RESEARCH NOTE

Association between vitamin D receptor gene polymorphism (TaqI) and obesity in Chinese population

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Introduction

Overweight and obesity have become rapidly growing threat to health worldwide. Comorbidities include, cardiovascular disease, hypertension, stroke, certain types of cancer and type 2 diabetes (Shen *et al.* 2012). The pathogenesis of obesity, including environment and genetic factors, is complex and gene variations accounting for as much as 60–70% of causes in obesity (Bell *et al.* 2005).

Although, the mechanisms underlying vitamin D in obesity is still incompletely explained, poor vitamin D status has been proved to be associated with obesity in humans, but vitamin D supplementation failed to decrease body weight (Bouillon *et al.* 2014). The active form of vitamin D acts as a ligand agonist for the vitamin D receptor (VDR), a member of nuclear receptor family of transcription factor (Uitterlinden *et al*. 2004). *VDR* gene, located at chromosome region 12q13, includes nine exons and eight introns, expressed in many tissues including parathyroid gland, intestine, kidney, adipose tissue, etc. VDR has been proven to have many functions in regulating bone density, insulin secretion, immune system, hair growth cycle and lipolysis (Haussler *et al.* 1998). VDR knockout mice were resistant to high fat diet-induced weight gain. The lean phenotype of VDR knockout mice was associated with reduced serum leptin and increased expression of uncoupling protein (Lementowski and Zelicof 2008). It is confirmed that VDR plays an important role in energy metabolism (Wong *et al.* 2011), and potentially modulates adipogenesis and preadipocyte differentiation (Ding *et al.* 2012).

VDR polymorphisms, such as ApaI (rs7975232), TaqI (rs731236), BsmI (rs1544410) and FokI (rs2228570) have been reported to be associated with various diseases. There are several papers reported in association between VDR polymorphisms and obesity, however, these studies are for just one VDR polymorphism (Vasilopoulos *et al.* 2013), for different ethnic group (Binh *et al.* 2011), and for type 2 diabetic subjects (Ye *et al.* 2001). These limitations may lead the results for *VDR* gene polymorphisms varied in the former studies (Uitterlinden *et al.* 2004; Mishra *et al.* 2013). Hence, the relationship between the VDR polymorphisms and obesity are still needed to be focussed on.

This study was designed to investigate whether the VDR polymorphisms (VDR rs731236 T>C (TaqI); VDR rs7975 232 G>T (ApaI); VDR rs2228570 C>T (FokI)) are associated with obesity in Chinese population or with biochemical parameters and physical conditions.

Materials and methods

Subjects

For this study, we selected 529 samples (245 obese subjects and 284 controls) of ethnic Han from Harbin People's Health Study of 2008. Participants were selected by stratified multistage random cluster sampling design. The inclusion criteria was as the follows: (i) BMI $\geq$ 28 kg/m^2 (case group) or 18.5 kg/m^2 $\leq$BMI $<$24 kg/m^2 (control group), (ii) no history of diabetes, cardiovascular disease, chronic lung disease, chronic liver disease, chronic renal disease, gastrointestinal disease, cancer, and (iii) no weight control, kept body weight stable during the six months before the study. The study protocol was approved by the Harbin Medical University Ethics Committee, and written informed consent was obtained from all participants. All investigators were trained by researchers or medical personnel from the Harbin Medical University.

DNA isolation and genotyping

Genomic DNA was extracted from peripheral venous blood samples by a TIANamp Blood Genomic DNA Purification Kit (Tiangen Biotech, Beijing, China) according to the

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Table 1. Basic characteristics and biochemical indexes of cases and controls.

