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Interleukin-38 promoter variants and risk of COVID-19 among Iraqis

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A R T I C L E   I N F O

Keywords: Coronavirus disease 19
Haplotype
Interleukin-38
Single nucleotide polymorphism
Susceptibility

A B S T R A C T

Coronavirus disease-19 (COVID-19) has recently emerged as a respiratory infection with a significant impact on health and society. The pathogenesis is primarily attributed to a dysregulation of cytokines, especially those with pro-inflammatory and anti-inflammatory effects. Interleukin-38 (IL-38) is a recently identified anti-inflammatory cytokine with a proposed involvement in mediating COVID-19 pathogenesis, while the association between IL38 gene variants and disease susceptibility has not been explored. Therefore, a pilot study was designed to evaluate the association of three gene variants in the promoter region of IL38 gene (rs7599662 T/A/G, rs28992497 T/C and rs28992498 C/A/T) with COVID-19 risk. DNA sequencing was performed to identify these variants. The study included 148 Iraqi patients with COVID-19 and 113 healthy controls (HC). Only rs7599662 showed a significant negative association with susceptibility to COVID-19. The mutant T allele was presented at a significantly lower frequency in patients compared to HC. Analysis of recessive, dominant and codominant models demonstrated that rs7599662 TT genotype frequency was significantly lower in patients than in HC. In terms of haplotypes (in order: rs7599662, rs28992497 and rs28992498), frequency of CTC haplotype was significantly increased in patients compared to HC, while TTC haplotype showed significantly lower frequency in patients. The three SNPs influenced serum IL-38 levels and homozygous genotypes of mutant alleles were associated with elevated levels. In conclusion, this study indicated that IL38 gene in terms of promoter variants and haplotypes may have important implications for COVID-19 risk.

1. Introduction

Coronavirus disease-19 (COVID-19) recently emerged as a respiratory infection that was initially reported in Wuhan, China, and then spread worldwide. It is caused by severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2), a single-stranded RNA virus believed to be transmitted from animals to humans (Pal et al., 2020). The infections can cause illnesses ranging from the common cold with flu-like symptoms to serious illnesses that may include pneumonia, kidney failure and even loss of life (Li et al., 2020).

A hallmark of SARS-CoV-2 infection is excessive inflammatory responses, which correlate with disease severity and even mortality (Rabaan et al., 2021). An effective inflammatory response against the virus involves provoking a cascade of immune responses by producing a range of inflammatory mediators, particularly cytokines (Notz et al., 2020). Although cytokines are powerful soluble mediators of the immune system, their elevated levels can lead to inflammation, infiltration of macrophages and neutrophils and lung injury in patients with COVID-19 (Rabaan et al., 2021). Studies have indicated that uncontrolled production of cytokines, particularly those with pro-inflammatory functions such tumor necrosis factor (TNF)-α, interleukin (IL)-1 and IL-6, is a prominent feature of the dysregulated immune response during SARS-CoV-2 infection (Costela-Ruiz et al., 2020). Dysregulated production of anti-inflammatory cytokines has also been described in COVID-19 patients including members of IL-1 family (Ahmed and Ad’hiah, 2021; Mardi et al., 2021). IL-1 family comprises both pro-inflammatory (IL-1, IL-18, IL-33 and IL-36) and anti-inflammatory (IL-36, IL-37 and IL-38) cytokines (Dinarello, 2018). IL-38 is a recently

Abbreviations: ACE, Angiotensin-converting enzyme; CI, Confidence interval; COVID-19, Coronavirus disease 2019; CT, Chest tomography; D’, Linkage disequilibrium coefficient; HC, Healthy controls; HWE, Hardy-Weinberg equilibrium; IL, Interleukin; LD, Linkage disequilibrium; MLR, Multinomial logistic regression; OR, Odds ratio; p, Probability; pc, Corrected probability; PCR, Polymerase chain reaction; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; SNP, Single nucleotide polymorphism; STAT3, Signal transducer and activator of transcription 3; TF, Transcription factor; TFBS, Transcription factor binding site; TNF, Tumor necrosis factor.

