Established in 1871
Swiss Medical Weekly
Formerly: Schweizerische Medizinische Wochenschrift
The European Journal of Medical Sciences

Original article | Published 16 July 2012, doi:10.4414/smw.2012.13636
Cite this as: Swiss Med Wkly. 2012;142:w13636

The GC, CYP2R1 and DHCR7 genes are associated with vitamin D levels in northeastern Han Chinese children

Yuling Zhang[a], *Xi Wang*[b], *Ye Liu*[c], *Hui Qu*[a], *Shuqiang Qu*[a], *Wei Wang*[a], *Lihong Ren*[a]

[a] Department of Pediatrics, Second Affiliated Hospital of Harbin Medical University, Harbin, Heilongjiang Province, China
[b] Department of Gastroenterology, the First Affiliated Hospital of Harbin Medical University, Harbin, Heilongjiang Province, China
[c] Department of Immunology, Harbin Medical University, Harbin, China

Summary

Vitamin D deficiency is associated with risk in several diseases. Vitamin D status has high heritability, yet the genetic epidemiology of vitamin D or its metabolites has not been well studied. Our objective was to identify the relationship among three vitamin D-related genes (*GC*, *CYP2R1* and *DHCR7*/*NADSYN1*) and the levels of 25(OH)D in northeastern Han Chinese children. A total of 506 northeastern Han Chinese children were enrolled in this study. Linear regression was used to examine the impact of 12 SNPs on 25(OH)D concentrations after adjustment for age, gender, BMI and regular usage of vitamin D, and Bonferroni's method was adopted for multiple corrections. The two SNPs in *GC* (rs222020, rs2298849), four SNPs in *CYP2R1* (rs10741657, rs10766197, rs12794714 and rs1562902) and two SNPs in *DHCR7*/*NADSYN1* (rs3829251, rs12785878) were significantly associated with plasma 25(OH)D concentrations under both additive and recessive models ($P < 0.05$). The genotypes of the *CYP2R1* rs2060793 polymorphism showed positive association with serum 25(OH)D status under all of the three genetic models even after correction for multiple comparison. This population-based study was the first to confirm the strong effects of the *GC*, *CYP2R1* and *DHCR7*/*NADSYN1* loci on circulating 25(OH)D concentrations in northeastern Han Chinese children.

Abbreviations

25(OH)D = 25-hydroxyvitamin D
UVB = ultraviolet B
GC = group specific component
CYP2R1 = Cytochrome P450, family 2, subfamily R, polypeptide 1
DHCR7/NADSYN1 = 7-dehydro-cholesterol reductase/ nicotinamide-adenine dinucleotide synthetase 1
SNPs = single nucleotide polymorphisms
GWAS = genome-wide association study
ELISA = enzyme linked immunosorbent assay
MAF = minor allele frequencies
HWE = Hardy-Weinberg equilibrium
VDR = vitamin D receptor

Key words: *CYP2R1; DHCR7/NADSYN1; GC; polymorphisms; Vitamin D*

Introduction

Vitamin D deficiency is a common public health problem throughout the world. It is associated with many medical outcomes, including rickets, osteoporosis [1], multiple autoimmune disease, renal diseases [2], type 1 diabetes [3], cardiovascular diseases [4], asthma [5] and more than a dozen types of cancer [6, 7]. The most stable and plentiful metabolite of vitamin D in human serum is 25-hydroxyvitamin D [25(OH)D], which has a half-life of approximately 3 weeks, making it the most suitable indicator of vitamin D status. Currently, serum 25(OH)D concentration is considered the best indicator of vitamin D status and determination of its deficiency [25(OH)D <20 ng/mL] or sufficiency [25(OH)D >30 ng/mL] [8].

