Independent and interactive associations of season, dietary vitamin D, and vitamin D-related genetic variants with serum 25(OH)D in Korean adults aged 40 years or older

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Abstract. Only limited information is available on the inter-relationships between genetic and non-genetic factors such as diet and sunlight exposure with serum 25-hydroxyvitamin D [25(OH)D] concentration. This cross-sectional study aimed to examine the independent and interactive associations of season, dietary vitamin D intake, and SNPs of 11 vitamin D-related candidate genes with serum 25(OH)D concentration among 2,721 adults aged ≥40 years at baseline from the Yangpyeong cohort, a part of the Korean Genome Epidemiology Study (KoGES). The interactions between season or dietary vitamin D and 556 SNPs were evaluated using 2-degree of freedom joint tests. Season was strongly (p<1.00 × 10⁻¹²) and dietary vitamin D intake was slightly but significantly associated with serum 25(OH)D concentration (p<0.0119). Among five SNPs (rs11723621-GC, rs7041-GC, rs10500804-CYP2R1, rs7129781-CYP2R1, and rs2852853-DHCR7) identified in the screening steps, only one, rs10500804-CYP2R1, significantly interacted with season (p=8.01 × 10⁻⁵). The inverse association between number of minor alleles of rs10500804-CYP2R1 and concentration of 25(OH)D was significant only in summer/fall. Conversely, dietary vitamin D intake was positively associated only in winter/spring. In conclusion, season, dietary vitamin D intake, and four SNPs in GC, CYP2R1, and DHCR7 are independently and rs10500804-CYP2R1 is interactively associated with serum 25(OH)D concentration. Serum 25(OH)D is influenced by genotype of rs10500804-CYP2R1 in summer/fall when sunlight exposure is high, while dietary vitamin D intake is an important determinant of serum 25(OH)D during the seasons with low cutaneous vitamin D synthesis.

Key words: Vitamin D, 25-Hydroxyvitamin D, Season, Dietary vitamin D intake

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Abbreviations: 25(OH)D, 25-hydroxyvitamin D; AI, Adequate intake; AMDHD1, Amidohydrolase domain containing 1; BMI, Body mass index; C10orf88, Chromosome 10 open-reading frame 88; CYP24A1, Vitamin D 24-hydroxylase; CYP27A1, Sterol 27-hydroxylase; CYP27B1, Vitamin D₃₂₄-hydroxylase; CYP2R1, Vitamin D 25-hydroxylase; DBP, Vitamin D binding protein; df, Degree of freedom; DHCR7, 7-dehydrocholesterol reductase; FFQ, Food frequency questionnaire; GC, Group-specific component (vitamin D binding protein, DBP); GLM, General linear model; GRS, Genetic risk score; GWAS, Genome-wide association study; kb, Kilobase; K-CHIP, Korean Chip; KNHANES, Korea National Health and Nutrition Examination Survey; KNIH, Korea National Institute of Health; KoGES, Korean Genome and Epidemiology Study; LD, Linkage disequilibrium; MAF, Minor allele frequency; NADSYN1, Glutamine-dependent NAD synthetase; QC, Quality control; RA, Rheumatoid arthritis; SEC23A, Sec23 homolog A, coat complex II component; SEI, SNP-environment interaction; SNP(s), Single nucleotide polymorphism(s); SUNLIGHT, Study of Underlying Genetic Determinants of Vitamin D and Highly Related Traits; UVB, Ultraviolet B; VDR, Vitamin D receptor; WC, Waist circumference; WHI-OS, Women’s Health Initiative Observational Study
with prevention of diseases such as bone disease, several types of cancer, autoimmune disease, diabetes, hypertension, and cardiovascular diseases have been previously reported, and the public health value of vitamin D is highlighted [3].

Vitamin D is primarily obtained through skin synthesis initiated by ultraviolet B (UVB) radiation exposure [4], and typically only small amounts are obtained through diet [5]. 25-Hydroxyvitamin D [25(OH)D] is considered the primary biomarker of vitamin D status and reflects the sum of vitamin D intake and vitamin D produced from sun exposure [6]. In general, old age, female sex, higher latitude, winter season, darker skin pigmentation, low sunlight exposure, dietary habits, and absence of vitamin D fortification are associated with lower 25(OH)D level [7]. In addition, genetic contribution to 25(OH)D concentration has been reported in previous twin and family studies, ranging from 23 to 80% [8, 9].

