Int J Mol Epidemiol Genet 2018;9(5):55-63
www.ijmeg.org /ISSN:1948-1756/IJMEG0081510

Original Article

Study on the association between vitamin D receptor gene fokI (T/C) polymorphisms and the susceptibility to type 2 diabetic kidney disease of Han nationality in south of China

Xia Dong, Dan Yang, Rui Han, Wei Yang, Wei Pang, Dianping Song, Rou Shi

The Diabetes Department of 1st Hospital Affiliated to Kunming Medical University, Kunming, Yunnan, China

Received June 20, 2018; Accepted August 12, 2018; Epub October 20, 2018; Published October 30, 2018

Abstract: Aims: To investigate the distribution of vitamin D receptor fokI gene polymorphism in Yunnan Han population, and to explore the relationship between SNP of fokI and type 2 diabetic kidney disease. Methods: We included 276 individuals of Han population of Yunnan in this study: 91 type 2 diabetes patients without kidney disease (DM group), their duration of diabetes is more than 10 years, 89 type 2 diabetes patients with diabetic kidney disease (DKD group), their duration of diabetes is less than 10 years and 96 healthy controls (NC group). We compared the concentration of 25 hydroxy vitamin D in different groups and used taqman probe to detect the genotype and allele of fokI, then analysed the relationship between the polymorphisms of fokI and the susceptibility of diabetic kidney disease. Results: (1) NC group had a significantly higher plasma concentrations of 25 (OH) D than DKD group and DM group ($P < 0.01$); (2) 25 (OH) D and age, BMI, HbA1c, TG showed a weak negative correlation ($P < 0.01$); (3) Genotype of fokI showed no differences in DM group and DKD group, same as in DM group and NC group; FF genotype in DKD group is relatively lower than NC group ($P < 0.05$), and there is no difference in Ff and ff genotype ($P > 0.05$); In DKD group, f allele was 53.4%, higher than DM group ($RR = 1.46$, $P < 0.05$); (4) Logistic regression analysis showed that ff genotype may be a susceptible factor for DKD ($P = 0.04$, $OR = 2.37$). Conclusion: FokI Ff genotype accounted for a larger proportion of the Han population in Yunnan, and ff genotype may be a susceptible factor for DKD in Yunnan Han population.

Keywords: Single nucleotide polymorphism, diabetes mellitus, diabetic kidney disease, receptor, calcitriol

Introduction

Diabetic kidney disease (DKD) is one of most common microvascular complications of diabetes, which is a progressive chronic inflammatory vascular disease mediated by the immune system in the multivariate pathological condition. In America and part of European countries. DKD is the main cause of end-stage renal disease (ESRD). Based on the investigations of Chinese dialysis patients in 2008, the percentage of DKD patients has increased up to 19%, and it is still on the increase compared to the previous years [1]. In China DKD is probably becoming the primary cause of ESRD instead of glomerular nephritis [2], which greatly reduced the quality of diabetics' life. According to domestic and foreign researches, the causes of DKD are related to not only the level of blood sugar or pressure, but also the genetic and environment factors [3].

The Epidemiological data showed that about 30%-40% type 2 diabetics develop into DKD [4]. It means that the hyperglycemia is not the only cause for DKD. Additionally, DKD are affected by blood pressure, blood fats, atherosclerosis angiocardiopathy and Vitamin D [5-7]. The deficiency of Vitamin D appears frequently in DKD patients. By comparing vitamin D concentrations in patients with type 2 diabetic kidney disease, researchers found that [8] the deficiency of 25 hydroxy vitamin D_3 accounted for 47.01% among those patients or even higher [7]. Vitamin D prevented kidney from injury caused by hyperglycemia in many ways. Animal tests show that Vitamin D and signal pathways of Vitamin D in glomerular podocyte played an important role in preventing diabetic kidney damage [9, 10]. 1,25 $(OH)_2$ D_3, as a signal molecule, combined with VDR in target cell, it forms hormone receptor complexes, then affected the expression of gene's structure by acting on

