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RESEARCH ARTICLE

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Association between variants in vitamin D-binding protein gene and vitamin D deficiency among pregnant women in china

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Abstract

Background: The prevalence of vitamin D deficiency and insufficiency is extremely high in pregnant women worldwide. However, the association between single nucleotide polymorphisms (SNPs) in vitamin D metabolic pathway genes and 25-hydroxyvitamin D (25(OH)D) concentration among Chinese pregnant women is seldom reported. The risk of adverse neonatal outcomes due to maternal vitamin D deficiency has not been well investigated.

Methods: A total of 815 pregnant women and 407 infants were enrolled in this study. Serum 25(OH)D concentration was detected. DNA was extracted from the maternal blood for genotyping genetic SNPs in vitamin D pathway. An XGBoost model was established based on SNPs combined with external variables.

Results: Mean serum 25(OH)D level was 15.67 ± 7.98 ng/mL among the pregnant women. Seventy-five percent of pregnant women had 25(OH)D deficiency in China. SNPs of *GC* (rs17467825, rs4588, rs2282679, rs2298850, and rs1155563) were significantly associated with maternal 25(OH)D concentration. The influence of variants of rs17467825, rs4588, rs2282679, and rs2298850 on maternal 25(OH)D might be modified by vitamin D supplementation and sunshine exposure. An XGBoost model was established for monitoring 25(OH)D status in pregnant women and provided clinical advice to reduce the risk of 25(OH)D deficiency. Mothers with 25(OH)D deficiency hinted a risk for macrosomia.

Conclusion: A high prevalence of vitamin D deficiency in China has been confirmed. A clinical model was established to guide pregnant women to supplement vitamin D according to genotype. Furthermore, we suggest the effect of maternal vitamin D status on the risk of macrosomia.

KEYWORDS
25(OH)D, birthweight, pregnant women, SNP

Dong and Zhou contributed equally to this work.

1 | INTRODUCTION

Vitamin D deficiency is widespread among different races worldwide.[1-4] Furthermore, the prevalence of vitamin D deficiency and insufficiency is extremely high in pregnant women, even reaching up to about 99% during pregnancy.[5-7] Due to maternal vitamin D deficiency, increased risk of malign effects on pregnant women and infants happened. Accumulating researches have indicated the associations of vitamin D deficiency with gestational diabetes, preeclampsia, and inflammatory disorders in pregnant women.[8-10] Besides, aberrations occurred in infants such as abnormal neurocognitive functions, hypertension susceptibility, and macrosomia.[11-15] The vital effects of vitamin D on infants are due to the role of vitamin D in participating in the synthesis of hormones which is essential for embryo implantation, gestation, and fetal development during pregnancy.[16]

Vitamin D, a steroid derivative, has been proved to play important roles in lots of physiological processes including regulating the metabolism of calcium and phosphorus, promoting skeleton growth, and monitoring cell differentiation.[17,18] Vitamin D contains vitamin D_2 and vitamin D_3 obtained from plant-based and animal-based foods, respectively. Vitamin D can be synthesized from skin sources through a series of hydroxylations in liver and kidney depending on various hydroxylases. The active dihydroxyvitamin D forms bind to the vitamin D-binding protein (DBP) encoded by *GC* gene and are transported to multiple target organs including the placenta.[16,19] Thus, the 25-hydroxyvitamin D (25(OH)D) concentration, the best biomarker of vitamin D status measured in blood, is mainly influenced by vitamin D pathway genes involved in vitamin D synthesis and metabolism. Researchers have indicated that SNPs in *GC*, *CYP2R1*, *VDR*, *CYP24A1*, *CYP27B1*, *CYP3A4*, and *LRP2* genes had a strong relationship with 25(OH)D level among non-pregnant persons.[20-24] Recent studies have reported that genetic variations in *GC*, *CYP2R1*, *CYP3A4*, *CYP24A1*, and *NADSYN1/DHCR7* involved in vitamin D pathway were significantly related to 25(OH)D concentration among pregnant women in China.[19,25] Additionally, serum 25(OH)D concentration is influenced by many environmental factors, such as sun exposure, dietary vitamin D supplementation, and race.[26]

Previous studies have demonstrated that 25(OH)D deficiency among pregnant women is a common situation in China.[3,27,28] However, the association between SNPs and 25(OH)D concentration among pregnant women in China is seldom reported. Moreover, it is of great importance to detect whether environmental factors would modify the link between 25(OH)D concentration and SNPs during pregnancy. Finally, we aim to construct an evaluation model to guide pregnant women to elevate 25(OH)D concentration properly.

2 | METHODS

2.1 | Participants and blood sample collection

The study was conducted in Xiangyang First people's hospital, Hubei province, China, since December 2017. Total of 900 pregnancy women were recruited and assigned to three groups by the number of gestational weeks: first trimester, 1-12 weeks; second trimester, 13-27 weeks; and third trimester, 28-40 weeks in this study. Clinical questionnaire including age, pre-pregnancy BMI, dietary habits, times of physical activities, sun exposure, smoking and drinking habits and vitamin D supplementation was filled in. The exclusion criteria were (a) a history of any serious disease; (b) a history of any mental disorder; (c) a history of drug abuse; (d) a history of use of drugs that dramatically alter enzymatic expression of the target genes; (e) abnormal fetal development; and (f) lack of clinical information. Thus, we enrolled 815 participants and their 407 infants in the analysis. All pregnant women had similar dietary habits in inland China. The vitamin D intake of seafood, milk, and eggs was evaluated by using questionnaire. Influence of diet, including seafood (three groups: no seafood consumption; occasional: 1-2 meal containing seafood per week on average; and frequent: at least three meal containing seafood per week), cow milk (three groups: no consumption; occasional: 1-500 mL per week on average; and frequent: at least 500 mL per week), and egg (three groups: no egg consumption; occasional: 1-2 eggs per week on average; and frequent: at least three eggs per week) were recorded according to the above classification. Research nurses were responsible for recording infants' birthweight, length, and femur length. The study protocol was approved by the Research Ethics Committee (201702170b) of Hubei Medical College. Informed consents were provided for all participants. With the participants' consent, fasting blood sample (8 hours) was drawn into a serum separator tube and an EDTA anticoagulant tube. Serum separator tubes were centrifuged (1509 *g*, 5 minutes, 4°C) to separate serum (used for measuring 25(OH)D concentration), and EDTA anticoagulant tubes were used to extract DNA.

