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SHORT COMMUNICATION

Genetic variant in vitamin D binding protein is associated with serum 25-hydroxyvitamin D and vitamin D insufficiency in southern Chinese

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Previous large-scale genome-wide meta-analysis identified four loci affecting 25-hydroxyvitamin D (25(OH)D) concentrations. However, whether these loci are associated with 25(OH)D concentration in southern Chinese remain unknown. Our primary aim was to examine whether the four top hits (rs2282679, rs10741657, rs12785878 and rs6013897) could be replicated in 712 southern Chinese women. The associations between these single-nucleotide polymorphisms (SNPs), serum 25(OH)D concentration (continuous variable) and vitamin D insufficiency (dichotomized variable) were examined using multivariable linear regression and logistic regression, respectively. Age, body mass index and season were adjusted in the model. Among these four SNPs, rs2282679 was associated with serum 25(OH)D levels ($\beta = -0.066$; $P = 9 \times 10^{-5}$) and vitamin D insufficiency (odds ratio (OR) = 1.51, 95% confidence interval (CI) 1.19–1.93; $P = 8.6 \times 10^{-4}$), whereas rs12785878 was nominally associated with vitamin D insufficiency only (OR = 0.79, 95% CI 0.63–0.99; $P = 0.042$). Genotype risk score (GRS), by summing risk variants of these two SNPs, had more significant association with vitamin D insufficiency (OR = 1.38; 95% CI 1.17–1.64; $P_{trend} = 1.76 \times 10^{-4}$) than the model that included only either SNP. The areas under receiver operating characteristic curves of rs2282679 and GRS were 0.561 ($P = 0.005$) and 0.576 ($P = 5 \times 10^{-4}$), respectively. Our study provides an independent evidence of the associations of rs2282679 and probably rs12785878 with 25(OH)D and vitamin D insufficiency in southern Chinese.
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Vitamin D deficiency leads to multiple adverse health consequences, such as growth retardation and skeletal deformities in children, precipitate osteoporosis, muscle weakness and risk of fracture in the later age.[1] Recently, studies suggested that vitamin D insufficiency is also a public health concern that has been associated with multiple diseases in addition to bone health, such as osteoarthritis,[2] diabetes[3] and cardiovascular disease.[4] It has been estimated that over 1 billion people worldwide have vitamin D deficiency or insufficiency.[5] Therefore, identification of determinants of vitamin D is particularly important.

Genetics contribute substantially in determining vitamin D metabolism,[6] with heritability ranging from 53 to 68.9%.[7,8] Using genome-wide association approach, we previously identified four 25-hydroxyvitamin D (25(OH)D) determining loci, 4p12 (rs2282679 in *GC*), 11p15 (rs10741657 in *CYP2R1*), 11q12 (rs12785878 in *DHCR7/NADSYN1*), and 20q13 (rs6013897 in *CYP24A1*), in 33 996 individuals of European origins.[9] In the current study, we aimed to replicate these four loci in Hong Kong southern Chinese.

In this replication study, 712 unrelated Hong Kong southern Chinese women with serum 25(OH)D data were selected from the Hong Kong Osteoporosis Study Database. The recruitment procedure and exclusion criteria have been detailed elsewhere.[10,11] Briefly, subjects with a history of chronic medical illness, premature menopause age below 40 years, malabsorption, previous major gastrointestinal surgery, metabolic bone disease, endocrine disorders including hyper- and hypothyroidism, or prescription of medication that may affect bone and calcium metabolism, hormone replacement therapy, anti-osteoporosis medication and active vitamin D3 metabolites were excluded from the analysis. Therefore, the primary sources of serum 25(OH)D were expected to be from diet and sunlight. Serum 25(OH)D was measured by a radioimmunoassay (RIA) (DiaSorin, Stillwater, MN, USA) after extraction with

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acetonitrile. The sensitivity of the assay was 1.5 ng ml^{-1} with an intra-assay coefficients of variability (CV) of 5.59% at 15.9 ng ml^{-1} and 11.62% at 58.9 ng ml^{-1}. The inter-assay CV at these two levels were 8.99% and 11.94%, respectively.

