ARTICLE

New evidence for associations between *vitamin D receptor* polymorphism and obesity: case-control and family-based studies

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Abstract

Association between *vitamin D receptor* (*VDR*) genetic polymorphism and obesity was observed in several case-control studies. This study hypothesized that these associations could be verified in family-based study. We aimed at investigating the associations between *VDR* SNPs and obesity (BMI ≥ 28 kg/m^2) by case-control study with 688 subjects and family-based study with 419 pedigrees. The results of case-control study suggested that rs3847987 (AC vs CC, Adjusted OR: 1.938, 95% CI: 1.359–2.763, $P = 0.000405$) was associated with obesity. Allele C of rs3847987 was risk factors for obesity ($P = 0.006$). Furthermore, association of rs3847987 with BMI was verified in family-based study ($Z = 2.077$, $P = 0.037811$). In addition, sibling with AC genotype of rs3847987 had significant higher BMI than CC genotype in the same family ($P = 0.03$). Therefore, it could be concluded that *VDR* genetic polymorphism (rs3847987) may be associated with obesity.

Introduction

The incidence of obesity increased rapidly in the past decade. More than 30% of adults are suffering from obesity [1]. Cumulative epidemiological evidence suggests that vitamin D deficiency is associated with obesity [2]. Martini and Wood reported that adipogenesis could be inhibited by 1,25 (OH)$_2$D [3]. SNPs of *vitamin D receptor* (*VDR*) were reported to be associated with obesity. Al-Daghri's work suggested that rs731236 and rs1544410 of *VDR* were significantly associated with obesity in Saudi and China by case-control studies [4, 5]. However, whether these associations could be found in family-based study remains unclear.

Case-control study is an epidemiologic method, which is the most frequently applied to investigate the association between risk factors and diseases [6]. Nevertheless, subject selection bias easily happens in a case-control study. Then false positive results caused by population stratification due to mismatched subject selection would be obtained [7]. In contrast, influence of population stratification on association test could be theoretically avoided in family-based study [8].

In this study, we hypothesized that these associations could be verified in family-based study. We aimed at investigating the associations between SNPs of *VDR* and obesity by both case-control and family-based studies. First, associations between SNPs and obesity were investigated by a 1:1 matched case-control study. Second, associations of the validated SNPs with body mass index (BMI) are verified by a family-based study. Finally, associations between verified SNPs and BMI were further verified between siblings. This work would provide stronger evidence for the association between SNPs in *VDR* and obesity.

Material and methods

Study subjects

Case-control study

Three hundred and forty-four subjects without any medical care and with obesity (BMI ≥ 28.0 kg/m^2) [9] were selected from the Henan Rural Cohort Study for case-control study

[10] (Registration number: ChiCTR-OOC-15006699, website: http://www.chictr.org.cn/showproj.aspx?proj=11375). Three hundred and forty-four nonobesity subjects (BMI < 28.0 kg/m^2) matched for sex and age with the cases were selected for case-control study by 1:1 matching. The matching parameters were set 0 for sex and 3 for age.

Family-based association study

419 pedigrees containing 1560 subjects in Wuzhi County, Henan Province, China were included for family-based study. Face-to-face interview was carried out to obtain the subject's information including smoking, drinking and physical activity. Their height and weight were measured three times by qualified investigator. Then BMI was calculated with average height and weight. The equation to calculate BMI was as follows:

$$BMI = Weight/Height^2 (kg/m^2)$$

Sibling study

From the 419 pedigrees described above, 29 families containing sibling with CC and AC genotypes of rs3847987, two families containing sibling with AA and CC genotypes of rs3847987 and ten families containing sibling with AA and AC genotypes of rs3847987 were included for sibling study.

SNP selection and genotyping

According to literature, seven SNPs (rs2228570, rs7975232, rs731236, rs2189480, rs3847987, rs2239179, and rs739837) in *VDR* being frequently reported to be associated with disorders were selected in this study [11, 12]. Taqman Assay combined with fluorescence quantitative PCR system (7500 Fast, Applied Biosystems, CA, USA) was applied for genotyping of all the selected SNPs. All the operations were according to the manufacturer manual.

