

ORIGINAL ARTICLE

VDR gene polymorphisms are associated with the increased susceptibility to COVID-19 among Iranian population: A case-control study

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Abstract

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), but the pathogenesis is unclear. Host genetic background is one of the main factors influencing the patients' susceptibility to several viral infectious diseases. This study aimed to investigate the association between host genetic polymorphisms of two genes, including vitamin D receptor (VDR) and vitamin D binding protein (DBP), and susceptibility to COVID-19 in a sample of the Iranian population. This case-control study enrolled 188 hospitalized COVID-19 patients as the case group and 218 suspected COVID-19 patients with mild signs as the control group. The VDR (rs7975232, rs731236 and rs2228570) and DBP (rs7041) gene single nucleotide polymorphisms (SNPs) were genotyped by Polymerase Chain Reaction Restriction – Fragment Length Polymorphism (PCR-RFLP) method. A significant association between rs2228570 SNP in the VDR gene and the susceptibility

Abbreviations: COPD, Chronic obstructive pulmonary disease; COVID-19, Coronavirus disease 2019; DBP, Vitamin D-binding protein; GAPDH, Glyceraldehyde 3-phosphate dehydrogenase; HCV, Hepatitis C virus; HWE, Hardy–Weinberg equilibrium; IL-1 β , Interleukin 1 beta; IL-6, Interleukin 6; IL-8, Interleukin 8; MCP-1, Monocyte chemoattractant protein-1; MERS-CoV, Middle East respiratory syndrome coronavirus; NGS, Next-generation sequencing; OR, Odds ratio; PCR-RFLP, Polymerase chain reaction restriction – fragment length polymorphism; RSV, Respiratory syncytial virus; RT-PCR, Reverse transcription polymerase chain reaction; SARS-CoV-1, Severe acute respiratory syndrome coronavirus 1; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; SNP, Single nucleotide polymorphism; TH1/TH2, Type 1 T helper/ Type 2 T helper; TNF- α , Tumour necrosis factor alpha; VDR, Vitamin D receptor; WHO, World Health Organization.

of COVID-19 was found between case and control groups. The CT genotype (Heterozygous) of rs2228570 C > T polymorphism showed significant association with a 3.088 fold increased odds of COVID-19 ($p < .0001$; adjusted OR: 3.088; 95% CI: 1.902–5.012). In addition, a significant association between CC genotype of rs2228570 CT polymorphism and increased odds of COVID-19 in male and female groups ($p = .001$; adjusted OR: 3.125; 95% CI: 1.630–5.991 and $p = .002$; adjusted OR: 3.071; 95% CI: 1.485–6.354 respectively) were determined. Our results revealed no significant differences in the frequency of genotype and allele of VDR (rs7975232 and rs731236) and DBP (rs7041) between SARS-CoV-2-infected patients and controls ($p > .05$). Our results showed that polymorphism of VDR (rs2228570) probably could influence individual susceptibility to COVID-19. The polymorphisms of VDR (rs7975232 and rs731236) and DBP (rs7041) were not associated with SARS-CoV-2 infection susceptibility.

KEYWORDS

COVID-19, DBP, genetic polymorphism, susceptibility, VDR

1 | INTRODUCTION

Acute respiratory disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), named by the World Health Organization (WHO) as Coronavirus disease 2019 (COVID-19) disease, which quickly was declared the first pandemic of the century. The SARS-CoV-2 can infect the lower respiratory tract and cause severe acute pneumonia in humans with a very high rate of spread and mortality rate of about 3.4% (Chen et al., 2020; Zhou et al., 2020).

The Coronavirus can target the upper and lower respiratory tract and cause disease with a wide range of clinical manifestations from asymptomatic to critical conditions in infected individuals. Interestingly, COVID-19 has no specific clinical symptoms compared to common viral respiratory diseases (Sheervalilou, Ahmadzadeh et al., 2021; Sheervalilou et al., 2022). Studies have shown that old age and having the underlying disease (some morbidity) are related to the severity of COVID-19 (Heidari Nia et al., 2022; Sargazi et al., 2021). An accurate and rapid diagnosis of COVID-19 can be beneficial in managing and treating this life-threatening disease (Sheervalilou Shirvaliloo et al., 2021).

The most common cause of death in COVID-19 is disruption of homeostasis of the host immune system and cytokine storm. Thus, the pathogenesis of the virus increases the production of some cytokines, especially the cytokines tumour necrosis factor- α (TNF- α), interleukin 1 beta (IL-1 β) and interleukin 6 (IL-6), and chemokines such as interleukin 8 (IL-8) and the monocyte chemotactic protein-1 (MCP-1), which rapidly leads to symptoms such as hypotension, fever, and oedema and can eventually lead to multiple organ failure and death of the host in severe cases (Blackwell et al., 2009; Dendrou et al., 2018).