A genome-wide association study (GWAS) and meta-analysis identified variants in three loci of GC (vitamin D binding protein, DBP), CYP2R1 (vitamin D 25-hydroxylase), and DHCR7 (7-dehydrocholesterol reductase), which are all in genes related with the vitamin D metabolic pathway, to be associated with 25(OH)D [10]. The larger meta-analysis by the SUNLIGHT (Study of Underlying Genetic Determinants of Vitamin D and Highly Related Traits) consortium confirmed these three loci and identified additional variants in CYP24A1 (vitamin D 24-hydroxylase) [11]. The expanded SUNLIGHT consortium identified two additional loci of SEC23A (sec23 homolog A, coat complex II component) and AMDHD1 (amidohydrolase domain containing 1) [12].

Although vitamin D deficiency has a high prevalence throughout the world, deficiency is more highly prevalent in the Middle east and Asia [13]. Previously, a study based on the Korea National Health and Nutrition Examination Survey (KNHANES) showed that vitamin D deficiency affected more than half of the population and increased from 2008 to 2014 [14]. In another KNHANES study, a positive relationship between dietary vitamin D intake and serum 25(OH)D was observed in men and women aged younger than 50 years [15]. The mean level of serum 25(OH)D in Koreans differed by season and amount of sunlight, being lowest in January and February and highest in August through October [16].

Most studies on determinants of serum vitamin D have focused on one aspect of genetic or non-genetic factors and have been for participants of European ancestry [10-12]. Although postulated that genetic factors may differently affect variation of serum 25(OH)D depending on environmental factors [17], supporting evidence on the interaction of lifestyle choices, such as outdoor activities, and dietary vitamin D intake with genetic variants related to vitamin D metabolism is lacking.

Therefore, we aimed to identify the independent and interactive associations of season (winter/spring and summer/fall), dietary vitamin D intake, and 556 single nucleotide polymorphisms (SNPs) located in vitamin D metabolic pathway genes with serum 25(OH)D concentration among Korean adults aged 40 or older.

**Methods**

**Study design and population**

This study was cross-sectional using data from the Yangpyeong Cohort, a part of community cohort of the Korean Genome and Epidemiology Study (KoGES 2004–2011), which was a cardiovascular disease prevention study in adults aged over 40 years as described in detail elsewhere [18]. The study participants were enrolled in a rural area of South Korea, Yangpyeong County located 45 km east of Seoul at latitude 37°33’01” N, between February 2005 and August 2011. A total of 3,266 participants who did not have cardiovascular disease or cancer, we excluded those with missing data on the main variables (genome data (n = 403), vitamin D blood sample data (n = 80), or dietary intake information (n = 32)). We also excluded those with missing covariate data: education level, exercise, smoking status, alcohol consumption, drinking status, body mass index (BMI), and waist circumference (WC) (n = 177). As a result, 2,721 participants (991 men and 1,730 women) were included in the final analysis. All procedures followed were in accordance with the ethical standards of the responsible Hanyang University Institutional Review Board committee and with the Helsinki Declaration of 1964, as revised in 2000. All participants provided written informed consent to voluntarily participate in this study.

**Assessment of dietary intake and covariates**

Dietary intake was assessed using the validated semi-quantitative food frequency questionnaire (FFQ) composed of 106 food items with nine frequency categories ranging from ‘never or rarely’ to ‘three times/day.’ Three or four portion sizes were specified for each food item [19]. For seasonal foods, data on consumption period were obtained using four categories (3, 6, 9, and 12 months). The survey was conducted by responding to the frequency and amount of food consumed on average over the past year. Dietary vitamin D intake and other nutrients were calculated using the nutrient database of Computer Aided Nutritional Analysis program (CAN-Pro) version 4.0 of the Korean Nutrition Society [20].

Information on covariates was collected using a
structured questionnaire to assess demographics (age, sex, and education level) and lifestyle factors (physical activity, smoking status, drinking status, and alcohol intake). Higher education level was defined as ≥12 years of schooling. Regular exercise was defined as ≥3 days per week and ≥30 min per session. Smoking status was classified as current smoker, past smoker, or never-smoker. Drinking status was classified as never-drinker, past drinker, or current drinker. Alcohol consumption was assessed using questionnaires that specified six alcoholic beverages (soju, takju, beer, refined rice wine, wine, and whisky). Soju and takju are the most popular alcoholic beverages in Korea; soju is made from distillation of grains such as rice or starches such as sweet potatoes, and takju is made by fermenting other grains. Current and past drinkers were identified, and total daily alcohol consumption was calculated based on the total volume of each alcoholic beverage consumed, expressed in milliliters of alcohol per day (mL/d).

