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Impact of vitamin D binding protein (GC) and vitamin D receptor (VDR) gene polymorphism on the risk of developing preeclampsia

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ABSTRACT

Objective: Preeclampsia is a multifactorial disease characterized by high blood pressure and protein in the urine. In this study, we investigated the association of vitamin D binding protein (GC) and vitamin D receptor (VDR) gene polymorphism with the risk of developing preeclampsia.
Methods: 25-hydroxyvitamin D was measured using High-performance Liquid Chromatography. Vitamin D binding protein and vitamin D receptor gene polymorphisms were determined by polymerase chain reaction-restriction fragment length polymorphism.
Results: The control subjects have significant higher level of 25-hydroxyvitamin D (33.5 ± 1.194 ng/mL) relative to patients (23.97 ± 1.604 ng/mL) ($p < 0.05$). Vitamin D receptor rs1544410 and rs2228570 dominant model (GA + AA; TC + CC) showed significant higher risk of developing Preeclampsia (OR = 4.11, 95% CI = 0.62–27.09, $p < 0.01$; OR = 3.58, 95%CI = 0.78–16.38, $p < 0.001$ respectively). Similarly, vitamin D binding protein rs7041 and rs4588, dominant model (TG + GG; CA + AA) showed higher risk of preeclampsia development compared to control people (OR = 1.69, 95%CI = 0.35–8.19, $p < 0.05$; OR = 1.06, 95%CI = 0.25–4.44, $p < 0.05$ respectively). AA genotype of rs4588 of GC gene was significantly associated with 25-hydroxyvitamin D level in serum relative to CC and CA ($p < 0.05$).
Conclusion: From our study, we can conclude that a low level of 25-hydroxyvitamin D, GC (rs1544410 and rs2228570), and VDR (rs4588 and rs7041) gene polymorphism is linked with an increased risk of developing preeclampsia.

1. Introduction

Preeclampsia is a multifactorial condition defined by denovo hypertension after 20 weeks of gestation, proteinuria, and/or signs of maternal acute kidney injury (AKI), liver failure, neurological symptoms, hemolysis, or thrombocytopenia, or fetal growth restriction (FGR) [1]. The risk factors of preeclampsia include prior preeclampsia, chronic hypertension, pregestational diabetes mellitus, maternal body mass index >30 kg/m^2, antiphospholipid syndrome, and receipt of assisted reproduction [1]. Vitamin D is a class of fat-soluble secosteroids that has a variety of biological effects, including enhancing intestinal absorption of calcium, magnesium, and phosphate [2]. Intervention research found that taking vitamin D supplements dramatically reduced the chance of developing preeclampsia [3]. According to one study, preeclamptic women have lower levels of vitamin D than the control group [4]. The association of vitamin D deficiency with the risk of developing preeclampsia during pregnancy in Bangladesh was reported by Ullah et al. [5]. According to a meta-analysis, vitamin D insufficiency is strongly related to preeclampsia, and vitamin D supplementation considerably lowers the risk of preeclampsia during pregnancy [6,7].

In humans, the GC gene encodes group-specific component protein (GC protein). It is the primary 25(OH)D and 1,25(OH)2D binding protein [8]. Several studies have shown changes in calcium and vitamin D metabolism during clinical illness in late pregnancy, including hypocalciuria, in preeclampsia [9–12]. It was also postulated that changes in vitamin D levels during preeclamptic pregnancy might be mediated by changes in circulation levels of GC protein [8]. Two single nucleotide variants (SNVs), rs7041 at codon 416 (Asp > Glu) and rs4588 at codon

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420 (Thr > Lys), have been thoroughly investigated and are linked to lower vitamin D binding affinity. As a result, GC gene polymorphism may alter the availability of vitamin D in circulation during pregnancy, increasing the risk of preeclampsia.

Vitamin D receptor protein is encoded by the VDR gene located in chromosome 12 (12q13.11). The active vitamin D metabolite calcitriol modulates its biological effects by binding to the vitamin D receptor (VDR), a steroid hormone receptor present in bone, kidney, small intestine, and neuronal cells [14–16]. VDR gene expression in the placenta controls several genes involved in implantation, fetal maturation, and bone development [17]. For our investigation, we chose two VDR gene SNVs *Bsm*I (rs1544410) and *Fok*I (rs2228570). Studies on the Iranian and Chinese populations identified an association between *Fok*I polymorphism, low level of vitamin D, and risk of preeclampsia [18–20]. The present study was conducted to explore the genetic inference of VDR and GC gene polymorphism on the risk of late-onset preeclampsia in Bangladeshi women.

