Genetic and environmental predictors of serum 25-hydroxyvitamin D concentrations among middle-aged and elderly Chinese in Singapore

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Abstract

Vitamin D is known for maintaining Ca homeostasis and bone structure, and may also decrease susceptibility to chronic and infectious diseases. However, data on vitamin D status and its predictors among Southeast Asian populations are limited. We evaluated the distribution and determinants (genetic and environmental) of serum 25-hydroxyvitamin D (25(OH)D) concentrations among 504 middle-aged and elderly participants (aged 45–74 years) in the Singapore Chinese Health Study. Data on dietary and other lifestyle factors were collected by trained interviewers. Serum 25(OH)D concentrations and genetic polymorphisms in vitamin D metabolism pathway enzymes (cytochrome P450 (CYP) 2R1, 3A4, 27B1, 24A1; vitamin D binding protein (also known as group-specific component, GC); and vitamin D receptor) were measured using stored biospecimens. Mean 25(OH)D concentration was 68·8 nmol/l. Serum 25(OH)D concentrations were positively associated with dietary vitamin D intake, and inversely associated with hours spent sitting at work. BMI was not associated with 25(OH)D concentrations. CYP2R1 rs10741657, rs12794714, rs1993116; CYP3A4 rs2242480; and GC rs4588, rs7041, rs16847015, rs2298849 were statistically significantly associated with 25(OH)D concentrations. Individuals with the Gc2-2 haplotype (rs4588 AA /rs7041 TT) had statistically significantly lower 25(OH)D concentrations compared to all other Gc haplotypes (P-trend, 0·001). The majority of participants (86 %) had 25(OH)D concentrations ≥ 50 nmol/l, which is consistent with the 2011 Institute of Medicine (US) recommendation for bone health, and 32 % had concentrations of ≥ 75 nmol/l that are thought to be required for broader health effects. Dietary vitamin D intake, hours spent indoors at work and genetic variation in CYP2R1, CYP3A4 and GC are significant predictors of 25(OH)D concentrations among Singapore Chinese.

Key words: 25-Hydroxyvitamin D: CYP2R1: CYP3A4: Group-specific component

Vitamin D (as the 1,25-dihydroxyvitamin D metabolite) is a steroid hormone that is well known for its role in maintaining Ca homeostasis and normal bone structure. Recent evidence suggests that in addition to Ca homeostasis, the vitamin may also play a role in a variety of other physiological processes such as modulation of inflammatory pathways and susceptibility to diabetes, cancer, and infectious and cardiovascular diseases. Thus, the nutrient could play a significant role in public health.

In the USA, the Institute of Medicine recently proposed ≥ 50 nmol/l (20 ng/ml) as the definition of vitamin D adequacy based solely on requirements to optimise bone health, due to

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; CYP, cytochrome P450; GC, group-specific component; NIST, National Institute of Standards and Technology; rs, reference SNP, SCHS, Singapore Chinese Health Study; VDR, vitamin D receptor.

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a lack of data to support recommendations for the prevention of other disease endpoints\(^{(6)}\). However, many leading vitamin D researchers continue to recommend serum 25-hydroxyvitamin D (25(OH)D) concentrations of \(\geq 75\) nmol/l (30 ng/ml) to achieve the broader health benefits\(^{(7-10)}\).

Season, UVB exposure, skin pigmentation, age, race, sex, obesity and dietary/supplemental vitamin D intake have all been previously reported to influence serum 25(OH)D concentrations\(^{(11)}\). However, the effect of genetic variation in the vitamin D synthesis and metabolism pathway on circulating concentrations is less well understood. Vitamin D enters the circulation through the activation of vitamin D precursors by UV radiation on the skin to produce cholecalciferol, or via absorption of dietary or supplemental ergo- or cholecalciferol from the intestinal tract. It is then converted to 25(OH)D via 25-hydroxylases (cytochrome P450 (CYP) 2R1, 27A1 and 3A4) in the liver\(^{(11)}\). Further hydroxylation of 25(OH)D via 1a-hydroxylase (CYP27B1) in the kidney or at the local tissue level produces 1,25-dihydroxycholecalciferol\(^{(12)}\). Catalysis of vitamin D metabolites occurs via 24-hydroxylase (CYP24A1)\(^{(11)}\). Vitamin D binding protein (also known as group-specific component, GC) is the transport protein for vitamin D metabolites in circulation\(^{(12)}\). Genetic variation in any of these steps has the potential to alter serum 25(OH)D concentrations.