Numerous studies have shown that the host genetic factors play an essential role in determining host sensitivity to human infectious

diseases such as malaria, human immunodeficiency virus (HIV), *Helicobacter pylori* gastric tuberculosis and pneumococcal diseases (Hill, 2006; Martins et al., 2018). It is well-acknowledged that the direct or indirect role of genetic polymorphisms in human genes could influence host immune response and disease outcomes (Bahrami et al., 2020).

Many studies show that vitamin D plays a role in regulating immune cells and modulating the immune response. In several studies, the anti-inflammatory and immunomodulatory effect of vitamin D on inflammatory diseases, particularly on respiratory viruses such as influenza, respiratory syncytial virus (RSV), Middle East Respiratory Syndrome Coronavirus (MERS-CoV), SARS-CoV-1 and rhinovirus, is shown (Greiller & Martineau, 2015; Lương & Nguyễn, 2013; Sutaria et al., 2014).

Furthermore, recent investigations indicated the essential role of vitamin D in the innate and acquired immune responses, which are widely involved in increasing the immune response and suppressing cytokine storms. The most vital role of vitamin D is to shift responses from type 1 T helper (TH1) to type 2 T helper (TH2) and to modulate the immune response and secretion of inflammatory cytokines (Bui et al., 2021; Grant et al., 2020; Shah Alam et al., 2021). In this regard, it is rational that any disruption in the delivery, activation, and function of vitamin D can directly and indirectly influence the efficient function of vitamin D on the host immune system. According to numerous official reports, vitamin D deficiency within the Iranian population is a serious concern and has been repeatedly warned about its devastating health consequences (Heshmat et al., 1970; Tabriz et al., 2018; Vatandost et al., 2018).

In recent studies, a strong association between severe vitamin D deficiency with COVID-19 severity and mortality in different populations was reported by several researchers (AlSafar et al., 2021; Demir et al., 2021; Weir et al., 2020).

Vitamin D receptor (VDR) and vitamin D-binding protein (DBP) are two principle molecules involved in the vitamin D pathway (Uitterlinden et al., 2004). Vitamin D is bound to DBP in the bloodstream and translocates to the liver and the kidneys to mature in the active form of vitamin D (Sutaria et al., 2014). VDR is the DNA-binding transcription factor that is activated in the presence of bioactive vitamin D (or calcitriol) and subsequently causes the transcription of abundant genes. VDR has regulatory effects on calcitriol in numerous tissues (Bahrami et al., 2020). DBP can play an essential role in the immune system by transporting fatty acids such as proteoglycans (Bouillon et al., 2019). Accordingly, DBP has no apparent direct effects on the inflammation process, but this role is performed by altering vitamin D availability levels in serum (Alshahawy, 2021). Previously the association between DBP's haplotype and RSV bronchiolitis and asthma development also was reported by Randolph et al. (2014). The two most common variants of DBP (rs7041 and rs4588) are presumed to be associated with chronic inflammatory lung disease such as chronic obstructive pulmonary disease (COPD) (Alshahawy, 2021).

The *DBP* gene polymorphisms could influence the bioavailability of vitamin D serum and subsequently lead to susceptibility to viral infections such as the relation of rs7041 locus polymorphism (DBP rs7041-G allele) with a high risk of susceptibility to hepatitis C virus (HCV) infection (Alshahawy, 2021). In this line of thought, it is noteworthy that several previous studies have shown that specific VDR polymorphisms increase resistance to some viruses, such as respiratory viruses. In contrast, others make their hosts more susceptible to infection with an enveloped virus such as HIV, dengue and RSV infection (Greiller & Martineau, 2015; Lafi et al., 2015; Laplana et al., 2018; Sutaria et al., 2014).

Accordingly, the evaluation of single nucleotide polymorphisms (SNPs) in the *VDR* and *DBP* genes can confirm the hypothesis of the functional impact of polymorphisms on the severity of COVID-19.

Therefore, the main aim of the present study was to examine this hypothetical relationship between *VDR* and *DBP* gene polymorphisms and the odds of the severity of the disease to COVID-19 in the Iranian population. It may help evaluate the personalized odds of certain diseases such as COVID-19 and the treatment response.

2 | MATERIALS AND METHODS

2.1 | Study design and subject criteria

This project was a cross-sectional retrospective case-control study. Samples were collected from different hospitals affiliated with the Iran University of Medical Sciences with the assistance of the Iranian Network for Research in Viral Diseases (INRVD). All samples were received from the Ghods Clinic affiliated with Tehran Medical Sciences, Islamic Azad University, Tehran, Iran, from March to June 2020.

These samples include pharyngeal and nasopharyngeal swab specimens from unvaccinated individuals. Samples that used wooden cotton

swabs, small sample volumes, or inappropriate conditions such as poor shipping were excluded from the screening step. Based on defined inclusion criteria, samples were chosen.