	Control (n=290)	Cases(n=245)	P value
Gender (male/female)	64/226	72/173	0.06
Age (year)	53.92 ± 10.40	54.85 ± 11.77	0.42
BMI (kg/m^2)	21.51 ± 1.50	30.31 ± 2.67	<0.001
TC (mmol/L)	4.98 ± 0.94	5.08 ± 1.02	0.33
TG (mmol/L)	1.65 ± 1.16	2.25 ± 1.84	<0.001
FBG (mmol/L)	4.44 ± 0.54	4.47 ± 0.70	<0.001
PBG (mmol/L)	5.26 ± 1.33	5.99 ± 1.77	<0.001
SBP (mmHg)	122.90 ± 19.65	140.86 ± 22.19	<0.001
DBP (mmHg)	75.45 ± 10.29	84.88 ± 11.44	<0.001
WC (cm)	75.89 ± 6.77	95.03 ± 8.48	<0.001
HC (cm)	91.44 ± 5.75	106.46 ± 7.34	<0.001

WC, waist circumference; HP, hip circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglyceride; FBG, fasting blood-glucose; PBG, postprandial blood-glucose. Data are shown as means ± SD.

manufacturer's instructions. We detected VDR genotyping by polymerase chain reaction-restriction fragment length polymorphism analysis (PCR-RFLP).

Standard PCR conditions were as follows: initial denaturation at 94°C for 5 min, 35 cycles of 94°C for 30 s, annealing temperature (ApaI and TaqI at 64°C, FokI at 60°C) for 30 s, 72°C for 30 s, and final extension of 72°C for 5 min. The primers were obtained for ApaI and TaqI (F) 5′-CAG AGC ATG GAC AGG GAG CAAG-3′, (R) 5′-GCA ACT CCT CAT GGC TGA GGT CTC A-3′; for FokI(F) 5′-AGC TGG CCC TGG CAC TGA CTC TGC TCT-3′, (R) 5′-ATG GAA ACA CCT TGC TTC TTC TCC CTC-3′ (Bai *et al.* 2009). For ApaI and TaqI, a fragment of 740 bp was amplified. Digestion with *Apa*I revealed two fragments of 515 and 225 bp in a 1.5% agarose gel which meant the presence of restriction site can be written as aa. Digestion with *Taq*I in a 2.5% agarose gel showed the fragments of 290, 245 and 205 bp in the presence of polymorphic site written as tt; fragments of 495 and 245 bp written as TT. For FokI polymorphism, a fragment of 265 bp was digested into two fragments 196 and 69 bp in presence of FokI site which recognized as ff.

Haplotype identifying

Two alleles could be detected for ApaI, TaqI and FokI polymorphisms. Haplotype is a set of single-nucleotide polymorphisms (SNPs) on a single chromosome. Haploview Software (Broad Institute of MIT, Harvard, USA) was used to estimate haplotype frequencies and eight possible haplotypes were inferred. Subjects with obesity or control were considered as dependent variant and haplotypes as independent variant to estimate odds ratios (OR) in logistic regression analysis.

Table 2. Association between genotypes and allele frequencies of VDR polymorphisms and obesity in case group (n = 245) and control group (n = 284).

SNP	Control (%)	Case (%)	Total (%)	P value	OR (95% CI)
ApaI (rs7975232)					
aa	110 (38.7)	111 (45.3)	221 (41.8)		1.00
Aa	132 (46.5)	100 (40.8)	232 (43.9)		0.75 (0.52–1.09)
AA	42 (14.8)	34 (13.9)	76 (14.4)	0.30	0.80 (0.48–1.35)
a	352 (62.0)	322 (65.7)	674 (63.7)		1.00
A	216 (38.0)	168 (34.3)	384 (36.3)	0.21	0.85 (0.66–1.09)
TaqI (rs731236)					
Tt	79 (27.8)	28 (11.4)	110 (20.2)		1.00
TT	205 (72.2)	217 (88.6)	422 (79.8)	**<0.001**	**2.99 (1.86–4.79)**
t	79 (13.9)	28 (5.7)	107 (10.1)		1.00
T	489 (86.1)	462 (94.3)	951 (89.9)	**<0.001**	**2.67 (1.70–4.18)**
FokI (rs2228570)					
ff	40 (14.1)	34 (13.9)	74 (14.0)		1.00
Ff	111 (39.1)	110 (44.9)	226 (41.8)		1.17 (0.69–1.98)
FF	133 (46.8)	101 (41.2)	235 (44.2)	0.37	0.89 (0.53–1.51)
f	191 (33.6)	178 (36.3)	369 (34.9)		1.00
F	377 (66.4)	312 (63.7)	689 (65.1)	0.36	0.89 (0.69–1.14)

Significant values: $P < 0.05$. Statistically differences have been shown in a more visualized way, I value and OR are easily detected in bold.