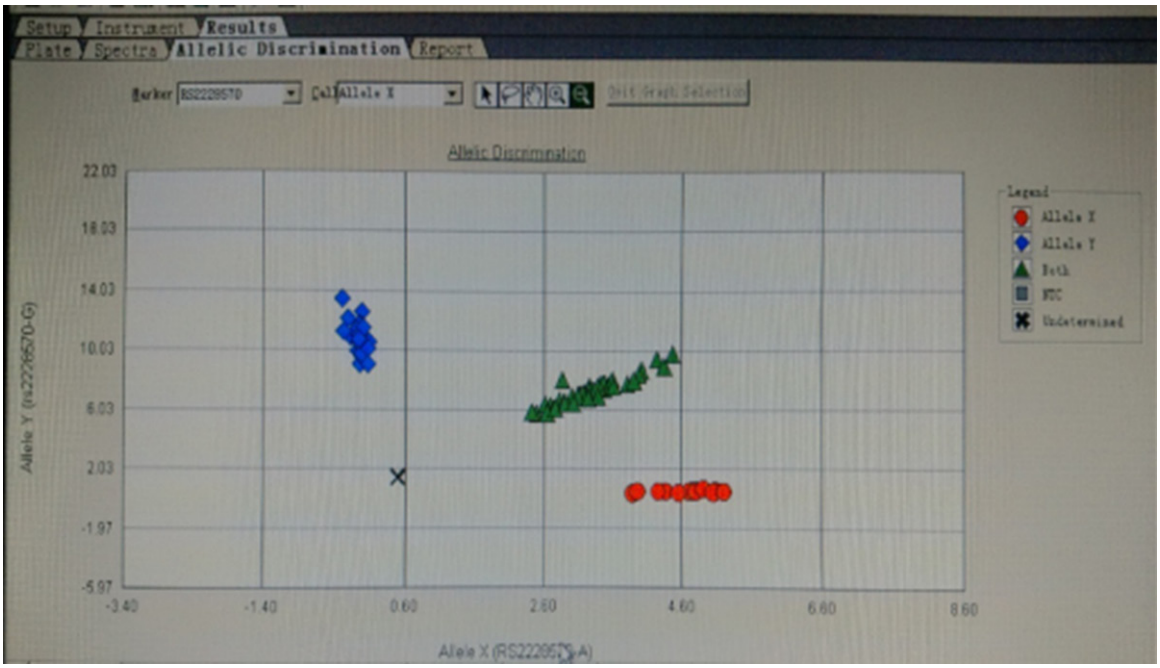

Figure 1. Photograph for fokI polymorphisms by Allelic Discrimination software in ABI 7300 real-time PCR Instrument.

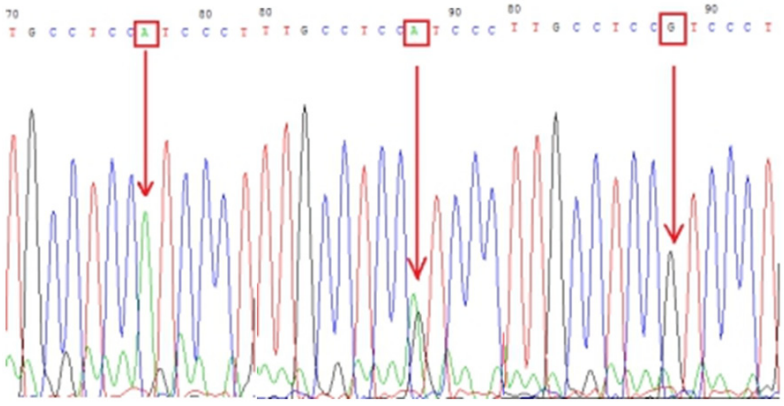

Figure 2. The sequencing results of foki polymorphisms. The left one is homozygote ff (T/T), the middle one is heterozygote Ff (T/C), the right one is homozygote FF (C/C).

specific DNA of target gene. The key is that the different activity of VDR determined the difference of transcriptional activity in the progress. Therefore, we infer that Vitamin D receptor gene is related to diabetic kidney disease closely.

The relatively common SNP sites of VDR gene are ApaI, BsmI, FokI and TaqI, polymorphisms of fokI can lead to the differences in transcriptional activity, thus affecting the VDR-mediated effect. There are some studies about fokI polymorphism in type 2 diabetes and diabetic kidney disease at home and abroad [6, 11-13], but the results of susceptibility to DKD were different, and the distribution of VDR gene in the Han population in Yunnan is not reported at present. Therefore, our study focused on the frequencies and distribution of VDR fokI allele and genotype in Yunnan to investigate the relationship between polymorphisms of fokI and the susceptibility for type 2 diabetic kidney disease.

Materials and methods

Study participants and clinical data

We included 276 individuals of Han population of Yunan from October 2014 to December 2015 in this study: 91 type 2 diabetes patients without kidney disease (DM group), their duration of diabetes is more than 10 years; 89 type 2 diabetic kidney disease patients (male ≥ 22 ug/mg, female ≥ 31 ug/mg) (DKD group), their duration of diabetes is less than 10 years and 96 healthy controls (NC group). The diagnosis

Table 1. The comparison of general data and biochemical indexes among the three groups [x ± s/M (QR)]

