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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 $<0{\cdot}001$). 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[1] and susceptibility to diabetes[2], cancer[3], and infectious[4] and cardiovascular[5] 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 ≥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 1α-hydroxylase (CYP27B1) in the kidney or at the local tissue level produces 1,25-dihydroxycholecalciferol[11]. Catabolism 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 with significant seasonal variation in UV exposure, and only a small number of studies have considered environmental vitamin D exposures or personal characteristics as potential effect modifiers.

Data on the distribution and determinants of serum 25(OH)D concentrations in Southeast Asian populations are limited. In this cross-sectional observational study, we evaluated the distribution of serum 25(OH)D concentrations, 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[36]. 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[38]. 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 *v.* 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[39]. 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[43–47]. For the NIST standard level 1 (58·0 nmol/l 25(OH)D_3 in unaltered

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human serum), the mean inter-batch CV was 6·2 %. Similarly, for the NIST standard level 2 (4·18 nmol/l 25(OH)D_2 and 30·0 nmol/l 25(OH)D_3 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) were used to identify haplotype tagging SNP for the Han Chinese. No HapMap tag SNP were identified for *CYP27A1* in the CHB population. Also, any SNP that had been documented in the literature as having functional and/or phenotypic relevance were included.

A total of fifty-five SNP and eight proxies met the inclusion criteria (by rs (reference SNP) number, proxies in parentheses): *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, 2239180, 2239182, 2239184, 2239186, 2254210, 2283342, 2525044, 2853564, 2853559, 3782905, 3847987, 4760658, 7305032, 739837, 757343, 7965281, 7975232 also known as *ApaI*, 10783215, 10875695 (7136534), 11168268 (11168266), 11168275, 11168287, 11574143, 12721364. *CYP2R1*: 1993116, 12794714 (10500804), 10741657. *CYP3A4*: 2242480, 2246709, 3735451. *CYP27B1*: 4646536. *CYP24A1*: 912505, 2181874, 2209314, 2296241 (2585428), 2762941, 3787555, 4809958 (6068816), 6022999. *GC*: 4588 (2298850), 7041, 10488854, 12512631, 16847015, 222016, 2298849 (3733359), 705117, 705120.

DNA extraction and genotype determinations

DNA extraction and genotype determinations were performed by the University of Minnesota's BioMedical Genomics Center. DNA was extracted from buffy coats using a Qiagen Kit (Qiagen, Inc.). Genotype determinations were performed on the commercially available high-throughput genotyping SequenomMassARRAY 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 purposes, 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 equilibrium, 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 analyses. 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 predictor 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 (R^2, 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 concentrations were evaluated individually in the multivariate-adjusted models. Trends in serum 25(OH)D concentrations across each SNP genotype were tested for statistical significance by including the SNP as a three-level variable (homozygous wildtype, heterozygous and homozygous variant).

All P values quoted are two-tailed, and significance was defined as $P<0{\cdot}05$. For the SNP analyses, statistical significance was defined as $P\leq 0{\cdot}01$ 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 vitamin 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, $P<0{\cdot}001$), and a greater percentage of women (18 %) had 25(OH)D concentrations <50 nmol/l compared to men (9 %).

Serum 25(OH)D concentrations were statistically significantly associated with dietary vitamin D, Ca and dairy product

Table 1. Characteristics of subjects within the Singapore Chinese Health Study, overall and by sex (Mean values, standard deviations, ranges, number of subjects and percentages)

	Overall		Men		Women		
	n	%	*n*	%	*n*	%	*P**
Subjects	504		220	43·7	284	56·3	–
Age (years)							0·12
Mean	55·7		56·3		55·2		
SD	7·8		7·6		8·0		
Dialect group							0·03
Cantonese	217	43·1	83	37·7	134	47·2	
Hokkien	287	56·9	137	62·3	150	52·8	
Post-menopause (yes)	–		–		197	69·4	–
Highest level of education							<0·001
No education	138	27·4	27	12·3	111	39·1	
Primary	206	40·9	104	47·3	102	35·9	
≥Secondary	160	31·8	89	40·5	71	25·0	
BMI (kg/m^2)							0·7
Mean	22·8		22·8		22·9		
SD	3·0		3·0		3·0		
Range	13·5–37·1		13·5–32·1		15·8–37·1		
Smoking status							<0·001
Never	368	73·0	98	44·6	270	95·1	
Ever	136	27·0	122	55·5	14	4·9	
Hours spent sitting at work, ≥3 h/d	106	21·0	68	30·9	38	13·4	<0·001
Vigorous work, ≥0·5 h per week	32	6·4	20	9·1	12	4·2	0·03
Cod liver oil supplements (weekly) (yes)	14	2·8	8	3·6	6	2·1	0·3
Dietary vitamin D (μg/d)							0·02
Mean	2·6		2·8		2·4		
SD	1·7		1·8		1·7		
Serum 25(OH)D (nmol/l)							<0·001
Mean	68·6		74·3		64·2		
SD	18·3		19·7		15·8		
Range	27·0–153·5		37·2–153·5		27·0–125·9		–
Categories							<0·001
< 50·0 nmol/l	72	14·3	20	9·1	52	18·3	
50·0–74·9 nmol/l	271	53·8	103	46·8	168	59·2	
≥75·0 nmol/l	161	31·9	97	44·1	64	22·5	

25(OH)D, 25-hydroxyvitamin D.
*χ^2 or *t* test *P*-values for differences by sex for categorical and continuous variables, respectively.