2. Materials and methods

2.1. Study population

For the study, 240 unrelated female subjects were selected from different hospitals in Dhaka city. Among them, 120 are preeclampsia patients, and 120 are healthy pregnant women having no history of preeclampsia and other chronic diseases. Samples were collected between November 2017 to October 2018 during the summer season from two different hospitals named "Azimpur maternity clinic" and "Dhaka medical college hospital" in Dhaka city (we collected cases from two hospitals to accelerate the collection speed). The study was done on a homogeneous ethnic group, and no tribal population was included in the study. Pregnant women who were normotensive before pregnancy but developed hypertension (blood pressure>140/90) during the second trimester of pregnancy or later and tested positive for proteinuria were included as preeclamptic cases for the study. All samples were included in the study after confirming the late onset of the disease. Blood samples were collected from patients with increased blood pressure between two measurements of 4 h and >1+ on dipstick urine analysis in two samples taken ≥6 h apart [21]. A similar procedure was followed for control selection. Subjects with normal blood pressure (around 120/80 ± 5) were kept in follow-up until the baby's birth. Baby birth weight was recorded during birth, and all babies were full term. Aspirin was not used at any time during pregnancy. Blood samples from age-matched healthy pregnant women were taken in the first week of the third trimester, similar to preeclampsia subjects. Initially, 400 cases and 400 controls were contacted and kept under follow-up. Finally, we had 120 cases and 120 control subjects in our study because some of the study subjects developed other complications; some lost their interest to be enrolled in the study; some changed their hospital. All participants were informed about the study, and written informed consent was taken. A structured questionnaire was completed, which includes information of patients like - age, systolic blood pressure, diastolic blood pressure, gestational age, protein in the urine, leg swelling, and the number of pregnancies. Serum 25-hydroxyvitamin D (25(OH)D) level was measured in all subjects by the HPLC method described earlier [22]. Serum glucose, ALP, and calcium were measured using the colorimetric method [23–25]. The study was approved by the faculty of biological science, University of Dhaka ethical committee (approval number – BMBDU-ERC/EC/17/013). The study was conducted under the rules in the Declaration of Helsinki [26].

2.2. Sample collection

Approximately 3.0 mL of blood was drawn from each individual under aseptic precautions using a disposable syringe. One mL of drawn blood was taken in an ethylene diamine tetraacetic acid (EDTA) containing tube. The remaining 2 mL of blood was taken in a plain tube. The serum was separated from clotted blood within 1 h after drawing blood. All plain and EDTA tubes were placed in an icebox during transportation to the laboratory. Whole blood and serum samples were stored at −20 °C until doing experiments. DNA was extracted within one month after collecting whole blood and after checking the purity and concentration of the extracted DNA, it was stored at −80 °C.

2.3. Analyzing biochemical parameters

25(OH)D level was measured using a method described earlier [27]. Initially, 350 μL methanol:2-propanol(80:20) was added with 500 μL serum and vortexed for a few seconds to complete the denaturation of proteins. 25(OH)D was extracted in hexane (6 mL) and centrifuged at 3000 rpm for 5 min to separate the two layers. The upper hexane layer was taken into another fresh screw-cap tube and dried under nitrogen gas. At all times during sample preparation, the tubes were covered using aluminum foil to protect them from light. After completion of drying, residue in the tube was dissolved in 100 μL methanol and was taken into an amber HPLC vial for analysis.

2.4. Vitamin D receptor (VDR) genotyping

VDR *Bsm*I polymorphism (rs1544410) was investigated according to the protocol of Sarkissyan et al. [28]. *Bsm*I digestion produces three fragments depending on the genotype; GG (wild type): 820 bp; GA (heterozygote mutant): 820, 650, 170bp; and AA (homozygote mutant): 650, 170bp (Fig. 1a).

Primer and PCR conditions for VDR *Fok*I polymorphic site (rs2228750) were described earlier [29]. The PCR product size was 270 bp. After digestion using *Fok*I RE for 1 h at 37 °C, digested PCR product was separated in 2% agarose gel and visualized by ethidium bromide staining. *Fok*I digestion products contain three fragments depending on the genotype; CC (homozygote mutant): 210, 60 bp; TT (wild type): 270 bp and TC (heterozygote mutant): 270, 210, and 60 bp (Fig. 1b). All PCR was done in triplicates and then digested with desired RE to ensure the accuracy of the genotyping. Negative control was used for all PCR.