Anthropometric measurements were conducted by trained research staff with the standard protocol. Height was measured with a stadiometer to the nearest 0.1 cm, and weight was measured with a metric scale to the nearest 0.01 kg while the subjects were wearing light clothing with no shoes. Body mass index (BMI) was calculated as body weight (kg)/height (m²). Waist circumference (WC) was measured at the midpoint between the iliac crest and the lower rib margin.

**Measurement of serum 25(OH)D**

Vitamin D status is commonly assessed using serum 25(OH)D, which reflects vitamin D both from dermal production by sun exposure and from dietary intake [6]. Serum 25(OH)D was assayed from frozen stored serum samples at −70°C using the chemiluminescent microparticle immunoassay (ARCHTECT i2000, Abbott, IL, USA). The coefficient of variation for total analytic precision of this assay was ≤10% for 25(OH)D. The lower detection limit of this assay was 3.0 ng/mL for 25(OH)D.

**Genotyping information and selection of candidate genes**

We selected candidate genes related to 25(OH)D from the recent genome-wide association study (GWAS) and SUNLIGHT consortium [10, 11] and from the expanded SUNLIGHT consortium [12]. We selected all candidate genes regardless of the results from those consortia. The SNPs from 25(OH)D-related genes displaying significant meta-analysis results, which were significantly associated with 25(OH)D in the GWAS, have been mostly studied in European (non-Asian) populations. Eleven genes were selected: GC (vitamin D binding protein, DBP), CYP2R1 (vitamin D 25-hydroxylase), DHCR7 (7-dehydrocholesterol reductase), NADSYN1 (glutamine-dependent NAD synthetase), C10orf88 (chromosome 10 open-reading frame 88), CYP24A1 (vitamin D₃ 24-hydroxylase), CYP27B1 (vitamin D₃-1α-hydroxylase), CYP27A1 (sterol 27-hydroxylase), VDR (vitamin D receptor), SEC23A (sec23 homolog A, coat complex II component) and AMDHD1 (amidohydrolase domain containing 1) (Supplementary Table S1).

The genotype data were generated using the Korean Chip (K-CHIP), which was designed by the Center for Genome Science, Korea National Institute of Health (KNIH), based on the platform of UK Biobank Axiom Array and manufactured by Affymetrix [21]. Sample quality control (QC) filtering of the genome data was performed (low call rate (<99%), excessive heterozygosity, cryptic first-degree relatives, and sex inconsistency). SNP QC was conducted: low quality SNPs (off-target variants, others categorized by SNPolisher), Hardy-Weinberg equilibrium (p < 1 × 10⁻⁶), and genotype call rates (>95%) were determined. SNP imputation was performed with IMPUTE v2 [22] with 1,000 Genomes project phase 3 as a reference panel. Further details regarding genotype calling and quality control processes were described in a previous study [21]. We removed all SNPs with a minor allele frequency (MAF) <0.05, and 556 SNPs located in those 11 genes were included in the analysis (UCSC Genome Browser on Human Feb. 2009 (GRCh37/hg19) Assembly, https://genome.ucsc.edu/cgi-bin/hgGateway?db=hg19 Assessed on Mar. 14, 2020).

Those 11 genes (7 genes; GC, CYP2R1, and DHCR7, NADSYN1, CYP24A1, SEC23A, and AMDHD1) were confirmed or (4 genes; C10orf88, CYP27B1, CYP27A1, and VDR) were considered but not confirmed to be associated with 25(OH)D in the SUNLIGHT [10, 11] or expanded SUNLIGHT [12] consortium in Caucasian and European populations.

**Statistical analysis**

Season of blood collection was used as a proxy measure of sunlight exposure, given that seasonal differences influence the extent to which UVB photons are absorbed by the ozone layer [23]. We modeled season using categorical variables for winter/spring (December-May) and summer/fall (June-November). Dietary vitamin D intake was adjusted for total energy intake using the residual method [24] and divided into two groups as low (≤1.44 μg/d) or high (>1.44 μg/d) based on median (1.44 μg/d). The characteristics of participants were expressed as mean and standard error for continuous variables and as percentage for categorical variables (Supplementary Table S2). All mean differences in 25(OH)D and continuous or categorical covariates (Yes = 1/No = 0 for dichotomous categorical variables).
according to season, dietary vitamin D intake group, and genotype were determined with the general linear model (GLM). To select potential confounders, we first considered variables that have been previously reported as factors for 25(OH)D concentration [7, 15, 25-27] such as age (years), sex (men and women), education level (higher education ≥12 years of schooling, yes/no), smoking and drinking status (current/past/never), alcohol consumption (mL/d), regular exercise (≥3 times/wk and 30 min/session), BMI (kg/m²), WC (cm), season (winter/spring and summer/fall), and dietary vitamin D intake (µg/d). Variables that showed significant mean differences according to season and dietary vitamin D intake groups in the sex- and age-adjusted GLM at p < 0.05 were selected as confounders. However, regular exercise, BMI, and WC were not included in the final analysis given that the exact mechanism by which physical activity and body fat are associated with serum 25(OH)D concentration is not known.