Previous studies have identified SNP in the vitamin D receptor (VDR)\(^{(13-20)}\), CYP2R1\(^{(21-26)}\), CYP27B1\(^{(22,23,27)}\) and GC\(^{(19,24-26,28-35)}\) genes to be associated with alterations in serum 25(OH)D concentrations. These studies were primarily conducted among Caucasian populations living at higher latitudes. In this cross-sectional observational study, we evaluated the distribution of serum 25(OH)D concentrations among Southeast Asian populations and identified genetic, dietary and lifestyle predictors of serum 25(OH)D concentrations among middle-aged and older Chinese men and women in Singapore. As Singapore is 1° north of the equator, this study population provides a unique opportunity to evaluate the factors associated with vitamin D status in the absence of seasonal variation in UV exposure, which confounds studies conducted at higher latitudes.

### Methods

The Singapore Chinese Health Study (SCHS) is a population-based prospective cohort study of 63,257 Singapore Chinese men and women (aged 45–74 years) that was assembled between 1993 and 1998 to elucidate the role of diet and genetic factors in the causation of human cancer. Participants in the study were recruited from among permanent residents or citizens of Singapore who resided in government-built housing estates, and were one of the two major dialect groups of Singapore Chinese, Hokkien or Cantonese. At recruitment, each study subject was interviewed in person by a trained interviewer using a structured questionnaire that included questions on lifestyle, health behaviours and sociodemographic factors, as well as a 165-item FFQ. The FFQ was specifically designed to assess the dietary habits of Chinese in Singapore, and was subsequently validated using multiple 24 h dietary recalls\(^{(50)}\). However, because vitamin D is technically challenging to measure in food\(^{(37)}\), data on vitamin D contained in the nutrient databases used to analyse FFQ for epidemiological studies (including the US Department of Agriculture database used for the SCHS vitamin D analysis) are known to be incomplete\(^{(50)}\). Thus, our estimates probably underestimate the participants’ actual dietary vitamin D intake.

The only form of supplemental vitamin D intake to be assessed was cod liver oil; data on frequency of use over the year before the interview were collected and considered for use in this analysis. Participants were not asked specifically about time spent indoors vs. outdoors; however, participants were asked about the average number of hours spent ‘sitting at work’ each day, and hours spent doing ‘vigorous work’ such as moving heavy furniture, loading or unloading trucks, shovelling or equivalent manual labour’. Responses to these questions were included in our analyses as surrogates for time spent indoors (‘sitting at work’) and outdoors (‘vigorous work’).

Beginning in April 1994, a random 3% sample of cohort participants was also asked to provide blood or buccal cells, and spot urine samples as a pilot study to determine the feasibility of a larger biospecimen collection effort within the cohort. Details of the biospecimen collection, processing and storage procedures have been described\(^{(59)}\). The first 504 SCHS participants who provided biospecimens were included in this substudy. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Institutional Review Boards of the National University of Singapore and the University of Minnesota. Written informed consent was obtained from all participants.