2.1.1 Inclusion criteria

The case group is categorized by hospitalized patients with fever or respiratory symptoms who have confirmed positive SARS-CoV-2 reverse transcription polymerase chain reaction (RT-PCR) results. Outpatients with a history of fever or respiratory symptoms who had a negative SARS-CoV-2 RT-PCR result were considered the control group.

This study has an approval number IRTUMS.VCR.REC.1399.378 from the Ethics Committee of Tehran University of Medical Sciences.

2.2 | Genomic DNA extraction

After defining the case and control groups, genomic DNA from all samples was extracted using genomic extraction kits and according to the kit manufacturer's protocol (Sinacolon DNA EXTRACTION Kit DNP, IRAN). The purity of the extracted DNA was quantified using UV spectroscopy (Eppendorf BioPhotometer), and genomic DNA extraction was evaluated using amplification of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene by PCR technique and electrophoresis of PCR product on 1% agarose gel. The extracted DNA of samples was stored at -20°C until further analysis.

2.3 | Targeted segment amplified by conventional PCR

The specific primers needed to multiply target genes were selected using previous research, the NCBI site, and the Blast program. The sequences of all primers were presented in Table 1. Then, PCR was performed to amplify the targeted segment using specific primers for each gene segment according to a previously published study (Bahrami et al., 2020). To confirm the presence of the desired band, the PCR product was electrophoresed on 1% agarose gel.

2.4 | Digestion by restriction enzymes

After confirming the targeted bands' amplification, enzymatic digestion was performed on the PCR product according to the manufacturer's protocol. We used *FokI* (Thermo Scientific™ ER1881), *Apal* (Thermo Scientific™ ER0821), *TaqI* (Thermo Scientific™ ER0821) and *BsuRI* (Thermo Scientific™ ER0821) for enzymatic digestion of rs2228570, rs7975232, rs731236 and rs7041, respectively. Polymorphisms were demonstrated on 2.5% gel electrophoresis and interpreted from DNA banding patterns for single-nucleotide polymorphism (SNP) identification based on Table 1. SNP is a variation at a

TABLE 1 The corresponding data for each analysed SNP

Gene name	SNP (enzyme)	Primer sequence (5'–3')	Genotype (type)	Fragment's size (bp)	PCR protocol (denaturation, cycles, extension)	Reference
VDR	rs2228570 (FokI)	F: 5'CTGGCACTGACTCTGGCTCT3' R: 5'GGGCTCACCTGAAGAAGCCT3'	CC (Homozygous Reference)	204	95°C/10 min (95°C/30 s, 56°C/40 s, 72°C/30 s) × 35 72°C/5 min	(Bahrami et al., 2020)
			CT (Heterozygote)	204, 156, 48		
			TT (Homozygous Mutant)	156, 48		
VDR	rs7975232 (ApaI)	F: 5'CAGAGCATGGACAGGGAGCAA3' R: 5'CACTTCGAGCACAAAGGGCGTTAG3'	GG (Homozygous Reference)	490	95°C/5 min (95°C/50 s, 53°C/50 s, 72°C/40 s) × 30 72°C/5 min	(Bahrami et al., 2020)
			GT (Heterozygote)	490, 280, 210		
			TT (Homozygous Mutant)	280, 210		
VDR	rs731236 (TaqI)	F: 5'CAGAGCATGGACAGGGAGCAA3' R: 5'CACTTCGAGCACAAAGGGCGTTAG3'	TT (Homozygous Reference)	490	95°C/5 min (95°C/50 s, 53°C/50 s, 72°C/40 s) × 30 72°C/5 min	(Bahrami et al., 2020)
			TC (Heterozygote)	490, 290, 200		
			CC (Homozygous Mutant)	290, 200		
DBP	rs7041 (BsuRI)	F: 5'AAATAATGAGCAAATGAAAGAAGAC3' R: 5'CAATAACAGCAAAGAAATGAGTAGA3'	AA (Homozygous Reference)	482	94°C/5 min (94°C/45 s-56°C/45 s, 72°C/45 s) × 30 72°C/5 min	(Bahrami et al., 2020)
			AT (Heterozygote)	482, 298, 184		
			TT (Homozygous Mutant)	298, 184		
GAPDH		F: 5' CTC TTG CTA CTC TGC TCT G3' R: 5'GCC TGC CTG GTG ATA ATC3'	-	179	94°C/5 min (94°C/40 s, 56°C/40 s, 72°C/45 s) × 30 72°C/5 min	(Parsania et al., 2021)

single nucleotide at a specific position (adenine, thymine, cytosine or guanine) in a DNA sequence among individuals.