2.5. Vitamin D binding protein (GC) genotyping

Vitamin D binding protein (GC) gene polymorphism (rs7041 and rs4588) was determined using the polymerase chain reaction followed by restriction fragment length polymorphism described earlier, and the same primer sets were used [27]. The amplified product size was 483 bp band. Complete (297, 186 bp fragment), incomplete (483, 297, 186 bp fragment), and absence of *Hae*III digestion (only 483 bp fragment) indicate GG, TG, and TT genotype (Fig. 1c). For *Sty*I restriction site, complete (305 and 178 bp fragment), incomplete (483, 305, and 178 bp fragment) and absence of digestion (only 483 bp fragment) indicate AA, CA, and CC genotype (Fig. 1d). All restriction enzyme-digested PCR products were resolved in 2% agarose gel followed by ethidium bromide staining. All PCR was done in triplicates and then digested with desired RE to ensure the accuracy of the genotyping. Negative control was used for all PCR.

2.6. Statistical analysis

Statistical analyses were carried out using Graphpad Prism (version 7.0). Results were expressed as mean ± SEM (Standard error mean). Odds Ratio (OR) and 95% Confidence Interval (CI) were measured using logistic regression model for cases relative to controls for different genotypes of SNVs. The odds ratio was adjusted for Age, Systolic blood press, Diastolic blood pressure, Gestational age, Protein in urine, Leg swelling, Parity, and Biochemical parameters. The statistical method used was the unpaired *t*-test (two-tailed), Chi-square test, and Fisher exact test. The plink software package was used to assess the allele