Table 3. Logistic regression analysis of traits associated with obesity.

Variable	B	*P* value	OR	95% CI Lower	95% CI Upper
Age	−0.024	0.02*	0.976	0.957	1.807
Gender	0.112	0.67	1.119	0.693	0.996
Smoke	−0.043	0.77	0.958	0.715	1.284
Drink	−0.079	0.74	0.924	0.577	1.480
FBG	0.338	0.06	1.402	0.987	1.992
PBG	0.224	0.001*	1.251	1.092	1.432
SBP	0.036	<0.001*	1.036	1.022	1.051
DBP	0.035	0.005*	1.036	1.011	1.061
TC	0.039	0.73	1.039	0.834	1.295
TG	0.151	0.05	1.163	0.999	1.353
TT genotype	1.332	<0.001	3.790	2.195	6.545

FBG, fasting blood glucose; PBG, postprandial blood glucose; SBP, systolic blood pressure; DBP, diastolic blood pressure, TC, total cholesterol; TG, triglyceride and TaqI polymorphism as independent variables, and the group of individuals as binary-dependent-variables in logistic regression analysis. *$P < 0.05$.

Statistical analysis

Genotype frequencies were analysed for Hardy–Weinberg equilibrium (HWE) by using a χ^2 good-of-fit test. Distribution of VDR genotypes in two groups were examined using chi-square test. Genetic haplotypes were compared in logistic regression.

Results

Association between genotype and allele frequencies of VDR polymorphisms and obesity

Student's *t*-test was performed to evaluate differences for the following variables between cases and controls (table 1). There was no difference of male/female scale, age and serum TC between cases and controls. However, WC, HP, serum TG, FBG, PBG, SBP, DBP were significantly lower in control group than in case. Body mass index (BMI) was significantly different between the case and control groups ($P < 0.001$).

Table 4. Association of VDR haplotype frequencies and obesity.

VDR haplotype	Control	Case	Total	OR (95% CI)	*P* value
FaT	0.375	0.411	0.392	1.00	
faT	0.209	0.224	0.216	0.99 (0.72–1.36)	0.93
FAT	0.159	0.199	0.178	1.14 (0.81–1.61)	0.44
fAT	0.115	0.109	0.112	0.87 (0.58–1.31)	0.52
FAt	0.096	0.017	0.058	**0.16 (0.07–0.34)**	<0.001
fAt	0.033	0.022	0.028	0.63 (0.29–1.35)	0.23
Fat	0.013	0.0006	0.010	0.41 (0.11–1.55)	0.19
fat	0	0.011	0.005	–	–
					<0.001

Bold characters are statistically significance.

All genotype distributions in all subjects were in HWE except for VDR-TaqI in obesity group. Linkage disequilibrium was observed between TaqI and ApaI ($D' = 0.64$).

