The Association between Vitamin D-Related Gene Polymorphisms and Serum 25-Hydroxyvitamin D Concentration: A Prospective Cohort Study in Pregnant Minangkabau Women, Indonesia

Arif Sabta Aji1-2, Yusrawati Yusrawati1, Safarina G Malik4 and Nur Indrawaty Liipoeto5

1Department of Public Health, Alma Ata Graduate School of Public Health, Alma Ata University, Yogyakarta 55183, Indonesia
2Department of Nutrition, Faculty of Health Sciences, Alma Ata University, Yogyakarta 55183, Indonesia
3Department of Obstetrics and Gynaecology, Faculty of Medicine, Andalas University, Padang 25127, Indonesia
4Eijkman Institute for Molecular Biology, Jakarta 10430, Indonesia
5Department of Nutrition, Faculty of Medicine, Andalas University, Padang 25127, Indonesia

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Summary Several candidate genes in vitamin D synthesis and metabolism have been reported to have a significant association with 25-hydroxyvitamin D (25(OH)D) in Caucasians and African Americans. Few studies have indicated this relationship among Asians, especially in pregnant Minangkabau women, Indonesia. This study was conducted among 180 singleton pregnant women of West Sumatran Vitamin D Pregnant Mother (VDPM) cohort study. Serum 25(OH)D obtained in the third trimester (T3). Genetic risk scores (GRS) were created based on six vitamin D–related SNPs and their association with 25(OH)D levels were tested. Informations on demographics, lifestyle, pregnancy profile, and physical activity were collected using questionnaire. The average of 25(OH)D concentration was 21.21 ± 10.41 ng/mL respectively. Vitamin D-GRS has significantly associated with serum 25(OH)D levels in the third trimester (p=0.006). However, the synthesis-GRS and metabolism-GRS group of vitamin D-related gene polymorphisms had no association with 25(OH)D concentration at T3 (p>0.05). A high prevalence of insufficient-deficient vitamin D status at T3 was common. We observed an association between vitamin D-GRS and 25(OH)D concentration. The results of this study provides additional support for possible role of genetic variants in vitamin D-related gene polymorphisms on 25(OH)D concentration during pregnancy. Further replication studies with larger sample sizes are needed to confirm the findings.

Key Words vitamin D, single nucleotide polymorphisms, 25-hydroxyvitamin D, Pregnancy, West Sumatra

The status of vitamin D insufficiency was found to have a high prevalence in different races and geographic locations (1). Based on recent studies, vitamin D insufficiency is common in adult women living in Indonesia (2, 3). Although Indonesia has an abundant frequency of sun exposure, vitamin D insufficiency occurs due to low physical activity, low frequency of sun exposure, type of work, and dietary intake of vitamin D sources (2, 3). Pregnant women with vitamin D insufficiency are associated with adverse pregnancy outcomes such as small-gestational-age (SGA), neuro-developmental and cognitive impairment, high blood pressure in mothers and infants, respiratory infections, and increased incidence of infants treated in NICU, and health side effects in infants such as asthma, atopic allergy, and autoimmunity disorders such as Diabetes Mellitus Type I (DM1) (6–11)).

Vitamin D is a major precursor of steroid hormone, 1,25-dihydroxy vitamin D (1,25(OH)D), which has roles in calcium homeostasis and bone mineralization. Serum 25(OH)D concentration is a clinical marker of individual vitamin D status (9). The biological process of vitamin D starts with the conversion of 25(OH)D to 1,25(OH)D in the kidney. The metabolic pathways and synthesis of vitamin D are regulated by the specific genes present in the pathway and are initiated from exposure of UVB rays in the skin and dietary intake of vitamin D sources. Vitamin D3 production is obtained from the synthesis process in the body, while vitamin D2 is obtained from daily dietary intake. Sources of vitamin D2 are found in food and vitamin D-fortified food in some countries, while vitamin D1 is synthesized in the skin from 7-dehydrocholesterol (10). Vitamin D from food and UVB exposure is in biologically inactive form before going through a series of hydroxylation process by enzymes in the liver (CYP2R1, CYP27A1, CYP3A4), kidney (CYP27B1), and cell nucleus (VDR). Calcitriol (1,25(OH)D) is formed in the kidneys by binding to the vitamin D binding protein (DBP), encoded by the GC gene and then transported to multiple target organs.