### Serum 25-hydroxyvitamin D assay

Quantitative determination of serum 25(OH)D concentrations was performed using a direct, competitive chemiluminescence immunoassay on the DiaSorin LIAISON platform\(^{(40)}\) by Heartland Assays, a laboratory participating in the Vitamin D External Quality Assessment Scheme\(^{(41)}\). This assay measures total 25(OH)D (both the ergocalciferol (D$_2$) and cholecalciferol (D$_3$) derived 25(OH)D metabolites; the assay does not discriminate between the two forms). Two types of blinded controls were randomly interspersed among the study samples for quality control purposes: (1) pooled blood samples (\(n = 10\)), and (2) vitamin D standard (standard reference material 972, Vitamin D in Human Serum) from the US National Institute of Standards and Technology (NIST)\(^{(42)}\) (\(n = 16\)). Mean inter- and intra-batch CV for the 25(OH)D concentrations in the pooled blood samples was 6.1 and 5.4%, respectively, which is consistent with those of previously published reports\(^{(13-47)}\). For the NIST standard level 1 (58.0 nmol/l 25(OH)D$_3$ in unaltered
human serum), the mean inter-batch CV was 6.2%. Similarly, for
the NIST standard level 2 (4.18 nmol/l 25(OH)D3 and 30.0 nmol/l
25(OH)D2 in diluted human serum), the mean inter-batch CV was
5.2%. The CV for the NIST standards were well below those
of previously published reports (48). A number of studies have
demonstrated that 25(OH)D is extremely stable with long-
term storage (as long as 40 years) and under a variety of storage
conditions (49–52). Thus, our samples, which were stored for an
average of 13 (sd 1-2) years, should have experienced little, if
any, 25(OH)D degradation, and any degradation would have
been consistent across all samples.

SNP selection

Using a candidate pathway approach, we included common
genetic variants (minor allele frequency ≥20%) of genes
involved in 25(OH)D synthesis (CYP2R1, CYP3A4, CYP27B1),
transport (GC), gene transcription (VDR) and catabolism
(CYP24A1). Data from the International HapMap Project
(Tagger Pairwise method, HapMap Data Release 27 Phase
II + III, February 2009, on NCBI B36 assembly, dbSNP b126
for the Han Chinese in Beijing, China (CHB) population).
No HapMap tag SNP were identified for the Han Chinese
population. Also, any SNP that had been documented in
the literature as having functional and/or phenotypic relev-
ance were included.

A total of fifty-five SNP and eight proxies met the inclusion
criteria (by rs (reference SNP) number, proxies in parenth-
eses): VDR: 731236 also known as TaqI, 1540339, 1544410
also known as BsmI, 2107301 (12717991), 2189480, 2228570
also known as FokI (and previously reported as rs10735810),
2238136, 2289180, 23071482, 23071480, 2254210,
2238342, 2525044, 2855359, 2855359, 3789205, 3847987,
4760658, 7305032, 7305032, 757343, 7965281, 7975232 also
known as Apal, 10783215, 10875609 (7136534), 11168268
(11168266), 11168275, 11168287, 11574143, 12721364.
CYP2R1: 1993116, 12794714 (10500804), 10741657. CYP3A4:
2242480, 2246709, 3735451. CYP27B1: 466536. CYP24A1:
912505, 21888854, 12512631, 16847015, 222016, 2298849
(3733359), 507117, 507120.

DNA extraction and genotype determinations

DNA extraction and genotype determinations were performed
by the University of Minnesota’s BioMedical Genomics Center.
DNA was extracted fromuffy coats using a Qiagen Kit
(Qiagen, Inc.). Genotype determinations were performed
on the commercially available high-throughput genotyping
Sequenom MassARRAY platform (Sequenom, Inc.). Uniquely
located negative controls were routinely included in each
plate. These wells were used as controls for genotyping
assays, and their unique locations serve as a fingerprint to
identify the plate and its orientation. For quality control pur-
poses, only SNP with >95% call rates were included in the
analyses. All SNP were found to be in Hardy–Weinberg
equilibrium, except GC rs4588. Genotyping for rs4588 was
repeated using a TaqMan assay (Invitrogen). The data from
the TaqMan assay were found to be in Hardy–Weinberg equi-
librium, and were used for all analyses.