2.2 | Serum 25(OH)D measurement

The serum 25(OH)D concentration was measured by Ultra-high Performance Liquid Chromatography-Tandem Mass Spectrometry (UPLC-MS/MS; UPLC-MS/MS ACQUITY UPLC I-class Xevo TQD, Waters Corporation) according to the manufacturer's protocol. The concentrations of 25(OH)D_2 and 25(OH)D_3 were measured separately, and the sum of 25(OH)D_2 and 25(OH)D_3 was calculated as the total 25(OH)D concentration. According to the National standard, a serum 25(OH)D level <20 ng/mL was defined as 25(OH)D deficiency, that from 20 to 30 ng/mL was defined as 25(OH)D insufficiency, and that >30 ng/mL was defined as 25(OH)D sufficiency.

2.3 | SNP genotyping

Single nucleotide polymorphisms (SNPs) involved in vitamin D synthesis and metabolism pathways were selected according to previous studies reported among pregnant or non-pregnant population. Besides, these SNPs locate in the functional domain of genes and play a vital role in vitamin metabolism included in

the database of NCBI *VDR* (rs10735810, rs3847987, rs1544410, rs7968585, rs2408876, rs10783219); *GC* (rs7041, rs4588, rs2282679, rs1155563, rs12512631, rs222020, rs16847015, rs17467825, rs2070741, rs2298849, rs16846876, rs842999, rs222035, rs3755967, rs11939173, rs2298850, rs12512631); *CYP2R1* (rs2060793, rs10741657, rs1993116, rs7116978, rs12794714, rs1562902, rs10766197, rs10500804, rs10766197, rs10877012, rs11023374); *CYP24A1* (rs6013897, rs927650, rs2248137, rs6068816, rs73913757, rs2209314, rs2762939, rs17470271); *CYP27B1* (rs4646536, rs703842, rs10877012, rs118204009); *CYP3A43* (rs680055, rs2242480); *NADSYN1/DHCR7* (rs3829251, rs1790349, rs12785878, rs7944926, rs12800438, rs3794060, rs4945008, rs4944957); and *RXRA* (rs9409929, rs11185644) were selected as candidate SNPs. DNA was extracted from the peripheral blood leukocytes using a Rapid DNA Extraction and Detection Kit (Tiangen) according to the manufacturer's protocol, and SNP genotyping was conducted and analyzed by using the MassARRAY iPLEX Gold platform (Sequenom).

2.4 | XGBoost classifier decision trees model

eXtreme Gradient Boosting (XGBoost) is a type of gradient boosting decision tree method and is tuned to search for an optimal prediction solution. An XGBoost Classifier decision trees model (XGBoost 0.81) was established to assess the risk of 25(OH)D deficiency among pregnant women. SNPs including rs17467825 (A/G)—rs4588 (G/T)—rs2282679 (T/G)—rs2298850 (G/C)—rs1155563 (T/C) combined with age, sun exposure, vitamin D intake, gestational week, and physical activity were taken into consideration. The score output by model was negatively associated with 25(OH)D deficiency risk.

2.5 | Statistical analysis

Statistical analyses were performed by using Python software (version 3.6). Continuous variables were written as mean ± SD, and categorical variables were written as frequency and percentage. Body mass index (BMI) was divided into four subgroups (<18.5, 18.5-23.9, 24-27.9, and ≥28) according to the standard in China (National Health and Family Planning Commission of the People's Republic of China, 2013). Pregnant women were assigned to three groups by the number of gestational weeks. Physical activity and sun exposure were also grouped according to time. The significant associations between selected SNPs and 25(OH)D concentration were tested by using Kruskal-Wallis *H* test. Association between clinical variables and 25(OH)D concentration was analyzed by using univariate and multivariate analyses. Bonferroni correction was conducted to avoid false positive in the analysis. When undertaking multiple hypothesis testing in the study, the type I error rate should be reduced and kept at approximately the original critical level of significance at 0.05 (5%). Bonferroni's correction adjusts the critical level of significance for each test by dividing the critical level of significance, typically 0.05 (5%), by the number of significance tests performed. The aim of the correction is to maintain the type I error rate at about 5% and thereby reduce the probability of a type I error occurring when multiple testing. For the current study, we undertook six significance tests for "age" and "pre-pregnancy BMI," three significance tests for "gestational week," and one significance test for physical activity, sun exposure, sampling season, and vitamin D Table S2, the new critical level of significance after Bonferroni's correction was 0.05 ÷ 6 = 0.008, 0.05 ÷ 3 = 0.017, and 0.05 ÷ 1 = 0.05, respectively. In Table 3, comparisons were between two genotypes, and the critical level of significance after Bonferroni's correction was still 0.05. In Table S1, comparisons were between two groups, and the critical level of significance after Bonferroni's correction was 0.05. In Table S3, the critical level of significance after Bonferroni's correction was 0.05 ÷ 3 = 0.017. In Tables S5 and S6, 6 and 12 variables were taken into account using multivariable logistic regression analysis, respectively, and the new critical level of significance after Bonferroni's correction was 0.05 ÷ 6 = 0.008 and 0.05 ÷ 12 = 0.004.

3 | RESULTS

3.1 | Clinical characteristics of participants

The clinical characteristics of the participants were shown in Table 1. A total of 815 pregnant women and 407 infants were enrolled in the study. The mean 25(OH)D concentration was 15.67 ± 7.98 ng/mL among the pregnant women. The prevalence of 25(OH)D deficiency was almost 75%. Among them, 26.9% was seriously deficient (Figure 1). A large part of pregnant women experienced fewer physical activity (≤4 times/wk and ≤0.5 h/time) and less sunshine exposure (≤3 h/wk and ≤0.5 h/d) during pregnancy. Vitamin D supplementation (>400 IU/d) was not common (11.90% vs 88.10%). There was no significant difference between 25(OH)D concentration and foods containing vitamin D, such as seafood, milk, and eggs (Table S1). The mean birthweight of infants was 3231.05 ± 474.65 g, and 3.93% of the infants were giant babies (>4000 g).

