European Journal of Clinical Nutrition (2020) 74:806–810
https://doi.org/10.1038/s41430-020-0594-7

ARTICLE

Minerals, trace elements, Vit. D and bone health

CYP27B1 as an instrument gene to investigate the causal relationship between vitamin D deficiency and obesity: a family-based study

Songcheng Yu[1] · Yinhua Feng[1] · Chenling Qu[2] · Wenqian Huo[1] · Zhenxing Mao[1] · Chongjian Wang[1] · Wenjie Li[1] · Xing Li[1]

Received: 27 July 2019 / Revised: 15 February 2020 / Accepted: 19 February 2020 / Published online: 3 March 2020

Abstract

Objectives Vitamin D deficiency was associated with obesity. However, the causal relationship remains controversial. We hypothesized that there would be family-based associations in both vitamin D deficient families and obese families for the SNPs associated with vitamin D deficiency, if vitamin D deficiency was a causal factor of obesity. We aimed to investigate the family-based association of SNPs in CYP27B1 with both vitamin D deficiency and obesity.

Methods Four hundred and nineteen pedigrees containing 1505 rural individuals aged from 18 to 79 years in Henan Province of China were included in this study. Family-based associations of rs10877012 and rs4646536 in CYP27B1 with vitamin D deficiency and obesity were investigated. Serum 25(OH)D3 concentration <20 μg/L was defined as vitamin D deficiency. BMI ≥ 28 kg/m^2 was applied for obesity definition. Taqman assays were applied for SNP genotyping. Family-based associations were investigated with FBAT software.

Results It was shown that vitamin D deficiency was a risk factor of obesity (adjusted OR: 1.332, 95% CI: 1.042–1.703, $P = 0.022$). Furthermore, there were family-based associations for allele T of rs10877012 and allele T of rs4646536 in both vitamin D deficient families and obese families ($P < 0.05$). Both rs10877012 and rs4646536 were associated with serum 25 (OH)D3 levels between siblings ($P < 0.05$), but not BMI ($P > 0.05$). In addition, there is linkage disequilibrium between rs10877012 and rs4646536 ($D' = 1.0$, $r^2 = 0.992$).

Conclusion Vitamin D deficiency may be a causal factor of obesity. Maintaining sufficient vitamin D is beneficial to obesity prevention.

Introduction

Cumulative epidemiological evidence supports the association between vitamin D deficiency and obesity. Overweight and obese people seem to be more likely to suffer from vitamin D deficiency. Pereira-Santos reported that the prevalence of vitamin D deficiency was 35% higher in obese subjects and 24% higher in the overweight group when compared with the control group [1]. However, the causal relationship between vitamin D deficiency and obesity still remains inconclusive. On one hand, it is suggested that obese people may have less sun exposure which inhibit vitamin D synthesis due to their less outdoor physical activity [1]. And fat-soluble vitamin D metabolites are easily retained by excess body fat [2]. On the other hand, evidence suggests that lipogenesis can be promoted under the condition of vitamin D deficiency [3]. According to the reports above, the causal relationship between vitamin D deficiency and obesity is controversial.

25-hydroxyvitamin D or 25(OH)D is the main circulating metabolite of vitamin D which is widely used as an indicator of vitamin D status. It was estimated that the heritability of 25(OH)D range from 23 to 80% [4]. As the key metabolic gene of 25(OH)D, *CYP27B1* plays direct roles in circulating 25(OH)D concentration. Results of the twin study from the database of the longitudinal,

✉ Xing Li
15093285830@163.com

[1] College of Public Health, Zhengzhou University, Zhengzhou 450001, China

[2] College of Grain Oil and Food Science, Henan University of Technology, Zhengzhou 450001, China

population-based Canadian Collaborative Project on the Genetic Susceptibility to Multiple Sclerosis (CCPGSMS) and the 1958 British birth cohort suggested that rs10877012 and rs4646536 in *CYP27B1* were associated with serum 25(OH)D levels [4, 5]. If vitamin D deficiency is a causal factor of obesity, there should be family-based associations for the SNPs of *CYP27B1* associated with vitamin D deficiency in both vitamin D deficient families and obese families. In this study, we aimed at investigating the family-based associations of rs10877012 and rs4646536 in *CYP27B1* with both vitamin D deficiency and obesity. In addition, we also investigated the associations between different genotypes of *CYP27B1* (rs10877012 and rs4646536) and serum 25(OH)D concentration and body mass index (BMI), respectively. The results would shed light on the causal relationship between vitamin D deficiency and obesity.