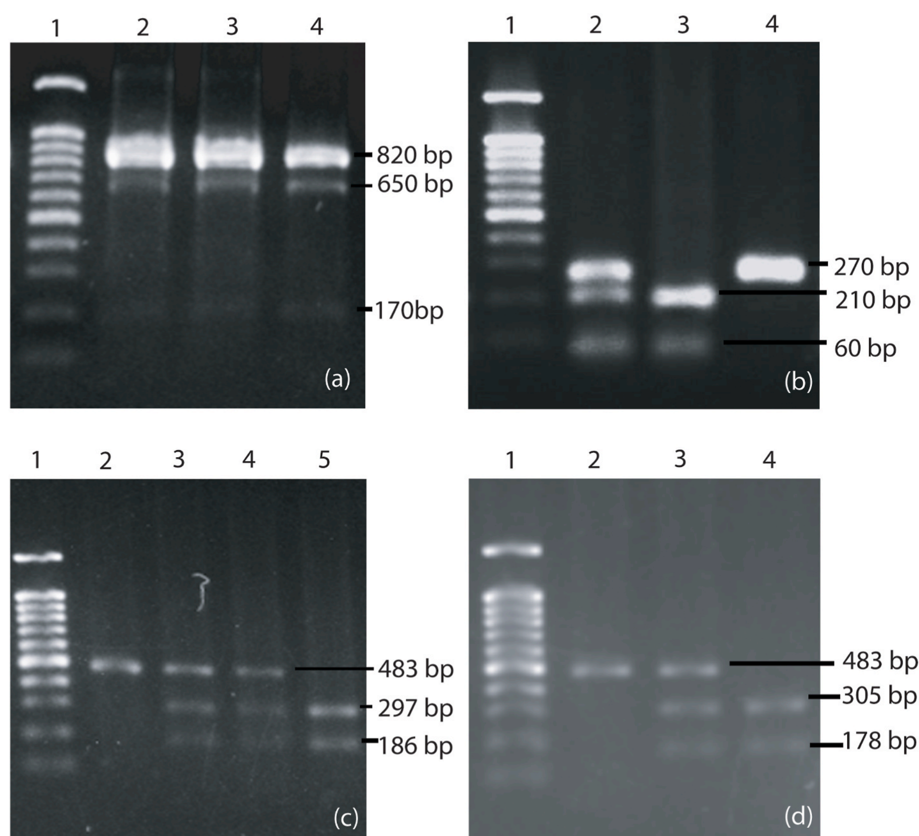

Fig. 1. The banding pattern of GC and VDR genotypes. (a) Banding pattern of VDR rs1544410 PCR product after digestion with *Bsm*I restriction enzyme (RE). Presence of 820 bp fragment represents GG genotype (lane 2). Presence of 820, 650, and 170 bp bands represent GA genotype (lane 1,3) and 650, 170 bp bands represent AA genotype (not present in this picture). 100 bp ladder in lane 1. (b) Banding pattern of VDR rs2228570 PCR product after digestion with *Fok*I RE. Absence of digestion (only 270 bp band; lane 1) represent TT genotype. Incomplete digestion represents TC genotype (270, 210, 60 bp band; lane 4). Complete digestion represents CC genotype (210, 60 bp band; lane 2). 100 bp ladder in lane 1. (c) Banding pattern of GC rs7041 polymorphism. Complete (297, 186 bp; lane 2,4), incomplete (483, 297, 186 bp; lane 1) and absence of digestion (only 483 bp; lane 3) represent GG, TG, and TT genotype. 100 bp ladder in lane 1. (d) Banding pattern on agarose gel of rs4588 polymorphism. *Sty*I digestion creates 1 band for CC (483 bp; lane 3), 2 band for AA (305, 178 bp; lane 2,4) and 3 band for CA (483, 305, 178 bp; lane 1) genotype. 100 bp ladder in lane 1.

association test and the Hardy–Weinberg equilibrium test [30]. Haplotype analysis and Linkage disequilibrium were calculated using SheSis Webtool [31]. $P < 0.05$ was considered significant.

3. Results

3.1. Baseline characteristics of the study population

The baseline characteristics of the study subjects include age, systolic blood pressure (SBP), diastolic blood pressure (DBP), gestational age (weeks), protein in the urine, leg swelling, and parity (Table 1). All parameters were significantly different between two groups except for fetal sex. Vitamin D level is significantly lower in preeclampsia patients having protein in urine relative to non-proteinuric patients (data not shown).

3.2. Association of preeclampsia with VDR rs1544410 and rs2228570 polymorphism

Table 2 represents the genotype frequency and risk of developing preeclampsia based on rs1544410 and rs2228570 genotypes. For rs1544410, the frequency of GG, GA, AA genotypes in control subjects were 88.33, 10.83, 0.84% respectively, and 72.5, 25, 2.5% in patients respectively. For rs2228570, the frequency of TT, TC, CC genotypes was 70, 22.5, 7.5% in control subjects and 42.5, 49.17, 8.33% in patients respectively. For rs1544410, there were approximately 3.5 times and 4.5 times higher risks of developing preeclampsia in GA and AA genotype carriers ($p < 0.01$ for GA genotype and p = ns for AA genotype). The dominant model showed that GA + AA carriers are at four times higher risk of developing preeclampsia compared to GG genotype carriers ($p < 0.01$).

For rs2228570, TC genotype showed five times more risk of developing preeclampsia compared to TT genotype ($p < 0.001$). In the

Table 1
Baseline characteristics and clinical parameters of the study subjects.

Parameters	Case (n = 120)	Control (n = 120)	P-value
Age (Years)	27.09 ± 0.44	25.6 ± 0.33	0.007
SBP (mmHg)	151.8 ± 1.86	116.2 ± 0.96	<0.0001
DBP (mmHg)	96.04 ± 1.0	78.38 ± 0.62	<0.0001
Gestational Age (Weeks)	29.66 ± 0.28	29.15 ± 0.22	0.1534
25(OH)D (ng/mL)^{ǂ}	23.97 ± 1.60	33.5 ± 1.19	0.0235
Baby birth weight (kg)	2.26 ± 0.11	2.67 ± 0.09	<0.01
Small for gestational age			
Yes	98 (81.6)	3 (2.5)	<0.0001
No	22 (18.4)	117 (97.5)	
Fetal sex			
Male	56	49	ns
Female	64	71	
Protein in Urine			
Yes	71 (59.17)	5 (4.17)	<0.0001
No	49 (40.83)	115 (95.83)	
Leg Swelling			
Yes	66 (0.55)	14 (11.67)	<0.0001
No	54 (0.45)	106 (88.33)	
Parity			
1	26 (21.67)	60 (50.0)	
2	44 (36.67)	42 (35.0)	<0.0001
>2	50 (41.66)	18 (15.0)	
25(OH)D tertiles			
<20 ng/mL	67 (55.83)	27 (22.50)	
20–40 ng/mL	32 (26.67)	50 (41.67)	<0.0001
>40 ng/mL	21 (17.50)	43 (35.83)	

Data represented as mean ± SEM and number (%). SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; 25(OH)D, vitamin D. *t*-test was done to assess significant difference between two groups. ǂValues were adjusted for age, SBP, DBP, gestational age, protein in urine, leg swelling, and parity.
$P < 0.05$ was considered significant ns, non-significant.

Table 2
Genotype frequency and risk assessment of rs1544410 and rs2228570 genotypes in study subjects.