Variables	Group		
	Group DM (n = 91)	Group DKD (n = 91)	Group NC (n = 96)
Age (years)	59.7 ± 8.7	53.8 ± 11.7★,△	57.9 ± 8.1
Gender (male/female)	44/47	57/32△	50/46
SBP (mmHg)	126 ± 19★	127 ± 17★	110 (11.5)
DBP (mmHg)	77.4 ± 9.6	81.6 ± 10.6★,△	76 (10)
BMI (kg/m^2)	24.2 ± 2.8★	23.9 ± 3.4★	22.4 ± 2.7
FPG (mmol/L)	6.8 (3.9)★	6.7 (3.7)★	4.8 ± 0.4
Glycosylated hemoglobin (%)	8.3 (2.7)★	8.8 (2.6)★,▲	5.98 ± 5.0
FCP (ng/ml)	1.1 ± 0.6★	1.05 (0.7)★	1.65 (0.54)
PCP (ng/ml)	2.0 (1.9)	2.0 (2.4)	-
LDL-C (mmol/L)	2.7 ± 0.7	2.5 (1.4)★	2.9 (0.8)
TG (mmol/L)	1.6 (1.3)★	1.7 (1.9)★	0.94 (0.6)
TC (mmol/L)	4.2 ± 0.9★	4.4 ± 1.0	4.6 ± 0.6
HDL-C (mmol/L)	1.0 ± 0.3★	0.9 ± 0.2★	1.5 ± 0.4
Ca^{2+} (mmol/L)	2.2 ± 0.1	2.3 ± 0.1	2.29 ± 0.1
25 (OH) D (ng/ml)	18.1 ± 6.8★	17.6 ± 4.7★	24.6 ± 6.1
eGFR	106.4 ± 23.6	111.3 ± 28.4☆	103.1 ± 19.6
AS (%)	67	57	--

△p < 0.05 for comparison to group DM; ▲p < 0.01 for comparison to group DM; ☆p < 0.05 for comparison to group NC; ★p < 0.01 for comparison to group NC.

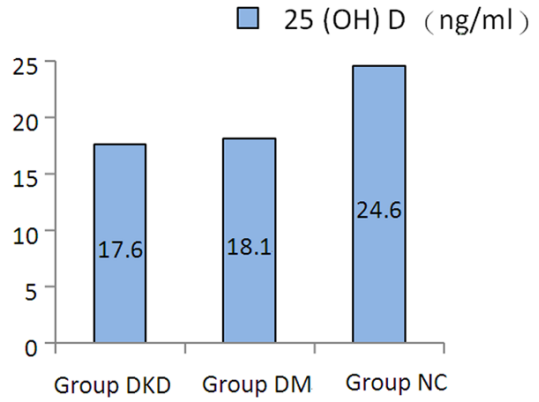

Figure 3. Comparition of 25 (OH) D among the three groups. 25 (OH) D_3 in group DKD is lower than DM group, but the difference was not statistically significant (P > 0.05). (25 OH) D_3 in NC group were significantly higher than group DKD and DM (P < 0.01).

of type 2 diabetes according to the criteria of WHO in 1999 [14], the diagnose of DKD refer to the prevention and treatment of type 2 diabetes in China (Chinese Diabetes Society, 2008) and type 2 diabetes duration of less than 10 years are included in diabetic kidney disease. Exclusion criteria: individuals with blood relationship involved in the study, impaired glucose tolerance, acute and chronic kidney disease, diabetic ketoacidosis, poisoning, urinary calculus, urinary tract infection recently, osteoporosis, cancer. The study is approved by the hospital ethics committee, and all subjects signed written informed consent.

Genotype determination

We used the blood genomic DNA Extraction Kit (supplied by Shanghai Lai Feng Biotechnology company) for DNA extraction, and strictly followed the instruction manual, and the purity and concentration of DNA were detected by Eppendorf AG spectrophotometer (Germany), and the extracted DNA was kept at -80°C until use. PCR reaction system was 15 ul in total, including DNA samples 1.5 ul, 2 × premix ex Taq (provided by Takara Bio company) 7.5 ul, Rox reference dye 0.3 ul and 40 × TaqMan SNP Genotyping Assay (supplied by the ABI) 0.4 ul and injection water 5.3 ul. The reaction conditions: initial denaturation at 95°C for 30 s, then performed 40 cycles: denaturation at 95°C for 5 s, annealing at 60°C for 31 s. Using Allelic discrimination Software to analysis all data and automatically read the genotype (**Figure 1**). Then selected three cases DNA with different genotypes to sequencing and to verify the SNP site (**Figure 2**).

Statistical analysis

SPSS 19.0 was used for statistical analysis. All of the data are applied to normality test, quantitative data of normal distribution were represented by mean ± sd (x ± s), quantitative data of skewness distribution were represented by median (interquartile range) M (Q_R). The qualitative data were represented by frequency and percentage. The comparison among groups were analyzed by two-independent-samples T test or variance analysis, the comparison of categorical data were analyzed by chi-square test, data with skewness distribution were ana-

Table 2. Pearson correlation analysis between 25 (OH) D and other variables

	FF	Ff	ff	age	BMI	gender	GH	FPC	LDL	HDL	TG	Ca^{2+}	AS
R	0.07	-0.02	-0.05	-0.25	-0.24	0	-0.35	0.13	-0.04	0.316	-0.29	-0.02	-0.05
p	0.26	0.68	0.43	0.00	0.00	0.98	0.00	0.05	0.51	0.00	0.00	0.85	0.61

Table 3. Genotype and allele frequencies of fokI gene polymorphisms among three groups