The experiments were repeated (all processes including DNA extraction, PCR, and digestion) in 10% of the randomly selected samples to validate data with the removal of procedural or observational errors.

2.5 | Statistical analysis

The collected data were analysed using a statistical test, and the results were reported separately for each gene compared to the control group. All statistical analysis of the data was performed using SPSS software v.24.0, and Pearson's chi-square tests were performed to assess the significance of identified genotypes-alleles relationships with COVID-19. The Bonferroni correction was done to determine the count of genotypes-alleles in each groups considered and to consider dependency in multiple-tests (0.05/n). The Bonferroni-corrected significance threshold for the case-control studies was considered .004167 (as shown in Tables 3 and 4). The count of genotypes and alleles of

each gene was examined using Hardy-Weinberg equilibrium (HWE) using the Pearson's chi-square goodness-of-fit test via SPSS and if p value < .05 the equilibrium is not in agreement with HWE. Logistic regression analysis calculated the 95% confidence interval (95% CI) and odds ratio (OR). The experiments were repeated in 10% of the randomly selected samples to validate procedural or observational error removal data, and p value < .05 was considered a statistically significant level.

3 | RESULTS

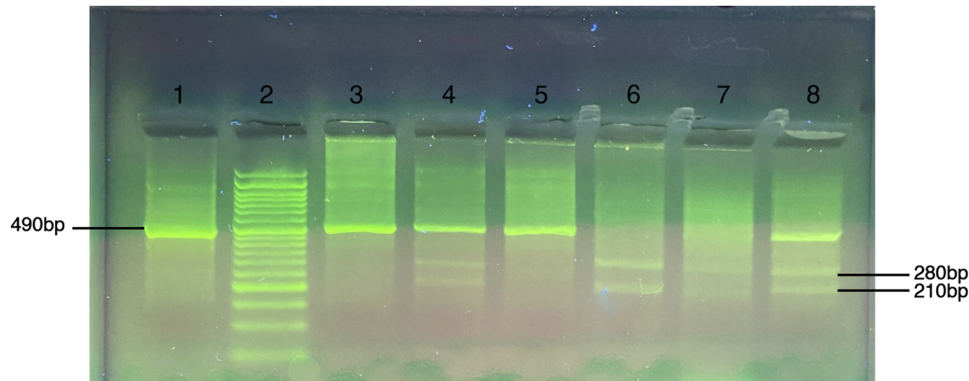
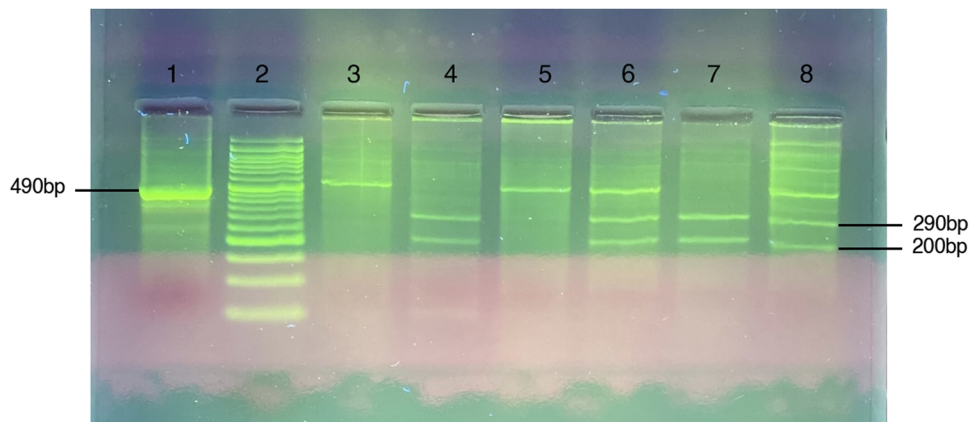
The study population for VDR (rs7975232, rs7975236 and rs2228570) and DBP rs7041 polymorphisms are shown in Table 2.

The allele and genotype frequencies of SNP of DBP rs7041 locus and three VDR loci (rs7975232, rs7312336 and rs2228570) were determined by the PCR-RFLP-based method (Figures 1–4). The results of genotype frequencies and allelic distribution for each gene locus and statistical analysis are exhibited in Table 3.

TABLE 2 Number of study population-based on gender in VDR (rs7975232, rs7975236 and rs2228570) and DBP rs7041 polymorphisms

	DBP rs7041	VDR rs2228570	VDR rs7975236	VDR rs7975232
Patients with COVID-19 (case group)	F: 110	F: 104	F: 121	F: 100
	M: 108	M: 114	M: 123	M: 120
	Total: 218	Total: 218	Total: 244	Total: 220
Suspected COVID-19 patients (control group)	F: 68	F: 70	F: 76	F: 76
	M: 120	M: 116	M: 92	M: 118
	Total: 188	Total: 186	Total: 164	Total: 194

F: female, M: male.

