Letter to the Editor

Polymorphisms in *CYP2R1* Gene Associated with Serum Vitamin D Levels and Status in a Chinese Rural Population*

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Vitamin D, a fat-soluble vitamin and endocrine hormone, and it impacts various bone and extra-bone health, such as osteoporosis, diabetes, and cancer. The main circulating form of vitamin D is 25-hydroxyvitamin D [25(OH)D] and it is a useful clinical biomarker of vitamin D status. The Institute of Medicine (IOM) defines as vitamin D deficiency (VDD) when serum 25(OH)D concentration is less than 20 ng/mL[1]. Worldwide, VDD is recognized as a severe public health problem. In 2007, Holick estimated that globally over one billion people suffered from VDD or vitamin D insufficiency (VDI). In China, it has been reported that the prevalence of VDD ranged from 38.8% to 91.2% in different regions[2,3].

The *CYP2R1* gene encodes 25-hydroxylase, which is the foremost enzyme in hepatic microsome to convert vitamin D to 25(OH)D. Tom reported that *CYP2R1* gene mutation impaired 25-hydroxylase activity and caused an atypical VDD. Genome-wide-association studies (GWAS) identified that the SNPs of *CYP2R1* were associated with vitamin D levels[4]. Also some previous studies have shown a significant association of *CYP2R1* variants with 25(OH)D levels[3,5]. However, to date, there is paucity in the literature addressing the involvement of vitamin D levels and status in the Chinese rural population. Therefore, we conducted this cross-sectional study to investigate the current status of VDD in rural area and the associations of genetic factors with vitamin D levels and status in a Chinese rural population.

From June to July in 2013, a total of 2,378 Han ethnic subjects were recruited from Zhengzhou, Luoyang and Jiaozuo cities in Henan province. All subjects completed a standardized questionnaire, detailed information has been described in our previous study[6]. After over-night fasting, samples of blood were drawn from the subjects for the measurements of glucose, lipid profile, and 25(OH)D concentrations.

Inclusion criterion were subjects aged between 14 and 80 years old and in good health. Subjects who suffered from diseases affecting vitamin D metabolism were excluded from the study. Other exclusion criterion were lacking of biological sample information and subjects who supplemented with vitamin D within the past 3 months. Based on these exclusion criterion, a total of 1,559 participants were included in the present analysis. The Ethics Committee of Zhengzhou University approved the study protocol according to the Declaration of Helsinki.

Four single nucleotide polymorphisms (SNPs) of *CYP2R1* (rs1279414, rs1993116, rs10766197, rs10741657) were selected for genotyping in this study. The basic characteristics of these SNPs are listed in Supplementary Table S1 (available in www.besjournal.com) in the supplementary material. Deviation from Hardy-Weinberg equilibrium (HWE) was tested for vitamin D sufficient subjects using Chi-square test. No significant deviation from HWE was observed. (Supplementary Table S2, available in www.besjournal.com).

Serum 25(OH)D concentrations were based on natural log transformed to approximate normality. When the serum 25(OH)D levels were seen as a continuous variable, all the analysis used the geometric mean to calculate the data and used the

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median to describe the distribution. We compared the baseline characteristics of the males and females using the *t* test or Chi-square test for continuous and categorical data, respectively. Multivariable adjusted linear regression was applied to investigate the association of individual SNP genotype with serum 25(OH)D levels. The odds ratio (*OR*) and 95% confidence intervals (*CI*s) were calculated to examine the association of individual SNP genotype with VDD by binary logistic regression adjusted for covariates. Covariates included gender (male and female), age, body mass index (BMI = weight in kilograms divided by height in meters squared, kg/m^2), alcohol status (drinking was defined as having consumed alcohol ≥ 12 times in the last year, or not drinking), smoking status (smoked defined as currently smoked, or not smoked) and physical activity (mild, moderate or severe physical activity). The genetic risk scores (GRS) were calculated by counting the number of CYP2R1-rs1279414 and rs10766197 risk alleles. Logistic regression was used to calculate the *OR* of vitamin D deficiency according to the GRS. All analyses were performed using SPSS software (22.0, SPSS, Chicago, IL, USA). Statistical significance was defined at *P* value < 0.05.