Data analysis

The distribution of serum 25(OH)D in the study population
was markedly skewed towards low values. Thus, the statistical
analyses were performed on logarithmically transformed
values, and geometric means are presented. ANOVA methods
were used to compare mean serum concentrations of
25(OH)D by potential lifestyle, sociodemographic, dietary
and genetic predictors of 25(OH)D concentration. Dialect
group, education level, menopausal status (women), BMI,
height, weight, body surface area, physical activity, smoking
status, hours spent sitting at work, season of blood draw,
use of cod liver oil supplements and dietary intake of vitamin
D, Ca, fish, dairy products and alcohol were considered as
potential predictors. Age, sex and time interval between last
meal and blood draw were included as covariates in all ana-
lyses. Dietary vitamin D and hours spent sitting at work
were also included as covariates in ANOVA for the assessment
of genetic predictors. To test for linear trend, the potential pre-
dicator was included as an ordinal variable in general linear
models. Each potential predictor was examined individually
by assessing its effect on the overall model fit (R2, F-test).
The final multivariate model for non-genetic predictors of
serum 25(OH)D concentrations included age, sex, dietary
vitamin D intake, hours spent sitting at work, and time interval
between last meal and blood draw.

Associations between single SNP and serum 25(OH)D con-
centrations were evaluated individually in the multivariate-
adjusted models. Trends in serum 25(OH)D concentrations
across each SNP genotype were tested for statistical signifi-
cance by including the SNP as a three-level variable (homozy-
gous wildtype, heterozygous and homozygous variant).
All P values quoted are two-tailed, and significance was
defined as P<0.05. For the SNP analyses, statistical signifi-
cance was defined as P<0.01 to minimise the likelihood of
reporting false-positive findings due to multiple comparisons.
Calculations were performed using the SAS statistical software
system (SAS Institute).

Results

The study population was 56% women, and the mean age of
study participants was 55.7 years (Table 1). Most women were
postmenopausal at baseline. Compared to men, women were
less educated, less likely to be a smoker, spent less time sitting
at work or in vigorous work, and consumed less dietary vita-
min D. Mean serum 25(OH)D concentration was 68.6 nmol/l
overall, and lower in women (mean: 64.2 nmol/l) than in
men (74.3 nmol/l, P<0.001), and a greater percentage of
women (18%) had 25(OH)D concentrations<50 nmol/l com-
pared to men (9%).

Serum 25(OH)D concentrations were statistically signifi-
cantly associated with dietary vitamin D, Ca and dairy product
intake among women, but not men (Table 2). Serum 25(OH)D levels decreased with increasing number of hours spent sitting at work for both men and women, although the linear relationship was not statistically significant for women. There were no associations with 25(OH)D concentrations for cod liver oil supplement use, fish intake or time between last meal and blood draw, regardless of sex (data not shown). Women engaging in vigorous work for at least half an hour per week showed significantly higher serum 25(OH)D level compared to their counterparts with no vigorous work, whereas there was no difference in serum 25(OH)D level between men with and without vigorous work. Age, BMI, alcohol intake and working status were not associated with serum 25(OH)D concentration in this population. When age, sex, dietary vitamin D intake, hours spent sitting at work, and time interval between last meal and blood draw were considered simultaneously in the final multivariate model, 10·2 % of the variation in serum 25(OH)D concentrations was explained (P,0·01).

Of the fifty-five SNP assessed, eight SNP in CYP2R1, CYP3A4 and GC were associated with 25(OH)D concentrations (Table 3). For five of the SNP, the major allele was associated with lower 25(OH)D concentrations (e.g. CYP2R1 rs10741657 and rs1993116; and GC rs7041, rs2298849 and rs1687015), while for the remaining three SNP, the major allele was associated with higher 25(OH)D concentrations (e.g. CYP2R1 rs12794714; CYP3A4 rs2242480; and GC rs4588). The strongest association was with the GC SNP rs4588, where the decrease in copies of the major allele (e.g. from 2 to 0) was associated with a 11·5 nmol/l decrease in serum 25(OH)D concentration (P,0·001). Including genotype information for individual SNP into the multivariable model explained an additional 0·8–3·7 % of the variation in serum 25(OH)D concentrations in this cohort (Table 3).

