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Research article

Variants in *PPARD-GLP1R* are related to diabetic kidney disease in Chinese Han patients with type 2 diabetes mellitus

Jinfang Song [a,b,*,1], Yongru Zhuang [b,1], Xiaojun Pan [c], Ya Chen [d], Fen Xie [a,**]

[a] Department of Pharmacy, Affiliated Hospital of Jiangnan University, Wuxi, 214000, China
[b] Jiangsu Key Laboratory of New Drug Research and Clinical Pharmacy, Xuzhou Medical University, Xuzhou, 221000, China
[c] Department of Pharmacy, Wuxi No.5 People's Hospital, Wuxi, 214000, China
[d] Department of Endocrinology, Affiliated Hospital of Jiangnan University, Wuxi, 214000, China

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ABSTRACT

Genetic susceptibility is an important pathogenic mechanism in diabetic kidney disease (DKD). Our previous studies have identified that PPARδ and GLP-1R are located in a pathway that is closely related to DKD. We aimed to explore the impacts of variants in *PPARD-GLP1R* on the susceptibility to DKD in Chinese Han patients with type 2 diabetes mellitus (T2DM). A total of 600 T2DM patients (300 with DKD and 300 without DKD) and 200 healthy control subjects were enrolled to identify *PPARD* (rs2016520, rs2267668 and rs3777744) and *GLP1R* (rs3765467, rs1042044 and rs9296291) genotype. The SNaPshot method was used to identify variants in *PPARD-GLP1R*. We performed correlation analysis between variants in *PPARD-GLP1R* and the susceptibility to DKD. We observed that *GLP1R* rs3765467 (G > A) was associated with DKD (OR = 3.145, 95 % CI = 2.128–6.021, P = 0.035). None of the other SNPs were associated with DKD. Regarding DKD related traits, rs3765467 was associated with UACR levels and TC, significant differences were observed among patients with different genotypes of rs2016520 in terms of BMI and TG, and patients with the rs3777744 risk G allele had noticeably higher PPG and HbA1c levels (P < 0.05). Moreover, the results showed the interactions between *PPARD* rs3777744 and *GLP1R* rs3765467 in the occurrence of DKD (OR = 4.572, P = 0.029). The results of this study indicate the potential relationship between variants in *PPARD-GLP1R* and the susceptibility to DKD in Chinese Han patients with T2DM.

1. Introduction

Diabetic kidney disease (DKD) is one of the most common chronic complications of diabetes mellitus, usually with a hidden onset. Type 2 diabetes mellitus (T2DM) accounts for more than 90 % of all diabetic patients, and international epidemiological studies have reported that about 40 % of type 2 diabetes mellitus eventually develops into DKD. Therefore, T2DM has become a leading cause of DKD [1–4]. Based on the data from the Diabetes Control and Complications Trial (DCCT), the development of DKD is mainly related to glycemic control, however, some patients with T2DM who have well-controlled glycemia showed elevated urinary albumin levels [5];

* Corresponding author. Affiliated Hospital of Jiangnan University, No.1000, Hefeng Road, Wuxi City, 224000, Jiangsu Province, China.
** Corresponding author. Affiliated Hospital of Jiangnan University, No.1000, Hefeng Road, Wuxi City, 224000, Jiangsu Province, China.
E-mail addresses: songjinfang1987@126.com (J. Song), 9862016026@jiangnan.edu.cn (F. Xie).
[1] Jinfang Song and Yongru Zhuang contributed equally to this paper.

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on the contrary, some patients with poor glycemic control and long duration of T2DM did not experience DKD [5]. DKD is a multifactorial disease that occurs and develops in response to a confluence of environmental and genetic factors, with genetic factors recognized as the major contributor to racial and individual differences in the development of DKD [6,7].