Sequenom MassARRAY System (Sequenom, San Diego, CA, USA) was used to genotype the single-nucleotide polymorphisms (SNPs; rs2282679, rs10741657, rs12785878 and rs6013897). Genotyping was repeated in 5% of the samples for verification and quality control; genotype data were confirmed to have a concordance rate of 99.9%. Information on the SNPs studied is provided in Supplementary Table 1. SNPs were tested for association (additive model) with the naturally log transformed serum 25(OH)D (as a continuous variable) and vitamin D insufficiency (categorical variable, defined as 25(OH)D <50 nmol l^{-1}) using a multivariable linear regression model and logistic regression model, respectively. All analyses were adjusted for age, body mass index and season on the measurement day as in our previous study.[9] We did not adjust sex because only women were analyzed in this study. We modeled season using categorical variables for summer + autumn (May–October), and winter + spring (November–April). The significance level was set to be 0.00625 in the current study, after correction for four SNPs and two phenotypes studied.

Characteristics of the study cohort are summarized in Table 1. Among 712 subjects studied, 41.8% of them were classified as vitamin D insufficiency. Subjects with vitamin D insufficiency were younger and the serum was less likely to be taken during summer or autumn.

Table 2 shows the association results. For the association with serum 25(OH)D levels, minor allele 'C' of rs2282679 near the *GC* showed a significant association with a β-value of -0.066 ($P = 9 \times 10^{-5}$), while it was also significantly associated with increased risk of vitamin D insufficiency with an odds ratio (OR) of 1.51 (95% confidence interval (CI) 1.19–1.93; $P = 8.6 \times 10^{-4}$). Previous study suggested that the effect of rs2282679 on 25(OH)D levels is stronger in summer,[12] however it was not observed in the current study ($P_{\text{interaction}} > 0.4$; Supplementary Table 2). Although no association was observed between rs12785878 and serum 25(OH)D levels (continuous variable), minor allele 'T' of rs12785878 was nominally associated with reduced risk of vitamin D insufficiency with an OR of 0.79 (95% CI 0.63–0.99; $P = 0.042$). We observed that rs2282679 and rs12785878 were independently associated with vitamin D insufficiency (Supplementary Table 3). In addition, likelihood ratio test showed that addition of rs12785878 in the logistic regression model significantly improved the model with rs2282679, age, body mass index and season ($P = 0.038$). Therefore, we derived a genotype risk score (GRS) by summing risk variants (minor allele of rs2282679 and major allele of rs12785878) for each individual. Each score of the GRS was associated with an increased risk of vitamin D insufficiency with an OR of 1.38 (95% CI 1.17–1.64; $P_{\text{trend}} = 1.76 \times 10^{-4}$; Figure 1). No significant association was observed for the other two SNPs.

We also assessed the predictive ability of rs2282679 and GRS for vitamin D insufficiency, and the area under receiver operating characteristic curve (AUC) was 0.561 ($P = 0.005$) and 0.576 ($P = 5 \times 10^{-4}$), respectively. Although GRS had the highest AUC, the difference between AUC of GRS and rs2282679 was not statistically significant ($P > 0.5$).

The SNP rs2282679 showed consistent and robust association with serum 25(OH)D traits. Although there is substantial difference in genetic background in Chinese residing in different administrative regions of China,[13] a recent replication study also showed that rs2282679 was significantly associated with serum 25(OH)D in northern and central Chinese.[14] We meta-analyzed the β-value of rs2282679 in northern, central and southern Chinese (Supplementary Figure 1) using inverse variance weighted meta-analysis, a stronger association with an overall β-value of -0.07 ($P_{\text{meta}} = 2.56 \times 10^{-27}$) was observed (Supplementary Table 4).