Genotype	Group					
	group DM (n = 91)	group DKD (n = 89)	group NC (n = 96)	P1	P2	P3
Ff (T/C)	50/54.9%	47/52.8%	45/46.9%	0.77	0.42	> 0.05
ff (T/T)	15/16.5%	24/27%	20/20.8%	0.08	0.4	> 0.05
FF (C/C)	26/28.6%	18/20.2%	31/32.3%	0.19	0.045	> 0.05
Allele						
f (T)	80/44%	95/53.4%	86/44.8%	0.046	0.09	0.36
F (C)	102/56%	83/46.6%	106/55.2%			

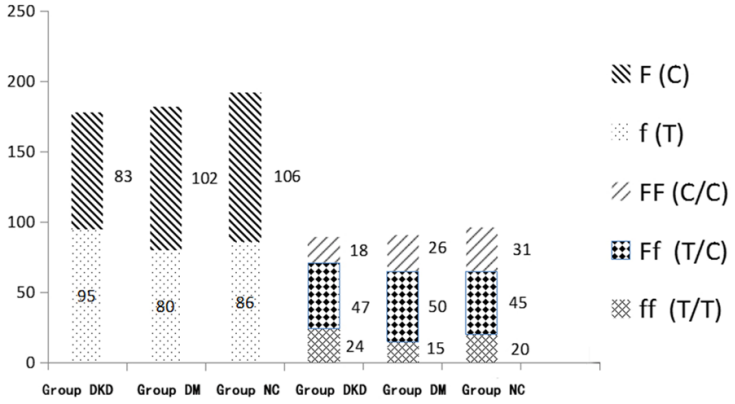

Figure 4. Comparison of genotype and allele among three groups. The genetype Ff, ff, FF accounted for 52.8%, 27%, 20.2% in group DKD, respectively, 54.9%, 16.5%, 28.6% in group DM and 46.9%, 20.8%, 32.3% in group NC. In group DKD genotype FF was relatively lower than group NC (χ^2 = 3.45, P = 0.045), and there is no difference in genotype ff, Ff (P > 0.05). The frequency of f was higher than group DM, which count for 53.4% in group DKD (P = 0.046).

Table 4. Relative risk of different genotypes and alleles of T2DM for DKD

Genotype	Group		RR
	group DM (n = 91)	group DKD (n = 89)	
Ff (T/C)	50/54.9%	47/52.8%	0.918
ff (T/T)	15/16.5%	24/27%	1.871
FF (C/C)	26/28.6%	18/20.2%	0.634
Allele			
f (T)	80/44%	95/53.4%	1.459
F (C)	102/56%	83/46.6%	0.685

lyzed by rank-sum test. Chi square test were used for hardy Weinberg equilibrium of genotype and allele, the comparison of genotype and allele were analyzed by chi-square test or Fisher exact test, and calculated the RR. We took fasting C peptide (FCP), gender, age, glycosylated hemoglobin (HbA1c), Triglyceride (TG), low density lipoprotein (LDL), High density lipoprotein (HDL), Body Mass Index (BMI), diastolic blood pressure (DBP), genotype as covariate, used binary logistic regression analysis to analyzed the relationship between fokI gene and diabetic kidney disease, and calculated odds ratio (OR) and 95% confidence interval (95% CI). P < 0.05 was considered statistically significant.

Results

There were no significant differences between group DM and DKD in BMI, FPG, LDL, FCP, HDL, Ca^{2+}, SBP, atherosclerosis (P > 0.05); there were statistical differences in age, SBP and HbA1C (P < 0.05), SBP and HbA1c in group DKD is higher than group DM; age in group DM is older than DKD (P < 0.05); SBP, BMI, FPG, HDL, TG in group NC were lower than other groups (P < 0.01), FCP in group NC was higher than other groups (P < 0.01); LDL in the three groups had no statistically difference; 25 (OH) D_3 in group DKD is lower than DM group, but the difference was not statistically significant (P > 0.05) (**Table 1**), (25 OH) D_3 in NC group were significantly higher than group DKD and DM (P < 0.01) (**Figure 3**). Pearson correlation analysis showed that 25 (OH) D_3 was negatively correlated with age, BMI, HbA1c, TG, but had a positive correlation with HDL (P < 0.01) (**Table 2**).

Table 5. Binary logistic regression analysis of risk factors for DKD

	B	S.E.	Wals	Sig.	Exp (B)	95% C.I.	
Genotypeff	0.86	0.43	4.03	0.04	2.37	1.02	5.53
DBP	0.06	0.02	6.43	0.01	1.06	1.01	1.11
TG	0.28	0.11	6.01	0.01	1.32	1.05	1.65
Age	0.05	0.01	7.28	0.01	1.05	1.01	1.09

The distribution of fokI genetypes and alleles conformed with Hardy-Weinberg equilibrium (P = 0.798, 0.904, 0.937). The genetype Ff, ff, FF accounted for 52.8%, 27%, 20.2% in group DKD, respectively; 54.9%, 16.5%, 28.6% in group DM and 46.9%, 20.8%, 32.3% in group NC. The genetype Ff, ff, FF was no statistical difference between group DM and NC (χ^2 = 3.6, P = 0.16), either in group DKD and DM (χ^2 = 2.03, P = 0.36). However, in group DKD genotype FF was relatively lower than group NC (χ^2 = 3.45, P = 0.045), and there is no difference in genotype ff, Ff (P > 0.05). The distribution of allele was significantly different between DM and DKD groups. The frequency of f was higher than group DM, which count for 53.4% in group DKD (P = 0.046) (**Table 3**; **Figure 4**). The relative risk (RR) of DKD for type 2 diabetics who carried the genotype ff or allele f was 1.87, 1.46, respectively (**Table 4**). Further more. Binary Logistic regression analysis showed that genotype ff might be the risk factor for DKD (P = 0.04, OR = 2.37). DBP, TG and age also may be the risk factors for DKD (P = 0.01, 0.01, 0.01, OR = 1.06, 1.31, 1.05) (**Table 5**).