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https://doi.org/10.1016/j.imbio.2022.152301
Received 26 June 2022; Received in revised form 13 October 2022; Accepted 5 November 2022
Available online 9 November 2022
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discovered cytokine in this family. It is produced in tissues of lymphoid organs (for instance, thymus, tonsil and spleen) and secreted by various cells (for instance, monocytes and macrophages), and its immunopathological role in infectious and autoimmune diseases has been proposed (Esmaeizadeh et al., 2021; Han et al., 2021). During viral infections and inflammatory conditions, IL-38 exhibits anti-inflammatory capabilities and exerts suppressive effects on the expression of various pro-inflammatory cytokines (Xia et al., 2021). Regarding COVID-19, a recent study showed higher serum IL-38 levels in patients versus controls, but when patients were categorized by disease severity, lower IL-38 levels were found in the severe group compared to the non-severe group (Gao et al., 2021).

Host genetic variants related to serious illness may identify some potential therapeutic targets to modulate immune response of the host to support survival (Bailie, 2014). For example, host-directed therapies have been for a long time the goal for treating the severe diseases caused by respiratory viruses (Bailie and Digard, 2013). Moreover, identification of genetic variants associated with susceptibility to COVID-19 could pave the way for particular targets to develop efficient therapies. Considerable attention has been paid to whether host genetic variation may influence COVID-19 risk and/or disease severity (Velavan et al., 2021). Several genetic variants of genes involved in controlling immune responses, inflammation or SARS-CoV-2 receptors have been studied and some significant findings have been obtained; for instance, IL-37, angiotensin-converting enzyme (ACE) and angiotensin-converting enzyme 2 (ACE2) genes (Ahmed and Ad‘hiah, 2022; Mahmood et al., 2022; Subh et al., 2022). In the case of IL-38, the coding gene is mapped to a region in human chromosome 2; 2q14.1 (https://www.ncbi.nlm.nih.gov/gene/84639). IL38 gene SNPs (single nucleotide polymorphisms) have been described, and there is evidence to suggest that these genetic variants not only regulate systemic levels of other IL-1 family cytokines such as IL-1ra (interleukin-1 receptor antagonist), but are also associated with inflammatory markers in the peripheral blood such as C-reactive protein (Dehghan et al., 2011; Herder et al., 2014). Besides, IL38 gene SNPs have been shown to influence susceptibility to several inflammatory and autoimmune disorders such as arthritic diseases (Xie et al., 2019). With regard to infectious diseases, it appears that the IL38 gene polymorphism has not been investigated.

A recent study we conducted revealed that COVID-19 did not influence IL-38 levels in the serum of patients, but the study indicated that classifying patients by some characteristics (demographic, anthropometric and clinical) may reveal a clearer role for IL-38 in COVID-19 pathogenesis (Al-bassam et al., 2022). The current pilot study included the same cohorts of patients and controls and was designed to evaluate the association of SNPs in the promoter region of IL38 gene with COVID-19 risk. In addition, severity of disease was considered in this evaluation. To the investigators’ knowledge, this study is the first to explore IL38 polymorphism in COVID-19.

2. Material and methods

2.1. Subjects

A case-control study was performed on 148 patients with COVID-19 (60.1 % male) and 113 healthy controls (HC; 59.3 % male) with mean age (± standard deviation [SD]) of 56.5 ± 14.3 and 37.8 ± 12.0 years, respectively. The study was conducted during the period from September to December 2020 and included patients admitted to COVID-19 care units in Baghdad. The RealLine SARS-CoV-2 kit (Bioron Diagnostics GmbH, REF BI1019-96) was used to diagnose SARS-CoV-2 in nasopharyngeal swabs. This was followed by a chest tomography (CT) scan. Inclusion criteria were a positive molecular test, CT scan indicating COVID-19 and age ≥ 18 years. Severity of COVID-19 was determined following the WHO Interim Guidance (World Health Organization, 2020; https://apps.who.int/iris/handle/10665/330893). Accordingly, patients were assigned into three groups: moderate (N = 45; mean age ± SD = 52.2 ± 14.8 years; males = 64.4 %), severe (N = 55; mean age ± SD = 58.1 ± 12.6 years; males = 49.1 %) and critical (N = 48; mean age ± SD = 58.8 ± 14.9 years; males = 68.8 %). The HC sample included blood donors and healthcare workers. They had no infectious or chronic disease at the time of investigation, and their sera were negative for COVID-19 IgG and IgM antibody testing. Written consent was provided by all participants to participate in study. Ethical approval was obtained from the Ethics Committee at the Ministry of Health.