E-mail: sabtaaji@almaata.ac.id
and tissues. 24-hydroxylase (CYP24A1) is an enzyme that catalyzes the conversion of both 25(OH)D and 1,25(OH)D from being inactive to active to keep the excess of vitamin D in the organ or tissue. In the nucleus, 1,25(OH)D is tied to VDR and has a function in transcription factors regulation, specifically in genes from organs or tissues for the biological function of vitamin D (11).

Hereditary factors are affecting 29% to 80% of serum 25(OH)D (12). Twin and family-based studies reported that vitamin D circulating levels are partially determined by genetic factors. It can be seen that genetic variants (SNPs) and mutation (deletion, amplification, and inversion) of genes involved in the synthesis, transport, metabolism, and receptor-linking processes may have an impact on vitamin D levels. With gene candidate analysis, five genes have been found, including GC, CYP24A1, CYP2R1, DHCRI7, and VDR (13). Two Genome-Wide Association Studies (GWAS) studies confirmed the association of these gene variants with 25OHD concentrations. Variants found near genes involved in cholesterol synthesis, hydroxylation, and vitamin D transport affect vitamin D status. The presence of these genetic variants identifies individuals who have substantially increased risk of vitamin D status deficiency (14, 15). Therefore, to increase the understanding in determining vitamin D status is mandatory and it is necessary to consider the relationship of hereditary traits.

Pregnancy is a crucial phase in the human life cycle. Disorders found in pregnant women, such as metabolic disorders of vitamin D may increase the risk of maternal high blood pressure which affects the adequacy of 25(OH)D to be transferred through the placenta and converted to 1,25(OH)D, and the growth and development of fetus is associated with the biological function of vitamin D (16). Therefore, the determination of vitamin D-related gene polymorphisms associated with serum 25(OH)D concentration during pregnancy is important. The purpose of this study was to examine the relationship between common SNPs in DHCRI7, CYP2R1, GC, CYP24A1, and VDR genes on serum 25(OH)D concentration in a prospective VDPM cohort study in the population of pregnant Minangkabau tribe women, Indonesia.

**MATERIALS AND METHODS**

**Study population.** In this study, we used samples from Vitamin D Pregnant Mother (VDPM) cohort study in West Sumatra that was conducted from July 2017 to April 2018 (17–20). Previously, some surveys were conducted to find the location of research based on high pregnancy rates in each location. This study was carried out at community health centers in West Sumatera. After the consecutive sampling based on the inclusion and exclusion criteria, some locations were obtained (Padang, Pariaman, Payakumbuh, Padang Pariaman, and Lima Puluh Kota).

The inclusion criteria of this study were pregnant women who performed the first ANC examination in maternal Clinic and public health centers with gestational age >27 wk or third trimester and willing to follow the research procedure by signing and informing consent. Meanwhile, mothers with multiple pregnancies, chronic illness (diabetes, hypertension, cardiovascular disease), took drugs that can interfere with vitamin D metabolism, suffered from hypothyroidism, had a miscarriage or stillbirth, and preeclampsia were excluded from this study. A total of 180 human DNA data were obtained which then would be analyzed for genotype to be associated with serum 25(OH)D levels. Subjects who agreed and were willing to follow the research procedure will be contacted and invited to the community health centers or maternal clinics to be interviewed.