**FIGURE 1** Electrophoresis results of VDR SNPs (rs7975232) after *Apal* restriction enzyme cleavage on 2.5% agarose gel. Lane 6 and 7, TT Homozygous Mutant, lanes 4 and 8, GT Heterozygote, lane 3 and 5, GG Homozygous Reference, lane 2 DNA ladder 50 bp and lane 1 PCR products**FIGURE 2** Electrophoresis results of VDR SNPs (rs731236) after *TaqI* restriction enzyme cleavage on 2.5% agarose gel. Lane 4 and 7, CC Homozygous Mutant, lanes 6 and 8, TC Heterozygote, lane 3 and 5, TT Homozygous Reference, lane 2 DNA ladder 50 bp and lane 1 PCR products

Our results revealed no significant differences in the frequency of genotype and allele of VDR *Apal* (rs7975232), *TaqI* (rs731236) and *BsuRI* (rs7041) between total COVID-19 patients and negative controls ($p > .004167$). Statistical analysis demonstrated a significant difference in genotype and allele frequencies of VDR gene *FokI* (rs2228570) between the case and control groups ($p < .0001$). In VDR gene *FokI* (rs2228570), CC, CT and TT genotypes, frequencies were 58.2%, 38.1% and 3.7% in SARS-CoV-2-infected patients, and

78.5%, 16.7% and 4.8% in negative controls, respectively and the frequency of C and T alleles in VDR *FokI* loci was 77.3% and 22.7% in case group and 86.9% and 13.1% in the control group, respectively (Table 3).

The allele frequency within female and male groups was insignificant, with a $p > .004167$. Among female subjects, the frequency of C and T alleles in VDR *FokI* loci was 75.6% and 24.4% in SARS-CoV-2-infected patients and 86.8% and 13.2% in negative controls,

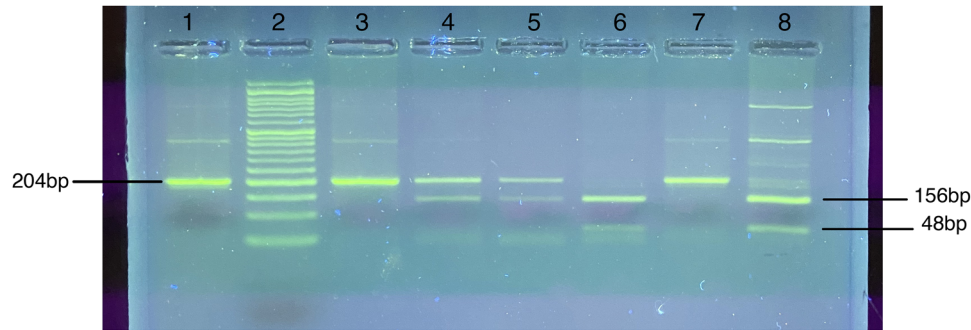


FIGURE 3 Electrophoresis results of VDR SNPs (rs2228570) after *FokI* restriction enzyme cleavage on 2.5% agarose gel. Lane 6 and 8, TT Homozygous Mutant, lanes 4 and 5, CT Heterozygote, lane 3 and 7, CC Homozygous Reference, lane 2 DNA ladder 50 bp and lane 1 PCR products

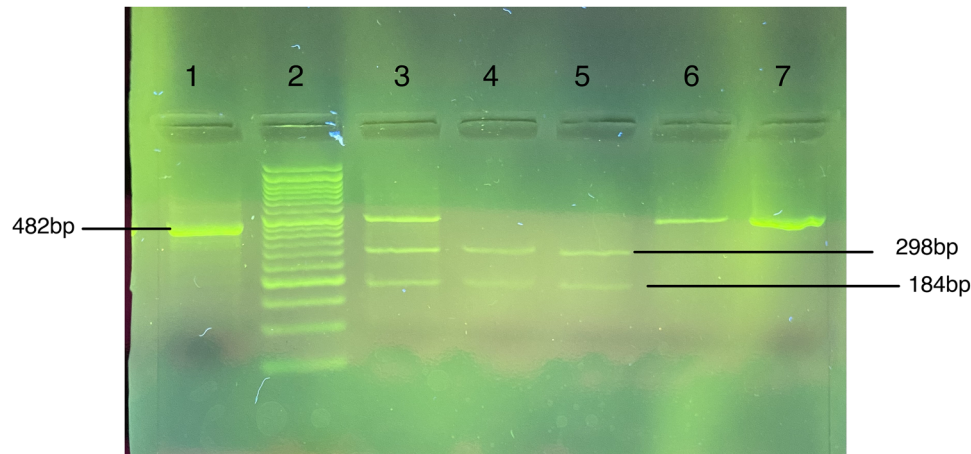


FIGURE 4 Electrophoresis results of DBP SNPs (rs7041) after *BsuRI* restriction enzyme cleavage on 2.5% agarose gel. Lane 4 and 5, TT Homozygous Mutant, lanes 3, AT Heterozygote, lane 6 and 7, AA Homozygous Reference, lane 2 DNA ladder 50 bp and lane 1 PCR products

respectively, and in the male subject, this frequency was 78.9% and 21.1% in case group and 87.0% and 13.0% in the control group, respectively (Table 4).