Materials and methods

Study subjects

In order to investigate the family-based associations of SNPs in *CYP27B1* with both vitamin D deficiency and obesity, 419 pedigrees containing 1505 rural individuals aged from 18 to 79 years in Henan Province of China were included in this study, which has been registered at Chinese Clinical Trial Register (Registration number: ChiCTR1800017362). Detailed information is available at the website: http://www.chictr.org.cn/showproj.aspx?proj=29473. BMI larger than 28 was defined as obesity according to the guidelines for prevention and control of obesity in Chinese adults [6].

This study complied with the Declaration of Helsinki. The protocol was reviewed and approved by Life Science Ethics Review Committee of Zhengzhou University. All the subjects signed an informed consent when they participated in this study.

Measurement of 25(OH)D_3

The concentration of serum 25(OH)D_3 was measured in a certificated third-party medical laboratory of Kingmed Center for Clinical Co., Ltd. (Guangzhou, China). The levels of serum 25(OH)D_3 below 20 μg/L was defined as vitamin D deficiency.

SNPs selection and genotyping

CYP27B1 plays direct roles in circulating the concentration of 25(OH)D. According to literature, rs10877012 and rs4646536 in *CYP27B1* were associated with serum 25(OH)D levels [4]. Therefore, they were selected as genetic instruments to investigate the causal relationship between vitamin D deficiency and obesity. Taqman assays together with fluorescence quantitative PCR instrument (7500 Fast, Applied Biosystems, California, US) were applied for SNP genotyping. Hardy-Weinberg Equilibrium and Mendelian Genetics were applied for quality control.

Statistical analysis

Chi-square test was used to compare the categorical variables. Continuous variables were compared with Student's *t* test. Association between vitamin D deficiency and obesity was investigated with logistic regression. Family-based associations for SNPs in both vitamin D deficient families and obese families were investigated with FBAT software which was developed by scientists in School of Public Health in Harvard University (V2.0.4Q, https://www.hsph.harvard.edu/fbat/fbat.htm). Student's *t* test was applied to investigate the differences of serum 25(OH)D_3 and BMI between siblings. Linkage disequilibrium between SNPs was analyzed with Haploview 4.2. Chi-square test, Student's *t* test and logistic regression were conducted with SPSS 21.0 (IBM SPSS, New York, US). Two-tailed P value < 0.05 was considered as statistical significance.

Results

Subject characteristics

The demographic characteristic of participants in this study was displayed in Table 1. It was shown that age was associated with obesity ($P = 0.008$). Obese people had less physical activity than non-obese population ($P = 0.027$). Gender, smoking and drinking were not associated with obesity ($P > 0.05$).

Association between vitamin D deficiency and obesity

Logistic regression was applied to investigate the association between vitamin D deficiency and obesity (Table 2). It was indicated that vitamin D deficiency could increase the risk of obesity after adjustment of age and physical activity (OR: 1.332, 95% CI: 1.042–1.703, $P = 0.022$). On the other hand, people with vitamin D deficiency (serum 25(OH)D_3 < 20 μg/L) had significantly higher BMI than those with serum 25(OH)D_3 levels above 20 μg/L ($P = 0.021$, Fig. 1).

Table 1 The demographic characteristic of participants in the study.

Variables		BMI < 28 kg/m^2 ($N = 1162$)	BMI ≥ 28 kg/m^2 ($N = 343$)	P
Male (%)		572 (78.4)	158 (21.6)	0.303
Age (years)		48.1 ± 19.9	51.2 ± 14.7	0.008*
Smoking (%)				
	Never	584 (78.3)	162 (21.7)	0.469
	Ever	67 (75.3)	22 (24.7)	
	Current	295 (78.0)	83 (22.0)	
	Passive	216 (74.0)	76 (26.0)	
Drinking (%)				
	Never	906 (77.2)	268 (22.8)	0.928
	Ever	88 (78.6)	24 (21.4)	
	Current	168 (76.7)	51 (23.3)	
Physical activity (%)				
	Low	425 (75.0)	142 (25.0)	
	Medium	223 (74.3)	77 (25.7)	0.027*
	High	514 (80.6)	124 (19.4)	
BMI (kg/m^2)		23.3 ± 3.3	30.4 ± 2.2	<0.001*

According to the guidelines for prevention and control of obesity in Chinese adults, BMI ≥ 28.0 kg/m^2 was defined as obesity. Categorical variable was tested by Chi-square test. Student's t test was applied for continuous variable with normal distribution.

