ORIGINAL ARTICLE

Genetic variations in the vitamin-D receptor (VDR) gene in preeclampsia patients in the Chinese Han population

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Previous studies have indicated that vitamin D deficiency is linked to a risk of preeclampsia (PE). The aim of our study was to investigate the association between genetic variations in the vitamin-D receptor (VDR) gene and the susceptibility to PE in the Chinese Han population. We examined the genotypes *VDR* rs2228570, rs11568820 and rs1544410 in 402 PE patients and 554 normal pregnant women in the third trimester by TaqMan allelic discrimination real-time polymerase chain reaction. The clinical data of the individuals were collected to enable genotype–phenotype analysis. A significant statistical difference in the genotypic frequencies of rs2228570 between cases and controls was found ($\chi^2 = 13.750$, $P = 0.001$). The G allele was the risk factor for the risk of PE ($\chi^2 = 9.456$, $P = 0.002$, OR = 1.137, 95% CI 1.111–1.610). There was no difference in the genotypic and allelic distributions of rs11568820 and rs1544410 between the two groups ($P > 0.05$). Our results provide evidence for a possible link between *VDR* and the development of PE in the Chinese Han population.

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INTRODUCTION

Preeclampsia (PE) is a common gestational disorder that is characterized by new-onset hypertension and proteinuria in pregnancy. It involves multiple organs and is a major cause of maternal and fetal mortality and perinatal morbidities.¹ The initiation and progression of PE are thought to be associated with several factors, such as placental ischemia, oxidative stress, inflammatory activation and maternal–fetal immune maladaptation.² Although many efforts have been taken to further understand PE, its etiology and pathogenesis have not been clearly identified. Recently, several studies have indicated that, among other factors, genetic involvement has an important role in the development of PE.³ Many candidate genes, such as the methylenetetrahydrofolate reductase gene (*MTHFR*), endoplasmic reticulum aminopeptidase 2 gene (*ERAP2*), catechol-O-methyltransferase gene (*COMT*), tumor necrosis factor- α (*TNF- α*) and interleukin 1- β (*IL1- β*) gene, are thought to be associated with susceptibility to PE.^{4–9} However, the results have not been consistent between populations from different regions or of different ethnicities.^{8,9}

Vitamin D is well known for its function in the metabolism of calcium and phosphorus.^{10,11} The active form of vitamin D, 1,25(OH)₂D, mediates its action by binding to the vitamin-D receptor (VDR) specifically. VDR belongs to the steroid receptor superfamily and contains two functional domains, a DNA-binding domain and a

ligand-binding domain.¹² 1,25(OH)₂D binds to ligand-binding domain to activate signaling cascades, which mainly regulate calcium metabolism and induce gene transcription mediated by DNA-binding domain binding to the vitamin-D response elements.^{12–14} VDR is expressed not only in classical target organs, like the bone, skin and kidney, but also in the placenta and decidua, where vitamin D may have an effect on the pregnancy and fetal growth.¹³ Through the modulation of the immune system, regulation of cytokines and delivery of calcium, vitamin-D aids in embryo implantation, regulates the synthesis of hormones involved in gestation and supports fetal growth during pregnancy.¹³ Moreover, many epidemiologic studies have suggested that vitamin-D deficiency is associated with the risk of PE^{15–17} and may affect the onset of PE through increased inflammation and/or the lack of immunosuppression.¹³

Considering the effect of vitamin D on pregnancy and the relationship between hypovitaminosis D with PE, we hypothesized that *VDR* might involve the pathological process of PE. *VDR* is located on the long arm of chromosome 12 (12q14) and regulates ~3% of the human genome, including the genes that are critical for the regulation of glucose, lipid metabolism and blood pressure.¹⁸ Several potentially functional polymorphisms have been identified in the *VDR* gene, and they have been reported to be associated with many diseases, such as hepatocellular carcinoma, ovarian cancer, breast cancer and so on.^{18–22}

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Therefore, in our present study, three functional loci in VDR, FokI (rs2228570), Cdx2 (rs11568820) and BsmI (rs1544410) were genotyped to evaluate the association between single-nucleotide polymorphisms (SNPs) of VDR and the risk of PE in the Chinese Han population. The individuals' demographic and clinical characteristics were also collected in order to analyze their relationship with the SNPs of VDR.