**Social, dietary, and lifestyle factors.** Information regarding sociodemographic factors, pregnancy profile, maternal anthropometry, and lifestyle was recorded by nutritionists as research assistants. All staff were given training at first. Health assessment was conducted to screen healthy pregnant women who will be selected as research subjects. Health checks were performed by the midwife or doctor at the local maternal clinic or public health service. Blood sampling of non-fasting subjects was performed to analyze the levels of serum 25(OH)D and genotyping analysis. These processes were conducted by skilled health analysts (phlebotomist).

All the average subjects were Minangkabau tribe women. Anthropometric data of pregnant women such as body weight before pregnancy, third trimester pregnancy weight, upper arm circumference, and pregnancy weight gain were recorded. Lifestyles such as the way of dressing, type of work, physical activity, use of sunscreen, duration of sun exposure per day, and smoking status were observed.

**Serum 25-hydroxyvitamin D measurements.** Maternal blood samples were collected in the non-fasting conditions at >27 wk gestation. Serum samples were stored at −80°C until being analyzed for serum 25(OH)D level. Serum 25(OH)D was assayed by using xMark Microplate Spectrophotometer (Bio-Rad Laboratories Inc, Hercules, California, USA). Serum concentrations of 25(OH)D were assessed using ELISA from Diagnostic Biochemistry Canada (DBC) 25-Hydroxyvitamin D ELISA kit (DBC, London, Ontario Canada). The assay has a sensitivity of 5.5 ng/mL, with intra- and inter-assay coefficient of variance of 5% and 8.1% respectively. The serum concentrations of 25(OH)D which were considered in the analyses as follows: <12 ng/mL (vitamin D deficient), 12–20 ng/mL (vitamin D insufficient), ≥20 ng/mL (vitamin D sufficient) (17).

**SNPs selection and genotyping process.** We selected 6 SNPs of candidate genes according to the following criteria: (1) biological importance in vitamin D synthesis, metabolism, transportation, or degradation; (2) SNPs with minor allele frequency of <5% were excluded, and (3) evidence of a significant association in previous GWASs. The selected genes were DHCRI7 (rs12785878), CYP2R1 (rs12794714), GC (2282679), CYP24A1 (rs6013897), and VDR (rs2288570 and rs7975232). The basic characteristics of these six genes are shown...
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The roles of these selected genes in the vitamin D cascade are shown in Fig. 1.

The main source of vitamin D in the body is obtained from UVB sunlight exposure against the surface of the skin. Skin exposure to UVB radiation triggers the conversion of 7-dehydrocholesterol to pre-vitamin D3. DHCR7 gene regulates the enzyme 7-dehydrocholesterol reductase, which converts 7-dehydrocholesterol to cholesterol by removing some substrates from the synthetic pathway of vitamin D3. The pre-vitamin D3 is converted to vitamin D3 from a heat-dependent process. Another form of vitamin D from food is vitamin D2, and both with vitamin D3 are transported into the liver, where it is converted by vitamin D-25-hydroxylase (CYP2R1) to 25-hydroxyvitamin D (25(OH)D). CYP2J2, CYP3A4, CYP27A1, CYP1A1, and CYP2C9 enzymes also contribute to the process. 25(OH)D is the main circulating form of vitamin D in the body and inactive, which is used to determine vitamin D status. Bounded with vitamin D-binding protein (GC), 25(OH)D is transported to the kidneys and converted by 25-hydroxyvitamin D-1-alpha-hydroxylase or 1α-hydroxylase deficiency (CYP27B1) to the biologically active form 1,25-dihydroxy vitamin D3 (Calcitriol). Calcitriol increases the expression of 24-hydroxylase (24-OHase) by the CYP24A1 gene to catabolize 25(OH)D to water-soluble form of biologically inactive calcitroic acid, which is excreted in the bile. In the nucleus, 1,25(OH)D is bound by a vitamin D receptor (VDR) to perform a transcriptional function to the targeted gene. DHCR7 and CYP2R1 function upstream of the production of 25(OH)D and hence, termed as 25(OH)D synthesis indicators, while GC, CYP27B1 and CYP24A1 function downstream of the 25(OH)D production and hence, termed as 25(OH)D metabolism indicators. The detailed functions of the candidate genes are described in the Discussion section. Genomic DNA was isolated from peripheral blood leukocytes using PureLink Genomic DNA Mini Kit (Invitrogen, Carlsbad, USA) with spin column method. The DNA concentration was determined using NanoDrop spectrophotometer (Isogen Life Science, De Meern, the Netherlands). Genotyping of six SNPs for validation in the replication set (n=180) was performed using Kompetitive Allele Specific (KASP) genotyping technology by LGC Genomics (Hoddesdon, UK). The genotyping result was visualized by SNPviewer version 1.98 (LGC Genomics, Hoddesdon, UK). Genotype frequencies were tested against the Hardy-Weinberg equilibrium (HWE) using the \( \chi^2 \) test or chi square test.