Statistical analysis

For demographic characteristics, Chi-square test was applied to compare the difference of smoking, drinking, and physical activity between case and control. And age was compared with Student's *t* test.

Different statistical methods were applied in 1:1 matched case-control study, family-based association study and sibling study. First, conditional logistic regression model in which estimates were conditional on the cases being linked to the controls in the 1:1 matched case-control study was

used to investigate the associations between *VDR* polymorphism and obesity [13]. Second, Family-based associations between SNPs in *VDR* associated with obesity and BMI were investigated with FBAT software [14] (V2.0.4Q, https://www.hsph.harvard.edu/fbat/fbat.htm). Finally, paired Student's *t* test was applied to compare the difference of BMI between siblings with different genotype in the same family.

SPSS 21.0 (IBM SPSS, New York, US) was applied to conduct all the statistical analysis except family-based association test. Two-tailed *P* value less than 0.05, as well as Bonferroni-corrected *P* value, was considered as statistical significance.

Results

Subject characteristics

The demographic characteristics of subjects in the 1:1 matched case-control study are shown in Table 1. Sex was exactly matched between case and control. There was no significant difference for age, smoking, and drinking (*P* > 0.05). Physical activity was associated with obesity (*P* = 0.021).

Table 1 Demographic characteristics of 1:1 matched case-control study.

Variables	BMI < 28 kg/m^2 (N = 344)	BMI ≥ 28 kg/m^2 (N = 344)	P
Male (%)	161 (46.8)	161 (46.8)	–
Age (years)	51.0 ± 14.7	51.0 ± 14.7	0.990
Smoking (%)			0.412
Never	159 (46.2)	160 (16.5)	
Ever	13 (3.8)	22 (6.4)	
Current	95 (27.6)	85 (24.7)	
Passive	77 (22.4)	77 (22.4)	
Drinking(%)			0.951
Never	263 (76.5)	266 (77.3)	
Ever	28 (8.1)	26 (7.6)	
Current	53 (15.4)	52 (15.1)	
Physical activity (%)			0.021*
Low	127 (36.9)	141 (41.0)	
Medium	57 (16.6)	77 (22.4)	
High	160 (46.5)	126 (36.6)	

BMI ≥ 28 kg/m^2 was defined as obesity. Categorical variable is described as frequency and percentage and compared by the Chi-square test. Continuous variable with normal distribution is presented as means ± SD and compared with the Student's *t* test

Asterisk denotes the *P* value below 0.05

Associations between *VDR* polymorphism and obesity by case-control study

The genotyping results were displayed in Table S1. All the tested SNPs of VDR were consistent with Hardy–Weinberg equilibrium ($P > 0.05$). The results of association between SNPs of *VDR* and obesity analyzed with conditional logistic regression model are shown in Table 2. It was suggested that rs3847987 (AC vs CC, Adjusted OR: 1.938, 95% CI: 1.359–2.763, $P = 0.000405$) was associated with obesity. Furthermore, allelic associations between SNPs of VDR and obesity were investigated (Table S2). It was suggested that allele C of rs3847987 (OR: 1.449, 95% CI: 1.111–1.889, $P = 0.006$) was risk factor for obesity.

Besides, the results of association between BMI and SNPs of *VDR* also suggested that rs3847987 was associated with BMI ($P < 0.05$, Table S3).

Associations between SNPs of *VDR* and BMI by family-based study

The results of family-based association test are shown in Table 3. There was transmission disequilibrium for allele C of rs3847987 in 133 informative families ($Z = 2.077$, $P = 0.037811$) in additive model. No significant transmission disequilibrium was found in dominant and recessive models ($P > 0.05$). rs7975232 and rs739837 were not associated with BMI in this family-based association study ($P > 0.05$).

Table 2 Association between SNPs of *VDR* and obesity by case-control study.