The associations between 556 SNPs and 25(OH)D were examined by sex- and age-adjusted linear regression under an additive genetic model using PLINK (http://pngu.mgh.harvard.edu/~purcell/plink/) (Supplementary Table S3). The interactions of season and dietary vitamin D intake with SNPs on 25(OH)D were analyzed using both a 2 degree of freedom (df) joint test of marginal association and a 1 df interaction test. A 2 df joint test was used as the screening step after adjusting for age, sex, and additional covariates; this step simultaneously considered regression coefficients for the SNPs and SNP-environment interaction (SEI) term (null hypothesis of βSNPs = 0 and βInteraction = 0 [28]). Bonferroni correction was used to screen SNPs at a significance level (p < 0.05/556 = 8.99 × 10⁻⁵) that considered the total number of SNPs (n = 556). After the screening step, we demonstrated 1 df interaction analysis for SNPs. A total of 63 SNPs for season and 60 SNPs for dietary vitamin D intake were selected in the screening step using the 2 df joint test at a significance level p < 0.05/556 = 8.99 × 10⁻⁵. A total of 5 SNPs remained after a clumping procedure among SNPs selected in the screening step for season analysis (rs11723621 and rs7041 in GC, rs10500804 and rs7129781 in CYP2R1, and rs2852853 in DHCR7) (Table 3). However, only one SNP, rs10500804-CYP2R1, significantly interacted with season (pinteraction = 8.01 × 10⁻⁵). We identified four independent SNPs in dietary vitamin D intake analysis (rs11723621 and rs7041 in GC, rs10500804 in CYP2R1, and rs2852853 in DHCR7); however, none of these SNPs showed interaction with dietary vitamin D intake.

Table 4 indicates the final associations of SNPs, season, and dietary vitamin D intake with 25(OH)D. The association of each individual SNP with 25(OH)D became more significant in the model considering season and dietary vitamin D intake (model 2), but the association between season and 25(OH)D was strongest. Because a significant interaction with season appeared only for rs10500804-CYP2R1, the interaction term was included only in the associated model. Serum 25(OH)D concentration decreased as the number of minor alleles of rs11723621-GC and rs10500804-CYP2R1 increased, but it increased as the number of minor alleles of rs7041-GC, rs7129781-CYP2R1, and rs2852853-DHCR7 (models 1 and 2) increased. Because only rs10500804-CYP2R1 interacted with season, it was the only included SNP-season interaction (model 3).

Accordingly, Table 5 shows the modified association between rs10500804-CYP2R1 or dietary vitamin D intake and serum 25(OH)D concentration by season. A negative association was found between the number of
minor alleles and serum 25(OH)D. Among other four SNPs that did not show a significant interaction with season, rs11723621-GC was negatively, and the other three SNPs were positively associated with serum 25(OH)D (Supplementary Table S4). The genetic association of rs10500804-CYP2R1 with serum 25(OH)D concentration was clear only in summer/fall ($p_{\text{difference}} < 0.0001$), while the positive association between dietary vitamin D intake and 25(OH)D was clear in winter/spring ($p_{\text{difference}} = 3.96 \times 10^{-3}$).

Table 1  Age- and sex-adjusted characteristics of study participants according to season and dietary vitamin D intake