Factors known to influence 25(OH)D levels include ultraviolet B (UVB) exposure (such as seasons and latitudes), diet, supplemental vitamin D intake and physical activity. Only approximately a quarter of the interindividual variability in 25(OH)D concentration, however, is attributable to all the factors known to influence 25(OH)D status [9]. Recent studies discovered that genetic factors may play an important role in the determination of serum 25(OH)D status. A few candidate genes have been identified, including group specific component (*GC*), Cytochrome P450, family 2, subfamily R, polypeptide 1 (*CYP2R1*) and 7-dehydrocholesterol reductase/nicotinamide-adenine dinucleotide synthetase 1 (*DHCR7*/*NADSYN1*). Kurylowicz et al. [10] examined three polymorphisms in the vitamin D binding protein gene (DBP), also known as *GC* gene, which encodes the DBP that binds to vitamin D metabolites and transports them to target tissues. They found an association between rs4588 and vitamin D deficiency in 110 Polish patients with Graves' disease. With respect to the *CYP2R1* gene, Ramos-Lopez et al. [11] examined five single nucleotide polymorphisms (SNPs) in the *CYP2R1* gene and discovered rs10741657 was significantly associ-

ated with 25(OH)D concentrations in type 1 diabetes patients. *DHCR7*/*NADSYN1* is the least studied gene. In a recent genome-wide association study (GWAS), Wang et al. [12] found multiple SNPs, including rs12785878 in the *DHCR7* gene and rs2282679 in the *CYP2R1* gene, that are significantly associated with 25(OH)D levels in 30000 subjects of European descent from 15 cohorts.

Although prior studies have demonstrated these genetic variants affecting vitamin D concentrations, the role of these loci in Chinese populations remains less clear, particularly in children and adolescents. Therefore, we conducted a study to investigate the association between serum 25(OH)D levels and the three candidate genes mentioned above in northeastern Han Chinese children.

Materials and methods

Participants

A total of 506 Han Chinese children (0–14 yrs) from Harbin, northeastern China, were enrolled in the current study. The study individuals were all stable residents of Harbin and were not genetically related in three generations. A standard informed consent procedure was included in the protocol and reviewed and approved by the Ethics Committee of Harbin Medical University. The parents of participants gave their consent after the nature of the study had been fully explained.

The DNA and serum samples of the 506 individuals were used in the present investigation. The study individuals were recruited from the outpatients department of the affiliated second hospital, Harbin Medical University. Subjects who had diseases that may affect vitamin D metabolism were excluded. These diseases included the following: (1) history of prematurity, (2) renal, liver, intestinal, cardiac or central nervous system disease, (3) chronic disease, (4) bone disease (with the exclusion of rickets), (5) tuberculosis, (6) family history of a hereditary forms of rickets, (7) all known cancers and (8) treatment with vitamin D or vitamin D supplements above 400 IU/d.

Measurement of vitamin D levels

Serum samples for 25(OH)D measurements were collected at the initial visit. At the time of collection, blood constituents were quickly stored in aliquots at –80 °C until analysed. The status of 25(OH)D was measured by enzyme linked immunosorbent assay (ELISA), which was conducted according to the manufacturer's instruction (R&D Systems, MN, USA). Other significant variables which could possibly influence serum 25(OH)D levels were also collected, including age, sex, height, weight, serum 25(OH)D measurement data and habitual vitamin D supplementation (400 IU/d). The clinical characteristics of the participants are presented in table 1.

Genotyping

In the present study, three candidate genes were selected according to the following criteria: (1) evidence of significant association in previous studies [12–14] and (2) biological significance in synthesising and transporting vitamin D. SNPs for analysis were selected from HapMap based on minor allele frequencies (MAF >10%). The genes selected were *GC*, *CYP2R1* and *DHCR7*/*NADSYN1*. The basic characteristics of the three genes are given in table 2.