METHODS

Subjects

We enrolled 402 PE patients (mean age 30.74 ± 5.70 years) and 554 normal pregnant women (mean age 30.67 ± 4.48 years) who were admitted to the Affiliated Hospital of Qingdao University, Linyi People's Hospital and Heze Municipal Hospital during the study period. The groups were matched for age. The controls were in the third trimester of normal pregnancy and excluded individuals with multiple pregnancy and pathological states, including placenta previa, premature rupture of membrane, poly or oligohydramnios, threatened abortion, hypertension, diabetes mellitus and other chronic diseases. If the fetus had congenital malformations or weighed >4 kg the maternal sample was also excluded from the control group. The clinical data of participants, such as pregnancy and family history, blood pressure, liver and renal function, blood clotting state, and complication(s) of the fetus, were also collected. The measurement of blood pressure was performed in the supine position. The biochemical variables were all determined according to standard protocol by Hitachi 7600 (Tokyo, Japan) automatic biochemical analyzer in different hospitals. The study was carried out after obtaining the consent of all the participants and approval from the Ethics Committee of the Affiliated Hospital of Medical College, Qingdao University.

The definition of PE was new-onset of hypertension and proteinuria at >20 weeks of gestation. Hypertension was defined as two or more readings of systolic blood pressure ≥ 140 mm Hg or diastolic blood pressure ≥ 90 mm Hg. Proteinuria was defined as the excretion of urine protein >300 mg in 24 h urine collection or at least 1+ on dipstick in random urine.

Techniques

DNA was extracted from the whole-blood specimens using a Qiagen DNA extraction kit (QIAGEN, Shanghai, China). The concentration and quality of DNA were tested by a spectrophotometer, and the purified DNA was kept at -20°C until genotyping was conducted. TaqMan allelic discrimination real-time PCR (Life Technologies, Grand Island, NY, USA) was used for genotyping the polymorphisms of rs2228570, rs11568820 and rs1544410 in VDR. The 25 μl reaction mixture contained 1.25 μl 20 $\times$ probe and primers, 12.5 μl 2 $\times$ PCR Master Mix and 11.25 μl DNA and DNase-free water. The Taqman probes were designed by Applied Biosystems (Foster City, CA, USA)

or Life Technologies. For rs2228570, the forward primer was 5'-GGAAGTGCTGGCCGCCATTGCCTCC-3', and the reverse primer was 5'-TCCCTGTAAGAACAGCAAGCAGGCC-3'. For rs11568820, the forward primer was 5'-ACCCATAATAAGAAATAAGTTTITA-3', and the reverse primer was 5'-TGTGACCTAGTTTACTCAGGAATAT-3'. For rs1544410, the sequences of the forward and reverse primers were 5'-GAGCAGAGCCTGAGTATTGGGAATG-3' and 5'-GCAGGCCTGTCTGTGGCCCCAGGAA-3', respectively. The amplifications were carried out with the following protocol: 95°C for 3 min, 45 cycles at 95°C for 15 s and 60°C for 1 min. For each cycle, the fluorescent signal from the VIC- or FAM-labeled probe was determined. The results of genotyping were analyzed with Bio-Rad CFX manager 3.0 software (Bio-Rad Laboratories, Inc., Richmond, CA, USA).

Statistical analysis

Hardy-Weinberg equilibrium was analyzed with a goodness-of-fit χ^2 test. The allelic and genotypic distributions of cases and controls were compared using Pearson's χ^2 test. If the expected values were below 5, Fisher's exact *t*-test was used. The comparisons of clinical data between groups were tested with Pearson's χ^2 or Student's *t*-test. Genotype-phenotype analysis was conducted with analysis of variance. A *P*-value <0.05 was considered statistically significant. All analyses were performed by statistical software package SPSS19.0 (IBM, Armonk, NY, USA).