2.2. SNP selection, DNA isolation and genotyping

The complete DNA sequence of IL38 gene (IL1F10 gene; Gene ID: 84639) with SNP data was downloaded (https://www.ensembl.org/Homo_sapiens/Gene/Sequence?g = ENSG00000136697;r = 2:113067970-113075843). Genious Prime software (www.geneious.com/prime) was used to design forward (5’-TCTTGGCAGTGGTCTTCCCT-3’) and reverse (5’-ACACGAGAGCTCACAAAAAGA-3’) primers that can amplify a promoter region of IL38 gene. In Silico validation was conducted to test efficiency and specificity of primers (genome.ucsc.edu/cgi-bin/hgPcr). Molecular size of the amplified region was found to be 977-bp (chr2:113067404 + 113068380). In this region, more than 150 SNPs were identified but those with a minor allele frequency ≥ 10 % were only three (rs7599662 T/A/C/G, rs28992497 T/C and rs288992498 C/A/T).

The ReliaPrep Blood gDNA Miniprep System Kit was used to isolate genomic DNA from EDTA blood following the manufacturer’s instructions (Promega, USA, Cat. No. A5081). PCR mix (total volume: 20 µL) consisted of 10 µL Master Mix (GoTaq Green Master Mix, Promega, USA, Cat. No. M7121), 1 µL forward primer, 1 µL reverse primer, 2 µL DNA and 6 µL nuclease free water. The thermal cycler (Thermo Fisher Scientific, USA) was programmed as follows: an initial denaturation cycle (95 °C; 5 min), followed by 30 cycles of denaturation (95 °C; 30 s), annealing (63 °C; 30 s) and extension (72 °C; 45 s). A final extension cycle (72 °C; 7 min) was also included. Amplified PCR products were electrophoresed in 1.5 % agarose gel (Fig. 1A) and then forward and reverse Sanger sequencing of PCR products was performed using the ABI3730XL Automated DNA Sequencer (Macrogen Corporation, Korea). Genious Prime software was used to perform DNA sequence alignment with reference sequences provided by the National Center for Biotechnology Information database (Sayers et al., 2022), and accordingly, SNP genotypes were assigned. Fig. 1B shows a representative chromatogram of SNP rs7599662.

2.3. IL-38 levels

Data for serum IL-38 levels were obtained from our previously published study (Al-bassam et al., 2022) and used to explore the impact of SNP genotypes on these levels.

2.4. TRANSFAC® MATCH analysis

To predict transcription factor binding sites (TFBSs) that might overlap with the studied SNPs, in silico analyses were performed using MATCH, a weight matrix-based tool for detecting putative TFBSs in DNA sequence of interest (Reb et al., 2003). The studied part of the IL-38 promoter sequence was submitted to TRANSFAC using the MATCH tool; the vertebrate database of TFs, high quality matrices and minimizing the false negative errors options were used. Results were manually filtered to choose the predicted TF binding sequences that overlap with the SNPs, which may explain the differential activity.

2.5. Statistical analysis

Hardy-Weinberg equilibrium (HWE) analysis was performed using the Pearson Chi-square goodness-of-fit test. Odds ratio (OR) and 95 %
confidence interval (CI) were calculated using the multinomial logistic regression (MR) test. Five genetic models were adopted in this test: allele, recessive, dominant, overdominant and codominant, and two-tailed Fisher exact test or Pearson Chi-square test was employed to assess significant differences. Median and interquartile range (IQR) were used to describe IL-38 levels, and significant differences were assessed using Mann-Whitney U test. Bonferroni correction was applied to adjust probability (p) and a p-value of 0.05 was taken significant. These analyses were performed using IBM SPSS Statistics version 25.0 (IBM Corp., Armonk, NY, USA). Linkage disequilibrium (LD) and haplotype construction were analyzed using SHEsis software (Shi and He, 2005). LD coefficient (D') was used to express LD between SNPs.