VDR SNVs	Control	Case	P-value[a]	P-value[b]	OR (95% CI)
rs1544410 genotype	(n = 120)	(n = 120)			
GG	106 (88.33)	87 (72.50)	–	<0.01	1 (Ref.)
GA	13 (10.83)	30 (25.00)	<0.01		3.46 (0.49–24.44)
AA	1 (0.84)	3 (2.50)	ns		4.65 (0.53–47.86)
GA + AA	14 (11.67)	33 (27.5)	<0.01		4.11 (0.62–27.09)
rs2228570 genotype					
TT	84 (70.00)	51 (42.50)	–	<0.001	1 (Ref.)
TC	27 (22.50)	59 (49.17)	<0.001		5.04 (0.93–27.17)
CC	9 (7.50)	10 (8.33)	ns		1.08 (0.07–16.12)
TC + CC	36 (30.00)	69 (57.50)	<0.001		3.58 (0.78–16.38)

Data represented as Number (%). OR (95% CI), Odds Ratio (95% Confidence Interval).
[a]P-value from fisher exact test.
[b]P-value from Chi-square test for significant difference in genotypic distribution.
Odds ratio adjusted for Age, Systolic blood press, Diastolic blood pressure, Gestational age, protein in urine, Leg swelling, Parity, and Biochemical parameters.

dominant model, TC + CC genotype showed a 3.5 times higher risk of developing preeclampsia, and it was significant ($p < 0.001$).

3.3. Association of preeclampsia with GC gene rs7041 and rs4588 polymorphism

Table 3 represents the GC gene rs7041 and rs4588 genotype frequency and risk assessment in the study population. For rs7041, the frequency of TT, TG, GG genotypes were 35, 41.67, 23.33% respectively in control and 20, 54.17, 25.83% respectively for patients. Similarly, for rs4588, the frequency of CC, CA, AA genotypes were 66.67, 30.83, 2.5% respectively in controls and 50.83, 43.33, 5.83% in patients respectively. For rs7041, TG genotype carrier people are 1.7 times more vulnerable to developing preeclampsia compared to TT genotype ($p < 0.05$). In the dominant model, TG + GG genotype carriers are 1.69 times more vulnerable to developing preeclampsia ($p < 0.05$) when compared to TT genotype carriers.

In the case of rs4588 polymorphism, for CA genotype, the risk was 0.98 times compared to CC genotype ($p < 0.05$). For the dominant model, CA + AA genotype carriers are 1.06 times more vulnerable to developing preeclampsia than CC genotype ($p < 0.05$).

Table 3
Significance of genotypes and risk of developing preeclampsia in rs7041 and rs4588 polymorphism in study subjects.

GC SNVs rs7041 genotype	Control (n = 120)	Case (n = 120)	P-value[a]	P-value[b]	OR (95% CI)
TT	42 (35.00)	24 (20.00)	–		1 (Ref.)
TG	50 (41.67)	65 (54.17)	<0.05	<0.05	1.73 (0.31–9.46)
GG	28 (23.33)	31 (25.83)	ns		1.63 (0.24–11.32)
TG + GG	78 (65.00)	96 (80.00)	<0.05		1.69 (0.35–8.19)
rs4588 genotype					
CC	80 (66.67)	61 (50.83)	–		1 (Ref.)
CA	37 (30.83)	52 (43.33)	<0.05	<0.05	0.98 (0.22–4.30)
AA	3 (2.50)	7 (5.83)	ns		2.01 (0.07–57.48)
CA + AA	40 (33.33)	59 (49.17)	<0.05		1.06 (0.25–4.44)

Data represented as Number (%). OR (95% CI), Odds Ratio (95% Confidence Interval).
Odds ratio adjusted for Age, Systolic blood press, Diastolic blood pressure, Gestational age, protein in urine, Leg swelling, Parity and Biochemical parameters.
[a]P-value from fisher exact test.
[b]P-value from Chi-square test for significant difference in genotypic distribution.

3.4. Analysis of minor alleles associated with the disease trait

Hardy-Weinberg equilibrium test was used to analyze the constancy of genotype frequency in study subjects (Online resource, Table S1). Minor allele association with disease condition and frequency of all major and minor alleles in cases and controls was done using plink allele association test (Online resource, Table S2). For rs4588, rs7041, rs1544410, and rs2228570, the minor allele was 'A', 'G', 'A', and 'C'. For rs4588, 'A' allele carriers are significantly 1.738 times more vulnerable to developing disease conditions compared to 'C' allele ($p < 0.05$). 'A' allele of VDR rs1544410 significantly increases the risk of disease 2.65 times compared to 'G' allele ($p < 0.01$). Similarly, the minor allele of rs2228570 increases the risk of disease 2.13 times compared to the dominant allele 'T' ($p < 0.001$).