Genomic DNA was extracted from peripheral blood leukocytes using the QIAamp DNA Blood Kit (Valencia, CA, USA). The SNaPshot assay was used to genotype 12 SNPs. Primers to amplify different sized fragments for each SNP within a multiplex reaction were designed using Primer3 (http://frodo.wi.mit.edu/), and extension primers that also differed in length within a multiplex reaction were picked from the sequence immediately up- or down-stream of the SNP. Primer interactions within the multiplex reaction were evaluated and minimised using the AutoDimer programme (http://www.cstl.nist.gov/div831/strbase/AutoDimer-Homepage/AutoDimerProgramHomepage.htm). The PCRs contained 10–50 ng DNA, 1X HotStarTaq buffer, 3 mM $MGCl_2$, 300 µM of each dNTP, 0.08 µM of each primer and 1U of HotStarTaq polymerase (Qiagen) in a 20 µl reaction volume. A touchdown PCR programme was used: denaturation at 95 °C for 15 min, then 11 cycles of 94 °C for 20 sec, annealing at 65 °C for 40 sec and extension at 72 °C for 90 sec, with a decrease in the annealing temperature by 0.5 °C per cycle. Those cycles were followed by 24 cycles of denaturation at 94 °C for 20 sec, annealing at 59 °C for 30 sec and extension at 72 °C for 90 sec and a final extension at 72 °C for 5 min. The PCR products were purified by treatment with exonuclease I (USB Corporation, Ohio, USA) and shrimp alkaline phosphatase (USB Corporation, Ohio, USA) at 37 °C for 1 hr followed by 75 °C for 15 min. The extension reaction contained 1X ABI Prism SNaPshot

Table 1: Clinical characteristics of the participants and the determinants of 25(OH)D concentrations.

Characteristic	Means ± SD
Number	506
Boys/girl (n/n)	303/203
Age (years)	6.05 ± 4.13
BMI (kg/m^2)	18.20 ± 3.56*
Calcium (mmol/L)	2.40 ± 0.14*
Phosphate (mmol/L)	1.68 ± 0.20
Alkaline phosphatase (IU/L)	226.66 ± 67.39
Regular vitamin D use [a] Yes No	 219 287
Serum 25(OH)D (ng/ml) [b]	21.14 ± 9.27

[a] Intake vitamin D 400 IU/d.

[b] Serum collected in November, December and January, 2009.

Linear regression models were used to test the association between plasma 25-hydroxyvitamin D [25(OH)D] concentrations and potential explanatory variables (*P <0.05).

Multiplex ready reaction mix (Applied Biosystems, CA, USA), 0.5 μM of each primer and 1 μl of each PCR product and was carried out as recommended (Applied Biosystems, CA, USA). The extension PCR products were purified using 1 unit shrimp alkaline phosphatase and then run on an ABI 3130 X l Genetic Analyzer. SNP calling was carried out using GeneMapper^{TM} software v.4.0 (Applied Biosystems, CA, USA). For quality control, genotyping was performed without knowledge of the status of the subjects, and a 5% random sample of subjects was genotyped twice for all of the SNPs by different persons; the reproducibility was 100%.

Statistical analysis

Hardy-Weinberg equilibrium (HWE) was evaluated by using Pearson's $\chi 2$ test. The association of vitamin D levels with genotypes was evaluated by t-test. To examine the impact of these variants on plasma 25(OH)D levels, linear regression was performed after adjusting for age, sex, BMI and regular usage of vitamin D as confounding factors. The analyses were performed using dominant, additive and recessive genetic models. Bonferroni's correction was also applied for multiple testing to avoid false positive findings. Statistical analyses were performed using SPSS for Windows software (version 17.0; SPSS, Chicago, IL, USA). The data were analysed using two-sided P values. Statistical significance was set at a probability P value <0.05.

Results

All of the subjects were genotyped for *GC* (rs4588, rs7041, rs222020, rs2282679 and rs2298849), *CYP2R1* (rs10741657, rs10766197, rs12794714, rs1562902 and rs2060793) and *DHCR7/NADSYN1* (rs3829251, rs12785878) SNPs and analysed for association with 25(OH)D concentrations. The clinical characteristics and the determinants of 25(OH)D concentrations are shown in table 1. The information on the genomic location of the investigated SNPs is summarized in table 2. The genotype distribution of all investigated SNPs was in Hardy-Weinberg equilibrium [shown in table 3 (i)].