FokI CT genotype (Heterozygous) was associated with an increased odds of susceptibility to COVID-19 infection compared to CC (Homozygous Reference) genotypes within male and female groups ($p = .001$ and $.002$, respectively).

This study showed no significant differences in the frequency of genotype and allele of DBP *BsuRI* (rs7041), VDR *Apal* (rs7975232), and VDR *TaqI* (rs731236) ($p > .004167$).

In addition, VDR rs2228570 polymorphisms frequencies were agreed to the Hardy-Weinberg equilibrium (HWE) in case and control groups ($p = .296$).

4 | DISCUSSION

The current study evaluated the possible relation between VDR and DBP polymorphisms and COVID-19 susceptibility. Eventually, the strong significant results were demonstrated in VDR rs2228570 results.

A rapid influx of research is currently being conducted on the relationship between VDR and DBP polymorphism and the susceptibility to COVID-19 and its outcome (AlSafar et al., 2021; Grant et al., 2020; Shah Alam et al., 2021).

Previously, the modulatory effects of active vitamin D on T cells include inhibiting T cell proliferation, shifting responses from Th1 to Th2 development, inhibiting Th17 cell growth, accelerating regulatory cell function, and inhibiting inflammatory cytokine production by T-monocytes has been indicated (Uitterlinden et al., 2004). All entire regulatory functions of vitamin D indirectly depend on VDR and transporter (DBP) (Bahrami et al., 2020). Variations in SNPs of VDR and DBP might be valuable indicators for predicting susceptibility to several diseases (Laplana et al., 2018).

Our results revealed that the *FokI* CT (Heterozygous) genotype significantly increases the odds of COVID-19 compared to CC (Homozygous Reference) ($p < .0001$; OR: 3.088; 95% CI: 1.902–5.012). T allele of VDR rs2228570 (*FokI*) was found to be associated with high susceptibility to SARS-CoV-2 infection ($p = .001$, OR: 1.954; 95% CI: 1.335–2.860). Interestingly, Laplana et al. (2018) study has shown a significant linkage between the frequency of

TABLE 3 Distributions and association analysis of all analysed genotypes and allele frequencies among case (COVID-19 positive) and negative control (COVID-19 negative)

		Control (%)	Case total: (%)	p Value	OR (95%, CI)
VDR rs7975232 (Apal) All subjects (188–218)	GG	128 (68.1)	147 (67.4)	–	ref (1.00)
	GT	60 (31.9)	69 (31.7)	.995	1.001 (0.658–1.523)
	TT	0 (0.0)	2 (0.9)	.501	1.014 (0.995–1.033)
	Total genotypes	188	218	.420	–
	G allele	316 (84.0)	363 (82.5)	–	ref (1.00)
	T allele	60 (16.0)	77 (17.5)	.557	1.117 (0.772–1.617)
VDR rs731236 (TaqI) All subjects (186–218)	TT	80 (43.0)	93 (42.7)	–	ref (1.00)
	TC	74 (39.8)	103 (47.2)	.404	1.197 (0.785–1.827)
	CC	32 (17.2)	22 (10.1)	.097	0.591 (0.318–1.099)
	Total genotypes	186	218	.079	–
	T allele	234 (62.9)	289 (66.3)	–	ref (1.00)
	C allele	138 (37.1)	147 (33.7)	.316	0.862 (0.646–1.152)
VDR rs2228570 (FokI) All subjects (168–244)	CC	132 (78.5)	142 (58.2)	–	ref (1.00)
	CT	28 (16.7)	93 (38.1)	<.0001	3.088 (1.902–5.012)
	TT	8 (4.8)	9 (3.7)	.929	1.046 (0.392–2.790)
	Total genotypes	168	244	<.0001	–
	C allele	292 (86.9)	377 (77.3)	–	ref (1.00)
	T allele	44 (13.1)	111 (22.7)	.001	1.954 (1.335–2.860)
DBP rs7041 (BsuRI) All subjects (194–220)	AA	50 (25.8)	45 (20.5)	–	ref (1.00)
	AT	100 (51.5)	110 (50.0)	.418	1.222 (0.752–1.986)
	TT	44 (22.7)	65 (29.5)	.080	1.641 (0.942–2.861)
	Total genotypes	194	220	.205	ref (1.00)
	A allele	200 (51.5)	200 (45.5)	–	1.277 (0.971–1.678)
	T allele	188 (48.5)	240 (54.5)	.080	–
Overall p value*		194	244	.0002 [#]	–

*Calculated by Bonferroni correction for 12 (4 for SNP and 3 for genotype) comparisons (p value threshold considered at level .004167).