SNP	Genotype	Crude OR		Adjusted OR	
		OR (95% CI)	*P*	*OR* (95% CI)	*P*
rs2228570					
	CC	Reference		Reference	
	CT	0.816 (0.575–1.157)	0.254	0.822 (0.578–1.169)	0.276
	TT	0.968 (0.622–1.506)	0.885	1.002 (0.642–1.565)	0.992
rs7975232					
	CC	Reference		Reference	
	CA	1.517 (1.097–2.099)	0.012	1.565 (1.127–2.173)	0.007
	AA	1.193 (0.609–2.336)	0.607	1.135 (0.575–2.241)	0.715
rs731236					
	CC	Reference		Reference	
	CT	1.412 (0.121–16.470)	0.783	1.343 (0.115–15.720)	0.814
	TT	2.000 (0.181–22.056)	0.571	1.881 (0.170–20.805)	0.606
rs2189480					
	CC	Reference		Reference	
	CA	1.042 (0.662–1.641)	0.859	1.027 (0.650–1.621)	0.911
	AA	1.056 (0.667–1.672)	0.815	1.021 (0.642–1.622)	0.931
rs3847987					
	CC	Reference		Reference	
	AC	1.904 (1.339–2.706)	0.000428*	1.938 (1.359–2.763)	0.000405*
	AA	1.087 (0.519–2.276)	0.826	1.056 (0.502–2.223)	0.885
rs2239179					
	AA	Reference		Reference	
	AG	0.865 (0.120–6.262)	0.886	0.781 (0.107–5.687)	0.807
	GG	0.969 (0.128–7.316)	0.976	0.922 (0.122–6.993)	0.938
rs739837					
	GG	Reference		Reference	
	GT	1.565 (1.130–2.167)	0.007	1.604 (1.155–2.229)	0.005
	TT	1.266 (0.652–2.459)	0.486	1.206 (0.616–2.360)	0.585

BMI ≥ 28 kg/m^2 was defined as obesity. Logistic regression was applied for risk assessment. Physical activity was adjusted to calculate the adjusted OR

CI confidence interval, *OR* odds ratio

Asterisk denotes *P* value below 0.0036 (Bonferroni corrected)

Table 3 Family-based association between SNPs of *VDR* and BMI.

Model	SNP	Allele	Afreq	Fam#	S–E (S)	Var (S)	Z	P
A								
	rs7975232	A	0.755	153	304.180	40181.98	1.517	0.129153
		C	0.245	153	−304.180	40181.98	−1.517	0.129153
	rs3847987	A	0.194	133	−388.650	35017.62	−2.077	0.037811
		C	0.806	133	388.650	35017.62	2.077	0.037811*
	rs739837	G	0.246	153	−244.710	40107.34	−1.222	0.221741
		T	0.754	153	244.710	40107.34	1.222	0.221741
D								
	rs7975232	A	0.755	47	116.652	8355.745	1.276	0.201903
		C	0.245	145	−187.527	27628.32	−1.128	0.259234
	rs3847987	A	0.194	129	−307.222	25450.19	−1.926	0.054131
		C	0.806	34	81.427	6159.371	1.038	0.299486
	rs739837	G	0.246	144	−151.805	27764.28	−0.911	0.362268
		T	0.754	46	92.905	8286.311	1.021	0.307441
R								
	rs7975232	A	0.755	145	187.527	27628.32	1.128	0.259234
		C	0.245	47	−116.653	8355.745	−1.276	0.201903
	rs3847987	A	0.194	34	−81.428	6159.371	−1.038	0.299486
		C	0.806	129	307.222	25450.19	1.926	0.054131
	rs739837	G	0.246	46	−92.905	8286.311	−1.021	0.307441
		T	0.754	144	151.805	27764.28	0.911	0.362268

Analysis was conducted with FBAT software (V2.0.4Q, https://www.hsph.harvard.edu/fbat/fbat.htm). S–E (S) and Var (S) are the expected value and variance of the test statistic. Z: the test statistic; *P*: significance level

A additive, *D* dominant, *R* recessive, *SNP* single nucleotide polymorphism, *Afreq* frequency of allele, *Fam#* number of informative families

Asterisk means significant association ($Z > 0$ and $P < 0.05$)

Associations between rs3847987 and BMI by sibling study

The association between rs3847987 and BMI is displayed in Fig. 1. Sibling with AC genotype of rs3847987 had significant higher BMI than CC genotype in the same family ($P = 0.03$).