| Characteristics | Season | Dietary vitamin D intake$^1$ | p value* |
|-----------------|--------|-----------------------------|----------|
|                 | Winter/Spring (Dec–May) | Low ($\leq 1.44 \mu g/d$) | High (>1.44 μg/d) |
|                 | Summer/Fall (Jun–Nov) | 1,001 | 1,361 | 1,360 | 0.63 (0, 1.435) | 2.66 (1.44, 18.46) | <.0001 |
| N               | 1,001 | 1,720 | 1,361 | 1,360 | 0.63 (0, 1.435) | 2.66 (1.44, 18.46) | <.0001 |
| Median (min, max) | --- | --- | 62.6 ± 0.28 | 58.4 ± 0.28 | --- | --- | --- |
| Age (year)      | 61.4 ± 0.33 | 60.1 ± 0.25 | 0.0013 | 62.6 ± 0.28 | 58.4 ± 0.28 | <.0001 |
| Women (%)       | 60.6 | 65.3 | 0.0144 | 62.4 | 64.7 | 0.2148 |
| Higher education (≥12 years, %) | 29.9 | 35.2 | 0.0009 | 26.6 | 39.9 | <.0001 |
| Smoking status (%) | Never smoker | 59.5 | 61.4 | 0.1437 | 60.5 | 60.9 | 0.6518 |
| Past smoker     | 22.0 | 21.3 | --- | 22.4 | 20.7 | --- |
| Current smoker  | 18.5 | 17.3 | --- | 17.1 | 18.4 | --- |
| Drinking status (%) | Never drinker | 41.5 | 40.9 | 0.9588 | 39.7 | 42.5 | 0.2716 |
| Past drinker    | 6.4 | 7.8 | --- | 8.2 | 6.4 | --- |
| Current drinker | 52.1 | 51.3 | --- | 52.1 | 51.1 | --- |
| Regular exercise (≥3 times and 30 min/wk, %) | 19.4 | 27.2 | <.0001 | 19.6 | 29.0 | <.0001 |
| Body Mass Index (kg/m$^2$) | 24.7 ± 0.10 | 24.4 ± 0.08 | 0.0042 | 24.5 ± 0.09 | 24.5 ± 0.09 | 0.7589 |
| Waist circumference (cm) | 86.8 ± 0.28 | 84.8 ± 0.21 | <.0001 | 85.9 ± 0.24 | 85.2 ± 0.24 | 0.0238 |
| Season (summer, %)$^2$ | 0 | 100 | <.0001 | 57.3 | 67.9 | <.0001 |
| Dietary vitamin D intake (μg/d)$^3$ | 1.67 ± 0.06 | 2.06 ± 0.04 | <.0001 | 0.68 ± 0.04 | 3.17 ± 0.04 | <.0001 |

All values are expressed as mean ± SE and adjusted for age and sex.

$^*$ P values were obtained using the general linear model (GLM).

$^1$ Dietary vitamin D intake was divided into low and high groups based on median (1.44 μg/d).

$^2$ Season was divided into two groups: winter (December to May) and summer (June to November).

$^3$ Dietary vitamin D intake was adjusted for total energy intake.

Table 2  Average serum 25(OH)D concentration according to season and dietary vitamin D intake

| Season | Dietary vitamin D intake$^1$ | p value* |
|--------|-----------------------------|----------|
|        | Winter/Spring (Dec–May) | Low ($\leq 1.44 \mu g/d$) | High (>1.44 μg/d) |
|        | Summer/Fall (Jun–Nov) | 1,001 | 1,361 | 1,360 | 0.63 (0, 1.435) | 2.66 (1.44, 18.46) | <.0001 |
| N      | 1,001 | 1,720 | 1,361 | 1,360 | 0.63 (0, 1.435) | 2.66 (1.44, 18.46) | <.0001 |
| Median (min, max) | --- | --- | 19.9 ± 0.23 | 21.4 ± 0.23 | 1.42E-06 |
| Serum 25(OH)D (ng/mL) | 24.7 ± 0.10 | 24.4 ± 0.08 | 0.0042 | 24.5 ± 0.09 | 24.5 ± 0.09 | 0.7589 |
| Age- and sex-adjusted model | 14.5 ± 0.21 | 24.3 ± 0.16 | 1.00E-12 | 19.9 ± 0.23 | 21.4 ± 0.23 | 1.42E-06 |
| Multivariable model$^2$ | 14.3 ± 0.22 | 24.0 ± 0.17 | 1.00E-12 | 18.8 ± 0.20 | 19.5 ± 0.19 | 1.19E-02 |

All values are expressed as mean ± SE.

$^*$ P values were obtained using the general linear model (GLM).

$^1$ Dietary vitamin D intake was divided into low and high groups based on median (1.44 μg/d).

$^2$ Multivariable model included age, sex, education level, and dietary vitamin D intake in analysis of season and dietary vitamin D intake.
In the present study, there were significant associations of season as a proxy of sunlight exposure and dietary vitamin D intake with serum 25(OH)D concentration. Five SNPs for season and four for vitamin D intake were selected in the 2 df joint test screening step (rs11723621 and rs7041 in GC, rs10500804 in CYP2R1, and rs2852853 in DHC7R7 for both season and vitamin D intake and rs7129781 in CYP2R1 for season), but only rs10500804-CYP2R1 showed a significant interaction with season. Increasing number of minor alleles of rs10500804 was associated with a descending trend of serum 25(OH)D concentration in summer/fall, but not in winter/spring, while dietary vitamin D intake positively associated with 25(OH)D concentration only in winter/spring.