3.2 | Association between serum 25(OH)D concentration and clinical characteristics

There was statistically significant difference in the respect of sun exposure, sampling season, daily vitamin D supplementation, and maternal 25(OH)D concentration (P = .0419, P = .0007, and P = .0354, respectively; Table 2). Less exposure to sunshine (≤3 h/wk and ≤0.5 h/d) and no vitamin D supplementation were associated with lower 25(OH)D concentration (15.60 ± 7.96 vs 19.74 ± 8.74 and 15.47 ± 7.96 vs 17.15 ± 8.07 ng/mL). More physical activity (>4 times/wk and >0.5 h/time) was associated with a much higher mean 25(OH)D concentration, but it was not of statistical significance in

TABLE 1 General characteristics of the participants

Variables	n (%)	Mean ± SD
Total pregnancy women	815 (100.00)	
25(OH)D (ng/mL)		
<10	219 (26.87)	15.67 ± 7.98
10-19.9	392 (48.10)	
20-29.9	154 (18.90)	
≥30	50 (6.13)	
Age, years		
0-25	182 (22.33)	28.19 ± 3.86
26-30	467 (57.30)	
31-35	130 (15.95)	
>35	36 (4.42)	
Gestational week, weeks		
≤12	179 (21.96)	14.84 ± 2.43
13-27	632 (77.55)	
≥28	4 (0.49)	
Pre-pregnancy BMI, kg/m^2		
<18.5	67 (8.22)	22.16 ± 3.24
18.5-23.9	575 (70.55)	
24-27.9	137 (16.81)	
≥28	36 (4.42)	
Physical activity (times/wk)		
≤4	632 (77.55)	2.88 ± 4.13
>4	183 (22.45)	
Sun exposure (h/wk)		
≤3	800 (98.16)	0.60 ± 0.86
>3	15 (1.84)	
Sampling season		
Winter	650 (79.75)	
Spring	165 (20.25)	
Smoker		
Yes	0 (0.00)	
No	816 (100.00)	
Drinker		
Yes	801 (98.28)	
No	14 (1.72)	
Vitamin D supplementation		
Yes	97 (11.90)	
No	718 (88.10)	
Total infants	407 (100)	
Infant weight (g)		
<4000	391 (96.07)	3231.05 ± 474.65
≥4000	16 (3.93)	
Infant length (cm)		49.34 ± 1.83
Femur length (cm)		7.41 ± 0.36

Abbreviations: BMI, body mass index; SD, standard deviation.

two groups. Otherwise, no statistically significant correlation of the 25(OH)D concentration with age, gestational week, and BMI was observed at the time of sample collection.

3.3 | The significant association between SNPs in *GC* gene and maternal 25(OH)D concentration

After adjustment for covariates, five SNPs in *GC* (rs17467825, rs4588, rs2282679, rs2298850, and rs1155563) were in significant associations with serum 25(OH)D concentration among pregnant women (Table 3). Pregnant women with their minor alleles had much lower 25(OH)D concentration compared with those with major alleles. Genetic Risk Score (GRS) was calculated to explore the association between associated SNPS with the outcome (25(OH)D deficiency). The sum of risk alleles from rs17467825, rs4588, rs2282679, rs2298850, and rs1155563 was calculated as GRS, and GRS was negatively associated with 25(OH)D concentrations. Individuals with GRS > 5 had significantly lower 25(OH)D concentrations, compared to individuals with GRS ≤ 5 (Table S2). However, the statistically significant associations of other selected SNPs in *VDR*, *CYP24A1*, *CYP27B1*, *CYP3A43*, *CYP2R1*, *NADSYN1*, and *RXRA* with 25(OH)D concentration were not observed.

3.4 | The effect of SNPs on 25(OH)D level is influenced by diet and sunshine

Owing to the significant association between 25(OH)D concentration and vitamin D supplementation as well as sun exposure mentioned above, a genotype-phenotype analysis was conducted to investigate the effect of diet and sunshine on the relationships between the 25(OH)D concentration and SNPs (Figure 2). Associations between SNPs in *GC* and 25(OH)D concentration were modified by vitamin D intake among pregnant women without vitamin D supplementation since pregnancy, but not among those with vitamin D supplementation. Pregnant women with two minor alleles in either of the four SNPs, rs17467825, rs4588, rs2282679, and rs2298850 had much lower 25(OH)D concentration than women with one or two major alleles. There was a significant increase in 25(OH)D level in women with "G" allele taking vitamin D supplements compared to those without supplementation($P < .01$), and daily vitamin D intake also dramatically improved the 25(OH)D concentration of women who carried the rs4588 "T" allele, or women who carried the rs2282679 "G" allele or rs2298850 "C" allele compared to women with the same alleles without vitamin D supplementation ($P < .0001$). Besides, higher sun exposure (>3 hours/w) showed an impact on regulating 25(OH)D concentration in terms of some SNPs. Carriers with genotype AG or GG of rs17467825 and women with genotype GT or TT of rs4588 exposed to more sunshine showed elevated 25(OH)D concentration compared to those with inadequate sunlight exposure ($P < .05$), while high sun exposure significantly increased 25(OH)D concentration in individuals with genotype GG

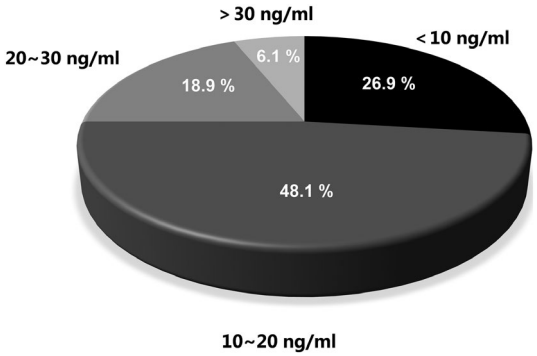

FIGURE 1 Distribution of 25-hydroxyvitamin D (25(OH)D) concentration in total study population. The proportion of pregnant women with different 25(OH)D concentration was shown in the pie chart

of rs2282679 and CC of rs2298850 ($P < .01$). However, there was no such association between sunlight exposure time and SNPs in women with one or two major alleles. Even without vitamin D supplementation, high sun exposure also significantly increased 25(OH)D concentration in pregnant women carrying two minor alleles of rs17467825, rs4588, rs2282679, and rs2298850 ($P < .05$), while no such effect was observed in women with one or two major alleles of such four SNPs.