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: rs4588*AA*, 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

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Table 2. Geometric means of 25-hydroxyvitamin D (25(OH)D) by potential predictors overall and by sex
(Mean values and standard deviations)

	Overall (*n* 504)			Men (*n* 220)			Women (*n* 284)		
		25(OH)D (nmol/l)			25(OH)D (nmol/l)			25(OH)D (nmol/l)	
Potential predictors	*n*	Mean*	SD	*n*	Mean*	SD	*n*	Mean*	SD
Median age (years)									
< 55·0	252	67·1	0·02	98	71·7	0·04	154	64·4	0·03
≥ 55·0	252	65·5	0·02	122	72·0	0·03	130	58·7	0·03
P		0·5			0·9			0·1	
Work status at blood draw									
Not working	276	66·5	0·02	128	72·4	0·02	148	62·3	0·02
Working	228	66·1	0·02	92	71·2	0·03	136	62·3	0·02
P		0·8			0·6			0·9	
BMI (kg/m^2)†									
≤ 23·0	244	65·9	0·02	109	72·6	0·02	135	61·0	0·02
> 23·0	260	66·7	0·02	111	71·2	0·02	149	63·5	0·02
P		0·6			0·6			0·2	
Vitamin D intake									
Tertile 1‡	183	64·6	0·02	85	72·1	0·03	98	58·9	0·02
Tertile 2	149	66·3	0·02	70	73·9	0·03	79	62·0	0·03
Tertile 3	172	68·2	0·02	65	69·9	0·03	107	66·6	0·03
P		0·04			0·5			< 0·001	
Ca intake									
Tertile 1§	160	64·8	0·02	84	71·4	0·03	76	59·8	0·03
Tertile 2	154	66·4	0·02	70	73·0	0·03	84	61·0	0·03
Tertile 3	190	67·5	0·02	66	71·4	0·03	124	65·9	0·03
P		0·18			0·9			0·03	
Dairy product intake									
Tertile 1¶	177	63·2	0·02	74	68·2	0·03	103	59·3	0·02
Tertile 2	147	68·4	0·02	74	77·5	0·03	73	61·7	0·03
Tertile 3	180	67·7	0·02	72	71·0	0·03	108	66·1	0·03
P		0·005			0·4			0·002	
Alcohol intake, drinks/week									
0	418	66·0	0·01	153	70·8	0·02	265	62·2	0·02
< 7	63	66·5	0·03	48	74·2	0·4	15	66·0‖	0·08
≥ 7	23	70·9	0·3	19	75·3	0·06	4		
P		0·3			0·2			0·5	
Vigorous work (h/week)									
0	472	66·0	0·01	200	71·7	0·02	272	61·9	0·02
≥ 0·5	32	71·0	0·05	20	73·7	0·06	12	72·4	0·07
P		0·1			0·7			0·03	
Hours spent sitting at work/d									
None	291	68·1	0·02	115	75·3	0·02	176	62·9	0·02
< 1	62	66·4	0·03	18	76·0	0·06	44	61·0	0·04
1–2	45	68·0	0·04	19	69·5	0·06	26	67·1	0·05
3–6	68	63·0	0·03	42	67·7	0·04	26	60·6	0·05
≥ 7	38	57·2	0·04	26	63·6	0·05	12	52·9	0·07
P		< 0·001			0·001			0·2	
Smoking status									
Never	368	66·0	0·01	98	70·7	0·03	270	62·4	0·02
Former	57	67·2	0·04	55	71·7	0·04	2	58·2	0·2
Current	79	67·2	0·03	67	73·7	0·03	12	59·8	0·07
P		0·6			0·3			0·5	
Season of blood draw									
February–April	124	64·5	0·02	51	68·3	0·04	73	61·6	0·03
May–July	142	67·6	0·02	63	76·7	0·03	79	61·2	0·03
August–October	131	66·8	0·02	58	70·9	0·03	73	63·8	0·03
November–January	107	66·1	0·02	48	70·9	0·04	59	62·7	0·03
P		0·6			0·9			0·5	

* 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/4·184 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/4·184 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/4·184 kJ) were: 1·3, 14·4 and 118, respectively, for all subjects; 1·1, 10·8 and 77·0, 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.

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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 range of health concerns[7]. However, the serum 25(OH)D concentrations for participants in this study were higher, on average, than has been reported for comparably aged men (58·0 nmol/l) and women (54·8 nmol/l) in the USA[53], which might be expected of individuals living near the equator.

