Location | Mutated allele | MAF (GnomAD) | MAF (cohort) |
|---|---|---|---|---|---|---|---|
| ***IL-12A*** | Chromosome 3 | rs2243136 | c.607-104T>C | Intron | C | 0.10 | 0.10 |
| | Chromosome 3 | rs35990253 | c.631 G>A (p.Val211Met) | Exon | A | 0.009 | 0.11 |
| ***IL-12B*** | Chromosome 5 | rs11574790 | c.856-22C>T | Intron | A | 0.13 | 0.19 |
| | Chromosome 5 | rs3212227 | c.*743A>C | 3′ UTR | G | 0.26 | 0.29 |
| | Chromosome 5 | rs919766 | c.483-36T>G | Intron | C | 0.14 | 0.19 |
| ***IL-6*** | Chromosome 7 | rs2069840 | c.324 + 147C>G | Intron | G | 0.27 | 0.18 |
| ***TNF*** | Chromosome 6 | rs3093662 | c.187-122A>G | Intron | G | 0.07 | 0.10 |

**Table 3.** Association of detected variants with COVID-19 susceptibility.

| Model | Genotype | Negative COVID-19 | Positive COVID-19 | Odd ratio (95% CI) | *p*-value |
|---|---|---|---|---|---|
| *IL-12A* rs2243136 | | | | | |
| Codominant | T/T | 90 (76.3%) | 167 (80.7%) | 1.00 | 0.6 |
| | T/C | 26 (22%) | 38 (18.4%) | 0.79 (0.45-1.38) | |
| | C/C | 2 (1.7%) | 2 (1%) | 0.54 (0.07-3.89) | |
| Dominant | T/T | 90 (76.3%) | 167 (80.7%) | 1.00 | 0.35 |
| | T/C-C/C | 28 (23.7%) | 40 (19.3%) | 0.77 (0.45-1.33) | |
| Recessive | T/T-C/C | 116 (98.3%) | 205 (99%) | 1.00 | |
| | C/C | 2 (1.7%) | 2 (1%) | 0.57 (0.08-4.07) | |
| ***IL-12A* rs35990253** | | | | | |
| Codominant | G/G | 103 (87.3%) | 187 (90.3%) | 1.00 | 0.48 |
| | G/A | 13 (11%) | 19 (9.2%) | 0.81 (0.38-1.70) | |
| | A/A | 2 (1.7%) | 1 (0.5%) | 0.28 (0.02-3.07) | |
| Dominant | G/G | 103 (87.3%) | 187 (90.3%) | 1.00 | 0.4 |
| | G/A-A/A | 15 (12.7%) | 20 (9.7%) | 0.73 (0.36-1.50) | |
| Recessive | G/G-G/A | 116 (98.3%) | 206 (99.5%) | 1.00 | 0.28 |
| | A/A | 2 (1.7%) | 1 (0.5%) | 0.28 (0.03-3.14) | |
| ***IL-12B* rs11574790** | | | | | |
| Codominant | G/G | 81 (68.6%) | 137 (66.2%) | 1.00 | 0.9 |
| | G/A | 33 (28%) | 62 (29.9%) | 1.11 (0.67-1.84) | |
| | A/A | 4 (3.4%) | 8 (3.9%) | 1.18 (0.35-4.05) | |
| Dominant | G/G | 81 (68.6%) | 137 (66.2%) | 1.00 | 0.65 |
| | G/A-A/A | 37 (31.4%) | 70 (33.8%) | 1.12 (0.69-1.81) | |
| Recessive | G/G-G/A | 114 (96.6%) | 199 (96.1%) | 1.00 | 0.83 |
| | A/A | 4 (3.4%) | 8 (3.9%) | 1.15 (0.34-3.89) | |
| ***IL-12B* rs3212227** | | | | | |
| Codominant | T/T | 55 (46.6%) | 109 (52.7%) | 1.00 | 0.52 |
| | T/G | 52 (44.1%) | 78 (37.7%) | 0.76 (0.47-1.22) | |
| | G/G | 11 (9.3%) | 20 (9.7%) | 0.92 (0.41-2.05) | |
| Dominant | T/T | 55 (46.6%) | 109 (52.7%) | 1.00 | 0.29 |
| | T/G-G/G | 63 (53.4%) | 98 (47.3%) | 0.78 (0.50-1.23) | |
| Recessive | T/T-T/G | 107 (90.7%) | 187 (90.3%) | 1.00 | 0.92 |
| | G/G | 11 (9.3%) | 20 (9.7%) | 1.04 (0.48-2.25) | |
| ***IL-12B* rs919766** | | | | | |
| Codominant | A/A | 76 (64.4%) | 136 (65.7%) | 1.00 | 0.82 |
| | A/C | 37 (31.4%) | 65 (31.4%) | 0.98 (0.60-1.61) | |
| | C/C | 5 (4.2%) | 6 (2.9%) | 0.67 (0.20-2.27) | |
| Dominant | A/A | 76 (64.4%) | 136 (65.7%) | 1.00 | 0.81 |
| | A/C-C/C | 42 (35.6%) | 71 (34.3%) | 0.94 (0.59-1.52) | |
| Recessive | A/A-A/C | 113 (95.8%) | 201 (97.1%) | 1.00 | 0.53 |
| | C/C | 5 (4.2%) | 6 (2.9%) | 0.67 (0.20-2.26) | |
| ***IL-6* rs2069840** | | | | | |
| Codominant | C/C | 68 (57.6%) | 135 (65.2%) | 1.00 | **0.02** |
| | C/G | 40 (33.9%) | 68 (32.9%) | 0.86 (0.53-1.39) | |
| | G/G | 10 (8.5%) | 4 (1.9%) | 0.20 (0.06-0.67) | |
| Dominant | C/C | 68 (57.6%) | 135 (65.2%) | 1.00 | 0.18 |
| | C/G-G/G | 50 (42.4%) | 72 (34.8%) | 0.73 (0.46-1.15) | |
| Recessive | C/C-C/G | 108 (91.5%) | 203 (98.1%) | 1.00 | **0.0063** |
| | G/G | 10 (8.5%) | 4 (1.9%) | 0.21 (0.07-0.69) | |
| ***TNF* rs3093662** | | | | | |
| Codominant | A/A | 96 (81.4%) | 1 (0.8%) | 1.00 | 0.33 |
| | A/G | 21 (17.8%) | 41 (19.8%) | 1.13 (0.63-2.02) | |
| | G/G | 1 (0.8%) | 0 (0%) | 0.00 (0.00-NA) | |