3.5 | A clinical model of monitoring 25(OH)D concentration in the pregnant women

We constructed an XGBoost model to predict the risk of 25(OH)D deficiency based on the interaction of SNPs and exogenous factors (Figure 3). Five significant SNPs including rs17467825 (A/G)—rs4588 (G/T)—rs2282679 (T/G)—rs2298850 (G/C)—rs1155563 (T/C) combined with age, sun exposure, vitamin D intake, gestational week, and physical activity were taken into consideration. ROC curves were depicted to describe the efficiency of this model. The area under the ROC curve (AUC) was 0.828 and 0.807 for the training (n = 733) and test sets (n = 82), respectively. The score output by model was negatively associated with the risk of 25(OH)D deficiency. Participants with score >0.5 had higher genetic risk of 25(OH)D deficiency compared to women with score <0.5. Four grades and corresponding measures according to the score were list in Table 4. Based on the SNPs, the adjustable parameters involved in this model like dose of vitamin D supplementation or sun exposure time can be recommended to participants to help them achieve normal 25(OH)D status, thus contributing to individualized monitoring and intelligent adjustment of vitamin D concentration in pregnant women.

3.6 | Relationship between maternal 25(OH)D deficiency and neonatal macrosomia risk

Although the number of macrosomia is very small, we found that 12 of the 16 macrosomia's mothers were 25(OH)D deficiency, and 4 of the 16 macrosomia's mothers were non-deficiency (75% vs 25.00%; Table S3). In Table S4, maternal 25(OH)D concentration was divided into four different subgroups. We observed the trend that the proportion of macrosomia decreased with the increase in maternal 25(OH)D concentration. Multivariable logistic regression analysis showed no significant linear correlation between allele type, vitamin D status, and macrosomia risk (Table S5). There was no statistically significant difference in the association of maternal 25(OH)D concentration and the mean length and femur length of the infants.

TABLE 2 Serum 25(OH)D concentration in participants according to their characteristics

Variables	25(OH)D concentration (ng/mL) (mean ± SD)	*P*-value
Age (y)		
0-25	15.24 ± 7.70	.0647
26-30	15.61 ± 8.23	
31-35	16.95 ± 7.73	
>35	14.12 ± 6.83	
Gestational week (wk)		
≤12	16.29 ± 8.28	.4230
13-27	15.52 ± 7.92	
≥28	12.38 ± 4.36	
Pre-pregnancy BMI (kg/m^2)		
<18.5	16.18 ± 8.26	.9465
18.5-24	15.75 ± 8.13	
24.1-28.9	15.19 ± 7.27	
≥29	15.40 ± 8.01	
Physical activity (times/wk)		
≤4	15.41 ± 7.85	.0524
>4	16.42 ± 8.39	
Sun exposure (h/wk)		
≤3	15.60 ± 7.96	.0419*
>3	19.74 ± 8.74	
Sampling season		
Winter	16.14 ± 8.01	.0007*
Spring	13.80 ± 7.57	
Vitamin D intake		
Yes	17.15 ± 8.07	.0354*
No	15.47 ± 7.96	

*Represented statistically significant difference (Bonferroni-adjusted), Kruskal-Wallis *H* test. For "age" and "pre-pregnancy BMI," statistically significant difference set as .008; "gestational week," statistically significant difference set as .017; others, statistically significant difference set as .05.

4 | DISCUSSION

25(OH)D deficiency could cause multiple metabolic disorders such as diabetes, obesity, hypertension, depression, and cardiovascular

TABLE 3 Association between SNPs and serum 25(OH)D levels

Genotype	n	25(OH)D levels (ng/mL) (mean ± SD)	*P*-value
GC (DBP)			
rs17467825			
AA	418	16.63 ± 8.10	AA:AG .003*
AG	324	15.09 ± 7.97	AG:GG .029*
GG	73	12.80 ± 6.40	AA:GG .000*
rs4588			
GG	409	16.83 ± 8.07	GG:GT .000*
GT	334	14.85 ± 7.89	GT:TT .048*
TT	72	12.92 ± 6.71	GG:TT .000*
rs2298850			
GG	401	16.84 ± 8.20	GG:GC .000*
GC	338	14.89 ± 7.79	GC:CC .047*
CC	76	13.01 ± 6.67	GG:CC .000*
rs2282679			
TT	482	16.30 ± 8.02	TT:TG .024*
TG	270	15.20 ± 8.12	TG:GG .050
GG	63	12.89 ± 6.38	TT:GG .024*
rs1155563			
TT	223	16.59 ± 7.81	TT:TC .122
TC	431	15.88 ± 8.21	TC:CC .005*
CC	161	13.86 ± 7.38	TT:CC .000*

*Statistically significant difference set at .05, Kruskal-Wallis *H* test.

syndromes.[29] Recent studies demonstrated that 25(OH)D deficiency or insufficiency was prevalent among pregnant women worldwide.[5-7] In China, a great majority of pregnant women had 25(OH)D deficiency was reported,[3,19,30,31] indicating the prevalence of 25(OH)D deficiency in pregnant women was extremely high. The present study indicated that the mean serum 25(OH)D level was 15.67 ± 7.98 ng/mL among the recruited pregnant women in Xiangyang, Hubei province. More than 93% of pregnant women had 25(OH)D insufficiency. Moreover, 75% of pregnant women had 25(OH)D deficiency. Though different dietary habits, geographical position, and genetic background could influence 25(OH)D status in pregnant women in different areas, there is a similar trend toward 25(OH)D deficiency or insufficiency among pregnant women globally. Because of the 25(OH)D deficiency, the incidence of abnormal infant and fetus increased.[32-34]