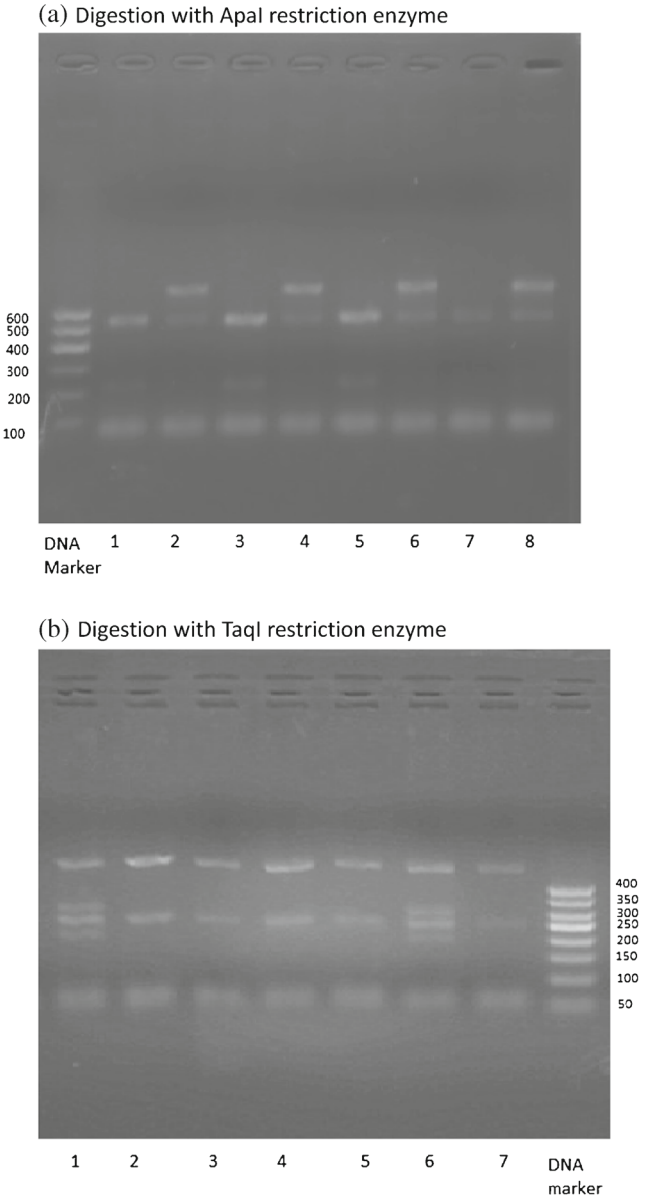

Figure 1. Digested products with restriction enzyme of ApaI and TaqI polymorphisms. Amplified DNA was digested using ApaI restriction enzyme at 25°C for 3 h using TaqI at 65°C for 6 h. The PCR product was applied to agarose gel containing 1 mg/mL ethidium bromide, electrophoresed at 120 V and photographed under ultraviolet light. (a) Digested products were applied to a 1.5% agarose gel, electrophoresis for 30 min. With reference to DNA marker, fragments of 740 bp could be written as 'AA', fragments of 515 and 225 bp as 'aa', and 740, 515, 225 bp as 'Aa'. For example, results in figure (a): 1, aa; 2, Aa; 3, aa; 4, Aa; 5, aa; 6, Aa; 7, aa; 8, Aa. (b) Digested products were applied to a 2.5% agarose gel, electrophoresis for 45 min. With reference to DNA marker, fragments of 495 and 245 bp could be written as 'TT', fragments of 290, 245, 205 bp as 'Tt' and 495, 290, 245, 205 bp as 'Tt'. For example, results in figure (b): 1, Tt; 2, TT; 3, TT; 4, TT; 5, TT; 6, Tt; 7, TT.

No significant difference between cases and controls was observed in genotype and allele frequencies of VDR-ApaI ($P = 0.30$ and 0.21) and VDR-FokI ($P = 0.37$ and 0.36) (table 2). For the TaqI polymorphism, 422 (79.8%) subjects were TT and 110 (20.2%) subjects were Tt. There were significant differences in genotype frequencies of VDR-TaqI between obesity and control groups (OR = 2.99; 95% CI: 1.86–4.79). The 'T' allele distribution in the obesity group was significantly higher than control group ($P < 0.001$), and the OR for 'T' was 2.67 (95% CI: 1.70–4.18). It still shows significant association between TaqI and obesity (OR = 3.79, 95% CI: 2.195–6.545; $P < 0.001$) in logistic model. Age, blood pressure, postprandial blood-glucose were also associated with obesity (table 3). Further, individuals with TT of TaqI had larger waist circumference ($P = 0.003$) and hip circumference ($P = 0.004$) than Tt (not shown), but no associa-