[#]The Bonferroni-corrected significance threshold for the case-control studies was considered 0.004167.

the TT *FokI* genotype (C > T) and the T allele in viral-infected patients.

Arai et al. (1997), based on a study on transfected HeLa cells with the appropriate VDR expression vector, were demonstrated that the *FokI* locus C allele (Reference Allele) tends to have high transcriptional activity compared to the T allele (Mutant Allele); consequently causing higher VDR expression and further binding to calcitriol. In our study, association between TT genotype and VDR expression was not well determined. One reason for this may be the low frequency of individuals with TT genotype in the case and control groups (3.7% and 4.8%, respectively). Concurrently, the *FokI* polymorphism (rs2228570) is located in the start codon, the only known VDR gene polymorphism that alters protein production rate with three longer amino acids that lead to a reduction in transcriptional activity. The short form of VDR protein is a more transcriptional activity function than the longer-length of VDR protein; any variation in VDR structure may lead to a decline in VDR production; subsequently, it may affect the host anti-

microbial response (Bahrami et al., 2020; Salimi et al., 2019; Triantos et al., 2018).

BsuRI, TaqI and Apal VDR polymorphisms could interrupt the interaction between vitamin D and VDR, subsequently reducing the vitamin D-related signalling pathways activities, and, consequently, pro-inflammatory cytokines release becomes uncontrolled. Numerous studies have reported a positive correlation between VDR polymorphism and higher pro-inflammatory cytokine levels, leading to the severity of the disease (Triantos et al., 2018). The association between SNPs of VDR loci, including, *Apal*, *TaqI*, *FokI* and *BsuRI*, in several diseases such as autoimmune thyroid disease, osteoporosis, and breast cancer has been investigated by Iranian researchers (Kazemi et al., 2022; Mohammadi et al., 2015; Zarrin et al., 2018). Several case-control studies reported the correlation between *FokI* polymorphism (rs2228570) and numerous diseases, such as intestinal inflammation, diabetes mellitus type I, and Lumbar disc degeneration (Mashayekhi et al., 2018; Naderi et al., 2008; Soroush et al., 2017).

TABLE 4 Distributions and association analysis of VDR (rs2228570, rs7975232 and rs731236) and DBP (rs7041) analysed genotypes and allele frequencies within female and male among case and control groups genotypes and allele frequencies within females and males among case and control groups

			Control (total: 168) N (%)	Case (total: 244) N (%)	p Value*	OR (95% CI)
VDR rs2228570 (FokI)	Male (92-123)	CC	72 (78.3)	72 (58.5)	-	ref (1.00)
		CT	16 (17.4)	50 (40.7)	.001#	3.125 (1.630-5.991)
		TT	4 (4.3)	1 (0.8)	.220	0.250 (0.027-2.292)
		C allele	160 (87.0)	194 (78.9)	-	ref (1.00)
		T allele	24 (13.0)	52 (21.1)	.031	1.787 (1.055-3.027)
	Female (76-121)	CC	60 (78.9)	70 (57.9)	-	ref (1.00)
		CT	12 (15.8)	43 (35.5)	.002#	3.071 (1.485-6.354)
		TT	4 (5.3)	8 (6.6)	.398	1.714 (0.492-5.976)
		C allele	132 (86.8)	183 (75.6)	-	ref (1.00)
		T allele	20 (13.2)	59 (24.4)	.008	2.128 (1.222-3.704)
DBP rs7041 (BsuRI)	Male (118-120)	AA	30 (25.4)	24 (20.0)	-	ref (1.00)
		AT	64 (54.2)	55 (45.8)	.828	1.074 (0.563-2.051)
		TT	24 (20.3)	41 (34.2)	.043	2.135 (1.023-4.458)
		A allele	124 (52.5)	103 (42.9)	-	ref (1.00)
		T allele	112 (47.5)	137 (47.1)	.036	1.473 (1.026-2.114)
	Female (76-100)	AA	20 (26.3)	21 (21.0)	-	ref (1.00)
		AT	36 (47.4)	55 (55.0)	.322	1.455 (0.692-3.058)
		TT	20 (26.3)	24 (24.0)	.759	1.143 (0.487-2.681)
		A allele	76 (50.0)	97 (48.5)	-	ref (1.00)
		T allele	76 (50.0)	103 (51.5)	.780	1.062 (0.696-1.619)
VDRrs7975232 (ApaI)	Male (120-110)	GG	88 (73.3)	77 (70.0)	-	ref (1.00)
		GT	32 (26.7)	33 (30.0)	.575	1.179 (0.664-2.093)
		TT	0 (0.0)	0 (0.0)	N/A	N/A
		G allele	208 (86.7)	187 (85.0)	-	ref (1.00)
		T allele	32 (13.3)	33 (15.0)	.608	1.147 (0.679-1.939)
	Female (68-108)	GG	40 (58.8)	70 (64.8)	-	ref (1.00)
		GT	28 (41.2)	36 (33.3)	.336	0.735 (0.392-1.377)
		TT	0 (0.0)	2 (1.9)	.537	1.029 (0.989-1.070)
		G allele	108 (79.4)	176 (81.5)	-	ref (1.00)
		T allele	28 (20.6)	40 (18.5)	.632	0.877 (0.511-1.503)
VDR rs731236 (TaqI)	Male (116-114)	TT	44 (37.9)	46 (40.4)	-	ref (1.00)
		TC	48 (41.4)	54 (47.4)	.800	1.076 (0.610-1.898)
		CC	24 (20.7)	14 (12.2)	.142	0.558 (0.526-1.215)
		T allele	136 (58.6)	146 (64.0)	-	ref (1.00)
		C allele	96 (41.4)	82 (36.0)	.234	0.796 (0.546-1.159)
	Female (70-104)	TT	36 (51.4)	47 (45.2)	-	ref (1.00)
		TC	26 (37.1)	49 (47.1)	.264	1.444 (0.758-2.748)
		CC	8 (11.4)	8 (7.7)	.626	0.766 (0.262-2.237)
		T allele	98 (70.0)	143 (68.7)	-	ref (1.00)
		C allele	42 (30.0)	65 (31.3)	.804	1.061 (0.666-1.689)