According to the Korean National Health and Nutrition Examination Survey (KNHANES) 2008–2014, the mean serum 25(OH)D was 45.7 nmol/L in men and 40.9 nmol/L in women (converted to 18.3 ng/mL and 16.4 ng/mL, respectively) [14], and for adults aged 40 or older, it was ranged from 21.1 to 23.1 ng/mL in men and 17.3 to 19.6 in women [27]. In our study participants whose average age was 60 years, the mean serum 25(OH)D concentrations were slightly higher than those of the KNHANES study, 23.1 ng/mL in men and 18.1 ng/mL in women. Those values were probably comparable with those of the KNHANES, which showed a tendency to increase with age in both sexes, but likely to be higher due to the relatively high proportion of farmers in the present study (40.2%). In a rural population with outdoor activities through farming, gardening, and other work, we expect sunlight exposure and vitamin D levels to be high. An Indonesian study also showed that the mean serum 25(OH)D among farmers was 20.2 ± 4.4 ng/dL, while that among indoor workers (non-government employees, nurses, and doctors) was 14.9 ± 3.6 ng/dL [29].

In the present study, we found a pronounced seasonal variation of 25(OH)D as shown in the KNHANES study [14]. The seasons in the Korea farming community are mainly divided into farming season when farming is possible and off-farming season when farming is minimal due to cold conditions. The Korea farming season is usually from mid-June to early November (summer/fall), and the off-farming season (winter/spring) is the remainder of the year [30]. Given that many of the participants in our study were farmers, a wide variation in time spent outdoors between the farming and off-farming seasons in our study participants was possible.

We found that five variants in three genes (rs11723621 and rs7041 in GC, rs10500804 and rs7129781 in CYP2R1, and rs2852853 in DHC7R7) were screened in 2-
Table 4  The associations of SNPs, season, and dietary vitamin D intake with serum 25(OH)D concentration (ng/mL) in the multivariable models

| Variables in the multivariable model | SNP | Season | SNP × Season | Dietary vitamin D intake |
|-------------------------------------|-----|--------|--------------|-------------------------|
|                                     | β   | p value* | β   | p value* | β   | p value* |
| **GC**                              |     |         |     |          |     |          |
| rs11723621                          |     |         |     |          |     |          |
| Model 1†                            | –1.5438 | 1.56E-10 | — | — | — | — |
| Model 2‡                            | –1.6856 | 5.64E-18 | 9.8068 | 6.07E-247 | — | — | 0.2293 | 1.29E-03 |
| rs7041                              |     |         |     |          |     |          |
| Model 1†                            | 1.1540 | 4.60E-06 | — | — | — | — |
| Model 2‡                            | 1.2090 | 3.20E-09 | 9.7639 | 1.06E-244 | — | — | 0.2316 | 1.22E-03 |
| **CYP2R1**                          |     |         |     |          |     |          |
| rs10500804                          |     |         |     |          |     |          |
| Model 1†                            | –0.8851 | 8.89E-05 | — | — | — | — |
| Model 2‡                            | –1.0149 | 3.02E-08 | 9.7883 | 1.58E-241 | — | — | 0.2352 | 1.14E-03 |
| Model 3§                            | –0.0652 | 8.29E-01 | 10.942 | 1.96E-149 | –1.4964 | 8.01E-05 | 0.2412 | 8.24E-04 |
| rs7129781                           |     |         |     |          |     |          |
| Model 1†                            | 0.7497 | 3.94E-02 | — | — | — | — |
| Model 2‡                            | 1.0810 | 2.51E-04 | 9.7789 | 1.36E-243 | — | — | 0.2356 | 1.05E-03 |
| **DHCR7**                           |     |         |     |          |     |          |
| rs2852853                           |     |         |     |          |     |          |
| Model 1†                            | 0.8758 | 1.45E-04 | — | — | — | — |
| Model 2‡                            | 0.9797 | 1.58E-07 | 9.7991 | 2.12E-242 | — | — | 0.2137 | 3.15E-03 |

* Association analyses were [SNP, additive] test with 1 df.
† Model 1 included age, sex, and education level.
‡ Model 2 included age, sex, education level, dietary vitamin D intake, and season.
§ Model 3 included model 2 and an additional interaction term [SNP × Season] for only rs10500804.