3.5. Haplotype and linkage disequilibrium (LD) analysis of rs7041, rs4588, rs1544410, and rs2228570 in study subjects

Table 4 represents haplotype analysis of rs7041, rs4588, rs1544410, and rs2225870. LD and R^2 for 4 SNVs of GC and VDR gene represented in Fig. 2. Gene interaction analysis using SheSis plus showed a significant difference between case interaction and control interaction for 'rs7041, rs2228570' and 'rs7041, rs4588, rs1544410' (Online resource, Table S3).

3.6. Impact of GC and VDR genotypes on serum 25(OH)D level

Fig. 3 represents the distribution of 25(OH)D levels in different genotypes of GC and VDR SNVs. For rs7041, 25(OH)D level was 31.63 ± 1.616, 29.20 ± 1.875, 27.19 ± 2.249 ng/mL for TT, TG, and GG genotypes, respectively. There were no significant difference in 25(OH)D levels within different genotypes ($p > 0.05$). However, for rs4588, a significant difference was found in 25(OH)D level in different genotypes (for CC, CA, and AA genotype, 25(OH)D level was 32.63 ± 1.774, 28.33 ± 1.750, and 19.71 ± 1.975 ng/mL respectively; $p < 0.05$). On the other hand, VDR rs1544410 genotypes GG, GA, and AA have 25(OH)D levels around 31.43 ± 1.417, 27.76 ± 2.805, and 28.29 ± 3.416 ng/mL, respectively. Similarly, for rs2228570, 25(OH)D levels for TT, TC, and CC genotypes were 31.55 ± 1.890, 30.58 ± 1.810, and 25.81 ± 3.627 ng/mL, respectively. For both SNVs (rs1544410 and rs2228570) of the VDR gene, there was no significant difference in 25(OH)D levels for different genotypes ($p > 0.05$ for both rs1544410 and rs2228570).

4. Discussion

In comparison to controls, preeclampsia patients had considerably lower levels of 25(OH)D, according to our study [5,32–35]. All of these investigations confirm our findings. In line with prior investigations, we additionally found that preeclamptic women had very low calcium levels [36].

In our study, the GC gene rs4588 and rs7041 dominant model showed a significant association and increased risk of developing preeclampsia compared to controls. Our research found a substantial

Table 4
Haplotype analysis of all 4 SNVs of GC and VDR gene using SheSis web tool.

Haplotypes	Case (freq.)	Control (freq.)	Chi-square value	Fisher's p value	Pearson's p value	OR (95% CI)
GC gene rs7041, rs4588						
GA	23.01(0.10)	18.21(0.08)	0.61	0.434027	0.433996	1.29(0.68–2.46)
GC	103.99(0.43)	87.79(0.37)	2.28	0.131264	0.131191	1.33(0.92–1.91)
TA	42.99(0.18)	24.79(0.10)	5.69	0.017108	0.017092	1.89(1.11–3.22)
TC	70.01(0.29)	109.21(0.46)	13.68	0.000219	0.000218	0.49(0.34–0.72)
VDR gene rs1544410, rs2228570						
AC	12.20(0.05)	5.12(0.02)	3.00	0.083102	0.083045	2.46(0.86–7.01)
AT	23.80(0.10)	9.88(0.04)	6.19	0.012914	0.012902	2.56(1.19–5.51)
GC	66.80(0.28)	39.88(0.17)	8.73	0.003140	0.003136	1.94(1.24–3.01)
GT	137.20(0.57)	185.12(0.77)	21.69	3.29e-006	3.26e-006	0.37(0.27–0.59)

[1]Global result:Total control = 240.0, total case = 240.0Global chi2 is 15.385927 while df = 3 (frequency<0.03 in both control & case has been dropped.)Fisher's p value is 0.001531Pearson's p value is 0.001516.
[2]Global result:Total control = 240.0, total case = 240.0Global chi2 is 22.563137 while df = 3 (frequency<0.03 in both control & case has been dropped.)Fisher's p value is 5.10e-005Pearson's p value is 4.98e-005.
[1]Global result for VDBP gene.
[2]Global result for VDR gene.
All OR adjusted for Age, Systolic blood press, Diastolic blood pressure, Gestational age, protein in urine, Leg swelling, Parity, and biochemical parameters.