*(continued)*

**Table 3.** (continued)

| Model | Genotype | Negative COVID-19 | Positive COVID-19 | Odd ratio (95% CI) | *p*-value |
|---|---|---|---|---|---|
| Dominant | A/A | 96 (81.4%) | 166 (80.2%) | 1.00 | 0.8 |
| | A/G-G/G | 22 (18.6%) | 41 (19.8%) | 1.08 (0.61-1.92) | |
| Recessive | A/A-A/G | 117 (99.2%) | 207 (100%) | 1.00 | 0.15 |
| | G/G | 1 (0.8%) | 0 (0%) | 0.00 (0.00-NA) | |

increased COVID-19 severity, aligning with existing literature on risk factors for COVID-19-related complications.[6,7]

*IL-12A*, *IL-12B*, *IL-6*, *TNF* genes are key inflammatory cytokine involved in COVID-19 pathogenesis, they exhibit varying degrees of genetic conservation and polymorphism. Several SNPs within these genes have been reported in the literature as correlated with the severity or susceptibility to COVID-19 in various populations. The *IL-6* gene remains highly polymorphic, with several studied SNP associated with COVID-19 outcomes. A GWAS and meta-analysis identified a global association of rs2069837,11,12 located in the intron of *IL-6* gene, with critical COVID-19 outcomes through its impact on IL-6 expression, this association was not limited to specific ethnic groups or region. While The G allele of rs2069837 has been shown to provide protection against the critical outcomes by decreasing IL-6 expression.[13] In our sequencing results rs2069837 was not identified, and most of identified variants in *IL-6* were rare, only rs2069840 was relatively common (MAF >10%) and showed a negative correlation conferring a protective effect to COVID-19 susceptibility. However, a study exploring biomarkers in COVID-19 patients of Moroccan ethnicity provide evidence of a significant association between IL-6 levels and COVID-19 severity. This underscores the presence of other parameters responsible for the elevated production of IL-6, which remain to be determined.[14] Other *IL-6* SNPs have shown to be protective against COVID-19 severity, particularly The rs1800796 C/C genotype conferring a lower risk of severe COVID-19 and rs1800795 G/C genotype potentially by moderating IL-6 excessive expression. However, there was a discrepancy in the results due to differences in study populations and genetic backgrounds.[15,16] rs1800795 and rs1800796 were identified in our population but relatively rare (MAF <10%).