RESULTS

Demographic and clinical characteristics

The clinical characteristics of the population enrolled and *P*-value for comparison are shown in Table 1. Compared with controls, PE patients had earlier gestational weeks at delivery ($P<0.001$), lower birth weight of offspring ($P<0.001$) and higher blood pressure ($P<0.001$). In addition, the levels of triglycerides, alanine aminotransferase, aspartate aminotransferase, urea nitrogen and creatinine were higher in PE cases than controls ($P<0.05$). There was no statistically significant difference in weight-gained during pregnancy, number of abortions, age of menarche and the serum level of total cholesterol between the two groups ($P>0.05$).

Genetic analysis

The genotypic distribution of the three SNPs followed Hardy-Weinberg equilibrium, as shown in Table 2. The genotypic and allelic frequencies of these polymorphisms in VDR between the two groups are presented in Table 3. For rs2228570, the difference of genotypic distribution was associated with a risk of PE ($\chi^2=13.750$, $P=0.001$), and the G allele was the risk factor for predisposition to PE

Table 1 The clinical characteristics of cases and controls

Characteristic	Cases (n = 402)	Controls (n = 554)	t-value	P-value
Age (years)	30.74 ± 5.70	30.67 ± 4.48	0.197	0.844
Gestational age (weeks)	34.48 ± 4.35	38.73 ± 1.81	-17.46	<0.001
Gestational age at delivery (weeks)	36.31 ± 2.93	39.23 ± 1.35	-16.68	<0.001
Weight gained during pregnancy (kg)	18.15 ± 6.65	17.68 ± 4.42	0.593	0.554
Birth weight of offspring (g)	2684 ± 909	3382 ± 394	-12.78	<0.001
Number of abortions	0.88 ± 1.10	0.76 ± 0.93	1.69	0.092
Age of menarche (years)	14.08 ± 1.32	14.19 ± 1.42	-1.21	0.226
Systolic blood pressure (mm Hg)	157 ± 22	113 ± 10	35.65	<0.001
Diastolic blood pressure (mm Hg)	103 ± 16	74 ± 8	33.52	<0.001
Triglycerides (mmol l $^{-1}$)	3.81 ± 1.86	2.94 ± 1.39	2.187	0.030
Total cholesterol (mmol l $^{-1}$)	6.56 ± 1.98	6.34 ± 1.66	0.521	0.603
Alanine aminotransferase (IU l $^{-1}$)	27.29 ± 45.04	16.53 ± 19.48	4.287	<0.001
Aspartate aminotransferase (IU l $^{-1}$)	31.01 ± 38.56	20.60 ± 16.96	4.842	<0.001
Urea nitrogen (mmol l $^{-1}$)	4.71 ± 2.01	3.15 ± 1.11	13.38	<0.001
Creatinine ($\mu\text{mol l}^{-1}$)	68.77 ± 20.07	55.38 ± 15.49	10.68	<0.001

($\chi^2 = 9.456$, $P = 0.002$, odds ratio = 1.137, 95% confidence interval = 1.111–1.610). There was no significant difference in the genotypic and allelic distributions of rs11568820 and rs1544410 between cases and controls ($P > 0.05$).

Genotype–phenotype analysis

Owing to the association between rs2228570 and the development of PE, a genotype–phenotype analysis was made to explore the effect

of genetic involvement. The results are listed in Table 4. For rs2228570, gestational age at diagnosis showed a significantly different distribution among the three genotypes ($P = 0.015$). Patients who had the GG genotype showed earlier gestational age at diagnosis than those who had the AA genotype ($P = 0.005$). Similarly, the difference of the serum creatinine level among the three genotypes was statistically significant ($P = 0.041$). Patients carrying the GG genotype had higher serum creatinine level compared with patients carrying the AG genotype ($P = 0.027$) or AA genotype ($P = 0.048$). Moreover, patients with the AA genotype showed higher TG levels than those with the GG genotype ($P = 0.045$) or AG genotype ($P = 0.028$).