In addition to the single-SNP associations with serum 25(OH)D concentrations, we also evaluated the combined effect of two well-described GC SNP, rs4588 and rs7041. These SNP have been previously shown to jointly determine three well-described protein transcripts: Gc1s, Gc1f and Gc2 (12). As shown in Table 4, the mean 25(OH)D concentration was the highest for individuals with two copies of the Gc1s allele (Gc1s-1s: rs4588 CC, rs7041 GG), the lowest for individuals with two copies of the Gc2 allele (Gc2-2: rs4588A4, rs7041 TT), and intermediate for those with any other Gc haplotype (P-trend <0·001). When stratified by median dietary vitamin D intake, the trends with 25(OH)D concentrations remained consistent across sex, diet and life-style parameters.
Table 2. Geometric means of 25-hydroxyvitamin D (25(OH)D) by potential predictors overall and by sex
(Mean values and standard deviations)

<table>
<thead>
<tr>
<th>Potential predictors</th>
<th>Overall (n 504)</th>
<th>Men (n 220)</th>
<th>Women (n 284)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25(OH)D (nmol/l)</td>
<td>25(OH)D (nmol/l)</td>
<td>25(OH)D (nmol/l)</td>
</tr>
<tr>
<td>Median age (years)</td>
<td>n</td>
<td>Mean*</td>
<td>SD</td>
</tr>
<tr>
<td>&lt; 55-0</td>
<td>252</td>
<td>67.1</td>
<td>0.02</td>
</tr>
<tr>
<td>≥ 55-0</td>
<td>252</td>
<td>65.5</td>
<td>0.02</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Work status at blood draw</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not working</td>
<td>276</td>
<td>66.5</td>
<td>0.02</td>
</tr>
<tr>
<td>Working</td>
<td>228</td>
<td>66.1</td>
<td>0.02</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)†</td>
<td>≤ 23-0</td>
<td>244</td>
<td>65.9</td>
</tr>
<tr>
<td>&gt; 23-0</td>
<td>260</td>
<td>66.7</td>
<td>0.02</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>Vitamin D intake</td>
<td>Tertile 1‡</td>
<td>183</td>
<td>64.6</td>
</tr>
<tr>
<td>Tertile 2</td>
<td>149</td>
<td>66.3</td>
<td>0.02</td>
</tr>
<tr>
<td>Tertile 3</td>
<td>172</td>
<td>68.2</td>
<td>0.02</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Ca intake</td>
<td>Tertile 1§</td>
<td>160</td>
<td>64.8</td>
</tr>
<tr>
<td>Tertile 2</td>
<td>154</td>
<td>66.4</td>
<td>0.02</td>
</tr>
<tr>
<td>Tertile 3</td>
<td>190</td>
<td>67.5</td>
<td>0.02</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>Dairy product intake</td>
<td>Tertile 1</td>
<td>177</td>
<td>63.2</td>
</tr>
<tr>
<td>Tertile 2</td>
<td>147</td>
<td>68.4</td>
<td>0.02</td>
</tr>
<tr>
<td>Tertile 3</td>
<td>180</td>
<td>67.7</td>
<td>0.02</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>Alcohol intake, drinks/week</td>
<td>0 &lt; 7</td>
<td>418</td>
<td>66.0</td>
</tr>
<tr>
<td>≥ 7</td>
<td>63</td>
<td>68.5</td>
<td>0.03</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Vigorous work (h/week)</td>
<td>0 ≤ 5</td>
<td>472</td>
<td>66.0</td>
</tr>
<tr>
<td>&gt; 5</td>
<td>32</td>
<td>71.0</td>
<td>0.05</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Hours spent sitting at work/d</td>
<td>None &lt; 1</td>
<td>291</td>
<td>68.1</td>
</tr>
<tr>
<td>1–2</td>
<td>62</td>
<td>66.4</td>
<td>0.03</td>
</tr>
<tr>
<td>3–6</td>
<td>45</td>
<td>68.0</td>
<td>0.04</td>
</tr>
<tr>
<td>≥ 7</td>
<td>68</td>
<td>63.0</td>
<td>0.03</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Smoking status</td>
<td>Never &lt; 1</td>
<td>368</td>
<td>66.0</td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>57</td>
<td>67.2</td>
<td>0.04</td>
</tr>
<tr>
<td>Current</td>
<td>79</td>
<td>67.2</td>
<td>0.03</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>Season of blood draw</td>
<td>February–April &lt; 1</td>
<td>124</td>
<td>64.5</td>
</tr>
<tr>
<td>May–July</td>
<td>142</td>
<td>67.6</td>
<td>0.02</td>
</tr>
<tr>
<td>August–October</td>
<td>131</td>
<td>66.8</td>
<td>0.02</td>
</tr>
<tr>
<td>November–January</td>
<td>107</td>
<td>66.1</td>
<td>0.02</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>0.8</td>
<td></td>
</tr>
</tbody>
</table>