The complete data set included 1,559 individuals (728 males and 831 females), the demographic and clinical characteristics were described in Table 1. The distribution of serum 25(OH)D, insulin and triacylglycerol (TG) concentrations were skewed, so natural log-transformations were performed before analysis. Male and female had similar characteristics, except ($P < 0.05$) that female had a higher glucose and high-density lipoprotein cholesterol (HDL-C).

Of the 1,559 subjects, approximately half of them (49.6%) were vitamin D deficient with 25(OH)D concentration < 20 ng/mL. VDD is a global public health problem. It has been estimated that approximately 45%-98% of general population are VDD in Asia. In China, it was reported that 64.6% of healthy adults in Gansu province had a blood level of 25(OH)D less than 20 ng/mL[7]. Recently, Lee and colleagues showed that the prevalence of VDD in healthy individuals in Taiwan of China was 22.4%[8]. These differences may be partially explained by geographical latitude, sun exposure and dietary habit. The additional explaination for the high prevalence of VDD in Henan rural area may include: 1) with the development of agricultural technology, local residents have reduced labor hours under sun exposure; 2) lifestyle of personal habits, such as whose diet is rich in pickles[6].

The associations of individual SNP genotype with serum 25(OH)D levels and vitamin D status (VDD or not) were presented in Table 2. All four SNPs near the *CYP2R1* gene (rs1279414, rs1993116, rs1076197, and rs10741657) were significantly associated with serum levels of 25(OH)D. These results are similar with other studies[3,9]. Interestingly, stratified analysis by gender and age (cutting point of 50 years old) showed that this association could be detected only in females and in the less than 50 years old subgroups. To demonstrate this specific age and gender difference, we further compared the serum vitamin D level at two age groups (cutting point as 50 years old). The serum vitamin D levels were significantly lower in the subjects aged > 50 years old than those aged ≤ 50 years old ($t = 6.62$, $P < 0.001$). This suggested that differences in vitamin D levels among different genotypes might be caused by age rather than genotype. However, in the stratified analysis by gender, the differences of vitamin D level

Table 1. Demographic and Clinical Characteristics of the Study Participants

Characteristics	Total (*n* = 1,559)	Males (*n* = 728)	Females (*n* = 831)	t/χ^2	*P*
Age, year	50.85 ± 14.92	51.52 ± 15.46	50.26 ± 14.42	1.659	0.099
BMI, kg/m^2	24.99 ± 3.76	25.10 ± 3.66	24.89 ± 3.85	1.105	0.269
$25(OH)D_3$, ng/mL	20.1 (15.5-30.1)	20.6 (15.7-31.9)	19.3 (15.5-28.1)	0.944	0.345
GLU, mmol/L	4.73 ± 0.79	4.67 ± 0.80	4.77 ± 0.79	2.490	**0.013**
INS, mIUL	10.95 (8.19-14.09)	10.80 (7.85-14.27)	11.05 (8.39-13.84)	1.207	0.224
TC, mmol/L	4.46 ± 0.99	4.50 ± 1.03	4.43 ± 0.97	1.546	0.122
TG, mmol/L	1.26 (0.82-1.95)	1.35 (0.86-2.11)	1.17 (0.78-1.84)	4.351	**< 0.001**
HDL-C, mmol/L	1.25 ± 0.31	1.22 ± 0.31	1.27 ± 0.31	3.316	**0.001**
LDL-C, mmol/L	2.51 ± 0.78	2.50 ± 0.77	2.52 ± 0.79	0.349	0.728
Vitamin D deficiency	773 (49.6%)	343 (47.1%)	430 (51.7%)	3.327	0.068

Note. The data are presented as the means ± SD, median (interquartile range), or *n* (%). BMI: body mass index; GLU: glucose; INS: insulin; TC: total cholesterol; TG: triacylglycerol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol.

in gender were not detected (t = 0.944, P = 0.345). Almesri also reported that VDR (rs12721377) gene polymorphism was associated with vitamin D levels in females, but not in males[10]. Effects of gender dimorphism on T2DM and obesity have been previously reported[11,12]. This specific-gender association may be related to the interaction between vitamin D and circulating sex hormone concentrations.