Discussion

Out of the seven selected SNPs of *VDR*, rs3847987 was found to be associated with obesity in 1:1 matched case-control study. And rs3847987 was further verified to be associated with BMI in family-based study. In addition, sibling with AC genotype of rs3847987 had significant higher BMI than CC genotype in the same family. These results indicated that *VDR* polymorphism (rs3847987) is associated with obesity.

In case-control study, allele A of rs7975232, allele C of rs3847987, and allele G of rs739837 may be risk factors for obesity ($P < 0.05$). And all the heterozygotes of rs7975232, rs3847987, and rs739837 were associated with obesity ($P <$
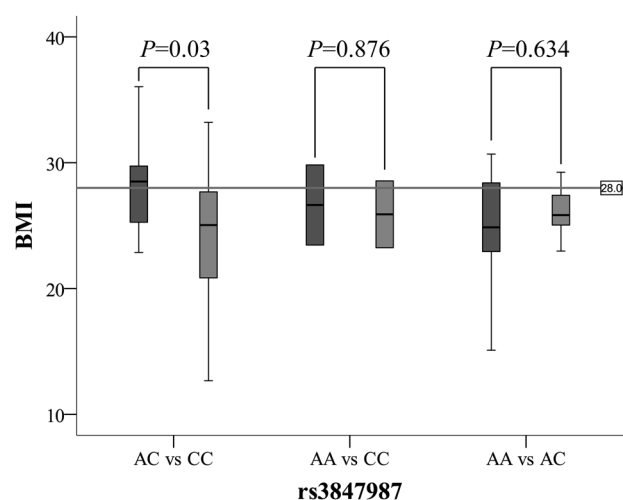

Fig. 1 Associations between rs3847987 and BMI by sibling study. 29 families containing sibling with CC and AC genotypes, two families containing sibling with AA and CC genotypes and ten families containing sibling with AA and AC genotypes were included. Paired Student's *t* test was applied to compare the difference of BMI between siblings with different genotypes in the same family. Sibling with AC genotype had higher BMI than CC genotype ($P = 0.03$).

0.05). There may be linkage disequilibrium among the three SNPs. Haploview was applied to examine the linkage disequilibrium among the seven SNPs of VDR (Fig. S1). Linkage disequilibrium was found among rs7975232, rs3847987, and rs739837. Therefore, rs3847987 is the main risk factor of obesity.

rs3847987 locates in the 3′ untranslated region (UTR) of *VDR*. 3′UTR is the target of miRNA to regulate the levels of mRNA expression, which could further influence the translational level of protein [15, 16]. Thus, variationCYP27B1s in the 3′ UTR of *VDR* may impact the expression level. Then the bioactivity of vitamin D would be influenced. It has been reported that rs3847987 was associated with colorectal cancer [17]. Therefore, rs3847987 may be associated with *VDR* expression and bioactivity of vitamin D, which may play role in development of obesity.

On the other hand, rs3847987 was reported to be associated with the levels of 25(OH)D [18]. Martini's work indicated that adipogenesis could be inhibited by $1,25(OH)_2D$ [3]. What is more, knock-out mouse model shows that *CYP27B1*, the gene converting 25(OH)D to active $1,25(OH)_2D$, has great effect on body weight gain [19]. Thus, rs3847987 may be associated with obesity through its effect on metabolism of vitamin D.

Compared with CC genotype of rs3847987, AC genotype increased the risk of obesity. On the other hand, the result of the sibling study also suggested that AC genotype of rs3847987 had higher BMI than CC genotype. Therefore, the association between AC genotype of rs3847987 and obesity was verified. However, no significant association between AA genotype of rs3847987 and obesity was observed when compared with CC genotype. This may be the limitation of small sample size in case-control study. Therefore, a further study with large sample size is required to verify the conclusion of this study.

To sum up, association between rs3847987 of *VDR* and obesity is validated by case-control and family-based studies. The function of rs3847987 and its related mechanism should be investigated in further study.

Compliance with ethical standards

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

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

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