Evidence from various studies supports the notion that *TNF* gene SNPs correlate with severity of COVID-19. Certain SNPs can predispose COVID-19 patients to heightened inflammatory responses leading to severe manifestation of the disease. *TNF* rs1800629, located in the promoter region, has been associated with increased expression of TNF-α, which may lead to a severe outcome of the disease. Studies have correlated the risk of susceptibility to severe outcomes with the rs1800629 G/A and A/A genotype, which is responsible for exacerbating inflammatory responses in COVID-19. Among the 21 SNP identified in our cohort, *TNF* rs1800629 was not detected. Only *TNF* rs3093662 had MAF >10% and did not show any correlation with either severity or susceptibility.[17–19] Association studies of *IL-12* SNPs and COVID-19 are less common; however, we chose to explore these genes given the crucial role that *IL-12A* and *IL-12B* play in the viral immune response. *IL-12 A* gene has been correlated to COVID-19 outcomes in a study involving Iraqi patients. *IL-12 A* rs568408 A/A and A/G genotypes had a significantly increased susceptibility to SARS-CoV-2 infection with an odds of 5.19. This highlights the need for further research to explore by which *IL-12A* SNPs affect the immune response to COVID-19.[8] Our investigation has shown no correlation genetic with COVID-19 outcomes.

Our results revealed that the majority of identified variants were rare, none of the SNPs previously mentioned in the literature were found to be associated with either severity or susceptibility to COVID-19 in our cohort. These results align with those obtained in our previous study, as no association was identified, and confirm the lack of association of several polymorphisms that had been previously recognized in the literature as being associated with severity and susceptibility to COVID-19 in diverse population.[20,21] These findings can explain the reduced impact and the low severity of COVID-19 in Morocco compared to other countries. This disparity might stem from ethnic diversity across countries, suggesting population-specific differences.

It is imperative to acknowledge that COVID-19 is a multifactorial disease influenced by a variety of genetic environmental and lifestyle factors, additional factors could elucidate the discrepancies between our findings and those of previously referenced studies, including the involvement of interactions with other polymorphisms or the impact of various risk and protective factors within individual populations. The involvement of multiple genes complicates the identification of specific genetic factors, as different gene variants from various parts of the genome may collectively increase overall risk interactions with other polymorphisms or the impact of various risk and protective factors within individual populations.[22]

The limitation of our study resides in the limited cohort size, which can be attributed to the stringent criteria