Clinical characteristics of the participants

The serum 25(OH)D levels were 21.14 ± 9.27 ng/ml (means ± SD; table 1), which were mostly near the level of vitamin D deficiency. Serum 25(OH)D levels were <20 ng/ml in 232 children (45.85%) and between 20 and 30 ng/ml in 210 children (41.50%). Only 64 (12.65%) children had 25(OH)D values >30 ng/ml.

The mean BMI was 18.20 ± 3.56 (means ± SD). BMI had a significant effect on serum 25(OH)D levels by using a linear regression analysis in our study (P = 0.045). The biochemical data revealed that serum calcium was statistically associated with 25(OH)D concentrations (P = 5.589×10-14). Other variables were not significantly associated with 25(OH)D status (data not shown).

Association between genotypes and 25(OH)D concentrations

We examined the association of vitamin D levels with genotype by t-test. The results are presented in table 3 (i). In unadjusted data, *GC*, *CYP2R1* and *DHCR7/NADSYN1* genes all showed significant effect on plasma 25(OH)D concentrations under one or two of the three genetic models (P <0.05).

We carried out linear regression analysis by comparing additive, dominant and recessive models with age, sex, BMI and regular usage of vitamin D as covariants. The results are given in table 3 (ii).

Concerning the five SNPs in *GC* gene, all loci were significantly associated with serum 25(OH)D status. After Bonferroni's correction for the 12 SNPs, only two SNPs (rs222020 , rs2298849) showed positive association with lower mean circulating levels of 25(OH)D under both additive and recessive models (P = 0.012 and P = 0.004 for rs222020, P = 0.024 for rs2298849 under both models, respectively). No other SNPs remained associated with 25(OH)D levels.

As to CYP2R1, after multiple comparisons, rs10741657, rs10766197, rs12794714 and rs1562902 were all associated with 25(OH)D status under additive and recessive genetic models (P = 4.686×10-7 and P = 1.315×10-9 for rs10741657, P = 0.012 and P = 2.748×10-5 for rs10766197, P = 0.001 and P = 3.022×10-5 for rs12794714, P = 0.001 and P = 2.376×10-8 for rs1562902, respectively). The genotypes of rs2060793 polymorphisms were significantly as-

Table 2: Basic characteristics of the three candidate genes.

Gene	Region	Length (kb)	Number of exons	SNP[a]	Locus	Location (bp)[b]	Function
GC(DBP)	4q13.3	63.8	13	rs4588	Exon	72618323	Missense Thr>Lys
				rs7041	Exon	72618334	Missense Glu>Asp
				rs222020	Intron	72636272	
				rs2282679	Intron	72608383	
				rs2298849	Intron	7264885	
CYP2R1	11p15.2	14.2	5	rs10741657	5′ Near gene	14914878	
				rs10766197	5′ Near gene	14921880	
				rs12794714	Exon	14913575	Synonymous
				rs1562902	5′ Near gene	14918216	Promoter
				rs2060793	5′ Near gene	14915310	
DHCR7/NADSYN1	11q13.4	42.5	10	rs3829251	Intron	71194559	
				rs1278587	Intron	71167449	

[a] SNP identifier based on NCBI dbSNP.
[b] Chromosomal location based on NCBI Human Genome Build 35 coordinates.

sociated with serum 25(OH)D concentrations under all of three genetic models (dominant, additive and recessive models) even after correction for multiple variables ($P = 1.463\times10^{-8}$, $P = 9.398\times10^{-14}$ and $P = 0.024$, respectively).

Table 3(i): The association between genotypes and 25(OH)D concentrations in studied individuals.

