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Association of vitamin D receptor gene polymorphisms with end-stage renal disease and the development of high-turnover renal osteodystrophy in a Chinese population

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ABSTRACT. Two single nucleotide polymorphisms (SNPs; *Taq*I and *Apa*I) in the vitamin D receptor (VDR) gene have been identified as risk factors for the progression of end-stage renal disease (ESRD). The purpose of our study was to confirm the reported association of these two SNPs with ESRD risk and progression of renal osteodystrophy in a Chinese Han population. A total of 452 ESRD patients and 904 matched-pair controls (based on age, gender, and body mass index) were included. Identification of VDR gene polymorphisms was performed using the polymerase chain reaction-restriction fragment length polymorphism method with *Taq*I and *Apa*I restriction enzymes.

There was no association of the *Taq*I polymorphism with ESRD risk. However, significant associations were seen between *Apa*I (rs7975232) polymorphism and ESRD risk in the heterozygote model (AC/ AA; P = 0.002; OR = 1.4, 95%CI = 1.14-1.83), homozygote model (CC/AA; P = 0.007; OR = 1.8, 95%CI = 1.17-2.85) genotypes for rs7975232, allelic model (P < 0.001; OR = 1.4, 95%CI = 1.15-1.64), dominant model (P = 0.001; OR = 1.5, 95%CI = 1.19-1.87), and recessive model (P = 0.046; OR = 0.6, 95%CI = 0.42-1.00) between cases and healthy controls Moreover, we found a significant correlation between the genotype and allele distribution of *Apa*I and intact parathyroid hormone (iPTH) levels, where allele C carriers have increased iPTH levels. The *Apa*I polymorphism in the VDR gene appears to be a susceptibility locus for ESRD in Chinese individuals, and allele C carriers may have an increased risk of high-turnover renal osteodystrophy.

Key words: *Apa*I; *Taq*I; End-stage renal disease; Polymorphism; Renal osteodystrophy

INTRODUCTION

Chronic kidney disease (CKD) is an important public health problem with a worldwide incidence of 10% (Sarnak et al., 2003). CKD can lead to decreased life expectancy and quality of life and to enormous costs for patients and health systems (Meguid El Nahas and Bello, 2005). End-stage renal disease (ESRD) is the partial or total loss of kidney function (Duseja et al., 2012). It is well recognized that CKD leading to ESRD is caused by complex pathogenic mechanisms that result from the interactions between multiple genes and environmental factors (Adler, 2006; Böger and Heid, 2011; Dwivedi et al., 2011). Due to the difficulty in stabilizing the disease, patients often develop various abnormalities in bone and mineral metabolism, which significantly increases morbidity and mortality (Erturk and DialGene Consortium, 2006). Therefore, the determination of causative genes leading to ESRD predisposition and its subsidiary complications could provide a better understanding for the pathogenesis of the disease and ensure optimal diagnosis and treatment.

Numerous studies have demonstrated that genetic factors are involved in the onset of ESRD (Borkar et al., 2011; George and Mittal, 2011; Prakash et al., 2012). As a disorder that impairs all metabolic processes associated with vitamin D, ESRD is unsurprisingly associated with vitamin D deficiency (Dusilová Sulková, 2012), which can lead to a series of complications in patients with renal disease. Hence, vitamin D supplementation is usually applied as an adjunct therapy in patients with renal disease, including ESRD (Dusilová Sulková, 2012). The functions of vitamin D in the body are initially mediated by vitamin D receptors (VDRs) (Gross et al., 1998) and clinical studies have reported a better prognosis in ESRD patients treated with VDR activators (Dusilová Sulková, 2012).

The VDR gene is mapped on chromosome 12q12-14 and consists of 11 exons, spanning 63,495 bp (Morrison et al., 1994). VDR has previously been investigated as a candidate gene involved in CKD risk. Non-BB (Bb + bb) variants of the VDR *Bsm*I gene polymorphism are reported to be associated with a higher risk of developing hypercalcemia in peritoneal dialysis patients (Akcay et al., 2005). Two other single nucleotide polymorphisms (SNPs), rs731236 (*Taq*I) and rs7975232 (*Apa*I), in the VDR gene have also been identified as risk factors in the

progression of CKD. However, the results remain controversial among the reported studies, and the effect of these SNPs in the VDR gene on ESRD risk in the Chinese population remains unclear (Fernández et al., 1997; Wang et al., 2004; El-Shehaby et al., 2013). Furthermore, vitamin D and its receptor have important roles in determining calcium-regulated parathyroid hormone (PTH) secretion (Brown et al., 1982). Few studies have reported the association of genetic variants of the VDR gene with the development of renal osteodystrophy (RO) in patients with ESRD. Therefore, the purpose of this study was to investigate whether two SNPs in the VDR gene (*Taq*I and *Apa*I) are associated with ESRD risk and RO progression.