Glucagon-like peptide-1 receptor (GLP-1R) belongs to the G protein-coupled receptor family and is expressed in pancreatic islets, kidneys, lungs, and endothelial cells, with high expression levels in the renovascular system and renal tubular epithelium of the kidney [8–11]. In the hyperglycemic state, agonism of GLP-1R activates the adenylate cyclase and protein kinase A, which exert the effect of reducing the glomerular filtration rate and sodium reabsorption by the proximal tubular epithelium [12–14]. The accepted main mechanisms of causing the development of DKD include tubular epithelial cell transdifferentiation, renal interstitial cell fibrosis and podocyte apoptosis, with studies showing that GLP-1R agonists have an important protective effect on glomerular injury, interstitial fibrosis, tubular epithelial cell inflammation and podocyte apoptosis [15–17]. Therefore, GLP-1R may have a key role in the development of DKD.

Peroxisome proliferator-activated receptor δ gene (*PPARD*) is located on chromosome 6p21.1-p21.2, and its coding product PPAR-δ (also named PPAR-β) is a member of the peroxisome proliferator activated receptor family, which is widely distributed in the liver, kidney, cardiac and skeletal muscle, adipose tissue, brain, pancreatic and vasculature [18]. The studies concerning the effects of PPAR-δ in the kidney have shown that PPAR-δ ± and PPAR-δ −/− mutant mice exhibit more severe renal dysfunction compared to wild-type mice in a model of acute renal failure caused by acute renal ischemia. These results suggest a potential nephroprotective role of PPAR-δ *in vivo* studies where agonistic PPAR-δ could act as an antiapoptotic agent by activating the Akt signaling pathway [19,20]. A recent study investigated the function of PPAR-δ in the inflammatory response of human mesangial cells and showed that PPAR-δ agonists inhibited activation of the nuclear factor-κB (NF-κB) pathway and apoptosis in response to advanced glycation end products and tumor necrosis factor-α (TNF-α) stimulation and increased the levels of cellular superoxide dismutase [21]. Therefore, PPAR-δ is considered to be closely related to the development, prevention and treatment of DKD.

In previous work, we revealed PPARδ binding sites in the promoter region of GLP-1R using the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/) and the JASPAR database (http://jaspar.genereg.net/), and experimentally demonstrated the role of PPARδ on regulation action of *GLP1R* [22]. Therefore, the hypothesis is that PPARδ and GLP-1R are located in a pathway that is closely related to DKD. Our study provides relevant information on the relationship between variants in *PPARD-GLP1R* and the susceptibility to DKD in Chinese Han patients with T2DM.

2. Materials and methods

2.1. Participants and study design

A total of 800 participants were recruited from the Department of Endocrinology and the Health Screening Center of the Affiliated Hospital of Xuzhou Medical University and the National Metabolic Disease Management Center of the Affiliated Hospital of Jiangnan University. All participants had Chinese Han ancestry and the patients with T2DM, according to the 1999 WHO criteria (fasting plasma glucose ≥7.0 mmol/L and/or 2 h plasma glucose ≥11.1 mmol/L) [23]. DKD was diagnosed on the basis of a persistent albuminuria, (microalbuminuria >30 mg/24 h; representing overt glomerular proteinuria) with or without elevated serum creatinine levels (reference range <1.3 mg/dL for males, <1.1 mg/dL for females) and in the absence of clinical evidence of non-diabetic renal disease. Among these participants, 300 patients with T2DM complicated with DKD were chosen as DKD case group, 300 patients with T2DM for more than 10 years without DKD were selected as T2DM group (microalbuminuria <30 mg/24 h and eGFR ≥90 mL/min per 1.73 m^2), and 200 healthy individuals were selected as healthy control group. Subjects with acute or severe chronic diabetic complications, serious comorbid diseases, New York Heart Function Scale (NYHA) III ~ IV, severe osteoporosis or a history of fractures, alanine aminotransferase (ALT) and aspartate transaminase (AST) ≥2.5 times of upper limit, or serum creatinine level ≥133 μmol/L, severe gastrointestinal dysfunction, ongoing use of weight-loss drugs, glucocorticoids, drugs that affecting gastrointestinal motility, transplant therapy drugs, any investigational drugs, a history of pancreatitis, or serum triglyceride level ≥5 mmol/L were excluded. The protocol was approved by the Ethics Committee of the Affiliated Hospital of Xuzhou Medical University and the Ethics Committee of the Affiliated Hospital of Jiangnan University. Written informed consent was obtained from each participant before taking part in the study.