*P value below 0.05.

Table 2 Association between vitamin D deficiency and obesity.

		BMI (kg/m^2)		Adjusted OR	
		<28	28≤	OR (95% CI)	P
25 (OH)D_3 (μg/L)	20≤	620	156	1.332 (1.042–1.703)	0.022*
	<20	542	187		

BMI ≥ 28.0 kg/m^2 was defined as obesity according to the guidelines for prevention and control of obesity in Chinese adults. Association between vitamin D deficiency and obesity was investigated with logistic regression. Age and physical activity were adjusted to estimate the adjusted OR.

*P value below 0.05.

Family-based associations of SNPs with vitamin D deficiency and obesity

Family-based associations between *CYP27B1* (rs10877012 and rs4646536) and vitamin D deficiency and obesity are shown in Table 3. It was indicated that family-based associations for both allele T of rs10877012 and allele T of rs4646536 were found in both vitamin D deficient families and obese families. In vitamin D deficient families, allele T of rs10877012 was significantly associated with vitamin D deficiency in both additive model ($Z = 2.6$, $P = 0.00933$) and recessive model ($Z = 2.746$, $P = 0.006037$).

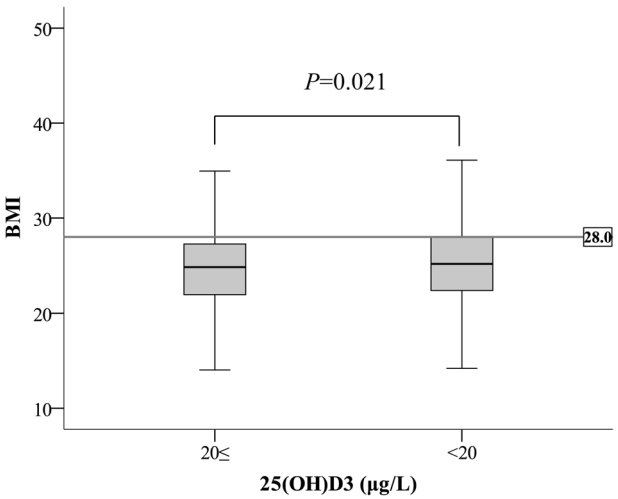

Fig. 1 Association between vitamin D deficiency and BMI. Student's t test was applied to investigate the difference. Serum 25(OH)D_3 < 20 μg/L was defined as vitamin D deficiency.

Meanwhile, allele T of rs4646536 was also significantly associated with vitamin D deficiency in both additive model ($Z = 2.248$, $P = 0.024589$) and recessive model ($Z = 2.512$, $P = 0.012002$). On the other hand, allele T of rs10877012 was significantly associated with obesity in both additive model ($Z = 2.94$, $P = 0.003283$) and recessive model ($Z = 3.381$, $P = 0.000723$) in obese families. And allele T of rs4646536 was also associated with obesity in both additive model ($Z = 2.673$, $P = 0.007526$) and recessive model ($Z = 3.138$, $P = 0.001699$).

Associations of SNPs with 25(OH)D_3 and BMI between siblings

Differences of serum 25(OH)D_3 and BMI between siblings with different genotypes of rs10877012 and rs4646536 were compared, respectively (Table 4). GG genotype of rs10877012 had significantly higher serum 25(OH)D_3 levels than GT genotype ($P = 0.028$). Serum 25(OH)D_3 levels in siblings with CC genotype of rs4646536 was significantly higher than those with GT genotype ($P = 0.011$). However, no significant difference of BMI was found between siblings for both rs10877012 and rs4646536 ($P > 0.05$).

Linkage disequilibrium of rs10877012 and rs4646536

rs10877012 and rs4646536 located in 5′UTR and intron with a distance of more than 4 kb. Linkage disequilibrium between them was investigated with Haploview software. The values for D' and r^2 were 1.0 and 0.992, respectively.

Table 3 The FBAT results of rs10877012 and rs4646536 in vitamin D deficient families and obese families.