3. Results

3.1. MLR analysis of SNPs

DNA sequence analysis of the amplified PCR products by alignment with reference SNP sequences identified three intergenic variants with a minor allele frequency ≥ 10 %, rs7599662, rs28992497 and rs28992498. Genotype frequencies of the three IL38 gene SNPs showed no significant deviation from HWE in patients or HC. The mutant allele of SNPs rs7599662 (TT vs CC), rs28992497 (CC vs TT) and rs28992498 (AA vs CC) exhibited significantly elevated IL-38 levels compared to the corresponding wild-type homozygous genotype (77.4 [IQR: 63.1–84.6] vs 67.7 [60.1–74.8] pg/mL, p < 0.05; 80.7 [67.5–102.3] vs 67.7 [59.8–75.6] pg/mL, p < 0.01; and 82.7 [60.8–95.1] vs 67.3 [59.4–76.5] pg/mL, p < 0.05, respectively) (Fig. 3).

3.2. IL38 gene SNPs and severity of COVID-19

Genotype frequencies of rs7599662, rs28992497 and rs28992498 SNPs showed no significant variation between patients classified by severity of COVID-19 (moderate, severe and critical illness) (Table 2).

3.3. LD and haplotype analysis

Pairwise LD analysis between IL38 gene SNPs demonstrated that rs7599662 had a weak LD with rs28992497 (D' = 0.2), while a strong LD was found with rs28992498 (D' = 0.68). Further, a stronger LD was found between rs28992497 and rs28992498 (D' = 0.76) (Fig. 2).

In terms of haplotypes (in order: rs7599662, rs28992497 and rs28992498), frequency of CTC haplotype was significantly increased in patients compared to HC (59.7 vs 46.4 %; OR = 1.71; 95 % CI = 1.21–2.43; p = 0.002; pc = 0.008), while a significant decrease in the frequency of TTC haplotype was observed in patients (16.2 vs 28.7 %; OR = 0.48; 95 % CI = 0.31–0.73; p = 0.001; pc = 0.004) (Table 3).

3.4. Impact of IL38 SNP genotypes on IL-38 levels

IL-38 levels were examined in all participants (COVID-19 patients and HC) after classification according to genotypes of each SNP. Individuals with a genotype homozygous for mutant allele of SNPs rs7599662 (TT vs CC), rs28992497 (CC vs TT) and rs28992498 (AA vs CC) exhibited significantly elevated IL-38 levels compared to the corresponding wild-type homozygous genotype (77.4 [IQR: 63.1–84.6] vs 67.7 [60.1–74.8] pg/mL, p < 0.05; 80.7 [67.5–102.3] vs 67.7 [59.8–75.6] pg/mL, p < 0.01; and 82.7 [60.8–95.1] vs 67.3 [59.4–76.5] pg/mL, p < 0.05, respectively) (Fig. 3).

3.5. Computational prediction of TFBSs

A possible explanation for the effects of SNPs on IL-38 promoter functions could be alterations in the TFBSs either by formation or disruption of TFBSs or through differential affinity for TFs. To examine this possibility, we used an in silico TFBS prediction tool, MATCH by TRANSFAC. Our data show that there are seven predicted TFBSs that interfere with the SNPs (rs7599662: COMP1; rs28992497: Myogenin and Hand1; rs28992498: COMP1, cp2, Pax6 and Pax4).