In the current study, we successfully replicated rs2282679 as a determinant of serum 25(OH)D levels in southern Chinese population, whereas the failure to replicate the associations of the other three SNPs could be due to insufficient power of the current study to detect SNPs with small effect size (Supplementary Figure 2). Nevertheless,

Table 1 Demographic data of the 712 subjects

Characteristics	*All subjects (N = 712)*	*Vitamin D sufficiency (N = 414)*	*Vitamin D insufficiency (N = 298)*	P-*value*
Age (year)	48.41 ± 15.34	50.98 ± 14.38	44.85 ± 15.93	<0.001
BMI (kg m^{-2})	22.76 ± 3.97	22.84 ± 3.71	22.65 ± 4.3	0.541
Serum 25(OH)D level (nmol l^{-1})	54.28 ± 16.2	64.61 ± 12.72	39.93 ± 6.94	<0.001
Season: summer or autumn	403 (56.6%)	251 (60.6%)	152 (51%)	0.011

Abbreviations: BMI, body mass index; 25(OH)D, 25-hydroxyvitamin D.

Table 2 Association between the four top loci, serum 25(OH)D concentration and vitamin D insufficiency

					Serum 25(OH)D concentration			*Vitamin D insufficiency*		
CHR	*SNP*	*Physical position*	*Nearest gene(s)*	*Minor allele*	β	*95% CI*	P-*value*	*OR*	*95% CI*	P-*value*
4	rs2282679	72827247	GC	C	−0.066	−0.100 to 0.033	9.7×10^{-5}	1.51	1.19–1.93	8.6×10^{-4}
11	rs10741657	14871454	CYP2R1	A	0.012	−0.021 to 0.044	0.474	0.93	0.74–1.18	0.566
11	rs12785878	70845097	DHCR7/NADSYN1	T	0.018	−0.013 to 0.050	0.248	0.79	0.63–0.99	0.042
20	rs6013897	52175886	CYP24A1	A	−0.018	−0.059 to 0.023	0.386	1.16	0.87–1.56	0.317

Abbreviations: BMI, body mass index; CI, confidence interval; OR, odds ratio; SNP, single-nucleotide polymorphism; 25(OH)D, 25-hydroxyvitamin D.
Models were adjusted for age, BMI and season.

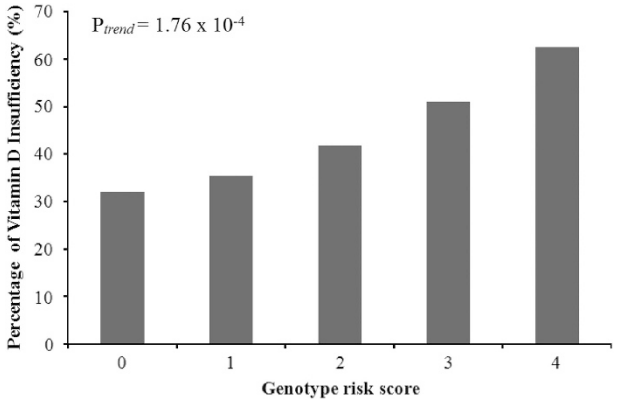

Figure 1 Prevalence of vitamin D insufficiency with different genotype risk score.

the meta-analysis of rs2282679 in southern, central and northern Chinese suggested that rs2282679 had a β-value −0.07 in Chinese population. Interestingly, the effect sizes of rs2282679 and rs12785878 were comparable between Europeans and southern Chinese. The OR of vitamin D insufficiency for those carriers of C allele of rs2282679 was 1.49 and 1.51 in Europeans and southern Chinese, respectively. On the other hand, for those carriers of G allele of rs12785878 was 1.21 and 1.27 in Europeans and southern Chinese, respectively.[9] We also showed that these two SNPs are significant predictors of vitamin D insufficiency, despite the predictive ability was only modest; whereas combining lifestyle factors and genetic information may improve the predictive ability.[15] Future development of predictive formula of vitamin D insufficiency will be important for early identification of subjects who are susceptible, and early intervention may help reducing its associated diseases.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on Journal of Human Genetics website (http://www.nature.com/jhg)