MATERIAL AND METHODS

The study protocol was approved by the review board of Tai'an City Central Hospital (China) and informed consent was obtained from each participant. A total of 452 Chinese hemodialysis patients (248 males and 204 females) with ESRD were enrolled from our hospital between January 2001 and December 2014. Patients were undergoing hemodialysis for periods ranging from 6 to 276 months (79 ± 68 months). Patients took calcium carbonate to retain a serum phosphorus concentration of less than 6 mg/dL, and calcitriol or alpha-calcidiol unless their serum calcium levels exceeded 11 mg/dL. Patients with liver disease or overt infections were excluded from the study based on their serum gamma glutamyl transferase (GGT) activity. All female patients were not pregnant. A 1:2 matched-pair control group included a total of 904 healthy individuals based on age, gender, and body mass index (BMI). These individuals were selected from those who requested general health examinations in the same hospital during the same period. Demographics of all subjects were collected from a questionnaire survey or medical records. These data included age, gender, BMI, blood pressure, blood biochemical markers [e.g., intact PTH (iPTH), triglycerides], and diabetes.

Blood sampling

A fasting blood sample was obtained from each patient mid-week on a non-dialysis day. Approximately 10 mL of venous blood was collected from each participant for DNA extraction and analysis of iPTH, vitamin D [1,25$(OH)_2$D3], calcium, phosphate, creatinine, blood urea nitrogen, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, and albumin. All samples were stored at -20°C until analysis.

Serum iPTH levels were determined using a two-site chemiluminometric assay (Magic Lite iPTH immunoassay, Ciba-Corning Diagnostics Corp., Medfield, MA, USA). Serum vitamin D levels were measured using a commercial competitive protein-binding assay with automated chemiluminescence.

Genotyping

Genomic DNA was extracted from the whole blood samples using a DNA extraction kit (QIAamp DNA mini Kit, Qiagen, Hilden, Germany). All PCR primers were designed based on sequences previously published (Narooie-Nejad et al., 2015). The *Taq*I and *Apa*I polymorphisms were verified by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. The primers sequences were as follows: *Taq*I, 5'-GGGACGAT GAGGGATGGACAGAGC-3' (forward) and 5'-GGAAAGGGGTTAGGTTGGACAGGA-3'

(reverse); and *Apa*I, 5'-AGAGCATGGACAGGGAGCAAGGCCAGGCAG-3' (forward) and 5'-GCGCAGGTCGGCTAGCTTCTGGATCATC-3' (reverse). The annealing temperatures for *Apa*I and *Taq*I SNP were 68° and 65°C, respectively. The two SNPs were distinguished by digestion with the *Apa*I and *Taq*I restriction enzymes. Approximately 10% of the samples analyzed were duplicated to confirm genotyping quality.

Statistical analysis

All statistical analyses were performed by using the statistical software SPSS 18 (SPSS, Chicago, IL, USA). The distribution of genotypes and alleles in healthy controls was assessed for deviation from Hardy-Weinberg equilibrium. Differences in genotype and allele distribution between patients and controls were analyzed using independent sample *t*-test and χ^2 test. The OR and 95%CI were also estimated. A P value < 0.05 was considered statistically significant.

RESULTS

For this study, we recruited 452 ESRD patients with a mean age of 58.2 ± 10.3 years and 904 unrelated healthy controls with a mean age of 57.9 ± 10.2 years. Forty-three ESRD patients had non-insulin-dependent diabetes mellitus and 312 had hypertension. All clinicopathological features of patients are displayed in Table 1. Genotype frequencies for *Taq*I and *Apa*I polymorphisms were in keeping with the Hardy-Weinberg equilibrium (P > 0.05).

Table 1. Demographics of ESRD patients included in the study.

Variables	ESRD cases (N = 452)
Age (years)	58.2 ± 10.3
Gender (M/F)	248/204
BMI	26.3 ± 3.81
Hypertension	312 (69.0%)
Diabetes mellitus	97 (21.5%)
LDL cholesterol (mM)	2.8 ± 1.44
HDL cholesterol (mM)	1.2 ± 0.65
Triglyceride (mM)	2.4 ± 1.41
Blood urea nitrogen (mM)	7.7 ± 4.54
Serum creatinine (μM)	152.9 ± 162.92
Plasma calcium (mM)	2.7 ± 0.21
Serum phosphate (mM)	1.7 ± 0.09
Serum albumin (g/L)	41 ± 6
Serum $1,25(OH)_2D3$ (ng/mL)	15 ± 2.91
Serum iPTH (pg/mL)	49.4 ± 42

M = male; F = female; BMI = body mass index; LDL = low-density lipoprotein; HDL = high-density lipoprotein; iPTH = intact parathyroid hormone.