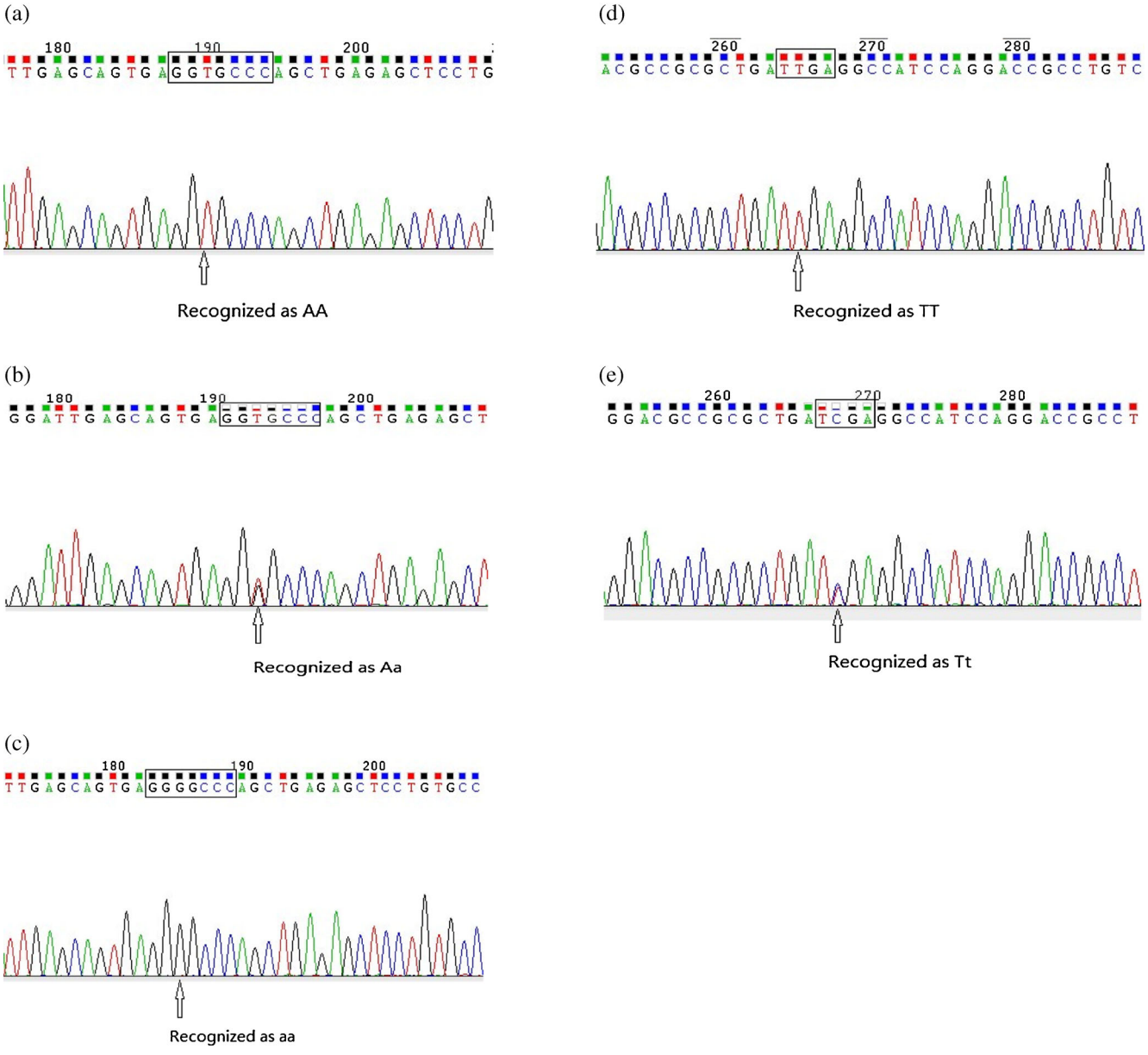

Figure 2. The sequence of VDR-ApaI and VDR-TaqI polymorphisms. *Apa*I restriction enzyme identified the sequence of 5′-GGGCC/C-3′, *Taq*I restriction enzyme identified 5′-T/CGA-3′. PCR products could be digested into separated fragments when restriction enzyme site existed, and the genotype could be recognized as aa or tt. PCR products could not be digested into separated fragments without any restriction enzyme site of ApaI and TaqI restriction enzyme, and could be recognized as AA or TT. (a) The sequence of ApaI polymorphism is 5′-GGTGCCC-3′ which could not be digested by *Apa*I restriction enzyme, so it could be written as 'AA'. (b) The sequence of ApaI polymorphism is 5′-GGT(C)GCCC-3′ which means it is a heterozygote and could be written as 'Aa'. (c) The sequence of ApaI polymorphism is 5′-GGGGCCC-3′ which could be totally digested by *Apa*I restriction enzyme, so the homozygote could be written as 'aa'. (d) The sequence of TaqI polymorphism is 5′-TTGA-3′ which could not be digested by TaqI restriction enzyme, and it could be written as 'TT'. (e) The sequence of TaqI polymorphism is 5′-TC(T)GA-3′ which means a heterozygote could be partly digested and could be written as 'Tt'.

tion between any polymorphism and insulin section (FBG, PBG), blood pressure (SBP, DBP), fat metabolism indexes (TC, TG) was observed.