4. Discussion

An impressive number of genome-wide association studies have reported that specific human genetic variants can influence susceptibility to various infectious diseases. These studies have provided valuable insights by identifying the associations of genetic variants of innate and adaptive immune responses with susceptibility to various infections (Mosetti et al., 2018). In COVID-19, studies of different ethnic groups have also indicated that host genetic variants, particularly those involved in immune response, are associated with disease risk and severity, as well as clinical outcomes (Barmenia et al., 2022; Chamnamphon et al., 2022; Ovsyannikova et al., 2020). A dysregulated immune response has been closely linked to COVID-19 pathogenesis, especially severity of disease, because the initial innate inflammatory response to SARS-CoV-2 leads to rapid recruitment of many immune response cells to the lungs, the primary site of infection, after which these cells are activated to produce high levels of cytokines (Wong and Perlman, 2022). The pathogenic role of cytokines in COVID-19 has received increasing attention across several studies. The cytokine storm, which is an exaggerated immune response leads to an uncontrolled elevation in circulating pro-inflammatory cytokines, has been shown to increase the risk of pulmonary failure, acute respiratory distress syndrome and death (Costela-Ruiz et al., 2020; Karaba et al., 2021; Rabaan et al., 2021). Besides, dysregulated production of some anti-inflammatory cytokines (for instance, IL-37 and IL-38) has also been
and severity of disease. Some cytokine SNPs (particularly those with a proposed role in the pathogenesis of COVID-19) have recently been examined in studies focusing on certain risk. Indeed, associations between genetic variants and susceptibility to COVID-19 cannot fully explain their association with COVID-19 susceptibility to COVID-19 (Ahmed and Al’hiah, 2021; Al-bassam et al., 2022; Gao et al., 2020; Suh et al., 2022; Velavan et al., 2021). The expressions of many cytokines are regulated at the transcription level, and promoter SNPs of many cytokines have been shown to be transcriptional regulators (de Graaf et al., 2021). However, due to the fact that cytokines are under genetic control, their serum profile cannot fully explain their association with COVID-19 risk. Indeed, associations between genetic variants and susceptibility to COVID-19 have recently been examined in studies focusing on certain cytokines, particularly those with a proposed role in the pathogenesis and severity of disease. Some cytokine SNPs (particularly those in the 384 promoter region) have been linked to interindividual heterogeneity in susceptibility to COVID-19 (Ahmed and Ad’hiah, 2022; Ovsyannikova et al., 2020; Suh et al., 2022; Velavan et al., 2021). The MLR analysis according to five genetic models indicated that only SNP rs7599662 may serve as an indicator of reduced susceptibility to COVID-19. Therefore, the current case-control study, the associations between three IL38 promoter SNPs (rs7599662, rs28992497 and rs28992498) and susceptibility to COVID-19 were examined.

**Table 1**

Multinomial logistic regression and Hardy-Weinberg equilibrium analyses of IL38 gene SNPs in COVID-19 patients versus controls.

| SNP/Genetic model | Allele/ genotype | Patients; N = 148 | OR (95 % CI) | p-value (pc) |
|-------------------|------------------|-----------------|--------------|-------------|
| rs7599662 T/A/C/G | Allele | C 239 80.7 | Reference | 0.44 (0.30-0.66) < 0.001 (0.001) |
|                   | T 57 19.3 | 79 35.0 | 0.30 (0.13-0.72) 0.006 (0.036) |
|                   | Recessive | CC + CT 140 94.6 | Reference | 0.42 (0.26-0.70) 0.001 (0.006) |
|                   | TT 8 5.4 | 18 15.9 | 0.52 (0.26-1.0) 0.083 (0.498) |
|                   | Dominant | CC 99 66.9 | Reference | 0.62 (0.37-1.05) 0.083 (0.498) |
|                   | Overdominant | CT + TT 49 33.1 | Reference | 0.62 (0.37-1.05) 0.083 (0.498) |
|                   | Codominant | CC 99 66.9 | Reference | 0.62 (0.37-1.05) 0.083 (0.498) |
|                   | HWE-p-value | 0.414 | 0.222 |
| rs28992497 T/C | Allele | C 241 81.4 | Reference | 0.89 (0.58-1.38) 0.655 (1.0) |
|                   | T 55 18.6 | 46 20.4 | 0.53 (0.16-1.71) 0.373 (1.0) |
|                   | Recessive | TT + TC 143 96.6 | Reference | 0.97 (0.58-1.62) 1.0 (1.0) |
|                   | CC 5 3.4 | 7 6.2 | 1.11 (0.65-1.89) 0.785 (1.0) |
|                   | Dominant | TT 98 66.2 | Reference | 1.11 (0.65-1.89) 0.785 (1.0) |
|                   | Overdominant | TT + CC 103 69.6 | Reference | 1.11 (0.65-1.89) 0.785 (1.0) |
|                   | Codominant | TT 98 66.2 | Reference | 1.11 (0.65-1.89) 0.785 (1.0) |
|                   | HWE-p-value | 0.998 | 0.404 |
| rs28992498 C/A/T | Allele | C 232 78.4 | Reference | 1.14 (0.74-1.75) 0.586 (1.0) |
|                   | A 64 21.6 | 44 19.5 | 0.50 (0.14-1.79) 0.337 (1.0) |
|                   | Recessive | CC + CA 144 97.3 | Reference | 1.35 (0.81-2.24) 0.302 (1.0) |
|                   | AA 4 2.7 | 6 5.3 | 1.54 (0.91-2.60) 0.115 (0.690) |
|                   | Dominant | CC 88 59.5 | Reference | 1.54 (0.91-2.60) 0.115 (0.690) |
|                   | Overdominant | CC + AA 60 40.5 | Reference | 1.54 (0.91-2.60) 0.115 (0.690) |
|                   | Codominant | CC 88 59.5 | Reference | 1.54 (0.91-2.60) 0.115 (0.690) |
|                   | HWE-p-value | 0.367 | 0.588 |