SNP	A/a	MAF	HWE	Subjects n (%)			mean 25(OH)D(ng/ml)			*P* value		
				CC	CR	RR	CC	CR	RR	Dominant	Additive*	Recessive
rs4588 *GC*	A/C	0.31	0.30	44 (8.69)	229 (45.26)	233 (46.05)	18.02±60.84	22.61±8.90	20.29±90.79	0.058	0.065 **0.008**	**0.003**
rs7041 *GC*	G/T	0.26	0.29	29 (5.74)	205 (40.51)	272 (53.75)	17.97±10.13	22.46±9.37	20.49±80.98	0.088	0.157 **0.020**	0.058
rs222020 *GC*	C/T	0.34	0.67	60 (11.86)	221 (43.67)	225 (44.47)	17.11±80.31	21.52±9.57	21.84±80.98	0.129	**0.000** 0.718	**0.000**
rs2282679 *GC*	C/A	0.32	0.22	44 (8.70)	232 (45.85)	230 (45.45)	18.07±60.90	22.45±9.15	20.41±90.59	0.109	0.058 **0.020**	**0.004**
rs2298849 *GC*	C/T	0.32	0.98	53 (10.47)	220 (43.48)	233 (46.05)	17.32±80.32	20.67±9.51	22.45±80.99	**0.003**	**0.000** **0.042**	**0.001**
rs10741657 *CYP2R1*	G/A	0.43	0.25	174 (34.38)	234 (46.25)	98 (19.37)	17.84±90.50	24.10±8.41	19.92±80.62	0.147	0.075 **0.000**	**0.000**
rs10766197 *CYP2R1*	A/G	1.00	0.36	64 (12.65)	233 (46.05)	209 (41.30)	16.76±70.94	23.21±8.54	20.18±90.83	0.054	**0.012** **0.001**	**0.000**
rs12794714 *CYP2R1*	A/G	0.55	0.36	61 (12.06)	240 (47.43)	205 (40.51)	16.39±10.20	21.90±9.15	21.66±8.74	0.298	**0.000** 0.775	**0.000**
rs1562902 *CYP2R1*	T/C	0.51	0.46	153 (30.24)	243 (48.02)	110 (21.74)	17.73±9.39	23.57±8.92	20.53±8.30	0.436	**0.013** **0.003**	**0.000**
rs2060793 *CYP2R1*	G/A	0.27	0.42	97 (19.17)	234 (46.25)	175 (34.58)	19.67±80.49	24.12±8.43	17.97±90.55	**0.000**	0.146 **0.000**	0.081
rs3829251 *DHCR7/NADSYN1*	A/G	0.28	0.59	41 (8.10)	196 (38.74)	269 (53.16)	16.64±80.11	20.97±8.95	21.95±90.49	**0.037**	**0.001** 0.264	**0.001**
rs12785878 *DHCR7/NADSYN1*	G/T	0.49	0.13	129 (25.50)	235 (46.44)	142 (28.06)	19.01±80.66	21.77±8.60	22.04±10.55	0.174	**0.010** 0.797	**0.002**

A/a, risk allele/non-risk allele.
CC, homozygous for risk allele; CR, heterozygous for risk allele; RR, homozygous for non-risk allele
P <0.05 indicates a significant difference.
MAF, minor allele frequency in the study population.
HWE, P values for Hardy–Weinberg Equilibrium test in the study population.
Additive*, P-value on the first line is the result (RR/CC), the second line is the result (CR/CC)

Table 3(ii): The adjusted P values for the genotype-phenotype relationship in studied individuals.