2.2. Clinical phenotypes

Plasma glucose and serum lipids, including triglycerides (TG), total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-c) and high-density lipoprotein-cholesterol (HDL-c), were detected using a Roche Cobas8000 analyser (Roche, Basel, Switzerland) with standard laboratory methods. Hemoglobin A1c (HbA1c) concentration was measured with a high performance liquid chromatography (HPLC) approach. Urinary levels of albumin (UAlb) and creatinine (UCr) were quantified with a CLINITEK Novus Automated Urine Chemistry Analyzer (Siemens, Germany), and the urine samples were collected as the first morning voids. Urinary albumin to creatinine ratio (UACR) was calculated as UAlb (mg/L)/UCr (g/L) or UAlb (mg/L)/UCr (umol/L) × 8840. Estimated glomerular filtration rate (eGFR) was calculated with a previously proposed formula that is more applicable to the Chinese population [24,25].

2.3. Tag SNP selection

Tag single nucleotide polymorphism (tag SNP) denotes that one SNP can represent the information and effects of all SNPs in a

region of linkage disequilibrium, and the tag SNP can simplify the study and achieve the efficacy of "one for many". This study was based on the Genotype-Tissue Expression (GTEx) database and the genetic information of Han Chinese in Beijing and Southern Han Chinese (CHB&CHS) from the 1000 Genomes Project database, and combined with SNPs functional annotation, linkage disequilibrium analysis, minimum allele frequency and literature reports to screen for tag SNPs. Tag SNPs were scoped to SNP sites between 20 kb upstream and 20 kb downstream of each candidate gene region.

2.4. DNA isolation and genotyping

Genomic DNA was extracted from peripheral blood using a TIANamp Genome DNA Kit (TIANGEN, Beijing, China), and DNA quality was measured using a Nanodrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA).

Genotyping was confirmed by SNaPshot using the ABI 3730XL Sequence Detection System (Applied Biosystems, USA) and raw data were analyzed with the Gene Mapper 4.1 (Applied Biosystems, USA). Standard quality control for the SNPs included Hardy-Weinberg equilibrium ($P > 0.05$), and call rate >90 %. Quality control procedures for individuals required a sample call rate >85 %.

2.5. Statistical analysis

All statistical calculations were performed using SPSS software (version 13.0 for Windows; SPSS Inc., Chicago, IL, USA). All continuous variables were summarized as mean ± standard deviation. Chi-square test was used to compare the Hardy-Weinberg equilibrium, allele frequency and genotype distribution among different groups. Linkage disequilibrium (LD) among SNPs was estimated in subjects using Haploview version 3.2. Parameters with normal distribution were analyzed by the *t*-test or the one-way ANOVA test, whereas those with abnormal distribution were analyzed by the Mann-Whitney test or the Kruskal-Wallis's test. Quanto software (Version 1.2.4.; written by: John Morrison and W.James Gauderman at the University of Southern California) was used to estimate the statistical power [26]. A value of $P < 0.05$ was considered significant.

3. Results

3.1. Clinical and laboratory characteristics of the study cohort

The demographic and clinical data of 200 healthy subjects (141 men and 59 women, mean age: 48.65 ± 12.67 years), 300 patients with T2DM (195 men and 105 women, mean age: 49.41 ± 12.91 years), and 300 patients with DKD (195 men and 105 women, mean age: 50.56 ± 10.70 years) are provided in Table 1. There was no statistically significant difference in the age and sex composition among the three groups ($P > 0.05$). Compared with healthy controls, the proportion of smoking, BMI, WHR, fasting plasma glucose (FPG), HbA1c, TC, TG, LDL-c, SBP, DBP, and BUN were significantly higher in the DKD and T2DM groups ($P < 0.05$). In addition, the

Table 1
Demographics and clinical laboratory data.