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/m^2)[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)[59], many Singaporeans avoid the heat of the midday sun. In our study, as the reported

Table 3. Geometric means of serum 25-hydroxyvitamin D (25(OH)D) by genotype
(Geometric mean values and 95 % confidence intervals)

			25(OH)D (nmol/l)				
	Genotype	*n*	Geometric mean*	95 % CI	*P* for trend	Variance explained by the model (%)†	Variance explained by genotype (%)‡
CYP2R1							
rs10741657	GG	253	64·9	62·9, 67·0	0·02	11·1	1·0
	GA	192	67·6	65·3, 70·0			
	AA	50	70·0	65·3, 75·3			
rs12794714	GG	197	69·2	66·8, 71·7	<0·001	13·3	3·1
	GA	242	66·0	64·0, 68·2			
	AA	58	58·6	54·9, 62·5			
rs1993116	CC	248	64·6	62·6, 66·7	0·04	11·4	0·8
	CT	201	67·8	65·5, 70·2			
	TT	42	68·5	63·5, 73·9			
CYP3A4							
rs2242480	CC	258	68·5	66·4, 70·6	0·008	11·5	1·3
	CT	199	63·9	61·7, 66·2			
	TT	40	64·5	59·6, 69·7			
GC							
rs4588	CC	267	68·7	66·7, 70·8	<0·001	13·3	3·7
	CA	173	64·3	61·9, 66·8			
	AA	39	57·2	52·8, 61·9			
rs7041	TT	226	64·0	61·9, 66·1	0·003	12·0	1·6
	TG	212	67·7	65·4, 70·0			
	GG	53	70·6	65·9, 75·6			
rs2298849	TT	176	63·9	61·6, 66·3	0·001	12·7	2·1
	TC	237	66·1	64·0, 68·3			
	CC	77	72·2	68·2, 76·4			
rs16847015	CC	275	64·1	62·2, 66·0	0·002	12·8	1·9
	CA	175	68·5	66·0, 71·1			
	AA	39	70·8	65·5, 76·6			

CYP2R1, cytochrome P450 2R1; rs, refSNP or reference SNP; *CYP3A4*, cytochrome P450 3A4, *GC*, group-specific component (vitamin D binding protein).
*25(OH)D concentrations are multivariate-adjusted for: age, sex, dietary vitamin D, hours spent sitting at work, and time interval between last meal and blood draw.
† 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.

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Table 4. Serum 25-hydroxyvitamin D (nmol/l) by group-specific component (*GC*) haplotype (*n* 467)†
(Adjusted geometric means, 95 % confidence intervals and number of subjects)

	rs4588 genotype												
	CC				CA				AA				
rs7041 genotype	Haplotype	Adjusted geometric mean	95 % CI	*n*	Haplotype	Adjusted geometric mean	95 % CI	*n*	Haplotype	Adjusted geometric mean	95 % CI	*n*	*P* for trend
GG	*Gc1s-1s*	70·9*	66·1, 76·2	49	*Gc1s-x*	–	–	–	*Gcx-x*	–	–	–	
TG	*Gc1s-1f*	67·6*	64·7, 70·6	132	*Gc2-1s*	67·3*	63·3, 71·5	69	*Gc2-x*	–	–	–	
TT	*Gc1f-1f*	68·9*	65·1, 72·8	80	*Gc2-1f*	62·3*	59·3, 65·5	100	*Gc2-2*	56·6	52·1, 61·4	37	0·0001

* Adjusted geometric mean value was significantly different from that of the Gc2-2 haplotype ($P<0·01$).
† From ANCOVA with adjustments for age, sex, dietary vitamin D intake, hours spent sitting at work and time interval between the last meal and blood draw.

number of hours spent sitting at work increased, serum 25(OH)D concentrations decreased (*P*-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.*[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) *v.* 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.*[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[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) compared to the men (mean: 56·2 nmol/l, 95 % CI 53·2, 59·2, $P<0·001$), 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.*[60] and Green *et al.*[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[19,21,26,28–32,34,35], including two large genome-wide association studies[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[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

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Table 5. Serum 25-hydroxyvitamin D (nmol/l) by group-specific component (*GC*) haplotype for subjects who reported no hours sitting at work† (*n* 268)
(Adjusted geometric means, 95 % confidence intervals and number of subjects)

	rs4588 genotype												
	CC				CA				AA				
rs7041 genotype	Haplotype	Adjusted geometric mean	95 % CI	*n*	Haplotype	Adjusted geometric mean	95 % CI	*n*	Haplotype	Adjusted geometric mean	95 % CI	*n*	*P* for trend
GG	*Gc1s-1s*	72·6	65·8, 80·1	27	*Gc1s-x*	–	–	–	*Gcx-x*	–	–	–	
TG	*Gc1s-1f*	69·2	65·2, 73·4	74	*Gc2-1s*	69·8	64·6, 75·4	43	*Gc2-x*	–	–	–	
TT	*Gc1f-1f*	66·9	62·0, 72·1	46	*Gc2-1f*	63·1	59·1, 67·4	61	*Gc2-2*	55·5	49·0, 62·8	17	0·0005

† From ANCOVA with adjustments for age, sex, dietary vitamin D intake, hours spent sitting at work and time interval between the last meal and blood draw.

the homozygous variant rs2242480*TT* 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. Future 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).

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