SNP: Single nucleotide polymorphism; HWE: Hardy-Weinberg equilibrium; OR: Odds ratio; CI: Confidence interval; pc: Bonferroni correction probability (significant p-value is indicated in bold).

**Table 2**

Frequencies of IL38 gene SNPs stratified by disease severity in COVID-19 patients.

| Disease severity | Genotype; N (%) | rs7599662 T/A/C/G | rs28992497 T/C | rs28992498 C/A/T |
|------------------|-----------------|------------------|-----------------|------------------|
|                   | CC | CT | TT | CC | TT | CC | CC | CA | AA |
| Moderate | r | 31 (68.9) | 13 (28.9) | 1 (2.2) | 26 (57.8) | 18 (40.0) | 1 (2.2) | 20 (44.4) | 24 (53.3) | 1 (2.2) |
| Severe | r | 37 (67.3) | 14 (25.5) | 4 (7.3) | 38 (69.1) | 15 (27.3) | 2 (3.6) | 37 (67.3) | 17 (30.9) | 1 (1.8) |
| Critical | r | 31 (64.6) | 14 (29.2) | 3 (6.3) | 34 (70.8) | 12 (25.0) | 2 (4.2) | 31 (64.6) | 15 (31.3) | 2 (4.2) |
| p-value (pc) | 0.828 (1.0) | 0.560 (1.0) | 0.127 (0.635) |

p: Pearson Chi-square test probability; pc: Bonferroni correction probability.
However, it may be too early to reach a productive conclusion: Bonferroni correction probability (significant $p_c$). Further studies are warranted regarding the role of the three SNPs in susceptibility to COVID-19 or their impact on IL-38 production and further studies are warranted.

Limited disease-association studies have explored the role of rs7599662 in disease susceptibility, and in fact only one study explored this SNP, as well as the SNPs rs28992497 and rs28992498. The homozygous genotype of mutant allele in each SNP (TT, CC and AA, respectively) was significantly effective in elevating IL-38 levels compared to wild-type genotype (CC, TT and CC, respectively). Mann-Whitney U test was used to compare medians: * $p < 0.05$, ** $p < 0.01$, ns $p$ greater than 0.05 (not significant).

As is well known, the genomic regulatory regions include different specific sequences of binding sites for TFs. Hence, the interaction between these TFs and their binding sites as well as the interplay of TFs with each other coordinate the effectiveness of genetic programs by activating or repressing the transcription of specific genes (Lambert et al., 2018). As a consequence, genetic variations (SNPs) within TFBSs that alter gene expression may play a significant role in the phenotypic variation in various traits, including the risk of developing diseases. Therefore, searching for functional non-coding genetic variants using Combining chromatin Immunoprecipitation (ChiP) assay, Electrophoretic Mobility Shift Assay (EMSA), DNA-affinity Precipitation Assay (DAPA), and others has become a major concern (Farh et al., 2015; Soderquest et al., 2017). For instance, a study by Tripathi and colleagues has shown that signal transducer and activator of transcription 3 (STAT3) is important for transcriptional regulation of human Th17 cell differentiation. They also found a number of SNPs associated with some immune-mediated disorders that were detected in STAT3-binding sites needed to induce Th17 cell specification (Tripathi et al., 2017). In the current study, in silico analysis was performed to indicate any potential TFBSs that may overlap with the identified SNPs. In fact, several TFBSs have been detected to be overlapped with the identified SNPs. These overlaps between the SNPs and the TFBSs suggest that the changes in promoter activity might be due to modifications in the TFBSs or in the binding affinity of TFs to their binding sites. However, further studies are needed to confirm these results and to determine the role of each putative TF in regulating promoter activity.