In the present study, we genotyped SNPs across key genes that encode protein to participate in the bioavailability of 25(OH)D metabolism.[35] Five maternal SNPs in *GC* were found to be closely associated with 25(OH)D concentration among pregnant women. *GC* gene encodes DBP, and previous researches have demonstrated that DBP concentration regulates the transport of 25(OH)D to target organs and vitamin D metabolism.[36,37] *GC* SNPs are reported to be associated with DBP levels and bioactivity, suggesting that SNPs in *GC* could influence serum 25(OH)D status.[38,39] In this study, variants of rs17467825, rs4588, rs2282679, rs2298850, and rs1155563 in *GC* were significantly associated with 25(OH)D concentration, consistent with previous identification.[20] For example, the T allele of *GC* rs4588 was associated with much lower 25(OH)D concentration, whereas the T allele of *GC* rs2282679 was associated with higher 25(OH)D concentration.[19,40] Other significant link of SNPs in *GC* and 25(OH)D concentration in this study was also consistent with previous studies among different races.[19,41-44] *CYP2R1* encodes 25-hydroxylase which can convert vitamin D into 25(OH)D.[45] Multiple evidences indicated that SNPs in the CYP2R1 gene might affect 25(OH)D concentration and induce disease occurrence and development caused by vitamin D deficiency.[20,24,41,46] Though polymorphisms in rs10766197 were reported to be closely linked to vitamin D deficiency in multiple ethnic populations,[47,48] the 25(OH)D level in this study was not strongly associated with pregnant women carrying the rs10766197 "G" allele and "A" allele. However, there was a significant relationship between increased 25(OH)D level with rs10766197 when the pregnant women were homozygous for the rs10766197 major allele ("GG" genotype) compared with the no-risk genotype "A" allele. This different finding was due to different genetic background and limited sample size. Another reasonable explanation might be that the location of rs10766197 in the intron region determined its synonymous mutations would not change the protein.

Studies have proved that there was a significant relationship between the sunshine duration and 25(OH)D concentration.[49,50] For example, the vitamin D concentration was lower in some populations with less sunshine due to geographical environment.[51,52] In addition, higher 25(OH)D concentration during the summer compared with lower 25(OH)D concentration during the winter further proves the importance of sun exposure.[53,54] As expected, sunshine duration was strongly associated with 25(OH)D concentration in our study. Pregnant women with more sun exposure had higher 25(OH)D concentration. Although the sampling season was concentrated in the winter and spring, we found that the serum 25(OH)D concentration in the winter was significantly higher than that in the spring group, which may be due to the fact that pregnant women in inland preferred sunbathe outside in the winter. Besides the duration of sun exposure, vitamin D supplementation was also closely linked with 25(OH)D concentration. The universal-recommended vitamin D intake for pregnant women is 400 IU/d, while recent studies have suggested better effect of supplementation with 2000 or 4000 IU/d on improving 25(OH)D concentration.[55] Scientists have also demonstrated that daily supplementation of vitamin D is more efficient than interval supplementation. In the present study, there was statistically significant difference in 25(OH)D concentration with or without vitamin D intake. Women with no vitamin D supplementation had lower 25(OH)D concentration (15.47 ± 7.96 vs 17.15 ± 8.07 ng/mL). Thus, a dose of at least 400 IU/d was recommended to ensure enough vitamin D support for Chinese pregnant women when taken into consideration limited sun exposure and outdoor activities. We also observed that

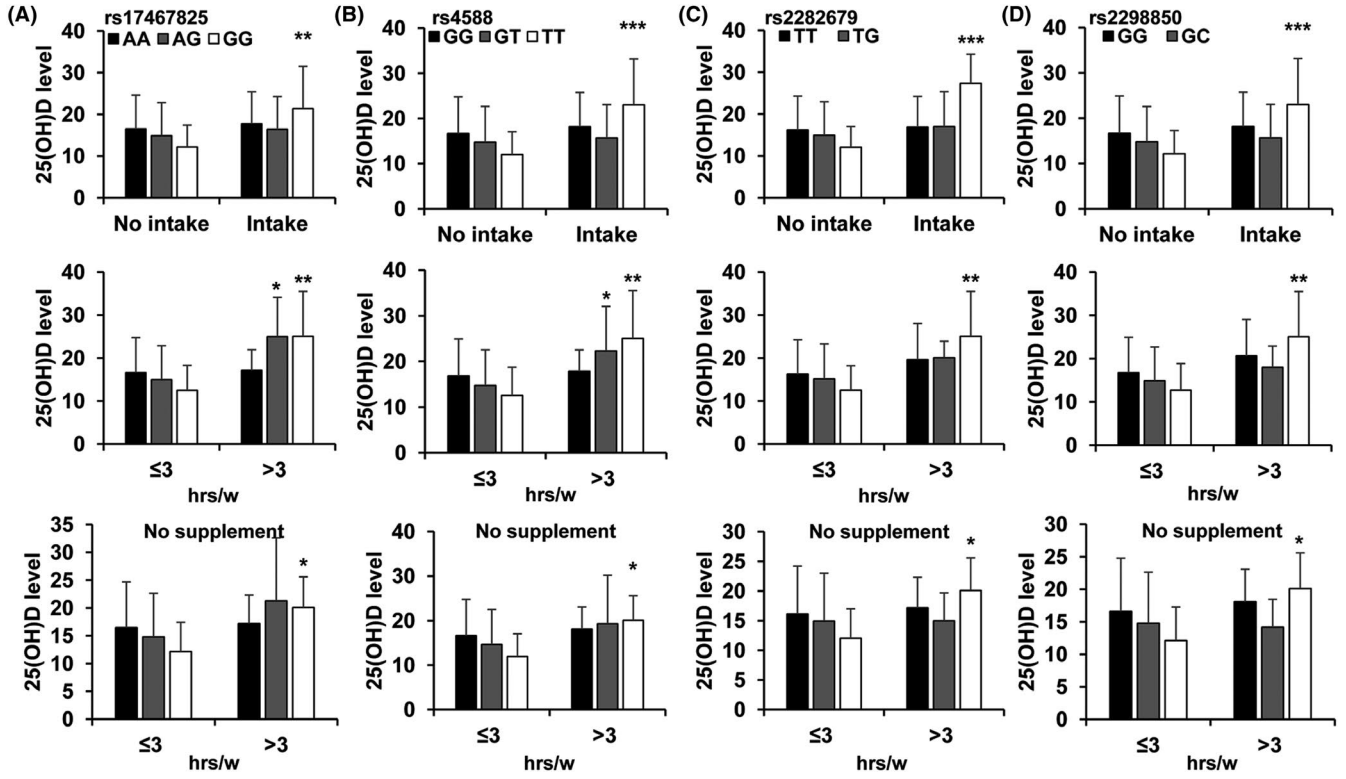

FIGURE 2 The association of rs17467825, rs4588, rs2282679, and rs2298850 with 25-hydroxyvitamin D (25(OH)D) concentration modified by vitamin D supplementation and sun exposure. Total serum 25(OH)D and (A), rs17467825 (B), rs4588 (C), rs2282679 (D), and rs2298850 exhibited interactions among SNPs with or without vitamin D supplementation and sun exposure. The top row represented the correlation between 25(OH)D concentration and alleles in response to vitamin D intake. The middle row represented the correlation between 25(OH)D concentration and alleles in response to sun exposure time. The bottom row represented the correlation between 25(OH)D concentration and alleles in response to sun exposure time when vitamin D is not supplemented. *, p<0.05; **, p<0.01; ***, p<0.001