Discussion

In recent years, with the changing in lifestyle and aging of population, the incidence of diabetes is rising, according to the latest statistics of International Diabetes Federation (IDF) in 2013, the number of diabetes patients in China has been ranking first in the world, and is still increasing. The diabetic kidney disease is the most common chronic vascular complications of diabetes, which is associated with many factors such as heredity and environment, The predisposing risk factors of diabetic kidney disease include hyperglycemia, dyslipidemia, hypertension, genetics, metabolic disorders of vitamin D [15, 16], unhealthy lifestyles etc. In clinical practice [17, 18], there are still some diabetics that have no difference in blood sugar and blood pressure control developing diabetic kidney disease. It shows that in addition to a number of controllable risk factors, genetic play an important role in the occurrence and development of diabetic kidney disease.

We found that the incidence of vitamin D deficiency and insufficiency is very high in patients with DKD in clinical observation [7, 8, 19], the researchers compared the vitamin D concentrations in patients with type 2 diabetic kidney disease, and found that the ratio of 25 hydroxy vitamin D_3 deficiency reached 47.01% [8], even more than 50% [7], and compared those patients with normal 25 hydroxy vitamin D, their vascular endothelial function decreased more significantly [20]. Vitamin D is a kind of fat-soluble group of steroid derivatives, the affinity of 25 (OH) D_3 with VDR is only 1% of 1,25 (OH) D_3 [21]. But the 1,25 $(OH)_2$ D_3 has a short half-life, and need higher detection technology, so we usually choose 25 (OH) D_3 vitamin D as the detection index reflecting the reserves of vitamin D in body.

Active vitamin D can reduce or inhibit a series of pathological reaction through several ways, such as RAAS system, ERK signaling pathway, mitochondria, and other inflammatory mediators, then protect renal injury from high glucose or delay or reduce the development of diabetic kidney disease. The role of vitamin D biological activity is mainly through binding with VDR, it can form hormone-receptor complexes, and the complexes effect on the specific DNA sequence of target gene, then regulates insulin secretion. For pancreas, VDR can change the VDR gene transcription of islet β cells, thereby regulating the secretion of insulin. Clinical studies [22] and animal experiments [23] indicated that VDR-mediated effects for diabetic kidney disease, to some extent, have a protective effect. vitamin D receptor gene are different among individuals, which can lead to different activity of VDR, and polymorphisms of fokI also affect the interaction between basal transcription factor IIB (TFIIB) and VDR, then affect the activity of VDR in the regulation of transcription [24]. Based on the above, we can speculate that the polymorphism of fokI may be the susceptible factors of diabetic kidney disease.

The fokI site is located in transcription initiation codon in second exon of VDR gene, compared to 3' of the non-function VDR gene, it is relatively more independent. f allele stands for the

restriction enzyme sites is present, F allele standing for lack of it. the SNPs of fokI (T > C) can change the length of amino acid sequence, when the initiation codon mutated from ATG to ACG (F allele), it lost the role of start the translation, and only wait for the next initiation codon to translate, which will result in lack of three amino acids in activation domain of VDR protein positions, the VDR protein lacked three amino acids is easier to activate its effector genes. In this study, we compared the frequency of fokI genotype among type 2 diabetes patients without kidney disease (DM group), type 2 diabetic patients with diabetic kidney disease (DKD group) and healthy controls (NC group) in Yunnan, China. We found that there are significant differences in allele frequencies between DM and DKD group, the frequency of F in DKD group was higher than DM group, genotype FF (C/C) in DKD group was lower than NC group, there is no significant difference in DM and NC group. Further more, we used binary Logistic regression to analysis the risk factors for type 2 diabetic kidney disease, finally genotype ff entered the regression equation (B = 0.72, P = 0.04, OR = 2.37). these results suggest that genotype ff may be associated with type 2 diabetic kidney disease, the risk of diabetic kidney disease for diabetic patients carrying f allele was 1.459 times higher than those without, which is similar to HM. Li's result in Nanjing, China [11]. We may explain those results from that Single nucleotide polymorphisms (SNPs) make the different activity of VDR, then lead to difference in activity of vitamin D, however, if only explain from this way, its reliability may be weaker. Whether there are another undiscovered loci linked to fokI caused this results still needs further study to prove. In addition, there are also differences in gene expression and epigenetic inheritance, such as methylation, RNA interference, histone modifications, etc., which is as important as the difference in gene.