As shown in Table 2, the allele and genotype frequencies of VDR SNPs (*Taq*I and *Apa*I) in ESRD patients were examined. When the *Taq*I TT homozygous genotype is used as the reference group, no associations were observed between ESRD risk and the CC and TC genotypes. Similarly, we found no associations between ESRD risk and allelic, dominant, or recessive models. For the *Apa*I SNP, there was a positive correlation between ESRD risk and the AC (P = 0.002; OR = 1.4, 95%CI = 1.14-1.83) and CC (P = 0.007; OR = 1.8, 95%CI = 1.17-2.85) genotypes when the AA genotype is used as the reference group. In addition, the frequency of the C allele of the *Apa*I polymorphism was significantly higher in ESRD patients than in healthy controls (P < 0.001; OR = 1.4, 95%CI = 1.15-1.64). Furthermore, we found

a significant difference in dominant (P = 0.001; OR = 1.5, 95%CI = 1.19-1.87) and recessive (P = 0.046; OR = 0.6, 95%CI = 0.42-1.00) models between ESRD cases and healthy controls.

Table 2. Distribution of genotypes and alleles of the VDR polymorphisms in patients with ESRD and healthy controls.

	ESRD (N = 452)	Control (N = 904)	OR (95%CI)	P value
*Taq*I				
Genotypic model:				
TT	215 (47.6%)	474 (52.4%)	1.00	Reference
TC	197 (43.6%)	358 (39.6%)	1.2 (0.96-1.54)	0.110
CC	40 (8.8%)	72 (8.0%)	1.2 (0.81-1.86)	0.342
Allelic model:				
T	627 (69.4%)	1306 (72.2%)	1.00	Reference
C	277 (30.6%)	502 (27.8%)	1.1 (0.96-1.37)	0.119
Dominant model:				
TT	215	474	1.00	Reference
TC+CC	237	430	1.2 (0.97-1.52)	0.091
Recessive model:				
CC	40	72	1.00	Reference
TC+TT	412	832	0.9 (0.60-1.34)	0.577
*Apa*I				
Genotypic model:				
AA	206 (45.6%)	502 (55.5%)	1.00	Reference
AC	207 (45.8%)	350 (38.7%)	1.4 (1.14-1.83)	0.002
CC	39 (8.6%)	52 (5.8%)	1.8 (1.17-2.85)	0.007
Allelic model:				
A	619 (68.5%)	1354 (74.9%)	1.00	Reference
C	285 (31.5%)	454 (25.1%)	1.4 (1.15-1.64)	<0.001
Dominant model:				
AA	206	502	1.00	Reference
AC+CC	246	402	1.5 (1.19-1.87)	0.001
Recessive model:				
CC	39	52	1.00	Reference
AA+AC	413	852	0.6 (0.42-1.00)	0.046

Subsequently, we investigated the relationship between the distribution of the *Apa*I polymorphism and clinicopathological features in ESRD patients (Table 3). We only found a significant correlation between the *Apa*I genotype or allele distributions and serum iPTH levels. On the basis of their median serum iPTH levels, these 452 patients were divided into two groups (low and high iPTH). Further analysis revealed that carriers of the C allele have increased levels of iPTH (P = 0.005).

Table 3. Distribution of genotypes and alleles of the *Apa*I SNP in the VDR gene in relation to clinicopathological features of ESRD patients.

Variables	AA (N = 206)	AC (N = 207)	CC (N = 39)	P value	A (N = 619)	C (N = 285)	P value
Age (<55/≥55)	90/116	98/109	24/15	0.089	278/341	146/139	0.077
Gender (M/F)	102/103	113/94	25/14	0.105	317/302	163/122	0.094
BMI	26.3 ± 3.79	26.7 ± 3.84	26.8 ± 4.13	0.525	26.4 ± 3.81	26.9 ± 3.98	0.317
Hypertension (Y/N)	92/114	100/107	20/19	0.468	284/335	140/145	0.364
Diabetes mellitus (Y/N)	45/161	42/165	10/29	0.793	132/487	62/223	0.884
LDL cholesterol (mM)	2.7 ± 1.43	2.9 ± 1.52	2.9 ± 1.62	0.481	2.7 ± 1.53	2.9 ± 1.72	0.248
HDL cholesterol (mM)	1.4 ± 0.55	1.3 ± 0.43	1.2 ± 0.49	0.328	1.4 ± 0.58	1.2 ± 0.39	0.334
Triglyceride (mM)	2.3 ± 1.52	2.5 ± 1.43	2.6 ± 1.66	0.587	2.4 ± 1.59	2.5 ± 1.62	0.493
Blood urea nitrogen (mM)	7.5 ± 4.34	7.7 ± 4.58	7.9 ± 4.61	0.283	7.6 ± 4.41	7.8 ± 4.66	0.145
Serum creatinine (μM)	149.2 ± 159.29	153.1 ± 161.87	153.9 ± 164.47	0.102	151.6 ± 161.62	154.1 ± 164.12	0.083
Plasma calcium (mM)	2.5 ± 0.19	2.7 ± 0.21	2.8 ± 0.23	0.101	2.6 ± 0.19	2.9 ± 0.22	0.095
Serum phosphate (mM)	1.9 ± 0.09	1.8 ± 0.06	1.6 ± 0.11	0.113	1.9 ± 0.08	1.7 ± 0.13	0.104
Serum albumin (g/L)	40 ± 5	42 ± 7	43 ± 6	0.426	40 ± 8	43 ± 5	0.378
Serum 1,25$(OH)_2$D3 (ng/mL)	16 ± 2.91	14 ± 2.89	14 ± 2.95	0.221	15 ± 2.93	14 ± 3.12	0.203
Serum iPTH (< 42/≥ 42)[a] (pg/mL)	100/106	90/117	11/28	0.038*	290/329	112/173	0.005*