SNP	*P* value$_1$			*P* value$_2$		
	Dominant	Additive*	Recessive	Dominant	Additive*	Recessive
rs4588 *GC*	0.624	**0.027** 0.800	**0.008**	7.488	0.324 9.600	0.096
rs7041 *GC*	0.065	0.354 **0.018**	0.179	0.780	4.248 0.216	2.148
rs222020 *GC*	0.531	**0.001** 0.619	**0.037×10^{-2}**	6.372	**0.012** 7.428	**0.004**
rs2282679 *GC*	0.532	**0.019** 0.897	**0.006**	6.384	0.228 10.764	0.072
rs2298849 *GC*	0.110	**0.002** 0.482	**0.002**	1.320	**0.024** 5.784	**0.024**
rs10741657 *CYP2R1*	**0.007**	0.251 **3.905×10^{-8}**	**1.096×10^{-10}**	0.084	3.012 **4.686×10^{-7}**	**1.315×10^{-9}**
rs10766197 *CYP2R1*	0.460	**0.001** **0.016**	**2.290×10^{-6}**	5.520	**0.012** 0.192	**2.748×10^{-5}**
rs12794714 *CYP2R1*	0.601	**9.223×10^{-4}** 0.352	**2.519×10^{-6}**	7.212	**0.001** 4.224	**3.022×10^{-5}**
rs1562902 *CYP2R1*	0.122	**0.034** **1.116×10^{-3}**	**1.980×10^{-9}**	1.464	0.408 **0.001**	**2.376×10^{-8}**
rs2060793 *CYP2R1*	**1.219×10^{-9}**	0.456 **7.832×10^{-15}**	**0.002**	**1.463×10^{-8}**	5.472 **9.398×10^{-14}**	**0.024**
rs3829251 *DHCR7/NADSYN1*	0.099	**4.111×10^{-3}** 0.601	**4.412×10^{-3}**	1.188	**0.005** 7.212	**0.005**
rs12785878 *DHCR7/NADSYN1*	0.058	**0.004** 0.354	**0.002**	0.696	**0.048** 4.248	**0.024**

P <0.05 indicates a significant difference.
P value$_1$ was adjusted for age, sex, BMI and regular vitamin D use
P value$_2$ was after the Bonferroni's correction.
Additive*, P-value on the first line is the result (RR/CC), the second line is the result (CR/CC)

With respect to the *DHCR7*, the individuals with the rs3829251 AA and rs12785878 GG genotypes presented, on average, lower levels of 25(OH)D in comparison with the other genotypes under both additive and recessive models, even after correcting for multiple variables (P = 0.005 for rs3829251 under both models, P = 0.048 and P = 0.024 for rs12785878, respectively).

Discussion

Vitamin D deficiency remains a major health problem in the world, although the importance of supplemental vitamin D is well known. Children and adolescents are also potentially at high risk for vitamin D deficiency. For example, 52% of Hispanic and black adolescents in a study in Boston [15] and 48% of white preadolescent girls in a study in Maine [16] had 25-hydroxyvitamin D levels below 20 ng/mL. In another study, 42% of black girls and women throughout the United States had 25-hydroxyvitamin D levels below 20 ng/mL at the end of the winter [17]. This observation was similar to our findings, which showed that serum 25(OH)D levels were <20 ng/mL in 232 children (45.85%).

In vitamin D pathway, a series of studies had reported on interaction between vitamin D-related genes and 25(OH)D levels. Among them, vitamin D receptor gene played an important role in vitamin D pathway. Previous studies had shown that *VDR* polymorphisms had a significant effect on vitamin D levels in many populations including the Chinese [18–20].

In recent years, important advances for other genetic determinants of vitamin D levels (*GC*, *CYP2R1* and *DHCR7*/*NADSYN1* genes, for example) have obtained with the implement of GWAS in populations of European race. Although these variants were newly considered as susceptible loci for vitamin D levels, it was necessary to evaluate their effects on the quantitative trait in multiple ethnic populations; because it was well known that the difference in genetic background, lifestyle and environment exposures may lead to inconsistent association results for quantitative trait among different populations. The aim of the present study was to ascertain the contribution of these SNPs in recently identified vitamin D susceptible loci with the vitamin D levels in Han population in northeast China. To our knowledge, this is the first report that demonstrates significant associations between three candidate genes and vitamin D characteristics in healthy Chinese children.