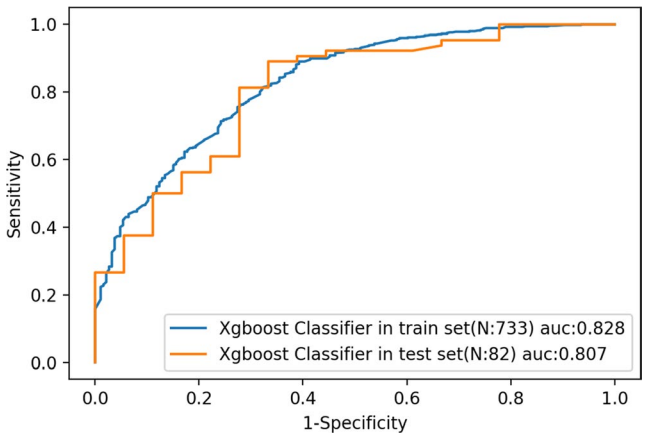

FIGURE 3 An XGBoost model of monitoring 25-hydroxyvitamin D concentration among pregnancy women. The AUC was 0.828 in the training set (n = 733) and 0.807 in the test set (n = 82)

external factors would influence the effects of SNPs on 25(OH)D status. rs17467825 "G" allele, rs4588 "T" allele, rs2282679 "G" allele, and rs2298850 "C" allele were in significant relation with declined 25(OH)D concentration at baseline, but they were associated with elevated 25(OH)D concentrations after vitamin D supplementation and/or more sun exposure regardless of seasonal influence.

The risk of adverse neonatal outcomes was higher in pregnant women with 25(OH)D deficiency than in women with adequate 25(OH)D. Wen et al reported that maternal vitamin D deficiency was associated with the risk of delivering a macrosomic infant, and they suggested that serum 25(OH)D concentration must be monitored during pregnancy.[14] Some researchers found that macrosomic infants had lower 25(OH)D concentration, suggesting the necessity of vitamin D supplementation for pregnant women to prevent macrosomia.[15] Controversially, other studies have indicated the association between low 25(OH)D concentration and low birthweight.[32,56] In this study, a high risk of macrosomia was observed when the pregnant women had 25(OH)D deficiency. We emphasized the influences of 25(OH)D status on the risk of macrosomia and suggested that clinical nutrition interventions are necessary during pregnancy for infant health. However, no standard guideline on vitamin D intake for pregnant women has been granted. Moreover, metabolism in vivo and environmental factors in vitro are different for each pregnant woman. Whether the recommended dose is proper should be evaluated with better indicators such as an ideal model.

Vitamin D concentration is affected by various factors including SNPs and clinical factors. A model based on multiple variables is better than a single risk factor in predicting 25(OH)D deficiency (Table S6). A clinical model was constructed to predict the genetic risk of

TABLE 4 Clinical application of the model

Score	Genetic risk	Clinical recommendations
0.71-1.0	High	Adjust the supplementary dose and sunshine duration according to the model
0.51-0.7	Medium-high	Detect 25(OH)D concentration and adjust the supplementary dose and sunshine duration according to the model
0.31-0.5	Medium	Monitor 25(OH)D concentration and prevent 25(OH)D deficiency
0.0-0.3	Low	Monitor 25(OH)D concentration

Note: SNPs, age, sun exposure, vitamin D intake, gestational week, and physical activity were put in the model as variables

serum 25(OH)D deficiency in pregnant women and provide clinical advice. SNPs, age, sun exposure, vitamin D intake, gestational week, and physical activity were input in the model as variables, and clinical guidance such as increasing sun exposure or vitamin supplementation, and the corresponding intensity and dose can be output according to the risk range. Moreover, an AUC > 0.8 in both training set and test set suggested that the model could serve as a better online system to ultimately help us to identify individualized functional variants on vitamin D endocrine system and provide suggestions suitable for individuals.

5 | LIMITATIONS

Limitations including ethnicity, sampling season, and sample size should be mentioned. The sample of both pregnant women and infants was relatively fewer. There is a big difference in the proportion of macrosomia and normal weight infants which may affect the statistical power of the study. Besides, the sunshine exposure and activity duration were collected by questionnaire. The cutoff hours for physical activities and sun exposure were set by the hospital lack of international references. Time bias might influence the statistical results. In future studies, it is necessary to further expand the sample size, especially for newborns, to study the association between SNP's in vitamin D-binding protein gene and neonatal macrosomia risk.

6 | CONCLUSIONS

This study provided evidence for the universality of vitamin D deficiency among pregnant women in China. Accordingly, maternal *GC* gene polymorphisms do influence on maternal vitamin D status. A model was constructed to predict the genetic risk of serum 25(OH)D deficiency in pregnant women and provide clinical advice to reduce the risk of maternal vitamin D deficiency.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS CONTRIBUTION

Wang-yang Xu and Jie Deng are responsible for the study design; Wang-yang Xu is responsible for writing of the manuscript; Jinju Dong, Qinhong Zhou, Jinxiu Wang, Yangqing Lu, Jun Li, Fei Li, Hongmei Zhou, Congli Liu, Ting Wang, Juan Wang, and Yi Mi are responsible for collection of samples and questionnaires; Lijun Wang is responsible for dealing with the data and model; Lingyun Wang and Peng Meng are responsible for drawing charts; all authors listed above were participated in processing patient information. All authors approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Allali F, El Aichaoui S, Khazani H, et al. High prevalence of hypovitaminosis D in Morocco: relationship to lifestyle, physical performance, bone markers, and bone mineral density. *Semin Arthritis Rheum*. 2009;38:444-451.
2. Ardawi MS, Sibiany AM, Bakhsh TM, Qari MH, Maimani AA. High prevalence of vitamin D deficiency among healthy Saudi Arabian men: relationship to bone mineral density, parathyroid hormone, bone turnover markers, and lifestyle factors. *Osteoporos Int*. 2012;23:675-686.
3. Hong-Bi S, Yin XU, Xiaowu Y, et al. High prevalence of vitamin D deficiency in pregnant women and its relationship with adverse pregnancy outcomes in Guizhou, China. *J Int Med Res*. 2018;46:4500-4505.
4. Yu S, Fang H, Han J, et al. The high prevalence of hypovitaminosis D in China: a multicenter vitamin D status survey. *Medicine*. 2015;94:e585.