The multivariable adjusted logistic regression for VDD confirmed the association of *CYP2R1* SNPs (rs1279414 and rs10766197) with VDD (*OR* = 1.39, 95% *CI*: 1.014-1.315, *P* = 0.037; *OR* = 1.164, 95% *CI*: 1.006-1.348, *P* = 0.041) (Table 2). This conclusion was supported by previous studies[13]. The GRS is used for evaluating the effects of genetic susceptible factors in risk prediction models. The contribution of multiple SNPs may be aggregated by GRS in order to evaluate the additive genetic effects on the risk of disease and improve the prediction of disease incidence from hereditary factors[14]. In our study, the GRS ranged from zero to four (Table 3). As the GRS increased, the individual's risk and proportion of VDD were increased. The results of the present study indicate that the GRS of *CYP2R1* variants are significantly associated with VDD. Carriers with two or more risk alleles could increase the association of VDD by 1.6 to 1.77-fold compared to non-risk alleles and which needs to be confirmed in other independent studies.

Table 2. Associations of Individual *CYP2R1* Polymorphism with Serum Levels of 25(OH)D and Vitamin D Deficiency

SNPs	Risk/No-risk Allele	Serum 25(OH)D Concentration Mean (*n*)			P^a	Vitamin D Deficiency	P^b
		AA	Aa	aa		*OR* (95% *CI*)	
rs1279414	G/A						
Whole		19.8 (591)	20.7 (749)	18.0 (219)	**0.003**	1.139 (1.014-1.315)	**0.037**
Male		20.7 (256)	20.7 (374)	18.7 (98)	0.419	1.118 (0.903-1.385)	0.305
Female		19.1 (335)	20.7 (375)	17.7 (121)	**0.003**	1.169 (0.952-1.435)	0.135
Age ≤ 50		20.2 (296)	21.7 (386)	18.4 (112)	**0.006**	1.042 (0.848-1.281)	0.695
Age > 50		19.3 (295)	19.7 (363)	17.9 (107)	0.366	1.224 (0.992-1.511)	0.059
rs1993116	G/A						
Whole		19.3 (612)	20.7 (724)	19.6 (223)	**0.003**	0.921 (0.796-1.067)	0.273
Male		19.8 (287)	21.3 (345)	20.6 (96)	0.094	0.886 (0.714-1.100)	0.272
Female		19.0 (325)	20.2 (379)	18.4 (127)	**0.010**	0.919 (0.750-1.126)	0.415
Age ≤ 50		20.3 (315)	22.6 (328)	19.3 (111)	**0.004**	1.012 (0.822-1.245)	0.912
Age > 50		18.6 (297)	19.6 (356)	20.3 (112)	0.402	0.841 (0.682-1.036)	0.104
rs10741657	G/A						
Whole		19.3 (613)	20.9 (724)	19.3 (222)	**0.002**	0.959 (0.699-1.280)	0.350
Male		19.7 (286)	21.4 (346)	20.4 (96)	0.084	0.924 (0.743-1.149)	0.477
Female		19.0 (327)	20.3 (378)	18.4 (126)	**0.010**	0.908 (0.739-1.115)	0.356
Age ≤ 50		20.3 (315)	22.6 (368)	19.3 (111)	**0.004**	1.037 (0.840-1.281)	0.732
Age > 50		18.6 (298)	19.9 (356)	20.2 (111)	0.320	0.830 (0.673-1.023)	0.080
rs10766197	A/G						
Whole		20.2 (595)	20.7 (743)	18.0 (221)	**0.018**	1.164 (1.006-1.348)	**0.041**
Male		20.9 (260)	20.7 (365)	18.8 (103)	0.525	1.188 (0.960-1.471)	0.113
Female		19.2 (335)	20.6 (378)	17.7 (118)	**0.007**	1.513 (1.081-2.117)	**0.016**
Age ≤ 50		20.3 (302)	21.7 (383)	19.0 (109)	**0.049**	1.041 (0.847-1.280)	0.702
Age > 50		20.1 (293)	19.6 (360)	17.8 (112)	0.238	1.149 (0.937-1.410)	0.183