Table 1. Characteristics of the Candidate Genes.

| Gene symbol | Location     | Gene name                        | Selected SNPs |
|-------------|--------------|----------------------------------|---------------|
| DHCR7       | 11q13.4      | 7-Dehydrocholesterol reductase   | 1             |
| GC          | 4q12–q13     | Vitamin D binding protein         | 1             |
| CYP2R1      | 11p15.2      | Vitamin D 25-hydroxylase          | 1             |
| CYP24A1     | 20q13        | Vitamin D 24-hydroxylase          | 1             |
| VDR         | 12q13.11     | Vitamin D receptor                | 2             |

SNPs: Single-nucleotide polymorphisms.
**Ethical statement.** This study was approved by the Ethics Committees of Medical Faculty, University of Andalas (No. 262/KEP/FK/2016). All mothers gave their written informed consent before data collection.

**Statistical analysis.** Data were analyzed using IBM SPSS Statistics for Windows (version 23.0; SPSS, Inc., Chicago, IL, USA). Continuous variables with normal distribution were presented as mean±SD and non-normal variables were presented as median (IQR). Categorical variables were presented as frequency and percentage. The normality of distribution of outcome variables (maternal serum 25(OH)D levels) was tested using Kolmogorov-Smirnov test. Since there was no normal distribution being observed, the data were log-transformed. Variance homogeneity was verified by Levene test.

The effect of SNPs on vitamin D concentrations was assessed using univariate general linear models after adjustment for covariates (age, pre-pregnancy BMI, vitamin D supplement, and geography status). The association of SNPs with vitamin D status was analyzed using logistic regression analyses. Odds ratios (OR) and corresponding 95% confidence intervals (95% CI) were given. Genetic risk score (GRS) was the sum of risk alleles from the most significant SNPs (DHCR7 (rs12785878), CYP2R1 (rs12794714), GC (2282679), CYP24A1 (rs6013897), and VDR (rs2228570 and rs7975232)). A crossover analysis was used to assess potential gene-environment interaction including lifestyle, and physical activity levels.

Serum 25(OH)D with concentration less than 12 ng/mL was defined as vitamin D deficiency. The relationship of maternal 25(OH)D concentration (outcome) with the independent variables such as lifestyle and confounders (maternal age, pre-pregnancy BMI, geography status, and vitamin D supplementation in pregnancy) were determined by univariate linear regression. A p-value<0.05 was considered as statistically significant.

**RESULTS**

In this study, 180 healthy singleton pregnancy (mean age, 29.59±5.57 y, BMI, 23.33±4.40 kg/m²) were included. The prevalence of maternal vitamin D status during the third trimester was 47.20% for sufficient, 35% for insufficient, and 17.80% for deficient of vitamin D status. We analyzed maternal characteristics based on maternal vitamin D status (Table 2).

During this analysis, the maternal status was divided into two categories, which were sufficient (≥20 ng/mL) and insufficient-deficient (<20 ng/mL). Maternal insufficient-deficient vitamin D status was significantly associated with the women of older age group (p=0.016), living in the mountainous areas (p=0.002), monthly family income (p=0.021), parity status (p=0.001), and type of work (p=0.047). An average of 25(OH)D concentration during pregnancy was 21.38±19.04 ng/mL. This result showed that the majority of these subjects had insufficient-deficient maternal vitamin D status of 52.80% compared to sufficient maternal vitamin D status of 47.20%.