Parameters	Groups			*P* value		
	NC	T2DM	DKD	NC to T2DM	NC to DKD	T2DM to DKD
N(men/women)	200(141/59)	300(195/105)	300(195/105)	0.199	0.199	1.000
Age (years)	48.65 ± 12.67	49.41 ± 12.91	50.56 ± 10.70	0.515	0.070	0.235
Smoking (yes/no)	32/168	71/229	94/206	0.038*	0.000*	0.035*
DR (yes/no)	–	77/223	188/112	–	–	0.000*
Duration of T2DM	–	11.03 ± 5.00	10.82 ± 5.09	–	–	0.616
BMI (kg/m^2)	22.37 ± 3.21	25.99 ± 3.84	25.48 ± 3.79	0.000*	0.000*	0.102
WHR	0.88 ± 0.07	0.94 ± 0.05	0.93 ± 0.07	0.000*	0.000*	0.123
FPG (mmol/L)	5.16 ± 0.53	10.52 ± 3.34	10.58 ± 2.89	0.000*	0.000*	0.801
PPG (mmol/L)	–	15.83 ± 4.38	16.12 ± 4.83	–	–	0.438
HbA1c (%)	5.47 ± 0.34	9.46 ± 1.74	9.34 ± 9.33	0.000*	0.000*	0.449
TG (mmol/L)	1.66 ± 1.06	2.41 ± 1.75	2.36 ± 2.61	0.000*	0.000*	0.809
TC (mmol/L)	4.55 ± 0.84	4.97 ± 1.32	5.11 ± 1.41	0.000*	0.000*	0.192
HDL-c (mmol/L)	1.20 ± 0.27	1.06 ± 0.27	1.02 ± 0.29	0.000*	0.000*	0.081
LDL-c (mmol/L)	2.97 ± 0.64	3.07 ± 1.13	3.25 ± 0.94	0.000*	0.000*	0.042*
SBP (mmHg)	122.50 ± 16.26	133.56 ± 17.65	133.05 ± 16.63	0.000*	0.000*	0.718
DBP (mmHg)	74.84 ± 11.74	84.05 ± 10.10	83.54 ± 9.15	0.000*	0.000*	0.520
Scr (μmol/L)	69.74 ± 12.42	52.73 ± 10.48	91.09 ± 76.82	0.003*	0.000*	0.000*
BUN (μmol/L)	5.02 ± 1.23	6.67 ± 12.84	8.43 ± 4.98	0.023*	0.000*	0.027*
eGFR (ml/min)	111.56 ± 13.26	119.76 ± 29.31	91.36 ± 31.99	0.000*	0.000*	0.000*
UACR (mg/g)	–	6.06 ± 5.47	98.01 ± 88.49	–	–	0.000*

Abbreviations: DR, diabetic retinopathy; BMI, body mass index; WHR, waist to hip ratio; FPG, fasting plasma glucose; PPG, postprandial plasma glucose; HbA1c, hemoglobin A1c; TG, triglyceride; TC, total cholesterol; HDL-c, high-density lipoprotein-cholesterol; LDL-c, low-density lipoprotein-cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure; Scr, serum creatinine; BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate; UACR, urine albumin creatinine ratio.
*$P < 0.05$ indicates statistical significance.

DKD group had significantly higher proportion of patients with smoking and diabetic retinopathy (DR), higher levels of UACR, Scr, BUN, LDL-c, but lower eGFR than the T2DM group ($P < 0.05$). However, there were no significant differences in age, sex, the duration of T2DM, BMI, WHR, FPG, postprandial plasma glucose (PPG), HbA1c, TC, TG, HDL-c, SBP and DBP between DKD and T2DM ($P > 0.05$).