Analysis of VDR haplotype frequencies and obesity

The FaT haplotype (FokI-C, ApaI-G, TaqI-T) was the most common haplotype in both controls and cases (table 4), and we used the FaT haplotype as the reference category to observe OR or 95% CI in logistic regression analysis. It revealed that the FAt of VDR haplotype was associated with obesity and FAt was inversely associated with obesity (OR = 0.16; 95% CI, 0.07–0.34).

Discussion

More than 25 polymorphisms have known to be present at the VDR gene (Uitterlinden *et al.* 2004). ApaI and TaqI are nonfunctional polymorphisms, ApaI located in intron 8 does not affect splicing site and transcription factor-binding site, TaqI located in exon 9 does not change the amino acid sequence of the encoded protein. These two polymorphisms located near the 3′ end of the gene, the 3′UTR is known to be involved in regulation of expression, especially through regulation of mRNA stability. FokI, located in exon 2, results in different translation initiation sites on VDR. This is the only known VDR polymorphism that translates in different protein products (Uitterlinden *et al.* 2004).

We analysed three VDR polymorphisms, including ApaI, TaqI and FokI. Our study provided evidence that one of the VDR gene polymorphisms, TaqI, was associated with obesity in Chinese population. Meanwhile, the OR for TT was 2.99 (95% CI: 1.86–4.79), which meant genotype of TT increased the risk of developing obesity. TT genotype also showed a significant association with obesity after adjustments for age, sex, smoking, drinking, blood pressure, blood glucose, triglyceride and cholesterol (OR = 3.79, 95% CI: 2.195–6.545). Individuals with TT of TaqI were in larger waist circumference and hip circumference. It was confirmed that 'T' allele of TaqI was associated with obesity. This is consistent with other studies in which TT genotype of the TaqI polymorphism presented a significantly higher weight and BMI (Ye *et al.* 2001; Binh *et al.* 2011). Similar results were shown in another study that T allele of TaqI polymorphism was in sigificant association with obesity (Vasilopoulos *et al.* 2013).

In terms of VDR gene no significant association was observed between the polymorphism of ApaI and FokI, and obesity. It is consistent with a previous study in Chinese male nuclear families that no significant association was obtained between genotypes or haplotypes of the VDR polymorphisms with fat mass and BMI (Gu *et al.* 2009). However, the former study (Binh *et al.* 2011) reported that the ApaI polymorphism of VDR gene was statistically significantly associated with being overweight and obesity in postmenopausal Vietnamese women. These results have pointed out the limitation of single polymorphism analysis, thus, we took further analysis of haplotypes. As a result, VDR haplotype FAt has been shown as a protective role in obesity as reference of FaT (OR = 0.16; 95% CI: 0.07–0.34), which means individuals with haplotype of FAt had decreased risk of obesity (figures 1 and 2).

Although vitamin D receptor is involved in regulating some genes related to cholesterol metabolism (He *et al.* 2011), its role in the regulation of serum cholesterol seems to be minimal (Wang *et al.* 2009), which was consistent with our study that none of VDR polymorphisms was related to the levels of blood cholesterol or triglyceride.

Different ethnic gene and allele variations are in different frequencies, studies have revealed that VDR polymorphisms across ethnics were correlated with different incidences of many diseases. Thus, it is necessary to investigate whether there was any association between the VDR polymorphisms and obesity in Chinese population. The discordance in our study with former ones may be resulted from different ethnics (Greek (Vasilopoulos *et al.* 2013) and Vietnam (Binh *et al.* 2011)). Although, in our study, it is confirmed that VDR gene polymorphism (TaqI) is associated with obesity, the possible role of VDR variants in obesity remains to be clarified by further studies.

In conclusion, our study suggests that TaqI of VDR polymorphisms is associated with obesity in Chinese population. The genotype TT and allele T of TaqI may be potential predictors related to obesity and the haplotype FAt appears to predispose protective role for obesity.