*Calculated by Bonferroni correction for 12 (4 for SNP and 3 for gender) comparisons (p value threshold considered at level .004167).

#The Bonferroni-corrected significance threshold for the case-control studies was considered 0.004167.

Triantos et al. have demonstrated that reference genotype in the VDR rs7975232 (Apal) gene is associated with significantly higher pro-inflammatory cytokines such as IL-1 β and IL-8. This finding suggests that Apal VDR polymorphism adversely had influenced the activity of vitamin D-related signalling pathways, then the disturbance of Th1/Th2 balance had occurred (Triantos et al., 2018). In the current study, we showed no significant association between VDR (rs7975232 and rs731236) and COVID-19 among the Iranian population, and this may be due to some factors such as sample size, genetic background and race diversity of the study population.

DBP is principally produced in the liver and is mainly regulated by glucocorticoids, oestrogen and inflammatory cytokines (Alshahawey, 2021). Our results indicated no significance in DBP rs7041 polymorphism between case and control.

Contrary to the current study, the association of rs7041 locus polymorphisms with increased odds of COVID-19 infection and mortality rate was demonstrated by Batur and Hekim (2021). Hence, the association of DBP polymorphisms and COVID-19 infection may depend on the DBP modulatory impact on the bioavailable vitamin D levels in fetal cases. It must be noticed that we did not involve any death-related data in our study.

This preliminary study was the first report on VDR and DBP gene polymorphisms frequencies among COVID-19 patients in the Iranian population. Perceptively, following any dysfunction and change in the expressed level of some gene can lead to the production of a different phenotype with biological function. The controller function of vitamin D on the immune system is impaired, and thus regulation of immune responses following severe immune reaction does not occur in response to infections in the host body. Therefore, it can cause severe, life-threatening immunopathological phenomenon, such as those seen in RSV or influenza cytokine storms and maybe in COVID-19. During this pandemic, numerous reports are being published about discovering genes that are claimed to have a fundamental role in the pathogenesis of SARS-CoV-2 infection. It must be considered that this difference in disease incidence is probably due to the interaction of a set of several genes (Hashemi et al., 2021). Accordingly, the interest to emphasize such fundamental epidemiological studies to find out any novel possible linkage disequilibrium that may be utilized in emerging prediction, response to treatment, or the prognosis of the diseases. Besides the VDR polymorphism frequency pattern in the Iranian population, due to vitamin D deficiency and the critical role of this vitamin in the regulating inflammatory cytokines in the host immune systems, the incidence of COVID-19 with high frequency and severity among the Iranian population is logically expected.

In conclusion, there was no association between VDR (rs7975232 and rs731236) and DBP genes polymorphism and COVID-19 among the Iranian population. The polymorphism rs2228570 C/T of the VDR gene was associated with increased odds of susceptibility to COVID-19. Also, the high COVID-19 incidence and death rate in the Iranian population may be associated with other genetic backgrounds. Further studies in specific groups with high-throughput data such as next-generation sequencing (NGS) analysis and measurement of calcitriol baseline are required.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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