3.2. Genotype and allele frequencies analysis

This study confirmed four SNPs (rs3765467, rs9296291, rs2016520 and rs3777744) consistent with HWE (P > 0.05) and two other SNPs (rs1042044 and rs2267668) were not analyzed remotely because they did not correlate with the HWE (P < 0.05). The genotype and allele frequencies of rs3765467, rs9296291, rs2016520 and rs3777744 for healthy controls, T2DM patients and DKD patients are presented in Table 2. The genotype and allele frequencies of rs3777744 were remarkably different between the T2DM and control groups, and the minor allele G of rs3777744 was significantly associated with an increased risk of T2DM (P < 0.05). However, rs3765467, rs9296291, and rs2016520 did not exhibit significant difference in genotype and allele frequencies between the T2DM and control groups. On the other hand, the genotype and allele frequencies of rs3765467 were significantly different between DKD and T2DM groups, and the minor allele A of rs3765467 was significantly associated with an increased risk of DKD (P < 0.05). For rs9296291, rs2016520 and rs3777744, there was no significant difference in the genotype and allele frequencies between the T2DM group and DKD group ($P > 0.05$).

3.3. Analysis of factors affecting DKD development

To further explore the correlation between candidate SNPs and the risk of DKD, binary logistic regression analysis was performed,

Table 2
Comparison of genotype and allelic frequencies of four SNPs polymorphism among healthy controls (n = 200), T2DM patients (n = 300) and DKD patients (n = 300).

Genotypes	Healthy subjects n = 200 (frequency, %)	T2DM patients n = 300 (frequency, %)	DKD patients n = 300 (frequency, %)	χ^2/P value		
				NC to T2DM	NC to DKD	T2DM to DKD
rs3765467						
GG	125 (62.50)	186 (62.00)	156 (52.00)			
GA	68 (34.00)	106 (35.33)	121 (40.33)			
AA	7 (3.50)	8 (2.67)	23 (7.67)	0.344/ 0.842	7.100/ 0.029*	10.881/ 0.004*
Alleles						
G	318 (79.50)	478 (79.67)	433 (72.17)			
A	82 (20.50)	122 (20.33)	167 (27.83)	0.004/ 0.949	9.230/ 0.002*	6.902/ 0.009*
rs9296291						
TT	99 (49.50)	143 (47.67)	150 (50.00)			
TC	90 (45.00)	131 (43.67)	128 (42.67)			
CC	11 (5.50)	26 (8.67)	22 (7.33)	1.758/ 0.415	0.767/ 0.681	0.535/0.765
Alleles						
T	288 (72.00)	417 (69.50)	428 (71.33)			
C	112 (28.00)	183 (30.50)	172 (28.67)	0.721/ 0.396	0.052/ 0.819	0.484/0.487
rs2016520						
TT	110(55.00)	152(50.67)	150 (50.00)			
TC	75(37.50)	126(42.00)	131 (43.67)			
CC	15(7.50)	22(7.33)	19 (6.33)	1.039/ 0.595	1.925/ 0.382	0.330/0.848
Alleles						
T	295(73.75)	421 (70.17)	431 (71.83)			
C	105(26.25)	179(29.83)	169 (28.17)	1.515/ 0.218	0.443/ 0.506	0.405/0.525
rs3777744						
AA	86(43.00)	96(32.00)	94 (31.33)			
AG	94(47.00)	152(50.67)	162 (54.00)			
GG	20(10.00)	52(17.33)	44 (14.67)	8.798/ 0.012*	7.727/ 0.021*	1.006/0.605
Alleles						
A	266(66.50)	344(57.33)	350 (58.33)			
G	134(33.50)	256(42.67)	250 (41.67)	8.477/ 0.004*	6.767/ 0.009*	0.123/0.726

*$P < 0.05$ indicates statistical significance.
This annotation may be beneficial for the unity of expression in the context.