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References

Bai Y., Yu Y., Yu B., Ge J., Ji J., Lu H. *et al.* 2009 Association of vitamin D receptor polymorphisms with the risk of prostate cancer in the Han population of Southern China. *BMC Med. Genet.* **10**, 125.

Bell C. G., Walley A. J. and Froguel P. 2005 The genetics of human obesity. *Nat. Rev. Genet.* **6**, 221–234.

Binh T. Q., Nakahori Y., Hien V. T., Khan N. C., Lam N. T., Mai le B. *et al.* 2011 Correlations between genetic variance and adiposity measures, and gene × gene interactions for obesity in postmenopausal Vietnamese women. *J. Genet.* **90**, 1–9.

Bouillon R., Carmeliet G., Lieben L., Watanabe M., Perino A., Auwerx J. *et al.* 2014 Vitamin D and energy homeostasis of mice and men. *Nat. Rev. Endocrinol.* **10**, 79–87.

Ding C., Gao D., Wilding J., Trayhurn P. and Bing C. 2012 Vitamin D signalling in adipose tissue. *Br. J. Nutr.* **108**, 1915–1923.

Gu J. M., Xiao W. J., He J. W., Zhang H., Hu W. W., Hu Y. Q. *et al.* 2009 Association between VDR and ESR1 gene polymorphisms with bone and obesity phenotypes in Chinese male nuclear families. *Acta Pharmacol. Sin.* **30**, 1634–1642.

Haussler M. R., Whitfield G. K., Haussler C. A., Hsieh J. C., Thompson P. D., Selznick S. H. *et al.* 1998 The nuclear vitamin D receptor: biological and molecular regulatory properties revealed. *J. Bone Miner. Res.* **13**, 325–349.

He Y. H., Song Y., Liao X. L., Wang L., Li G., Alima *et al.* 2011 The calcium-sensing receptor affects fat accumulation via effects on antilipolytic pathways in adipose tissue of rats fed low-calcium diets. *J. Nutr.* **141**, 1938–1946.

Lementowski P. W. and Zelicof S. B. 2008 Obesity and osteoarthritis. *Am. J. Ortho.* **37**, 148–151.

Mishra D. K., Wu Y., Sarkissyan M., Sarkissyan S., Chen Z., Shang X. *et al.* 2013 Vitamin D receptor gene polymorphisms and prognosis of breast cancer among African–American and Hispanic women. *PloS One* **8**, e57967.

Shen J., Goyal A. and Sperling L. 2012 The emerging epidemic of obesity, diabetes, and the metabolic syndrome in China. *Cardiol. Res. Pract.* **2012**, 178675.

Uitterlinden A. G., Fang Y., Van Meurs J. B., Pols H. A. and Van Leeuwen J. P. 2004 Genetics and biology of vitamin D receptor polymorphisms. *Gene* **338**, 143–156.

Uitterlinden A. G., Fang Y., Van Meurs J. B., Van Leeuwen H. and Pols H. A. 2004 Vitamin D receptor gene polymorphisms in relation to vitamin D related disease states. *J. Steroid Biochem. Mol. Biol.* **89–90**, 187–193.

Vasilopoulos Y., Sarafidou T., Kotsa K., Papadimitriou M., Goutzelas Y., Stamatis C. *et al.* 2013 VDR TaqI is associated with obesity in the Greek population. *Gene* **512**, 237–239.

Wang J. H., Keisala T., Solakivi T., Minasyan A., Kalueff A. V. and Tuohimaa P. 2009 Serum cholesterol and expression of ApoAI, LXRbeta and SREBP2 in vitamin D receptor knock-out mice. *J. Steroid Biochem. Mol. Biol.* **113**, 222–226.

Wong K. E., Kong J., Zhang W., Szeto F. L., Ye H., Deb D. K. *et al.* 2011 Targeted expression of human vitamin D receptor in adipocytes decreases energy expenditure and induces obesity in mice. *J. Biol. Chem.* **286**, 33804–33810.

Ye W. Z., Reis A. F., Dubois-Laforgue D., Bellanne-Chantelot C., Timsit J. and Velho G. 2001 Vitamin D receptor gene polymorphisms are associated with obesity in type 2 diabetic subjects with early age of onset. *Eur. J. Endocrinol.* **145**, 181–186.

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