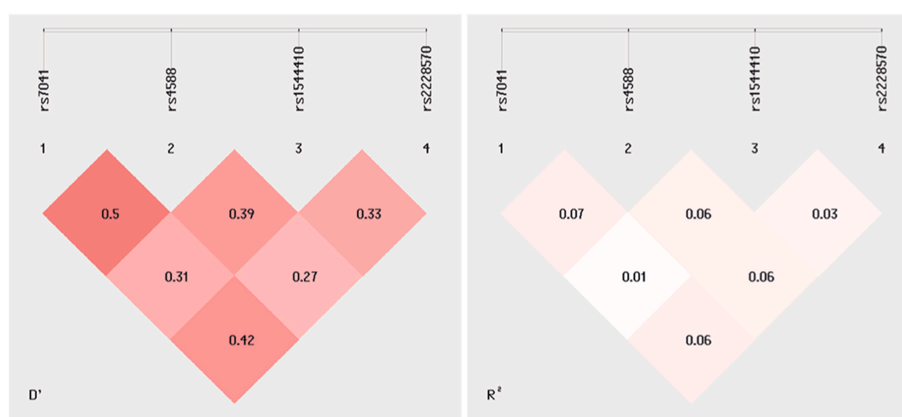

Fig. 2. Linkage disequilibrium (LD) and R2 for 4 SNVs of VDR and GC gene. D′ represents the value of disequilibrium for a pair of SNVs. R2 represents the goodness of LD calculation for a specific pair of SNVs.

correlation between the risk of preeclampsia and the dominant model of rs1544410 and rs2228570. Our findings are corroborated by case-control research that looked at the relationship between a substantial difference in vitamin D levels and a carrier of the rs2228570 C-allele's higher risk of developing preeclampsia [18].

We found a significant association of 3 haplotypes ('TA' of the GC gene and 'AT' & 'CG' of VDR gene) with an increased risk of developing preeclampsia. When compared to controls, the 'TA' haplotype of the GC gene showed an increased risk of preeclampsia. The 'GC' and 'AT' haplotypes for VDR also demonstrated an elevated risk of preeclampsia. In comparison to the haplotype of the two major alleles, the haplotype for both minor alleles of VDR showed a higher risk (3.5 fold). This high risk reflects the negative effects of having both minor alleles present in the preeclampsia development risk.

We found a significant association of 25(OH)D levels in different genotypic groups of rs4588 of the GC gene. Studies have shown that GC genetic polymorphisms affect blood vitamin D levels by lowering vitamin D affinities [13]. According to a different research, the rs7041 GG genotype considerably lengthens the serum half-life of vitamin D in comparison to the AA genotype [37]. Thus, by altering the plasma vitamin D level, which is linked to many diseases, GC gene polymorphism can be linked to disease risk. The amount of vitamin D in pregnant women is considerably affected by the GC gene polymorphism, according to a research on the Chinese population [38].

In contrast, no correlation between 25(OH)D levels and genotypic groups of the VDR gene's rs1544410 and rs2228570 SNVs was discovered. The VDR gene is linked to disease risk, however, there is no genotypic relationship between circulating 25(OH)D levels and disease risk. One explanation might be that the VDR gene affects 25(OH)D downstream signaling, independent of 25(OH)D synthesis and blood transport. According to a study conducted on the Egyptian population, obese women with VDR mutations have lower levels of circulating vitamin D [39]. So, the relationship between vitamin D levels and VDR genotypes is controversial and needs a large population study to draw a significant conclusion.

There is compelling evidence that the risk of preeclampsia and vitamin D status are connected. Therefore, the preeclampsia risk associated with vitamin D deficiency can be determined using GC genotypes. We can modify the advised dose of vitamin D to be taken during pregnancy based on the patient's GC genotype. Preeclampsia risk was shown to be higher for certain polymorphic variations. Therefore, additional research should be conducted to confirm VDR genotype as a preeclampsia risk factor and its potential use as a biomarker of the risk of developing preeclampsia.

Finally, our study has some limitations. The sample size is by far the most important. The association of GC and VDR gene polymorphism with preeclampsia risk may be better understood with a larger sample size. *Apa*I was left out of the research because it shares intron 8 with *Bsm*I at the 3′ terminus of the VDR gene. It was crucial to observe both of their impacts, but due to a lack of resources, we were unable to do so.