**Table 4.** Association of detected variants with COVID-19 severity.

| Model | Genotype | Non-hospitalized | Hospitalized | Odd ratio (95% CI) | *p*-value |
|---|---|---|---|---|---|
| *IL-12A* rs2243136 | | | | | |
| Codominant | T/T | 82 (81.2%) | 85 (80.2%) | 1.00 | 0.29 |
| | T/C | 19 (18.8%) | 19 (17.9%) | 0.96 (0.48-1.95) | |
| | C/C | 0 (0%) | 2 (1.9%) | NA (0.00-NA) | |
| Dominant | T/T | 82 (81.2%) | 85 (80.2%) | 1.00 | 0.89 |
| | T/C-C/C | 19 (18.8%) | 21 (19.8%) | 1.07 (0.53-2.13) | |
| Recessive | T/T-C/C | 101 (100%) | 0 (0%) | 1.00 | 0.10 |
| | C/C | 0 (0%) | 2 (1.9%) | NA (0.00-NA) | |
| ***IL-12A* rs35990253** | | | | | |
| Codominant | G/G | 89 (88.1%) | 98 (92.5%) | 1.00 | 0.34 |
| | G/A | 11 (10.9%) | 8 (7.5%) | 0.66 (0.25-1.72) | |
| | A/A | 1 (1%) | 0 (0%) | 0.00 (0.00-NA) | |
| Dominant | G/G | 89 (88.1%) | 98 (92.5%) | 1.00 | 0.29 |
| | G/A-A/A | 12 (11.9%) | 8 (7.5%) | 0.61 (0.24-1.55) | |
| Recessive | G/G-G/A | 100 (99%) | 106 (100%) | 1.00 | 0.23 |
| | A/A | 1 (1%) | 0 (0%) | 0.00 (0.00-NA) | |
| ***IL-12B* rs11574790** | | | | | |
| Codominant | G/G | 70 (69.3%) | 67 (63.2%) | 1.00 | 0.61 |
| | G/A | 27 (26.7%) | 35 (33%) | 1.35 (0.74-2.48) | |
| | A/A | 4 (4%) | 4 (3.8%) | 1.04 (0.25-4.35) | |
| Dominant | G/G | 70 (69.3%) | 67 (63.2%) | 1.00 | 0.35 |
| | G/A-A/A | 31 (30.7%) | 39 (36.8%) | 1.31 (0.74-2.34) | |
| Recessive | G/G-G/A | 97 (96%) | 102 (96.2%) | 1.00 | 0.94 |
| | A/A | 4 (4%) | 4 (3.8%) | 0.95 (0.23-3.91) | |
| ***IL-12B* rs3212227** | | | | | |
| Codominant | T/T | 53 (52.5%) | 56 (52.8%) | 1.00 | 0.83 |
| | T/G | 37 (36.6%) | 41 (38.7%) | 1.05 (0.59-1.88) | |
| | G/G | 11 (10.9%) | 9 (8.5%) | 0.77 (0.30-2.02) | |
| Dominant | T/T | 53 (52.5%) | 56 (52.8%) | 1.00 | 0.96 |
| | T/G-G/G | 48 (47.5%) | 50 (47.2%) | 0.99 (0.57-1.70) | |
| Recessive | T/T-T/G | 90 (89.1%) | 97 (91.5%) | 1.00 | 0.56 |
| | G/G | 11 (10.9%) | 9 (8.5%) | 0.76 (0.30-1.92) | |
| ***IL-12B* rs919766** | | | | | |
| Codominant | A/A | 68 (67.3%) | 68 (64.2%) | 1.00 | 0.71 |
| | A/C | 31 (30.7%) | 34 (32.1%) | 1.10 (0.61-1.98) | |
| | C/C | 2 (2%) | 4 (3.8%) | 2.00 (0.35-11.29) | |
| Dominant | A/A | 68 (67.3%) | 68 (64.2%) | 1.00 | 0.63 |
| | A/C-C/C | 33 (32.7%) | 38 (35.9%) | 1.15 (0.65-2.05) | |
| Recessive | A/A-A/C | 99 (98%) | 102 (96.2%) | 1.00 | 0.44 |
| | C/C | 2 (2%) | 4 (3.8%) | 1.94 (0.35-10.84) | |
| ***IL-6* rs2069840** | | | | | |
| Codominant | C/C | 66 (65.3%) | 69 (65.1%) | 1.00 | 0.54 |
| | C/G | 32 (31.7%) | 36 (34%) | 1.08 (0.60-1.93) | |
| | G/G | 3 (3%) | 1 (0.9%) | 0.32 (0.03-3.14) | |
| Dominant | C/C | 66 (65.3%) | 69 (65.1%) | 1.00 | 0.97 |
| | C/G-G/G | 35 (34.6%) | 37 (34.9%) | 1.01 (0.57-1.79) | |
| Recessive | C/C-C/G | 98 (97%) | 105 (99.1%) | 1.00 | 0.28 |
| | G/G | 3 (3%) | 1 (0.9%) | 0.31 (0.03-3.04) | |
| ***TNF* rs3093662** | | | | | |
| Alleles | A | 185 (92%) | 188 (89%) | 1.00 | 0.34 |
| | G | 17 (8%) | 24 (11%) | 1.38 (0.72-2.60) | |
| Codominant | A/A | 84 (83.2%) | 82 (77.4%) | 1.00 | 0.29 |
| | A/G | 17 (16.8%) | 24 (22.6%) | 1.45 (0.72-2.89) | |

concerning the lack of vaccination of our cohort, this approach aimed to eliminate the influence of vaccination on our patients outcomes. Sample size calculation was not performed since recruitment for our study was halted early due to Morocco's early and widespread introduction of vaccination. However, the three study groups are of similar sizes. Despite these limitations, our study sheds light on the involvement of *IL-12A*, *IL-12B*, *IL-6* and *TNF* in the severity and susceptibility to COVID-19.

## Conclusion

Polymorphisms in *IL-12A*, *IL-12B*, *IL-6*, and *TNF* have shown no correlation with the severity and susceptibility to COVID-19. Our study elucidates a correlation wherein the rs2069840 G allele provides protection against susceptibility to COVID-19. Further research involving larger patient cohorts and homogeneous ethnic backgrounds are recommended to validate or refute the association of various SNPs with COVID-19 severity and susceptibility.

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### Declaration of conflicting interests

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### Ethical statement

#### *Ethical approval*

Ethical approval for this study was obtained from Cheikh Zaid Hospital of Rabat (ID: CEFCZ/AB/PR_RFG).

#### *Informed consent*

Written informed consent was obtained from all subjects before the study and Written informed consent was obtained from legally authorized representatives before the study.

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