Two models (linear and logistic regression) of analysis were conducted to test the association between vitamin D-related gene polymorphisms and 25(OH)D concentration. Out of six genotyped SNPs, three SNPs in the CYP2R1 (rs12794714), GC (rs2282679), and VDR (rs7975232) were significantly associated with 25(OH)D concentration at T3 of pregnancy. However, SNP rs22282679 for GC and rs1279714 for CYP2R1 genes remain significant (p≤0.008) after multiple correction with Bonferroni-correction at significance of α/n of SNPs tested=0.05/6=0.008 (Table 3).

Three additional SNPs, rs2228570 in VDR, rs12785878 in DHCR7, and rs6013897 in CYP24A1, were not associated with 25(OH)D concentration at T3 of pregnancy. All 6 of these SNPs were adjusted with potential modification covariate variables such as maternal age, pre-pregnancy BMI, sun exposure status, geography status, and supplement consumption during pregnancy. After tested by logistic regression (model 2), the difference was not significant. The association remained significant between SNP rs12794714 in CYP2R1 and SNP rs22282679 in GC gene (p=0.008). Allele frequencies were estimated by gene counting and shown in Table 4.

The chi-square test was used to compare the proportions of genotype frequencies. All 6 SNPs of this study were in the Hardy Weinberg Equilibrium (HWE) (p>0.05). We observed the association between GRS and 25(OH)D concentration. GRS category summed up by total allele risk score of all 6 SNPs in this study. We divided GRS into two groups, which were women who have less than or equal to three risk alleles and more than or equal to four risk alleles. There were statistically significant (p=0.006). Women who carry more risk alleles score had lower 25(OH)D concentration compared to women who had less risk allele score (Table 5).

There was no significant association between mean serum 25(OH)D concentration at T3 for each genetic risk scores (p=0.073). However, women who carry 0 genetic risk score significantly had higher serum 25(OH)D concentration at T3 compared to women who carry more score of risk alleles (p<0.05) (Fig. 2).

The genetic risk score was calculated as the sum of the number of T alleles for DHCR7-rs12785878, T alleles for CYP2R1-rs12794714, C alleles for GC-rs2282679, T alleles for CYP24A1-rs6013897, C alleles for VDR-rs2228570, and G alleles for VDR-rs7975232. Plots of the mean adjusted serum 25(OH)D (ng/mL) at T3 (y-axis) for each genetic risk score category (x-axis). There was no significant association between mean serum 25(OH)D concentration and each GRS (p=0.073). However, there was one subject who had the highest genetic risk score. Women who carry 0 genetic risk score significantly had higher serum 25(OH)D concentration at T3 compared to women who carry more score of risk alleles (p<0.05).
Table 2. The characteristics of study participants based on vitamin D status.