				Vitamin D deficiency					Obesity				
Model	SNP	Allele	Afreq	Fam#	S-E (S)	Var (S)	*Z*	*P*	Fam#	S-E (S)	Var (S)	*Z*	*P*
A													
	rs10877012	G	0.354	74	−14.00	29.000	−2.600	0.009330	38	−11.00	14.000	−2.940	0.003283
		T	0.646	74	14.00	29.000	2.600	0.009330*	38	11.00	14.000	2.940	0.003283*
	rs4646536	C	0.352	74	−12.00	28.500	−2.248	0.024589	37	−10.00	14.000	−2.673	0.007526
		T	0.648	74	12.00	28.500	2.248	0.024589*	37	10.00	14.000	2.673	0.007526*
D													
	rs10877012	G	0.354	67	−11.75	18.313	−2.746	0.006037	34	−10.00	8.750	−3.381	0.000723
		T	0.646	31	2.25	7.313	0.832	0.405381	16	1.00	3.750	0.516	0.605577
	rs4646536	C	0.352	68	−10.75	18.313	−2.512	0.012002	34	−9.25	8.688	−3.138	0.001699
		T	0.648	30	1.25	6.813	0.479	0.632000	16	0.75	3.688	0.391	0.696118
R													
	rs10877012	G	0.354	31	−2.25	7.313	−0.832	0.405381	16	−1.00	3.750	−0.516	0.605577
		T	0.646	67	11.75	18.313	2.746	0.006037*	34	10.00	8.750	3.381	0.000723*
	rs4646536	C	0.352	30	−1.25	6.813	−0.479	0.632000	16	−0.75	3.688	−0.391	0.696118
		T	0.648	68	10.75	18.313	2.512	0.012002*	34	9.25	8.688	3.138	0.001699*

Analysis was conducted with FBAT software (V2.0.4Q, https://www.hsph.harvard.edu/fbat/fbat.htm). Transmission disequilibrium test (TDT) is applied to investigate the family-based associations of SNPs with vitamin D deficiency and obesity. TDT measures the transmission of maker alleles of heterozygous parents to the affected offspring. In the FABT software, informative families could be detected automatically for TDT. S-E (S) and Var (S) are the expected value and variance of the test statistic.

Serum 25(OH)D3 < 20 μg/L was defined as vitamin D deficiency. BMI ≥ 28.0 kg/m^2 was defined as obesity.

Z: the test statistic, P: significance level.

*Significant association ($Z > 0$ and $P < 0.05$).

A additive, *D* dominant, *R* recessive, *SNP* single nucleotide polymorphism, *Afreq* frequency of allele, *Fam#* number of informative families. Serum 25(OH)D_3 < 20 μg/L was defined as vitamin D deficiency. BMI ≥ 28.0 kg/m^2 was defined as obesity.

Table 4 Differences of serum 25(OH)D_3 and BMI between siblings.

SNP	Genotypes	Fam#	25(OH)D_3		BMI	
			Mean difference	*P*	Mean difference	*P*
rs10877012						
	GG/GT	13	18.4	0.028*	0.96	0.471
	GG/TT	6	21.5	0.850	1.87	0.472
	GT/TT	40	1.2	0.650	0.03	0.967
rs4646536						
	CC/CT	14	21.3	0.011*	2.39	0.144
	CC/TT	6	21.5	0.850	1.87	0.472
	TT/CT	40	1.2	0.650	0.03	0.967

Only siblings with two different genotypes in the same family were included in one test. Student's *t* test was applied to investigate the differences of serum 25(OH)D_3 and BMI between siblings.

Fam# number of informative families.

**P* value below 0.05.

Discussion

Vitamin D deficiency was associated with obesity. In order to investigate the causal relationship, family-based association between them was conducted in this study. The results suggested that family-based associations for rs10877012 and rs4646536 were found in both vitamin D deficient families and obese families. In addition, both rs10877012 and rs4646536 were associated with serum 25(OH)D_3 levels between siblings, but not BMI. It was indicated that vitamin D deficiency may be a causal factor of obesity.

Association between *CYP27B1* polymorphism and vitamin D deficiency

rs10877012 and rs4646536 locate in the promoter and the 6th intron of *CYP27B1*, respectively. There was linkage disequilibrium between them. The two SNPs were reported to be associated with and applied to predict the circulating levels of 25(OH)D [5, 7]. They were also found to be associated with diversified vitamin D related disorders [8]. These evidence supported that both rs10877012 and rs4646536 were associated with vitamin D deficiency, which was consistent with the FBAT results in this study.

Association between *CYP27B1* polymorphism and obesity

1α-hydroxylase converting 25(OH)D to active metabolite ($1,25(OH)_2D$) was encoded by *CYP27B1*, which was strongly associated not only with vitamin D status but also with obesity. It was suggested that adipogenesis could be inhibited by $1,25(OH)_2D$ [9]. Knockout mouse model showed that *CYP27B1* had great effect on body weight gain [10]. Thus, variation in *CYP27B1* was observed to be associated with obesity.