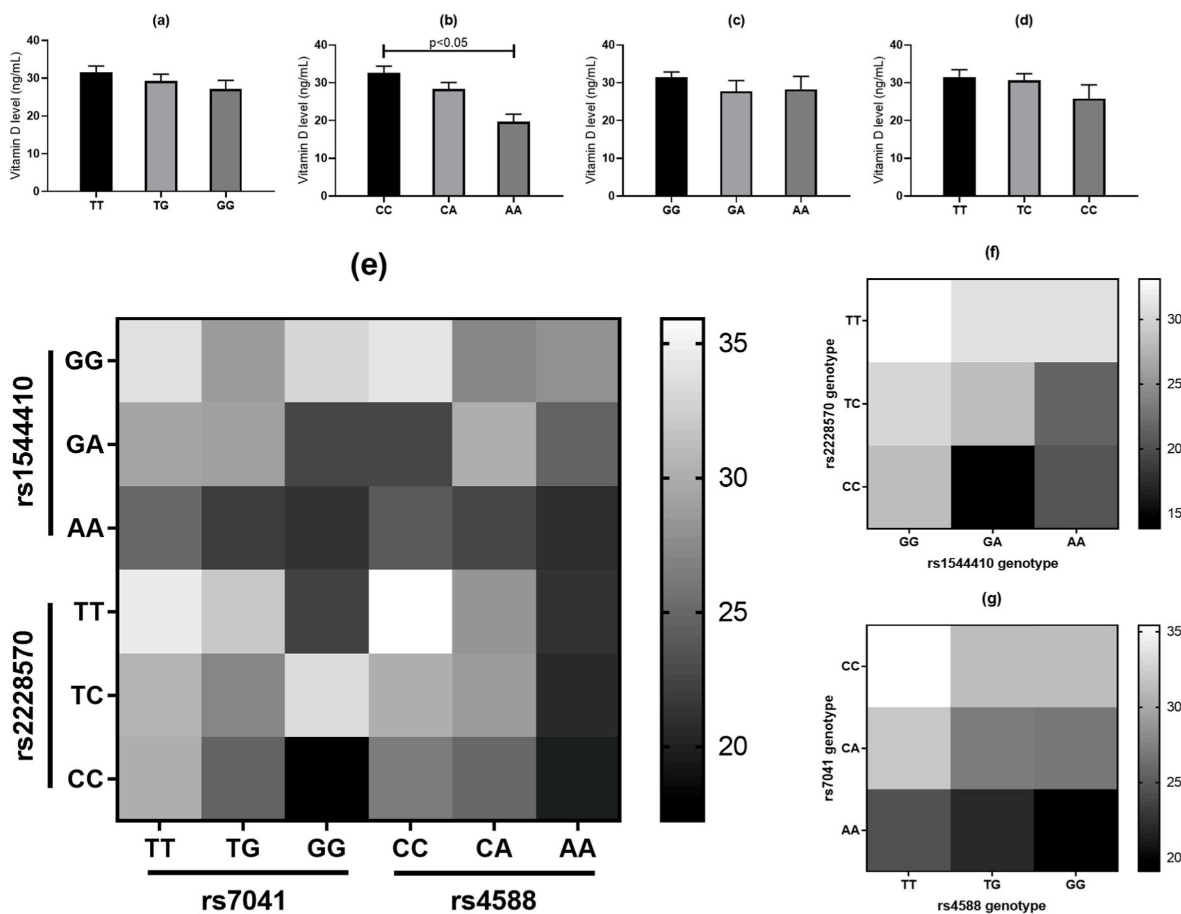

Fig. 3. 25(OH)D level in different genotypic groups of studied SNVs. a) 25(OH)D level in different genotypic groups of rs7041 of GC gene. b) 25(OH)D level in different genotypic groups of rs4588 of GC gene. c) 25(OH)D level in different genotypic groups of rs1544410 of the VDR gene. d) 25(OH)D level in different genotypic groups of rs2228570 of the VDR gene. e) Combined genotypic effect of VDR (rs1544410 and rs228570) and GC (rs7041 and rs4588) gene on vitamin D level in preeclampsia patients. f) VDR and g) GC gene genotypes and combined effect of SNVs on vitamin D level. One-way ANOVA was done to assess the significant difference in vitamin D levels among genotypic groups. $P < 0.05$ was considered significant. Heatmap was generated using the mean vitamin D value of each SNV pair.

5. Conclusion

From the study, we found a significant association between VDR and GC gene polymorphism and the risk of developing preeclampsia in the Bangladeshi population. Minor alleles of rs4588, rs1544410, and rs2228570 were significantly associated with an increased risk of pre-eclampsia. 25(OH)D level is considerably lower in preeclamptic women, and AA genotype of rs4588 of the GC gene is significantly associated with a low level of 25(OH)D in serum.

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Ethical approval

The research was approved by the ethical committee of the Department of Biochemistry and Molecular Biology, University of Dhaka (approval number – BMBDU-ERC/EC/17/013).

Consent to participate

Written consent was taken from each participant of the study.

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

The data that has been used is confidential.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2023.101526.

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