* Mean values were adjusted for age (years), sex (among overall) and time interval from last meal to blood draw.
† Asian-specific BMI cut-points were used [54].
‡ Median values of dietary vitamin D intake for tertiles (µg/4184 kJ) were: 0–8, 1–4 and 2–5, respectively, for all subjects; 0–9, 1–4 and 2–4, respectively, among men; and 0–8, 1–6 and 2–8, respectively, among women.
§ Median values of dietary Ca intake for tertiles (mg/4184 kJ) were: 177, 214 and 362, respectively, for all subjects; 168, 217 and 309, respectively, among men; and 185, 260 and 395, respectively, among women.
† Median values of dairy product intake (g/4184 kJ) were: 1–3, 4–14 and 18–25, respectively, for all subjects; 1–11, 10–19 and 20–77, respectively, among men; and 1–5, 17–3 and 134, respectively, among women.
| Among women, only geometric means for none or any alcohol and beer intake is presented, as only nine women reported drinking any alcohol.
by haplotype are similar to the overall pattern currently presented in Table 4 for both high and low dietary vitamin D intake (data not shown). However, when stratified by hours spent sitting at work, the trend of decreasing 25(OH)D concentration from Gc1s-1s to Gc2-2 haplotype was only evident among those who reported no hours spent sitting at work (n = 268, P-trend < 0.001, Table 5). The trend was less evident and was not statistically significant among those who reported any sitting hours at work (data not shown), although the interaction between Gc haplotype and hours spent sitting at work was not statistically significant (P-interaction = 0.24).

Discussion

On average, participants in this study had serum 25(OH)D concentrations that would be considered sufficient for optimal bone health according to the Institute of Medicine recommendations (6), but somewhat below the recommendations from leading vitamin D researchers of 75 nmol/l to address a broader action between vitamin D and bone health according to the Institute of Medicine recommendations (58). Given the average daily high temperatures of 31°C (88°F) (59), many Singaporeans avoid the heat of the midday sun. In our study, as the reported BMI was not associated with serum 25(OH)D concentrations in this population, mostly probably due to the fact that the mean BMI for the study participants was within the normal range for Asian populations (<23 kg/m²) (54), and the range was narrow. Mean dietary vitamin D intake for these middle- and older-aged Singaporean men (2.8 (SD 1.8) µg/d) and women (2.4 (SD 1.7) µg/d) was lower than comparably aged men (5.1 (SD 0.3) µg/d) and women (3.9 (SD 0.4) µg/d) in the USA according to the 2005–6 National Health and Nutrition Examination Survey data (55), and somewhat lower than adult men (3.1 (SD 0.1) µg/d) and women (2.7 (SD 0.1) µg/d) in the UK according to the 2008–9 National Diet and Nutrition Survey data (56).

Sun exposure is known to be a major determinant of vitamin D status. Singapore receives 12 h sunlight/d throughout the year, with a midday solar zenith angle that ranges from a minima of 0–3° (March, September) to a peak of 22–25° (June, December) (57). Despite a small amount of variation in solar zenith angle, we did not observe significant seasonal variation in serum 25(OH)D concentrations in this cohort. The UV index ranges from 10 (December) to 13 (March/April), indicating very high ambient UV radiation levels (58). Given the average daily high temperatures of 31°C (88°F), many Singaporeans avoid the heat of the midday sun.