| Variables                          | Vitamin D Sufficiency (≥20 ng/mL; n=85) | Vitamin D Insufficiency (<20 ng/mL; n=95) | p value |
|------------------------------------|------------------------------------------|-------------------------------------------|---------|
| Age (y)                            | 30.19±6.10                               | 29.05±5.02                                | 0.178   |
| Age groups                         |                                          |                                           | 0.016   |
| a. <20                             | 5 (5.90)                                 | 0 (0.0)                                   |         |
| b. 21–25                           | 15 (17.60)                               | 29 (30.50)                                |         |
| c. 26–30                           | 22 (25.90)                               | 29 (30.50)                                |         |
| d. >30                             | 43 (50.60)                               | 37 (38.90)                                |         |
| Education level                    |                                          |                                           | 0.286   |
| a. Primary                         | 26 (30.60)                               | 23 (24.20)                                |         |
| b. Secondary                       | 36 (42.40)                               | 36 (37.90)                                |         |
| c. Tertiary                        | 23 (27.10)                               | 36 (37.90)                                |         |
| Geography status                   |                                          |                                           | 0.002   |
| a. Coastal                         | 45 (52.90)                               | 28 (29.50)                                |         |
| b. Mountainous                     | 40 (47.10)                               | 67 (70.50)                                |         |
| Monthly family income (IDR in million) | 2,321±1.335                             | 3,734±5.431                              | 0.021   |
| Height (cm)                        | 153.89±6.68                              | 154.64±5.82                               | 0.425   |
| Pre-pregnancy BMI (kg/m²)          | 23.52±4.30                               | 23.15±4.50                                | 0.578   |
| a. Underweight                     | 10 (11.80)                               | 12 (12.60)                                |         |
| b. Normal                          | 35 (41.20)                               | 46 (48.40)                                |         |
| c. Overweight                      | 11 (12.90)                               | 11 (11.60)                                |         |
| d. Pre-obese                       | 20 (23.50)                               | 15 (15.80)                                |         |
| e. Obese                           | 9 (10.60)                                | 11 (11.60)                                |         |
| GWG (kg)                           | 8.22±2.63                                | 8.98±3.05                                 | 0.071   |
| Parity status                      |                                          |                                           | 0.001   |
| a. Nulliparous                     | 10 (11.80)                               | 32 (33.70)                                |         |
| b. Primiparous                     | 75 (88.20)                               | 63 (66.30)                                |         |
| Gestational age (wk)               | 30.09±2.94                               | 30.43±3.16                                | 0.455   |
| Vitamin D supplements              |                                          |                                           | 0.495   |
| a. No                              | 57 (67.10)                               | 58 (61.10)                                |         |
| b. Yes                             | 28 (32.90)                               | 37 (38.90)                                |         |
| Physical activity                  |                                          |                                           | 0.445   |
| a. Low-moderate                    | 63 (74.10)                               | 75 (78.90)                                |         |
| b. High                            | 22 (25.90)                               | 20 (21.10)                                |         |
| Length of sun exposure (h)         | 63.00±53.67                              | 59.68                                     | 0.666   |
| a. <1 h                            | 42 (49.40)                               | 49 (51.60)                                |         |
| b. ≥1 h                            | 43 (50.60)                               | 46 (48.40)                                |         |
| Smoking status                     |                                          |                                           | 0.301   |
| a. No                              | 73 (85.90)                               | 73 (76.80)                                |         |
| b. Active                          | 2 (2.40)                                 | 4 (4.20)                                  |         |
| c. Passive                         | 10 (11.80)                               | 18 (18.90)                                |         |
| Type of work                       |                                          |                                           | 0.047   |
| a. Indoor                          | 70 (82.40)                               | 65 (68.40)                                |         |
| b. Outdoor                         | 15 (17.60)                               | 30 (31.60)                                |         |
| Sunscreen application              |                                          |                                           |         |
| a. Yes                             |                                        |                                           |         |
| b. No                              |                                        |                                           |         |
| Dressing style                     |                                          |                                           |         |
| a. Covered                         | 72 (84.70)                               | 85 (89.50)                                |         |
| b. Uncovered                       | 13 (15.30)                               | 10 (10.50)                                |         |
| Serum 25(OH)D concentration (ng/mL)| 29.05±7.73                               | 13.73±3.98                                | 0.001   |

BMI, body mass index; GWG, gestational weight gain; 25(OH)D, 25-hydroxyvitamin D. The data are expressed as mean±SD or n (%). Bold numbers are expressed as significant association. p-values are based on chi-square test or Fisher’s exact for categorical variables and independent sample t test for continuous variables. Differences were considered statistically significant at p<0.05 level.
Table 3. Association between SNPs and serum 25(OH)D concentration at T3.