Note. [a]The analyses were performed under multi-linear regression adjusted for age, gender, BMI, alcohol status, smoking status and physical activity. [b]The analyses were performed under logistic regression adjusted for age, gender, BMI, alcohol status, smoking status and physical activity in the allele genetic models. AA was selected to be the most common homozygous genotype; Aa refers to the heterozygous genotype; aa refers to the homozygous genotype; *n*: number of genotype in individual SNP; *OR*: odds ratio; *CI*: confidence interval.

Table 3. Association and Percentage of Genetic Variants and Vitamin D Deficiency

Variants	*OR* (95% *CI*)	*N*	VDD (%)	*P*
Individual variants				
CYP2R1-rs1279414(G)	1.139 (1.014-1.315)			**0.037**
CYP2R1-rs10766197(A)	1.164 (1.006-1.348)			**0.041**
Number of risk alleles from rs1279414 and rs10766197				
0	1.0 (reference)	188	46.1%	
1	1.02 (0.56-1.85)	60	49.1%	0.961
2	1.60 (1.15-2.23)	668	60%	**0.006**
3 + 4	1.77 (1.27-2.47)	643	61%	**0.001**

***Note*.** The analysis were performed under logistic regression adjusted for age, gender, BMI, alcohol status, smoking status and physical activity. *OR*: odds ration; *CI*: confidence interval, VDD: vitamin D deficiency.

We report here that VDD is highly prevalent in rural population of Henan province in China. Our results support the current evidence that the *CYP2R1* variants are significantly associated with serum 25(OH)D levels and VDD. In conclusion, due to single geographical limitations, further well-designed and large scale studies are needed to reveal the association of *CYP2R1* gene variants with vitamin D levels in people situated in diverse rural areas in China.

The authors declare no conflicts of interest.

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Supplementary Table S1. The Basic Characteristics of CYP2R1 Gene

Chromosome	Location	Gene	Full Name	Selected SNPs
11	11p15.2	*CYP2R1*	Cytochrome P450, family 2, subfamily R, polypeptide 1	4 rs1279414 rs1993116 rs10766197 rs10741657

Note. SNP: signal nucleotide polymorphism; *CYP2R1*: cytochrome P450, family 2, subfamily R, polypeptide 1 (vitamin D 25-hydroxylase).

Supplementary Table S2. The HWE Test in the Vitamin D Sufficient Participants

SNP	Genotype	Observed Frequency	Expected Frequency	χ^2	*P*
rs1279414	GG	294 (37.4%)	313 (39.8%)	4.629	0.10
	AG	405 (51.5%)	366 (46.6%)		
	AA	87 (11.1%)	107 (13.6%)		
rs1993116	GG	292 (37.2%)	299 (38.0%)	0.467	0.792
	AG	385 (49.0%)	372 (47.3%)		
	AA	109 (13.8%)	115 (14.7%)		
rs10741657	GG	291 (37.0%)	299 (38.0%)	0.707	0.702
	AG	388 (49.4%)	372 (47.3%)		
	AA	107 (13.6%)	115 (14.7%)		
rs10766197	GG	302 (38.4%)	318 (40.6%)	3.094	0.213
	AG	396 (50.4%)	364 (46.2%)		
	AA	88 (11.2%)	104 (13.2%)		

Note. HWE: Hardy-Weinberg equilibrium.