Table 3. Geometric means of serum 25-hydroxyvitamin D (25(OH)D) by genotype

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>Geometric mean*</th>
<th>95 % CI</th>
<th>P for trend</th>
<th>Variance explained by the model (%)†</th>
<th>Variance explained by genotype (%)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2R1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>rs10741657 GG 253</td>
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</table>

CYP2R1, cytochrome P450 4R1; rs, refSNP or reference SNP; CYP3A4, cytochrome P450 3A4; GC, group-specific component (vitamin D binding protein).

* Geometric mean* 95% CI

† Variance explained is the model $R^2 \times 100$. The partial $R^2$ for each of the covariates in the individual multivariate models was: age ($<0.001$), sex (0.070–0.076), dietary vitamin D (0.005–0.008), hours spent sitting at work (0.020–0.026), and time interval between last meal and blood draw ($<0.001$).

‡ The variance explained by genotype is the partial $R^2 \times 100$ for the individual genotype in a linear regression model with variables for age, sex, dietary vitamin D, hours spent sitting at work, and time interval between last meal and blood draw.
number of hours spent sitting at work increased, serum 25(OH)D concentrations decreased ($P_{\text{trend}} < 0.001$).

Three other reports have described serum 25(OH)D concentrations among healthy adults living within 10° of the equator. Rahman et al.\textsuperscript{(60)} evaluated 276 post-menopausal women living near Kuala Lumpur, Malaysia (2°N). They found that ethnic Chinese women had significantly higher mean 25(OH)D concentrations compared to Malay women (68.8 (SD 15.7) vs 44.4 (10.6) nmol/l, $P<0.05$). Dietary vitamin D intake did not differ between the two ethnic groups. The Chinese women had a significantly lower mean BMI, and reported more regular physical activity than the Malay women. The Malay also tend to have more skin pigmentation than the Chinese, and many Malay women follow Muslim dress codes that further limit UV exposure. Serum 25(OH)D concentrations were significantly correlated with BMI, fat mass, parathyroid hormone concentrations and physical activity scores. Green et al.\textsuperscript{(61)} evaluated 378 younger women living in Kuala Lumpur (mean age: 25.2 years) and 126 women living in Jakarta, Indonesia (6°S, mean age: 30.0 years). Among the women in Malaysia, they also found higher serum 25(OH)D concentrations among the ethnic Chinese (mean: 58.0 nmol/l, 95 % CI 55.0, 61.0) compared to the Malay (mean: 43.0 nmol/l, 95 % CI 40.0, 46.0) or Indian (mean: 45.0 nmol/l, 95 % CI 43.0, 48.0) women ($P<0.01$). The Indonesian women had serum 25(OH)D concentrations that were comparable to the Malay and Indian women (mean: 46.0 nmol/l, 95 % CI 43.0, 48.0). Moy & Bulgiba\textsuperscript{(62)} recently reported on the vitamin D status of 380 Malay study participants (158 men, 222 women) in a voluntary health screening programme in Kuala Lumpur. The women had significantly lower serum 25(OH)D concentrations (mean: 36.2 nmol/l, 95 % CI 34.5, 38.0, $P_{\text{trend}} < 0.001$), which could be partially explained by differences in religious dress codes. Age, sex, BMI and abdominal obesity were found to be statistically significantly associated with vitamin D insufficiency in this study cohort. The women in our study had serum 25(OH)D concentrations that were similar to the ethnic Chinese participants in both the Rahman et al.\textsuperscript{(60)} and Green et al.\textsuperscript{(61)} studies.

Genetic variants in CYP2R1, CYP3A4 and GC were significantly associated with serum 25(OH)D concentrations in our study. The CYP2R1 rs10741657, rs12794714, and rs1993116 and GC rs4588 and rs7041 findings are consistent with several recent reports\textsuperscript{(19,21,26,28–32,34,35)} including two large genome-wide association studies\textsuperscript{(24,25)}. Several CYP enzymes have been shown to have 25-hydroxylase activity, and CYP2R1 has emerged as the predominant 25-hydroxylase, with the highest binding affinity and specificity for vitamin D\textsuperscript{(63)}. CYP2R1 rs10741657 lies in the promoter region, rs12794714 is a synonymous SNP in exon 1, and rs1993116 is in intron 1. To our knowledge, no previous studies have evaluated the association between genetic variation in CYP3A4 and serum 25(OH)D concentrations in human subjects. CYP3A4 rs2242480 is in intron 10 near the exon/intron boundary. Although the functional relevance of this SNP is unclear, a recent pharmacokinetic study suggests that individuals with