Table 5  The average serum 25(OH)D concentration (ng/mL) according to genotype of rs10500804, dietary vitamin D intake, and season and the interaction rs10500804 and dietary vitamin D intake with season in relation to serum 25(OH)D concentration

| Variables                      | N       | Total       | Winter/Spring (Dec–May) | Summer/Fall (Jun–Nov) | pseason* | pinter** |
|--------------------------------|---------|-------------|-------------------------|------------------------|----------|----------|
| SNP rs10500804_G                |         |             |                         |                        |          |          |
| TT                             | 1,003   | 20.0 ± 0.22* | 14.6 ± 0.39*            | 25.3 ± 0.31*           | 3.75E-11 | 2.54E-04 |
| GT                             | 1,263   | 18.9 ± 0.20b | 14.0 ± 0.31*            | 23.7 ± 0.24b           | 1.24E-11 |          |
| GG                             | 418     | 18.0 ± 0.33b | 14.5 ± 0.50c            | 22.0 ± 0.38c           | 1.00E-12 |          |
| pSNP*                          | 1.79E-07| 4.31E-01    | 6.56E-09                |                        |          |          |

Dietary vitamin D intake‡

|        | N       | Total       | Winter/Spring (Dec–May) | Summer/Fall (Jun–Nov) | pseason* | pinter** |
|--------|---------|-------------|-------------------------|------------------------|----------|----------|
| Low    | 1,361   | 18.84 ± 0.20 | 13.65 ± 0.30            | 23.85 ± 0.27           | 7.07E-12 | 6.84E-02 |
| High   | 1,360   | 19.50 ± 0.19 | 14.95 ± 0.34            | 24.24 ± 0.23           | 7.11E-12 |          |
| pDiet* | 1.19E-02| 3.96E-03    | 6.56E-09                |                        |          |          |

All results values are expressed as mean ± SE adjusted for age, sex, education level, dietary vitamin D intake, and season; and adjusted mean values with different superscripts (a, b, c) within a row were significantly different among the three groups on Tukey’s multiple comparison test.

* P difference values were obtained using the general linear model (GLM).
** P interaction was tested using a cross product term.
† SNP_minor allele
‡ Dietary vitamin D intake was divided into low and high groups based on median (1.44 μg/d).
§ P values for dietary vitamin D intake
family encoding cytochrome P450 proteins, encodes the liver [38]. Interestingly, in the present study, only alleles of rs2852853 in the DHCR7 gene (7-dehydrocholesterol reductase that converts 7-dehydrocholesterol to cholesterol in skin, thereby removing the substrate from the synthetic pathway of vitamin D3 [35]. Previously, in a case-control study of Hispanic women and non-Hispanic White women, rs12785878-DHCR7 demonstrated a significant interaction with time spent in outdoor activities [33]. However, in the present study, the number of minor alleles of rs2852853 in the DHCR7 gene was positively associated with serum 25(OH)D concentration. Only one study has previously identified rs2852853-DHCR7, but it was show in association with rheumatoid arthritis (RA), not with serum 25(OH)D [36]. Interestingly, the RA study suggested that the association between rs2852853-DHCR7 and RA may be due to some causal aspects of vitamin D metabolism in RA, based on evidence that vitamin D deficiency is common in RA patients, and that DHCR7 is moderately associated with sero-positive RA [37]. Third, the CYP2R1 gene, a member of the CYP2 family encoding cytochrome P450 proteins, encodes the key enzyme that converts vitamin D to 25(OH)D in the liver [38]. Interestingly, in the present study, only rs10500804-CYP2R1 showed a significant interaction effect, and a significant inverse association between the number of minor alleles and serum 25(OH)D concentration was observed only in summer/fall. In the present study, only rs10500804-CYP2R1 showed a significant interaction effect, and significantly lower serum 25(OH)D concentration with increase of the number of minor alleles was observed only in summer/fall. Our finding of a season difference in serum 25(OH)D according to vitamin D-related genetic variants is in line with previous studies of WHI-OS including rs10500804-CYP2R1 [39] and Danish children (rs7041-GC) [39, 40]. A Swedish twin study at northern latitude 60° [17] reported a moderate genetic impact during the summer season but undetectable heritability during the winter season when UVB radiation is minimal and cutaneous synthesis of pre-vitamin D is low [17]. Our participants were residents living at latitude 37°, a relatively low latitude, but the seasonal difference was the same. All SNPs in the surrounding regions of the 11 genes, extracting all SNPs within ±10 kilobase (kb), 20 kb, and 50 kb were also analyzed. A total of 10 more SNPs near the CYP2R1 locus was identified to interact with season, but they all were in strong LD with rs10500804-CYP2R1 (r² ≥ 0.78).