The distributions of VDR fokI gene and allele gene are different among people in different race, region, lifestyle, etc., and also the susceptibility to type 2 diabetic kidney disease is not same. Researchers did not find that fokI is associated with type 2 diabetes and its complications in Poland, Turkey, and North Indian [12, 13, 25], For Caucasian, healthy people with genotype FF are more sensitive to insulin than the genotype ff, Ff groups [24], Zhelong Liu, et al. suggest that the polymorphisms of fokI may affect the susceptibility to diabetic kidney disease in Caucasians by mata analysis, but it is not just for the type 2 diabetic kidney disease [26]. Most of the association between SNP and type 2 diabetic kidney disease is found based on the Asian population. In addition, the distribution of fokI genotype in the healthy people is different among different countries and ethnic groups. For healthy people, Ff genotype accounted for the highest proportion in Europe, Finland, France, North Indian, Poland and Australia [12]. In England and Black Pennsylvania, genotype FF account for the highest proportion [12]. According to the current researches in China, genotype FF is the most proportion in Han nationality in Jiangsu, and it is the genotype Ff for Han Chinese in Beijing [27]. In our reseach, we observed that Ff genotype accounted for 46.9% in Han population in Yunnan, FF accounted 32.3%. Because of the different history of ethnic development, gene also has the very big difference in the same race. Therefore, the variation of SNP in different races, regions, ethnic groups will be different.

According to the index in DM and DKD group we found that the mean value of BMI in the two groups is lower than Caucasians (Chinese Diabetes Society, 2014), We usually take BMI ≥ 24 as the boundary value of overweight, this means that for the Chinese diabetics, the insulin resistance and insulin secretion deficiency have already appeared in this state of BMI, due to the duration of diabetes is an independent risk factor for DKD, especially the course of more than 10 years, it is more significantly associated with albuminuria [3, 28, 29], we chose patients in group DKD required duration of diabetes less than 10 years (5.2 ± 3.4 years), duration of diabetes is more than 10 years (13.6 ± 4.5 years) in group DM, to eliminate type 2 diabetic kidney disease caused by long duration, not genetic polymorphisms, so it is a large difference in age between the two groups.

In this study, we also compared the levels of 25-hydroxy vitamin D among three groups, and found that 25-hydroxy vitamin D in group NC were significantly higher than the other groups. Furthermore, we use Pearson correlation analysis to find the relationship between 25-hydroxy vitamin D and other variables, found that a negative correlation between the concentra-

tion of 25 hydroxy vitamin D and age, BMI, glycated hemoglobin, TG showed a weak negative correlation. Clinical researches at home and abroad suggested that vitamin D deficiency can cause the injury in β cell of islet and insulin resistance, and vitamin D is associated with body fat and BMI [10]. In addition, our study observed that the level of 25-hydroxy vitamin D in group DM is higher than group DKD, but the difference was not statistically significant. This suggest that diabetic kidney disease was not directly affected by the level of 25-hydroxy vitamin D. At present, the results about relationship between fokI gene polymorphism and vitamin D were not consistent. Molecular forms of vitamin D on the protective mechanism of DKD is 1,25 $(OH)_2$ D_3, 25 (OH) D is less mentioned. Studies have found the relevance between 1,25 $(OH)_2$ D_3 and polymorphisms of fokI gene, but didn't find the correlation with 25 hydroxy vitamin D [6]. while another study found that 25 hydroxy vitamin D of patients who carried genotype ff was lower than the other genotype [30], which may eventually be explained from the difference of the fokI gene in different countries, races, environment, and there are seasonal differences in concentration of vitamin D [6, 31], and VD is influenced by diet, environment, more important, the relationship between the concentration of vitamin D and allele may be affected by other uncertain variables, such as different ways of life, the abnormal RAAS system, and the level of cGMP, calcium and protein kinase C activity. So we need to explore the relationship of fokI gene SNP and vitamin D from many aspects.

From the results of the comparison among the three groups of fokI genotype, DN genotype frequency of FF was higher than DM group, but no statistical significance, if we expand the sample size, the result may have statistical difference. Due to 1,25 $(OH)_2$ D_3 has a short half-life and need higher testing requirements, so we chose 25 (OH) D, not 1,25 $(OH)_2$ D_3, if we choose 1,25 $(OH)_2$ D_3, the persuasion may be higher.

Taken together, our study found that the genotype Ff accounted for the most in Yunnan population, and genotype ff may be the susceptible gene for type 2 diabetic nephropathy in Yunnan. We also observed that 25-hydroxy vitamin D levels in T2DM were significantly lower than the normal population, and 25-hydroxy vitamin D had the negatively correlation with age, BMI, glycated hemoglobin, TG. Overall, diabetes and its complications were related to many factors such as heredity and environment, and the cost of treatment is relatively high for patients with diabetes, there is still much room for improvement in the effectiveness of treatment, so researches for genetics in diabetes and its complications will continue to bring benefits. Vitamin D was associated with many functions and disease in human body, and there is much room for exploration, and the research between SNP in fokI VDR gene and type 2 diabetes and diabetic nephropathy will provide some data and theoretical basis in many fields such as epidemiological data, therapeutic targets for diabetes and its complications, and information of some diseases related to VDR gene.