M = male; F = female; Y = yes; N = no. [a]ESRD patients were divided into two groups according to the median value of iPTH levels. *P value was significant.

DISCUSSION

In this study, we investigated the potential effect of two SNPs (*Taq*I and *Apa*I) in the VDR gene on ESRD risk and development of RO in a Chinese population. This case-control association study involved 452 patients with ESRD and 904 matched-pair healthy controls. The comparison of genotype and allele distribution between these two groups revealed that the C allele of the *Apa*I polymorphism is significantly associated with ESRD risk. This is in accordance with previous findings on the association of VDR polymorphisms with renal function and progression of CKD (Fernández et al., 1997; Cozzolino et al., 2006; Pourfarzam et al., 2014). Moreover, the presence of the C allele of the *Apa*I polymorphism may dictate susceptibility for RO.

Vitamin D receptor gene polymorphisms are identified by the presence or absence of a restriction site for the enzymes *Bsm*I, *Apa*I, and *Taq*I at the 3' untranslated region and *Foq*I at the N-terminal region of the gene by RFLP. VDR gene polymorphisms were initially associated with bone metabolism and prediction for bone mass in subjects without renal disease (Morrison et al., 1994). Since then, several studies have explored the influence of VDR gene polymorphisms on bone and mineral metabolism abnormalities in patients with ESRD. The association of the *Apa*I polymorphism in the VDR gene with bone and mineral metabolism has been investigated in hemodialysis patients. Yokoyama et al. (1998) reported that serum PTH and osteocalcin levels among patients with the CC genotype were almost twice as high as in patients carrying the A allele. Consistent with their results, our study revealed that the C allele of the *Apa*I polymorphism is significantly associated with ESRD risk. In addition, patients carrying the C allele were more sensitive to changes in serum calcium levels than those with the A allele (Yokoyama et al., 2001). Therefore, the presence of the C allele in the *Apa*I SNP is a positive indicator for the development of secondary hyperparathyroidism.

In CKD, abnormal calcitriol function and metabolism play a critical role in the development of secondary hyperparathyroidism and RO (Slatopolsky et al., 1999). Several mechanisms associated with an impaired calcitriol/VDR interaction have been identified in CKD. Due to decreased renal mass and serum phosphate retention in ESRD, renal production of calcitriol significantly decreases, and reduced expression of both VDR and the retinoid X receptor has also been found in the kidney and parathyroid gland (Dusso, 2003). The effect of VDR gene polymorphisms on the response of the parathyroid gland to calcitriol has also been investigated (Alvarez-Hernández et al., 2003). High-turnover bone diseases are characterized by rapid bone turnover stimulated by high serum PTH levels and abnormal mineralization. Alvarez-Hernández et al. (2003) reported that the response of the parathyroid glands to calcitriol was not related to VDR gene polymorphisms *Apa*I or *Taq*I. However, in our study, we observed that allele C of the *Apa*I polymorphism in the VDR gene was associated with increased iPTH levels, and we concluded that this allele may be indicative of RO. To clarify this, further studies on the influence of VDR genetic variants on RO are needed.

There were several limitations in this case-control study. First, since it is a retrospective case-control study, inherent selection bias cannot be excluded. Second, we did not replicate another investigation in other independent subject panels or in other ethnic groups to confirm the validation of our findings in this study. Furthermore, the sample size in our study was relatively small and therefore some bias is inevitable.

In conclusion, the *Apa*I polymorphism of the VDR gene may be a susceptibility locus for ESRD in Chinese individuals, and allele C carriers appear to have an increased risk of RO.

Genotype determination of these SNPs may be more informative for assessment of the genetic risk of ESRD in Chinese individuals.

Conflicts of interest

The authors declare no conflict of interest.

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