as shown in Fig. 1. After adjusting for confounders including age, sex, baseline BMI, baseline HbA1c, and duration of T2DM, the polymorphism at the *GLP1R* rs3765467 locus was associated with the risk of DKD in Chinese Han patients with T2DM (OR = 3.145, 95 % CI = 2.128–6.021, P = 0.035). Genetic polymorphism of *PPARD* rs3777744 was associated with the risk of T2DM in Chinese Han population (OR = 2.062, 95 % CI = 1.434–2.911, *P* = 0.019). No obvious relationship between the other two SNPs and the risk of DKD or T2DM was identified under these genetic models.

3.4. Association of genotypes with the clinical indicators of DKD

The present study carried out additional analysis of the correlation between genotypes of SNPs and clinical indicators in Chinese Han T2DM patients with DKD. Compared to patients with GG genotype, patients genotyped for GA and AA of *GLP1R* rs3765467 had significantly higher levels of UACR and TC ($P < 0.05$) (Fig. 2, Supplementary Table S1). As for the *PPARD* rs2016520 and rs3777744, there were no significant differences in gender, age, postprandial serum insulin (PINS), HOMA-B, HDL-c, and LDL-c between different genotype groups. However, significant differences were observed among patients with different genotypes of *PPARD* rs2016520 in terms of BMI and TG ($P < 0.05$) (Fig. 3, Supplementary Table S3). Compared to patients with the AA genotype, patients with the rs3777744 risk G allele had noticeably higher PPG and HbA1c levels ($P < 0.05$) (Fig. 4, Supplementary Table S4). Besides, no additional clinical indicators were found that differed significantly ($P > 0.05$) between the different genotype groups (Supplementary Tables S1–S4).

3.5. SNP-SNP interactions

Plink software was employed to analyze the SNP-SNP interactions between DKD and T2DM groups. As a result, rs3777744 significantly interacted with rs3765467 ($P < 0.05$), as illustrated in Table 3. The minor allele A of rs3765467 had a negative effect on DKD (OR = 3.145, P = 0.035). Besides, the combination of rs3765467 and rs3777744 further increased the risk of DKD (OR = 4.572, *P*