5. Arora S, Goel P, Chawla D, Huria A, Arya A. Vitamin D status in mothers and their newborns and its association with pregnancy outcomes: experience from a tertiary care center in Northern India. *J obstet Gynaecol India*. 2018;68:389-393.
6. Dawodu A, Wagner CL. Mother-child vitamin D deficiency: an international perspective. *Arch Dis Child*. 2007;92:737-740.
7. Holmes VA, Barnes MS, Alexander HD, McFaul P, Wallace JM. Vitamin D deficiency and insufficiency in pregnant women: a longitudinal study. *Br J Nutr*. 2009;102:876-881.
8. Bodnar LM, Catov JM, Simhan HN, Holick MF, Powers RW, Roberts JM. Maternal vitamin D deficiency increases the risk of preeclampsia. *J Clin Endocrinol Metab*. 2007;92:3517-3522.
9. Lee CL, Ng BK, Wu LL, Cheah FC, Othman H, Ismail NAM. Vitamin D deficiency in pregnancy at term: risk factors and pregnancy outcomes. *Horm Mol Biol Clin Investig*. 2017;31.20170005.
10. Zhang Y, Gong Y, Xue H, Xiong J, Cheng G. Vitamin D and gestational diabetes mellitus: a systematic review based on data free of Hawthorne effect. *BJOG*. 2018;125:784-793.
11. Al-Ajlan A, Al-Musharaf S, Fouda MA, et al. Lower vitamin D levels in Saudi pregnant women are associated with higher risk of developing GDM. *BMC Pregnancy Childbirth*. 2018;18:86.
12. Meems LM, Mahmud H, Buikema H, et al. Parental vitamin D deficiency during pregnancy is associated with increased blood pressure in offspring via Panx1 hypermethylation. *Am J Physiol Heart Circ Physiol*. 2016;311:H1459-H1469.
13. Murthi P, Davies-Tuck M, Lappas M, et al. Maternal 25-hydroxyvitamin D is inversely correlated with foetal serotonin. *Clin Endocrinol*. 2017;86:401-409.
14. Wen J, Kang C, Wang J, et al. Association of maternal serum 25-hydroxyvitamin D concentrations in second and third trimester with risk of macrosomia. *Sci Rep*. 2018;8:6169.
15. Yilmaz S, Aktulay A, Demirtas C, Engin-Ustun Y. Low cord blood serum levels of vitamin D: cause or effect of fetal macrosomia? *Clin Exp Obstet Gynecol*. 2015;42:501-504.
16. Shin JS, Choi MY, Longtine MS, Nelson DM. Vitamin D effects on pregnancy and the placenta. *Placenta*. 2010;31:1027-1034.
17. Holick MF. Vitamin D deficiency in 2010: health benefits of vitamin D and sunlight: a D-bate. *Nat Rev Endocrinol*. 2011;7:73-75.
18. Norman AW. From vitamin D to hormone D: fundamentals of the vitamin D endocrine system essential for good health. *Am J Clin Nutr*. 2008;88:491S-S499.
19. Shao B, Jiang S, Muyiduli X, et al. Vitamin D pathway gene polymorphisms influenced vitamin D level among pregnant women. *Clin Nutr*. 2018;37:2230-2237.
20. Ahn J, Yu K, Stolzenberg-Solomon R, et al. Genome-wide association study of circulating vitamin D levels. *Hum Mol Genet*. 2010;19:2739-2745.
21. Barry EL, Rees JR, Peacock JL, et al. Genetic variants in CYP2R1, CYP24A1, and VDR modify the efficacy of vitamin D3 supplementation for increasing serum 25-hydroxyvitamin D levels in a randomized controlled trial. *J Clin Endocrinol Metab*. 2014;99:E2133-E2137.
22. Gupta RP, Hollis BW, Patel SB, Patrick KS, Bell NH. CYP3A4 is a human microsomal vitamin D 25-hydroxylase. *J Bone Miner Res*. 2004;19:680-688.
23. Nykjaer A, Dragun D, Walther D, et al. An endocytic pathway essential for renal uptake and activation of the steroid 25-(OH) vitamin D3. *Cell*. 1999;96:507-515.
24. Wang TJ, Zhang F, Richards JB, et al. Common genetic determinants of vitamin D insufficiency: a genome-wide association study. *Lancet*. 2010;376:180-188.
25. Duan L, Xue Z, Ji H, Zhang D, Wang Y. Effects of CYP2R1 gene variants on vitamin D levels and status: a systematic review and meta-analysis. *Gene*. 2018;678:361-369.
26. Holick MF. Vitamin D deficiency. *N Engl J Med*. 2007;357:266-281.
27. Chen Y-H, Fu L, Hao J-H, et al. Influent factors of gestational vitamin D deficiency and its relation to an increased risk of preterm delivery in Chinese population. *Sci Rep*. 2018;8:3608.
28. Wang J, Yang F, Mao M, Liu DH, Yang HM, Yang SF. High prevalence of vitamin D and calcium deficiency among pregnant women and their newborns in Chengdu, China. *World J Pediatr*. 2010;6:265-267.
29. Neyestani TR, Hajifaraji M, Omidvar N, et al. High prevalence of vitamin D deficiency in school-age children in Tehran, 2008: a red alert. *Public Health Nutr*. 2012;15:324-330.
30. Hu YC, Chen J, Li M, et al. Study on anemia and vitamin A and vitamin D nutritional status of Chinese urban pregnant women in 2010–2012. *Zhonghua Yu Fang Yi Xue Za Zhi*. 2017;51:125-131.
31. Yun C, Chen J, He Y, et al. Vitamin D deficiency prevalence and risk factors among pregnant Chinese women. *Public Health Nutr*. 2017;20:1746-1754.
32. Elsori DH, Hammoud MS. Vitamin D deficiency in mothers, neonates and children. *J Steroid Biochem Mol Biol*. 2018;175:195-199.
33. Özdemir AA, Ercan Gündemir Y, Küçük M, et al. Vitamin D deficiency in pregnant women and their infants. *J Clin Res Pediatr Endocrinol*. 2018;10:44-50.
34. Woolcott CG, Giguère Y, Weiler HA, et al. Determinants of vitamin D status in pregnant women and neonates. *Can J Public Health*. 2016;107:e410-e416.
35. Bahrami A, Sadeghnia HR, Tabatabaeizadeh S-A, et al. Genetic and epigenetic factors influencing vitamin D status. *J Cell Physiol*. 2018;233:4033-4043.
36. Lauridsen AL, Vestergaard P, Hermann AP, et al. Plasma concentrations of 25-hydroxy-vitamin D and 1,25-dihydroxy-vitamin D are related to the phenotype of Gc (vitamin D-binding protein): a cross-sectional study on 595 early postmenopausal women. *Calcif Tissue Int*. 2005;77:15-22.
37. Sinotte M, Diorio C, Berube S, Pollak M, Brisson J. Genetic polymorphisms of the vitamin D binding protein and plasma concentrations of 25-hydroxyvitamin D in premenopausal women. *Am J Clin Nutr*. 2009;89:634-640.
38. Medlej-Hashim M, Jounblat R, Hamade A, et al. Hypovitaminosis D in a young lebanese population: effect of GC gene polymorphisms on vitamin D and vitamin D binding protein levels. *Ann Hum Genet*. 2015;79:394-401.
39. Moy KA, Mondul AM, Zhang H, et al. Genome-wide association study of circulating vitamin D-binding protein. *Am J Clin Nutr*. 2014;99:1424-1431.
40. Chan Y-H, Yiu K-H, Hai JJ, et al. Genetically deprived vitamin D exposure predisposes to atrial fibrillation. Europace : European pacing, arrhythmias, and cardiac electrophysiology : journal of the working groups on cardiac pacing, arrhythmias, and cardiac cellular electrophysiology of the European Society of. *Cardiology*. 2017;19:iv25-iv31.
41. Elkum N, Alkayal F, Noronha F, et al. Vitamin D insufficiency in Arabs and South Asians positively associates with polymorphisms in GC and CYP2R1 genes. *PLoS ONE*. 2014;9:e113102.
42. Mezzavilla M, Tomei S, Alkayal F, et al. Investigation of genetic variation and lifestyle determinants in vitamin D levels in Arab individuals. *J Transl Med*. 2018;16:20.
43. Xu W, Sun J, Wang W, et al. Association of genetic variants of vit D binding protein (DBP/GC) and of the enzyme catalyzing its 25-hydroxylation (DCYP2R1) and serum vit D in postmenopausal women. *Hormones*. 2014;13:345-352.
44. Zhang Y, Wang X, Liu Y, et al. The GC, CYP2R1 and DHCR7 genes are associated with vitamin D levels in northeastern Han Chinese children. *Swiss Med Wkly*. 2012;142:w13636.
45. Cheng JB, Motola DL, Mangelsdorf DJ, Russell DW. De-orphanization of cytochrome P450 2R1: a microsomal vitamin D 25-hydroxilase. *J Biol Chem*. 2003;278:38084-38093.