Clue for causal relationship between vitamin D deficiency and obesity

Although association between vitamin D deficiency and obesity has been observed in several studies [1], the causal relationship remains unclear. On one hand, overweight and obese people seem to be more likely to suffer from vitamin D deficiency [2]. On the other hand, lipogenesis can be promoted under the condition of vitamin D deficiency [3]. Therefore, the causal relationship between vitamin D deficiency and obesity still remains controversial.

In this study, both rs10877012 and rs4646536 of *CYP27B1* were found to be family-based associated with vitamin D deficiency and obesity. Due to the direct metabolism from 25(OH)D to $1,25(OH)_2D$ by CYP27B1, as well as the association between $1,25(OH)_2D$ and obesity, family-based associations of rs10877012 and rs4646536 in both vitamin D deficient families and obese families suggested that vitamin D deficiency may be a causal factor of obesity. Furthermore, both rs10877012 and rs4646536 were associated with serum $25(OH)D_3$ levels between siblings, but not BMI. Considering the function of *CYP27B1* and its associations with serum $25(OH)D_3$ levels and BMI among siblings in this study, more light has been shed on the causal relationship between vitamin D deficiency and obesity.

Therefore, maintaining sufficient vitamin D status is beneficial to the prevention and control of obesity. This is consistent with the results of randomized clinical trial reported by Salehpour et al. [11].

Acknowledgements This work was supported by National Natural Science Foundation of China (Nos. 81703270, 81573151, and 81573243) and the National Key Research and Development Program "Precision Medicine Initiative" of China (No. 2016YFC0900803).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

1. Pereira-Santos M, Costa PRF, Assis AMO, Santos CAST, Santos DB. Obesity and vitamin D deficiency: a systematic review and meta-analysis. Obes Rev. 2015;16:341–9. https://doi.org/10.1111/obr.12239.
2. Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF. Decreased bioavailability of vitamin D in obesity. Am J Clin Nutr. 2000;72:690–3.
3. Wood RJ. Vitamin D and adipogenesis: new molecular insights. Nutr Rev. 2008;66:40–46. https://doi.org/10.1111/j.1753-4887.2007.00004.x.
4. Bahrami A, Sadeghnia HR, Tabatabaeizadeh SA, Bahrami-Taghanaki H, Behboodi N, Esmaeili H, et al. Genetic and epigenetic factors influencing vitamin D status. J Cell Physiol 2018;233:4033–43. https://doi.org/10.1002/jcp.26216.
5. Orton SM, Morris AP, Herrera BM, Ramagopalan SV, Lincoln MR, Chao MJ, et al. Evidence for genetic regulation of vitamin D status in twins with multiple sclerosis. Am J Clin Nutr. 2008;88:441–7.
6. Zhou BF. Effect of body mass index on all-cause mortality and incidence of cardiovascular diseases–report for meta-analysis of prospective studies open optimal cut-off points of body mass index in Chinese adults. Biomed Environ Sci. 2002;15:245–52.
7. Signorello LB, Shi JJ, Cai QY, Zheng W, Williams SM, Long JR et al. Common variation in vitamin D pathway genes predicts circulating 25-hydroxyvitamin D levels among African Americans. PloS ONE. 2011;6. https://doi.org/10.1371/journal.pone.0028623.
8. Bailey R, Cooper JD, Zeitels L, Smyth DJ, Yang JHM, Walker NM, et al. Association of the vitamin D metabolism gene CYP27B1 with type 1 diabetes. Diabetes. 2007;56:2616–21. https://doi.org/10.2337/db07-0652.
9. Martini LA, Wood RJ. Vitamin D status and the metabolic syndrome. Nutr Rev. 2006;64:479–86. https://doi.org/10.1301/nr.2006.nov.479-486.
10. Mutt SJ, Hypponen E, Saarnio J, Jarvelin MR, Herzig KH. Vitamin D and adipose tissue-more than storage. Front Physiol. 2014;5. https://doi.org/10.3389/Fphys.2014.00228.
11. Salehpour A, Hosseinpanah F, Shidfar F, Vafa M, Razaghi M, Dehghani S et al. A 12-week double-blind randomized clinical trial of vitamin D-3 supplementation on body fat mass in healthy overweight and obese women. Nutr J. 2012;11. https://doi.org/10.1186/1475-2891-11-78.