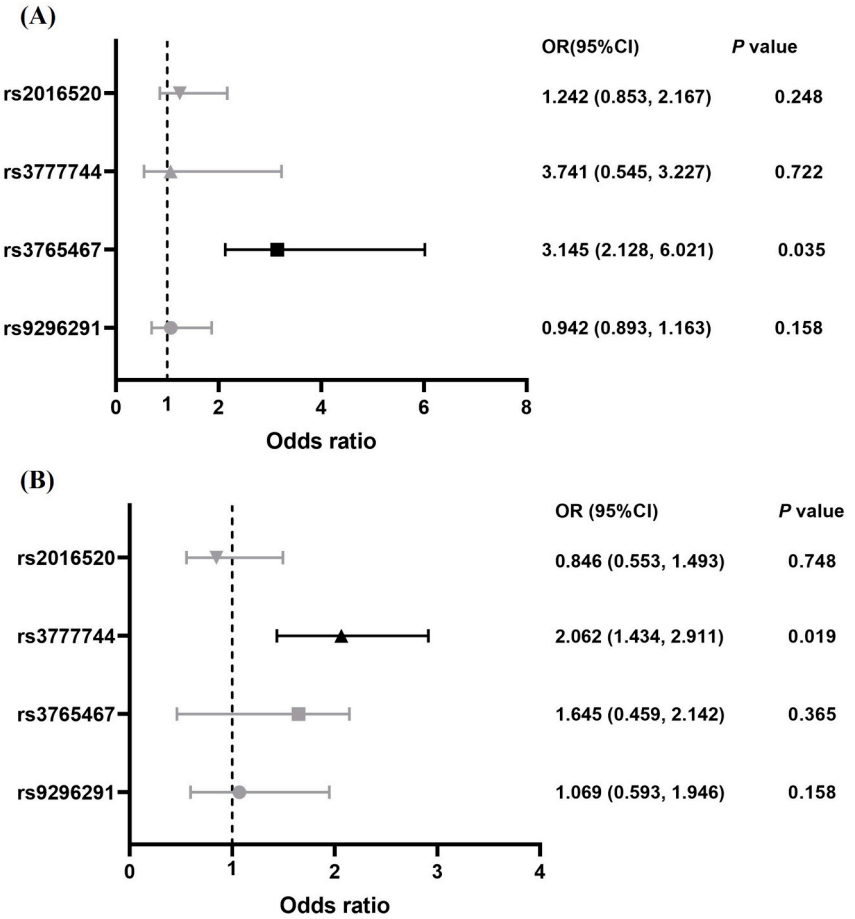

Fig. 1. Binary logistic regression analysis of factors affecting DKD development. (A) The correlation between SNPs and DKD. (B) The correlation between SNPs and T2DM.
DKD, diabetic kidney disease; SNPs, single nucleotide polymorphisms; T2DM, type 2 diabetes mellitus.

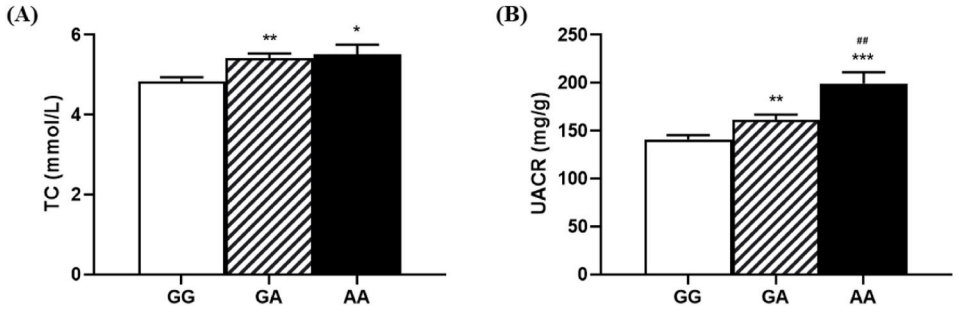

Fig. 2. Baseline levels of TC (A) and UACR (B) in DKD patients with different *GLP1R* rs3765467 genotypes. Data are expressed as mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with the GG genotype group, $^{\#\#}P < 0.01$ compared with the GA genotype group (n = 300). TC, total cholesterol; UACR, urinary albumin to creatinine ratio; DKD, diabetic kidney disease; *GLP-1R*, Glucagon-like peptide-1 receptor gene.

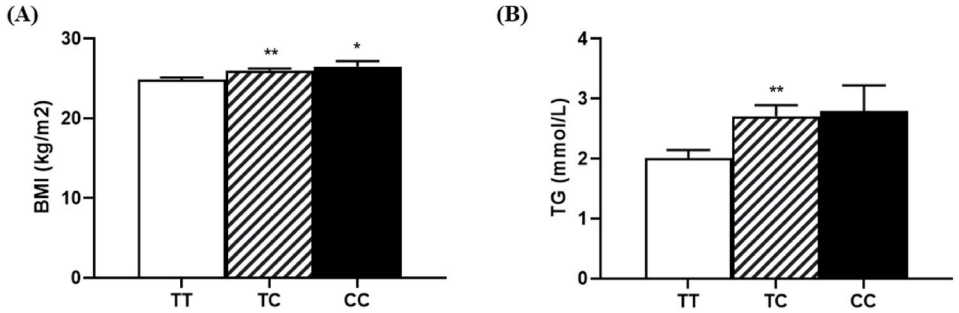

Fig. 3. Baseline levels of BMI (A) and TG (B) in DKD patients with different *PPARD* rs2016520 genotypes. Data are expressed as mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the TT genotype group (n = 300).
BMI, body mass index; TG, triglyceride; DKD, diabetic kidney disease; *PPARD*, peroxisome proliferator-activated receptor δ gene.