<table>
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<th>rs4588 genotype</th>
<th>Haplotype</th>
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<th>95% CI</th>
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<td>59-72.6</td>
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<td>Gc2f-1f</td>
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</table>

\* Adjusted geometric mean value was significantly different from that of the Gc2-2 haplotype ($P<0.01$).
the homozygous variant rs2242480TT genotype have significantly lower CYP3A4 activity\(^{(64)}\).

The GC SNP, rs4588 and rs7041, are both in exon 11. Consistent with the findings of several previous studies\(^{(19,28,65–68)}\), we also observed that individuals with two copies of the Gc2 allele (Gc2-2) have significantly lower 25(OH)D concentrations compared to other GC genotypes. In vitro data have shown that the Gc2 protein has a significantly lower affinity constant for 25(OH)D\(_3\) compared to the Gc1s or Gc1f proteins\(^{(12)}\). GC rs16847015 and rs2298849 both lie in intron 1, and their functional relevance is unclear.

While several previous reports identified genetic variants in the VDR\(^{(13–20)}\) and CYP27B1\(^{(22,23,27)}\) genes as being associated with serum 25(OH)D concentrations, we did not observe any statistically significant associations for those genes in our study population. The studies reporting significant findings for VDR and CYP27B1 variants tended to be among smaller study populations, conducted at higher latitudes, and many failed to adjust for factors known to alter serum 25(OH)D concentrations such as season of blood draw, BMI and dietary/supplemental vitamin D intake.

The present study has several strengths including being the largest study of vitamin D status among Southeast Asians to date, lack of seasonal UV variation, and consideration of dietary vitamin D exposures, lifestyle and sociodemographic factors as well as genetic variation as potential factors contributing to serum 25(OH)D concentrations. We also took a comprehensive approach to assessing the effect of genetic variation in the entire vitamin D metabolism pathway, as opposed to the evaluation of single candidate genes or SNP.

Limitations of the present study include incomplete assessment of time spent outdoors during daylight hours and degree of skin pigmentation, factors which may contribute to variation in serum 25(OH)D concentrations. Due to technical challenges in accurately measuring the vitamin D content of foods\(^{(37,38)}\), our assessment of the participants’ dietary vitamin D intake is probably underestimated. This underestimation of dietary vitamin D intake should occur to all study subjects non-differentially, which would result in underestimating the association between dietary vitamin D intake and serum 25(OH)D concentrations. Supplemental vitamin D intake, other than cod liver oil, was not specifically assessed. However, only 8% of our study cohort reported taking any vitamins or minerals at least once a week.

Our findings confirm the growing body of literature documenting an association between GC and CYP2R1 genetic variation and serum 25(OH)D concentrations. Further studies of both predictors of 25(OH)D concentrations and disease outcomes thought to be associated with vitamin D status should include an assessment of GC and CYP2R1 genotype. Further research is needed to confirm our findings related to CYP3A4 rs2242480.

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for study supervision; K. R., K. B. B., D. W., W.-P. K. and J.-M. Y. for the acquisition of data; L. M. B., R. W., K. R. and J.-M. Y. for the data analysis and interpretation; and K. R. and L. M. B. for drafting of the manuscript. All authors contributed to critical revision of the manuscript. None of the authors reported a conflict of interest. The authors thank Siew-Hong Low of the National University of Singapore for supervising the fieldwork of the SCHS, and Kazuko Arakawa for the development and maintenance of the cohort study database. Further, the authors acknowledge the founding, long-standing Principal Investigator of the SCHS – Mimi C. Yu. This study was funded by the University of Minnesota Masonic Cancer Center and the National Cancer Institute (R01 CA144034).

References