Acknowledgements

The authors would like to thank the practitioners, nurses and study coordinators in the Diabetes Department of 1st Hospital Affiliated to Kunming Medical University for help to complete this study. This study was supported by a grant from the foundation of Education Department of Yunnan Province (Grant No: 2013Y286); The project of Science and Technology Department of Yunnan Province (Grant No: 2014FB029); High-level Health and Technical Talent training Program of Yunnan Province (Grant No: D-201625); The funding program of Young and middle-aged reserve talents for academic and technical leaders (Grant No: 2017HB045).

Disclosure of conflict of interest

None.

Address correspondence to: Rui Han, The Diabetes Department of 1st Hospital Affiliated to Kunming Medical University, NO.295, Xichang Road, Kunming 650000, Yunnan, China. Tel: +008613529365266; E-mail: hanrui_201207@163.com

References

[1] Zuo L, Wang M. Current burden and probable increasing incidence of ESRD in China. Clin Nephrol 2010; 74: 20-22.

[2] Zhang LX, Wang HY. Developing trend and countermeasures of diabetic nephropathy in China from epidemiological point of view. Chin J Intern Med 2010; 49: 804-805.

[3] Ayodele OE, Alebiosu CO, Salako BL. Diabetic nephropathy--a review of the natural history,

burden, risk factors and treatment. J Natl Med Assoc 2004; 96: 1445-1454.
[4] Liu ZH. Study on the genetic background of diabetic nephropathy. Internal Medicine Bibliometrics Chinese Journal of Internal Medicine 2002; 41: 561-562.
[5] Ravani P, Malberti F, Tripepi G, Pecchini P, Cutrupi S, Pizzini P, Mallamaci F, Zoccali C. Vitamin D levels and patient outcome in chronic kidney disease. Kidney Int 2009; 75: 88-95.
[6] Yokoyama K, Nakashima A, Urashima M, Suga H, Mimura T, Kimura Y, Kanazawa Y, Yokota T, Sakamoto M, Ishizawa S, Nishimura R, Kurata H, Tanno Y, Tojo K, Kageyama S, Ohkido I, Utsunomiya K, Hosoya T. Interactions between serum vitamin D levels and vitamin D receptor gene fokipolymorphisms for renal function in patients with type 2 diabetes. PLoS One 2012; 7: e51171.
[7] Fernándezjuárez G, Luño J, Barrio V, de Vinuesa SG, Praga M, Goicoechea M, Lahera V, Casas L, Oliva J; PRONEDI Study Group. 25 (OH) vitamin D levels and renal disease progression in patients with type 2 diabetic nephropathy and blockade of the renin-angiotensin system. Clin J Am Soc Nephrol 2013; 8: 1870-1876.
[8] Zang L, Fu P, Huang YQ, Wu M, Li L, Zang J, Liu F. [Vitamin D deficiency and carotid artery intima-media thickness and coronary calcification in patients with diabetic nephropathy]. Journal of Sichuan University (Medical Science Edition) 2012; 43: 420-424, 450.
[9] Wang Y, Zhou J, Minto AW, Hack BK, Alexander JJ, Haas M, Li YC, Heilig CW, Quigg RJ. Altered vitamin D metabolism in type II diabetic mouse glomeruli may provide protection from diabetic nephropathy. Kidney Int 2006; 70: 882-891.
[10] Wang Y, Deb DK, Zhang Z, Sun T, Liu W, Yoon D, Kong J, Chen Y, Chang A, Li YC. Vitamin D receptor signaling in podocytes protects against diabetic nephropathy. J Am Soc Nephrol 2012; 23: 1977-1986.
[11] Li HM, Miao H, Lu YB, et al. Association between the polymorphism of human vitamin D receptor gene and the susceptibility of diabetic nephropathy in Chinese Han population. Chinese Journal of Clinical Rehabitation 2005; 9: 1-4.
[12] Bid HK, Mishra DK, Mittal RD. Vitamin-D receptor (VDR) gene (Fok-I, Taq-I and Apa-I) polymorphisms in healthy individuals from north Indian population. Asian Pac J Cancer Prev 2005; 6: 147-152.
[13] Bid HK, Konwar R, Aggarwal CG, Gautam S, Saxena M, Nayak VL, Banerjee M. Vitamin D receptor (FokI, BsmI and TaqI) gene polymorphisms and type 2 diabetes mellitus: a North Indian study. Indian J Med Sci 2009; 63: 187-194.
[14] Saracci R. The world health organisation needs to reconsider its definition of health. BMJ 1997; 314: 1409-1410.
[15] Gluhovschi GH, Gluhovschi C, Vlad A, Timar R, Bob F, Velciov S, Bozdog G, Petrica L. Diabetic nephropathy and multiorgan protection. Rom J Intern Med 2011; 49: 237-49.
[16] Nakai K, Fujii H, Kono K, Goto S, Kitazawa R, Kitazawa S, Hirata M, Shinohara M, Fukagawa M, Nishi S. Vitamin D activates the Nrf2-Keap1 antioxidant pathway and ameliorates nephropathy in diabetic rats. Am J Hypertens 2014; 27: 586-595.
[17] Antic M, Jotic A, Radovic M, Seferović JP, Lalić NM, Jovanović D, Lezaić V. [Risk factors for the development of diabetic nephropathy]. Srp Arh Celok Lek 2009; 137: 18-26.
[18] Lizicarova D, Krahulec B, Hirnerova E, Gaspar L, Celecova Z. Risk factors in diabetic nephropathy progression at present. Bratisl Lek Listy 2014; 115: 517-521.
[19] Li DM, Zhang Y, Ding B, Liu BL, Jiang LL, Xing CY, Ma JH. [The association between vitamin D deficiency and diabetic nephropathy in type 2 diabetic patients]. Zhonghua Nei Ke Za Zhi 2013; 52: 970-974.
[20] Munisamy S, Kamaliah MD, Suhaidarwani AH, Zahiruddin WM, Rasool AH. Impaired microvascular endothelial function in vitamin D-deficient diabetic nephropathy patients. J Cardiovasc Med (Hagerstown) 2013; 14: 466-471.
[21] Zhou JS, Zhang J. Progress in clinical application of vitamin D. Laboratory Medicine 2013; 28: 539-543.
[22] de Zeeuw D, Agarwal R, Amdahl M, Audhya P, Coyne D, Garimella T, Parving HH, Pritchett Y, Remuzzi G, Ritz E, Andress D. Selective vitamin D receptor activation with paricalcitol for reduction of albuminuria in patients with type 2 diabetes (VITAL study): a randomised controlled trial. Lancet 2010; 376: 1543-1551.
[23] Sanchez-Niño MD, Bozic M, Córdoba-Lanús E, Valcheva P, Gracia O, Ibarz M, Fernandez E, Navarro-Gonzalez JF, Ortiz A, Valdivielso JM. Beyond proteinuria: VDR activation reduces renal inflammation in experimental diabetic nephropathy. Am J Physiol Renal Physiol 2012; 302: F647-657.
[24] Palomer X, González-Clemente JM, Blanco-Vaca F, Mauricio D. Role of vitamin D in the pathogenesis of type 2 diabetes mellitus. Diabetes Obes Metab 2008; 10: 185-197.
[25] Malecki MT, Frey J, Moczulski D, Klupa T, Kozek E, Sieradzki J. Vitamin D receptor gene polymorphisms and association with type 2 diabetes mellitus in a Polish population. Exp Clin Endocrinol Diabetes 2003; 111: 505-509.
[26] Liu Z, Liu L, Chen X, He W, Yu X. Associations study of vitamin D receptor gene polymor-