| Genotype       | n  | 25(OH)D Mean±SD (ng/mL) | Model 1a  | Model 2b  |
|----------------|----|--------------------------|-----------|-----------|
|                |    |                          | \(\beta\) (SE) | \(p\) value | OR | 95% CI | \(p\) value |
| **DHCR7**      |    |                          |           |           |    |        |           |
| rs12785878     |    |                          |           |           |    |        |           |
| GG             | 104| 20.67±10.10              | -1.288    | 0.383     | 1.00|        | 0.456     |
| GT/TT          |  76| 22.36±10.15              |           |           | 0.61| 0.32–1.15|
| **CYP2R1**     |    |                          |           |           |    |        |           |
| rs12794714     |    |                          |           |           |    |        |           |
| CC             |  97| 23.63±11.30              |  4.804    | 0.001     | 1.00|        | 0.001     |
| CT/TT          |  83| 18.75±7.78               |           |           | 2.38| 1.21–4.32|
| **GC**         |    |                          |           |           |    |        |           |
| rs2282679      |    |                          |           |           |    |        |           |
| AA             | 116| 23.24±10.65              |  5.977    | 0.001     | 1.00|        | 0.001     |
| AC/CC          |  64| 18.00±8.10               |           |           | 3.15| 1.56–6.38|
| **CYP24A1**    |    |                          |           |           |    |        |           |
| rs6013897      |    |                          |           |           |    |        |           |
| AA             |  92| 21.82±10.23              |  0.903    | 0.536     | 1.00|        | 0.244     |
| AT/TT          |  88| 20.92±10.02              |           |           | 1.45| 0.77–2.73|
| **VDR**        |    |                          |           |           |    |        |           |
| rs2228570      |    |                          |           |           |    |        |           |
| TT             |  73| 21.02±8.89               | -1.220    | 0.414     | 1.00|        | 0.801     |
| TC/CC          | 107| 21.63±10.89              |           |           | 1.08| 0.58–2.04|
| rs7975232      |    |                          |           |           |    |        |           |
| TT             |  78| 23.65±10.42              |  3.096    | 0.037     | 1.00|        | 0.105     |
| TG/GG          | 102| 19.64±9.55               |           |           | 1.67| 0.89–3.23|

Adjusted for age, pre-pregnancy BMI, duration of sunlight exposure, supplement consumption, and geography status.

a. Univariate general linear model.
b. Logistic regression model of vitamin D insufficiency-deficiency status.

Table 4. The genetic variants frequency.

| Gene and SNPs          | Frequency (n=180) | HWE  
|------------------------|-------------------|------|
|                        | HR | HET | HV | \(p\) value |
| **DHCR7-rs12785878**   | 104 | 64 | 12 | 0.615 |
| **CYP2R1-rs12794714**  |  97 | 65 | 18 | 0.157 |
| **GC-rs2282679 (A)**   | 116 | 61 |  3 | 0.110 |
| **CYP24A1-rs6013897**  |  92 | 69 | 19 | 0.269 |
| **VDR-rs2228570**      |  74 | 73 | 33 | 0.053 |
| **VDR-rs7975232**      |  78 | 86 | 16 | 0.259 |

SNPs, single nucleotide polymorphisms; HWE, hardy Weinberg equilibrium; HV, homozygous variant genotype; HET, heterozygous genotype; HR, homozygous referent genotype. \(p\) value was analysed by chi square test. Genotype frequency was expressed (n). All genotype frequency was in HWE after comparing the proportion of observed and expected frequency (\(p>0.05\)).
DISCUSSION

There was a finding in maternal vitamin D-related genetic variants predicted maternal serum 25(OH)D concentration at T3 of pregnancy. Three of six genes were observed to have significant association with 25(OH)D concentration. To our knowledge, no previous studies have estimated the relationship between vitamin D-related gene polymorphisms and maternal serum 25(OH)D in pregnant Minangkabau women, Indonesia.