Table 2 The measurement of Hardy–Weinberg equilibrium among the cases and controls

	χ^2	P-value
<i>rs11568820</i>		
Cases	2.027	0.155
Controls	0.660	0.416
<i>rs2228570</i>		
Cases	1.784	0.182
Controls	2.462	0.117
<i>rs1544410</i>		
Cases	0.057	0.811
Controls	3.485	0.062

DISCUSSION

The active form of vitamin D, 1,25(OH)₂D, regulates many physiological and pathological activities by binding to VDR during gestation, such as aiding embryo implantation, regulating synthesis of hormones involved in gestation, supporting fetal growth and has roles in immunomodulatory networks.¹³ It also inhibits the proliferation of Th1 cells and B lymphocytes and, consequently, the levels of cytokines that are produced by those cells (such as interferon- γ , IL-2) are decreased.¹³ Perhaps because of the suppression of the adaptive immune system, vitamin D and the vitamin-D agonists are effective against autoimmune disorders in several animal models, including systemic lupus erythematosus, rheumatoid arthritis, inflammatory bowel disease, and type 1 diabetes.^{15,23} As many epidemiological studies show, hypovitaminosis D might affect the onset of PE

Table 3 The genotypic and allelic frequencies of four SNPs of VDR in two groups

Genotypes				χ^2	P-value	Alleles		χ^2	P-value	OR (95% CI)
<i>rs11568820</i>				4.467	0.107	C	T	1.881	0.170	1.140 (0.945–1.375)
Cases	CT	CC	TT			511	293			
Controls	173	169	60			670	438			
<i>rs2228570</i>				13.750	0.001	G	A	9.456	0.002	1.337 (1.111–1.610)
Cases	AG	GG	AA			502	302			
Controls	274	198	82			614	494			
<i>rs1544410</i>				3.808	0.149	C	T	2.050	0.152	0.807 (0.602–1.083)
Cases	CT	CC	TT			710	94			
Controls	84	313	5			1001	107			

Table 4 Associations between genotypes and characteristics among PE patients

	(1)GG (n = 163)	(2)AG (n = 176)	(3)AA (n = 63)	(1) vs. (2) vs. (3) P-value	(1) vs. (2) P-value	(1) vs. (3) P-value	(2) vs. (3) P-value	(1)+(2) vs. (3) P-value	(1) vs. (2) +(3) P-value
<i>Demographic characteristics (mean $\pm$ s.d.)</i>									
Age (years)	31.01 $\pm$ 6.06	30.78 $\pm$ 5.56	29.92 $\pm$ 5.06	0.435	0.711	0.199	0.307	0.216	0.431
Gestational age at diagnosis (weeks)	31.47 $\pm$ 5.73	32.48 $\pm$ 5.61	34.03 $\pm$ 5.14	0.015	0.114	0.005	0.083	0.015	0.020
Age of menarche (years)	13.99 $\pm$ 1.35	14.21 $\pm$ 1.34	13.93 $\pm$ 1.15	0.211	0.134	0.765	0.160	0.223	0.287
Gestational age at delivery (weeks)	35.92 $\pm$ 3.24	36.47 $\pm$ 2.77	36.87 $\pm$ 2.34	0.092	0.356	0.078	0.674	0.070	0.053
<i>Clinical characteristics (mean $\pm$ s.d.)</i>									
Triglycerides (mmol l ⁻¹)	3.71 $\pm$ 1.94	3.60 $\pm$ 1.67	4.56 $\pm$ 1.92	0.076	0.721	0.045	0.028	0.025	0.552
Total cholesterol (mmol l ⁻¹)	6.59 $\pm$ 1.83	6.36 $\pm$ 2.17	6.95 $\pm$ 1.91	0.397	0.467	0.402	0.180	0.250	0.839
ALT (IU l ⁻¹)	26.93 $\pm$ 28.8	29.41 $\pm$ 60.7	22.15 $\pm$ 21.4	0.574	0.627	0.495	0.296	0.349	0.900
AST (IU l ⁻¹)	30.45 $\pm$ 30.9	32.15 $\pm$ 45.6	29.27 $\pm$ 35.4	0.864	0.697	0.843	0.626	0.708	0.816
Urea nitrogen (mmol l ⁻¹)	4.94 $\pm$ 2.40	4.60 $\pm$ 1.79	4.42 $\pm$ 1.25	0.646	0.398	0.121	0.789	0.092	0.085
Creatinine (μ mol l ⁻¹)	71.91 $\pm$ 21.9	66.87 $\pm$ 19.8	65.68 $\pm$ 13.6	0.041	0.027	0.048	0.703	0.099	0.012
Systolic pressure (mm Hg)	156.7 $\pm$ 24.0	155.9 $\pm$ 19.4	159.2 $\pm$ 23.6	0.615	0.985	0.867	0.710	0.350	0.967
Diastolic pressure (mm Hg)	103.5 $\pm$ 17.3	102.8 $\pm$ 13.9	104.3 $\pm$ 16.3	0.806	0.673	0.761	0.535	0.615	0.831