phisms with diabetic microvascular complications: a meta-analysis. Gene 2014; 54: 6-10.
[27] Xia Z, Hu Y, Zhang H, Han Z, Bai J, Fu S, Deng X, He Y. [Association of vitamin D receptor Fok I and Bsm I polymorphisms with dyslipidemias in elderly male patients with type 2 diabetes]. Nan Fang Yi Ke Da Xue Xue Bao 2014; 34: 1562-1568.
[28] Alwakeel JS, Isnani AC, Abdulkareem A, Alharbi A, Shaffi SA, Almohaya S, Al Ghonaim M. Factors affecting the progression of diabetic nephropathy and its complications: a single-center experience in Saudi Arabia. Ann Saudi Med 2011; 31: 236-242.
[29] Al-Rubeaan K, Youssef AM, Subhani SN, Ahmad NA, Al-Sharqawi AH, Al-Mutlaq HM, David SK, AlNaqeb D. Diabetic nephropathy and its risk factors in a society with a type 2 diabetes epidemic: a Saudi national diabetes registry-based study. PLoS One 2014; 9: e88956.
[30] Neyestani TR, Djazayery A, Shab-Bidar S, Eshraghian MR, Kalayi A, Shariátzadeh N, Khalaji N, Zahedirad M, Gharavi A, Houshiarrad A, Chamari M, Asadzadeh S. Vitamin D receptor Fok-I polymorphism modulates diabetic host response to vitamin D intake: need for a nutrigenetic approach. Diabetes Care 2013; 36: 550-556.
[31] Schöttker B, Jorde R, Peasey A, Thorand B, Jansen EH, Groot Ld, Streppel M, Gardiner J, Ordóñez-Mena JM, Perna L, Wilsgaard T, Rathmann W, Feskens E, Kampman E, Siganos G, Njølstad I, Mathiesen EB, Kubínová R, Pająk A, Topor-Madry R, Tamosiunas A, Hughes M, Kee F, Bobak M, Trichopoulou A, Boffetta P, Brenner H; Consortium on Health and Ageing: Network of Cohorts in Europe and the United States. Vitamin D and mortality: meta-analysis of individual participant data from a large consortium of cohort studies from Europe and the United States. BMJ 2014; 348: g3656.