In this study, we replicate findings in the GC and CYP2R1 genes that were previously shown in the last large two GWAS meta-analyses of the Caucasian cohort (14, 15). The results were similar with to previous study, which was examination of 1,204 women of European descent from the Carotenoids in Age-related Eye Disease Study and tested the association between 2 SNPs in the GC gene and 4 SNPs in the CYP2R1 (18). Another study remains consistent with our results, namely rs4588 in the GC gene was associated with serum 25(OH)D concentration in the Canadians population. However, interaction with season of blood sample used to stratify the results and showed that its association found in the fall, but not in the winter (19).

In this study, SNPs in DHCR7 and CYP24A1 genes reported to be associated with 25(OH)D concentration in the last two large GWAS meta-analyses, were not significantly associated with 25(OH)D (14, 15). The role of these SNPs in the genome showed that it had no consequences. SNPs rs12785878 in DHCR7 and SNP rs6013897 in CYP24A1 genes located in the intron region which was lack of lacked effect during the translation process (20). The other two SNPs of this study from VDR (rs2228570 and 7975232) gene revealed the same result that it had no association with 25(OH)D concentration. However, rs7975232 was found to be associated with 25(OH)D concentration before being tested by multiple testing using Bonferroni-correction test. There were three previous studies reported similar findings that women who have heterozygote and homozgyote mutant more likely have a higher 25(OH)D concentration in Turkey (21), Brazil, and Egypt (22, 23).

The results of this study may have a potentially important implications for the government in creating policies, implementation of public health recommendations, and clinical practice guideline. We found that lower 25(OH)D concentration followed by increasing the number of risk alleles score. Subjects with more risk alleles score may achieve lower 25(OH)D concentration (Fig. 2). Furthermore, due to vitamin D-related gene variants were less prone to confounding factors. Modified healthy lifestyle and balanced nutrition during pregnancy are necessary to overcome for those who carry genetic variants. Many factors can determine the maternal vitamin D status. Our previous studies revealed that non-genetic factors such as women working status, supplement consumption, physical activity, age, the length of sun exposure levels, and living in the mountainous area were associated with maternal vitamin D status (24, 25).

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Table 5. Association between genetic risk score and serum 25(OH)D concentration at T3.

| Genetic risk score | n   | 25(OH)D Mean±SD (ng/mL) | β (SE) | p-value |
|-------------------|-----|-------------------------|--------|---------|
| a. ≤ 3 risk score | 93  | 23.38±10.70             | 4.012 (1.438) | 0.006   |
| b. ≥ 4 risk score | 87  | 19.24±9.01              |

GRS, genetic risk score; 25(OH)D, 25-hydroxyvitamin D.

a. Adjusted for age, pre-pregnancy BMI, lifestyle, duration of sunlight exposure, supplement consumption, and geography status.

b. Genetic risk score was put in the linear regression model as a continuous variable.

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Fig. 2. Mean serum 25(OH)D concentration at T3 for each genetic risk score category.
Additional research is needed to replicate and confirm our findings. This study conducted to pregnant Minangkabau women and more researches are needed beyond strictly Minangkabau population, including other ethnic or ancestral groups in the Indonesian population, since Indonesia is one of the most diverse countries in the world. Individuals with dark skin colour might have less prevalence in vitamin D deficiency (26). This study had a relatively small sample size: environmental factors were not observed in this study, and the chance of imperfect measurement of non-genetic factors, including sun exposure measurement, skin colour, food intake, and other biochemistry components related to vitamin D metabolism pathway. Future studies should be considered to accurately measure non-genetic factors.

In conclusion of this study, the prevalence of vitamin D deficiency was common in pregnant Minangkabau women, West Sumatra. Beside of environmental factors, genetic factors also play a vital role in concentration of 25(OH)D in the population of pregnant Minangkabau tribe women. Recommendations and policies about the detection and prevention of vitamin D insufficiency during pregnancy should be developed and taken into the account of the associated factors. However, further studies with larger samples are needed to confirm the findings in this study.

Disclosure of state of COI
No conflicts of interest to be declared.

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