It has been indicated that combinations of polymorphisms in the form of haplotypes can provide more information about disease susceptibility than a single locus polymorphism and offer a promising approach to revealing genetic variations that are responsible for complex human diseases including COVID-19 (Ahmed and Ad’hiah, 2022; Diao and Lin, 2020; Liu et al., 2008). Therefore, in the next step of this study, we constructed haplotypes integrating the three SNPs of IL38 gene (in the order: rs7599662, rs28992497 and rs28992498). A strong LD was found between rs7599662 and rs28992498, as well as between rs28992497 and rs28992498. A strong LD was found between rs7599662 and rs28992498, as well as between rs28992497 and rs28992498. A strong LD was found between rs7599662 and rs28992498, as well as between rs28992497 and rs28992498. A strong LD was found between rs7599662 and rs28992498, as well as between rs28992497 and rs28992498. A strong LD was found between rs7599662 and rs28992498, as well as between rs28992497 and rs28992498. A strong LD was found between rs7599662 and rs28992498, as well as between rs28992497 and rs28992498.

**Table 3**

Haplotype analysis of IL38 gene SNPs (rs7599662-rs28992497-rs28992498) among COVID-19 patients and controls.

| Haplotype | Patients (296 chromosomes) N | % | Controls (226 chromosomes) N | % | OR (95 % CI) | p-value (pc) |
|-----------|-----------------------------|---|-----------------------------|---|--------------|-------------|
| CCA       | 46                          | 15.5 | 29                          | 12.8 | 1.25 (0.76-2.06) | 0.450 (1.0) |
| CTC       | 177                         | 59.7 | 105                         | 46.4 | 1.71 (1.21-2.43) | 0.002 (0.008) |
| TTC       | 48                          | 16.2 | 65                          | 28.7 | 0.48 (0.31-0.73) | 0.001 (0.004) |
| CTA       | 15                          | 5.0  | 9                           | 3.9  | 1.29 (0.55-2.99) | 0.674 (1.0) |

OR: Odds ratio; CI: Confidence interval; p: Two-tailed Fisher exact probability; pc: Bonferroni correction probability (significant p-value is indicated in bold).

TT genotype of rs7599662 may have a protective role against the development COVID-19. Limited disease-association studies have explored the role of rs7599662 in disease susceptibility, and in fact only one study explored this SNP, as well as the SNPs rs28992497 and rs28992498, in patients with systemic juvenile idiopathic arthritis and no significant association was found (Stock, 2011). However, it was interesting to note that the homozygous genotype of the mutant allele of the three SNPs was associated with up-regulated IL-38 levels compared to the homozygous genotype of the wild-type. This finding may indicate that these promoter SNPs may influence IL-38 levels and the observed association with COVID-19 risk may be due to this effect. Consistent with our observation, promoter polymorphisms within IL6 gene influenced serum levels of IL-6 in patients with osteoarthritis (Singh et al., 2020). However, it may be too early to reach a productive conclusion regarding the role of the three SNPs in susceptibility to COVID-19 or their impact on IL-38 production and further studies are warranted.
infection. These results suggest the contribution of IL38 haplotypes to susceptibility to COVID-19, with one haplotype associated with a protective effect, while the other haplotype confers susceptibility to disease. Accordingly, we can point out that haplotype assessment can provide valuable information that helps explain gene-disease associations and polymorphism interactions within haplotypes.

Regardless of these intriguing findings, they should be interpreted with caution because the study encountered the limitation of relatively low number of patients and HC. Besides, gene expression of IL38 was not determined and its relationship to SNP genotypes and/or haplotypes may better explain IL38 role in COVID-19 pathogenesis.

5. Conclusions

IL38 gene in terms of the rs7599662 promoter SNP and haplotypes (rs7599662-rs28992497-rs28992498) may have important implications for susceptibility to COVID-19. Besides, the examined SNPs may influence serum IL38 levels. However, further investigations are recommended to uncover the proposed significance of IL38 gene polymorphism in disease risk.

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement

Bithal A. Al-Karaawi: Conceptualization, Visualization, Methodology, Investigation, Validation, Writing – review & editing. Wasan W. Al-bassam: Conceptualization, Visualization, Methodology, Investigation, Validation, Writing – review & editing. Haneen M. Ismaeel: Conceptualization, Visualization, Methodology, Investigation, Supervision, Software, Validation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgement

We appreciate the cooperation and kind assistance of the medical staff at COVID-19 care units in Baghdad hospitals.

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