Genes considered in the present study are mostly related to the metabolism of vitamin D in skin or after intestinal absorption. Until now, it is accepted that vitamin D is absorbed from a micelle with fat by a simple passive diffusion [41]. And thus conditions related to fat malabsorption can negatively influence vitamin D absorption efficiency [41]. Recently, it was suggested that vitamin D intake is probably absorbed through a mechanism involving membrane carriers, especially such as cholesterol transporter [42], although evidence in humans is scarce and unclear [43]. Given this, we analyzed the interaction of dietary vitamin D intake with 100 SNPs within and near (50 kb) NPC1L1 (Niemann-Pick C1-like 1 carrier) which is mainly suggested as the most important cholesterol membrane carrier [42]. However, no SNP passed the significance threshold (p < 0.05) in a 2 df joint test for all participants and for participants in winter/spring, the lowest p-value of 2 df joint test was 3.72 × 10⁻². Moreover, we performed additional analyses using all genotyped and imputed SNPs to identify whether there is any other novel gene, but no additional novel gene was found.

The Swedish twin study investigated a shared environmental impact on serum 25(OH)D concentration and showed a predominant influence only in winter [17]. Their findings were supported in the present study, which showed a significant positive association between dietary vitamin D intake and serum 25(OH)D only in winter/spring (P_{interaction} = 3.96 × 10⁻⁴). This has potentially important implications for public health and clinical practice guidelines when considered with the genetic impact seen only in summer/fall. Dietary vitamin D intake (1.81 ± 1.67 μg/d in men and 1.99 ± 1.91 μg/d in women) in the present study mostly originated from dairy products, mushrooms, and egg yolks and were far below current Korean adequate intake (AI) guidelines of vitamin D 10 μg/d in both men and women aged 30–64 years and 15 μg/d in those aged ≥65 years [44]. Particularly in winter/spring, higher vitamin D intake may be required to achieve adequate 25(OH)D. Moreover, based on genetic risk score (GRS), which was calculated with the number of risk alleles (“0” for absence of the risk allele, “1” for heterozygote, and “2” for homozygote) of four SNPs other than rs10500804-CYP2R1 (Supplementary Table S5), individuals with a larger number of risk alleles may need higher intake of vitamin D, particularly for indoor workers.
Some limitations should be considered in the interpretation of our findings. First, this study was cross-sectional in design, which precludes causal inference. Additionally, to ensure sufficient sample size, we did not stratify our study participants by sex. Another important limitation is that we did not fully consider all vitamin D-related covariates; for example, physical activity and obesity index (such as BMI and WC) are determinants of vitamin D. However, we did not include these as confounders because physical activity may be related to sunlight exposure or body fat [45]. It is not clear whether physical activity and body fat are directly associated with vitamin D metabolism regardless of sunlight exposure. When we conducted sensitivity analysis including these potential confounders, the results were unchanged (data not shown). Third, vitamin D supplementation may have a large impact on serum 25(OH)D concentration. The present study included only one current user of vitamin D supplement, as well as 271 current users of multinutrient supplements that might have contained vitamin D. However, vitamin D intake from supplements could not be calculated due to lack of information on supplements such as brand name. Fourth, we used season of blood collection as a proxy measure of sunlight exposure; this may not accurately reflect amount of sunlight. Further studies with more accurate sunlight exposure data are needed for confirmation of our results. Finally, although we considered 556 SNPs of 11 genes related to vitamin D metabolism, we could not consider polygenicity and rare or low-frequency variants. Regardless of these limitations, it is worth to note that the present study demonstrated the independent associations of non-genetic factors for genetic predisposition with 25(OH)D concentration.

In conclusion, our findings provide informative evidence that season, dietary vitamin D intake, and variants in CYP2R1 (rs10500804 and rs7129781), GC (rs11723621 and rs7041), and DHCR7 (rs2852853) genes contribute to variation of serum 25(OH)D concentration in Korean adults. There is a seasonal difference in 25(OH)D according to variant of rs10500804-CYP2R1. The influence of rs10500804-CYP2R1 is clear only in summer when sunlight exposure is high, and that of dietary vitamin D intake with serum 25(OH)D is seen only in winter/fall when sunlight exposure is minimal. Our findings suggest that genetic predisposition and environmental factors independently and interactively affect 25(OH)D. Further studies are needed to investigate the influential environmental determinants of serum 25(OH)D, particularly during the winter season, for public health recommendations and clinical practice guidelines regarding achievement of adequate vitamin D.

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Authorship

Writing - original draft: JL, HWW, MKK. Formal analysis: JL, HWW, JK. Methodology: JL, HWW, JK, MKK. Supervision: MKK. Funding acquisition: BYC, MKK. Investigation: HWW, MKK, MHS, BYC. Writing - review and editing: JL, HWW, MKK.

Disclosure

The authors declare that they have no conflicts of interest.

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