46. Wang Y, Yu F, Yu S, et al. Triangular relationship between CYP2R1 gene polymorphism, serum 25(OH)D3 levels and T2DM in a Chinese rural population. *Gene*. 2018;678:172-176.
47. Ramos-Lopez E, Bruck P, Jansen T, Herwig J, Badenhoop K. CYP2R1 (vitamin D 25-hydroxylase) gene is associated with susceptibility to type 1 diabetes and vitamin D levels in Germans. *Diabetes Metab Res Rev*. 2007;23:631-636.
48. Xu X, Mao J, Zhang M, et al. Vitamin D deficiency in uygurs and kazaks is associated with polymorphisms in CYP2R1 and DHCR7/NADSYN1 Genes. *Med Sci Monit*. 2015;21:1960-1968.
49. Baggerly CA, Cuomo RE, French CB, et al. Sunlight and vitamin D: necessary for public health. *J Am Coll Nutr*. 2015;34:359-365.
50. Eggemoen ÅR, Falk RS, Knutsen KV, et al. Vitamin D deficiency and supplementation in pregnancy in a multiethnic population-based cohort. *BMC Pregnancy Childbirth*. 2016;16:7.
51. Alp H, Tekgunduz KS, Akkar MK. Maternal and cord blood vitamin D status in high-altitude pregnancy. *J Matern Fetal Neonatal Med*. 2016;29:571-575.
52. Di Marco N, Kaufman J, Rodda CP. Shedding light on vitamin D status and its complexities during pregnancy. infancy and childhood: an australian perspective. *Int J Environ Res Public Health*. 2019;16:538.
53. Azarpeykan S, Dittmer KE, Gee EK, et al. Influence of blanketing and season on vitamin D and parathyroid hormone, calcium, phosphorus, and magnesium concentrations in horses in New Zealand. *Domest Anim Endocrinol*. 2016;56:75-84.
54. Levis S, Gomez A, Jimenez C, et al. Vitamin d deficiency and seasonal variation in an adult South Florida population. *J Clin Endocrinol Metab*. 2005;90:1557-1562.
55. Hollis BW, Johnson D, Hulsey TC, Ebeling M, Wagner CL. Vitamin D supplementation during pregnancy: double-blind, randomized clinical trial of safety and effectiveness. *J Bone Miner Res*. 2011;26:2341-2357.
56. Agarwal S, Kovilam O, Agrawal DK. Vitamin D and its impact on maternal-fetal outcomes in pregnancy: a critical review. *Crit Rev Food Sci Nutr*. 2018;58:755-769.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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