through inflammatory and immune mechanisms.^{15–17} Moreover, the protein expression of VDR was previously observed to be reduced in the preeclamptic placentas.²⁴ Therefore, the abnormal changes in gene expression in the vitamin-D pathway may result in the susceptibility to PE.¹³

VDR is highly polymorphic, and the frequencies of its alleles are highly variable among different races. It has been suggested that the stability, quantity and activity of VDR protein may be affected by its polymorphisms.¹² Amal et al.¹⁸ demonstrated that the level of 25(OH) D was significantly lower in carriers with haplotype Taq -IC/Apa -I C compared with the non-carriers in polycystic ovary syndrome. In our study, we selected three functional loci (rs2228570, rs1544410 and rs11568820) to explore their relationship with the risk of PE.

The rs2228570 SNP is located in the coding region of VDR, the variant of which results in the production of protein with three extra amino acids, which is functionally less effective.²⁵ There are hypotheses that the less active form of VDR may be associated with the predisposition to some diseases, such as cancer or other more aggressive diseases.²⁶ We found a relationship between the polymorphism of rs2228570 and the susceptibility to PE in our study, and the G allele was the risk factor for the development of PE. Patients with the GG genotype showed earlier gestational age at diagnosis of PE than those with the AA genotype. Furthermore, serum creatinine levels were higher in patients carrying the GG genotype than those carrying the AG or AA genotype.

The rs11568820 SNP is located in the promoter region of VDR, and it alters the binding site of a CDX transcription factor, leading to higher-transcriptional activity of allele A.^{25,27,28} The rs1544410 SNP is in the 3' untranslated region of the VDR, and may alter stability of the mRNA.²⁵ In our study, the distributions of genotypes and alleles of rs11568820 and rs1544410 showed no difference in cases and controls. As such, there is no evidence that the rs11568820 and rs1544410 SNPs are linked with an increased risk of PE. A previous study on 529 individuals in a mostly Caucasian population suggested that VDR polymorphisms (rs2228570, rs1544410 and rs7975232) are not associated with PE.²⁹

Owing to the spasm of the systemic small vessel, PE is often accompanied by multi-organ disturbances, such as the activation of the clotting system, and impaired liver and renal function, particularly in cases of severe PE.^{13,30} Women with a history of PE are observed to be at increased risk of future cardiovascular disease.³¹ In our study, PE patients had higher serum levels of ALT, AST, TG, urea nitrogen and creatinine, compared with the controls. Furthermore, earlier gestational weeks at delivery and lower birth weight of offspring were found in PE patients. This is likely because of the fact that PE patients usually have to terminate their pregnancy in advance to relieve the clinical symptoms, thus leading to premature delivery.

Our study was based on a relatively large sample from multiple hospitals, which were genotyped by TaqMan allelic discrimination real-time PCR. The demographic and clinical characteristics of the participants were also collected to investigate the influence of genetic involvement. There are several limitations to the current study that should be noted. On one hand, all subjects enrolled were ethnic Han Chinese, and as such, the population is not representative of other races. On the other hand, our clinical data did not include the serum level of 1,25(OH)₂D, and the quantity and activity of VDR protein was not assayed. In spite of these limitations, our study provided evidence for the associations between a polymorphism of VDR and the susceptibility to PE in the Chinese Han population. This needs to be validated in a larger sample and in other ethnicities,

together with functional analyses, to clarify the potential mechanisms underlying this association.

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Author contributions: YZ and ML carried out the initial analyses, performed the experiments, and drafted the initial manuscript. YY, YZh, JW and XW contributed to study design and data collection. SL and XL conceptualized and designed the study and approved the final manuscript.

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