GC, a member of the albumin family [21], encodes a vitamin D binding protein synthesised in the liver that binds and transports vitamin D and its metabolites. Several studies examined the relationship between more than ten SNPs in *GC* and 25(OH)D levels [13, 22–25]. The rs4588 and rs7041 polymorphisms were most consistently associated with 25(OH)D levels. In the present study, however, both of these SNPs were not associated with lower levels of 25(OH)D after applying Bonferroni's correction. The potential explanations include the following: (1) the influence of the sample size and different racial or ethnic groups; (2) several of the aforementioned studies were based on case-control studies, in which disease-related SNPs may confound the interpretation of the results; (3) false-negative results after Bonferroni's correction. Rs222020 and rs2298849 remained significantly associated with low levels of 25(OH)D after Bonferroni's correction, which was consistent with other studies. Feng-Xiao Bu et al. [26] researched the association of nine candidate genes with serum 25(OH)D levels among healthy Caucasian subjects and found that rs222020 was associated with the serum status of 25(OH)D even after correcting for multiple variables. Moreover, recent association studies revealed other loci in the *GC* gene associated with serum 25(OH)D variation. In a recent GWAS study for serum 25(OH)D in 4501 persons of European ancestry, Ahn et al. found that the rs2282679 SNP in the *GC* gene was the most significant one [14]. In summary, while the evidence linking variants in *GC* with 25(OH)D is strong, the underlying mechanism of action remains unclear. Understanding this mechanism requires further investigation.

CYP2R1, a member of the CYP2 family encoding cytochrome P450 proteins, is a key vitamin D 25-hydroxylase which hydroxylates vitamin D at the 25-C position for 25(OH)D synthesis in the liver [27]. Our study found that variant genotypes of SNPs in the *CYP2R1* gene, rs10766197, rs12794714, rs10741657, rs2060793 and rs1562902, were all significantly associated with plasma 25(OH)D levels. These results were consistent with the results in other studies. In the study of Wjst et al. [28], SNP rs10766197 in the *CYP2R1* gene was significantly associated with 25(OH)D status in 872 participants of the German Asthma Family Study. In addition, Ramos-Lopez et al. [11] found that rs10741657 in the *CYP2R1* gene was associated with the serum status of 25(OH)D in 609 participants from 203 type 1 diabetes families. rs10741657, located in a 2-kb *CYP2R1* mRNA transcript, is a coding SNP that could change the activity of the enzyme and subsequently cause a relative lack of 25(OH)D. In a recent genome-wide association study (GWAS), Wang et al. [12] found rs10741657 was significantly associated with 25(OH)D levels in 30000 subjects of European descent from 15 cohorts. All these studies indicate that genetic variants of the *CYP2R1* gene have a strong effect on serum 25(OH)D levels.

The third gene, *DHCR7*/*NADSYN1*, encodes the enzyme 7-dehydrocholesterol (7-DHC) reductase, which transforms 7-DHC to cholesterol, thereby removing that substrate from the synthetic pathway of vitamin D3, a precursor of 25(OH)D. Herein, we found that the two variant genotypes of *DHCR7*/*NADSYN1* (rs3829251, rs12785878) were both associated with serum 25(OH)D levels. These results were in agreement with those of Cooper et al., who showed that rs12785878 T allele carriers were significantly associated with lower levels of 25(OH)D in type 1 diabetic patients [29]. These findings suggest that this enzyme could play a more important role in the regulation of vitamin D status than previously thought.

In conclusion, our research suggests that the *GC*, *CYP2R1* and *DHCR7*/*NADSYN1* genes may be important in regulating serum 25(OH)D levels in healthy Chinese children. One limitation of the present study is that the sample size is relatively small. Further well-designed investigations with larger sample sizes and representing different ethnicities are warranted to confirm our findings.

Acknowledgments: We gratefully acknowledge the numerous sample donors for making this work possible.
Funding / potential competing interests: This work was funded by the Public Health Department of Heilongjiang Province (No.2011-056) of China.
Authors' contribution: The first two authors contribute equally to this work.

Correspondence: *Lihong Ren, PhD, Department of Pediatrics, Second Affiliated Hospital of Harbin Medical University, CN-Harbin, 150081, China, zhangyuling0000[at]yahoo.com.cn; renlihong1[at]126.com*

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