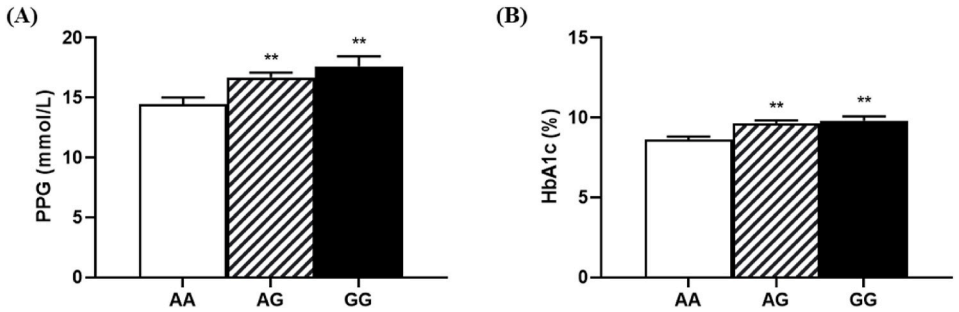

Fig. 4. Baseline levels of PPG (A) and HbA1c (B) in DKD patients with different *PPARD* rs3777744 genotypes. Data are expressed as mean ± SEM. $^{**}P < 0.01$ compared with the AA genotype group (n = 300).
PPG, postprandial plasma glucose; HbA1c, hemoglobin A1c; DKD, diabetic kidney disease; *PPARD*, peroxisome proliferator-activated receptor δ gene.

= 0.029).

4. Discussion

In the current study, we detected the potential impact of four SNPs (rs9296291, rs3765467, rs2016520 and rs3777744) of *GLP1R* and *PPARD* on risk of DKD susceptibility in the Chinese Han population. The study had an estimated > 85 % power (for α = 0.05) to detect such a difference in allelic frequencies and genotypes distribution. The results of this study suggested that the rs3765467 locus polymorphism can influence sensitivity to DKD. The minor allele A of rs3765467 was associated with an increased risk of DKD. On the other hand, we found that the risk G allele of rs3777744 in patients with T2DM had a higher frequency than that in the control group. Additionally, as presented in the analysis of clinical characteristics analysis of DKD patients, *GLP1R* rs3765467 A allele was responsible for higher UACR and TC. Whereas rs9296291, rs2016520 and rs3777744 have been not shown to be linked to risk of DKD, the *PPARD*

Table 3
Correlation between SNP-SNP interaction and DKD risk.

Number	SNP-SNP interaction	The minor allele	Correlation with DKD before interaction		Correlation with DKD after interaction	
			OR	*P*	OR	*P*
1	rs3765467	A	3.145	0.035*		
	rs9296291	C	0.942	0.158	2.063	0.311
2	rs3765467	A	3.145	0.035*		
	rs2016520	C	1.242	0.248	1.883	0.156
3	rs3765467	A	3.145	0.035*		
	rs3777744	G	3.741	0.722	4.572	0.029*
4	rs9296291	C	0.942	0.158		
	rs2016520	C	1.242	0.248	0.965	0.562
5	rs9296291	C	0.942	0.158		
	rs3777744	G	3.741	0.722	1.689	0.497
6	rs2016520	C	1.242	0.248		
	rs3777744	G	3.741	0.722	1.984	0.658

OR odds ratio, *$P < 0.05$ indicates statistical significance.

rs2016520 locus was associated with higher BMI and TG levels in patients with DKD, and higher PPG and HbA1c levels in *PPARD* rs3777744 G allele carriers meant poorer postprandial glycemic control. The most remarkable and interesting discovery of the present study was the SNP-SNP interaction between rs3765467 and rs3777744 further elevated the risk of DKD.

Glucagon-like peptide-1 (GLP-1) is an enteric insulin that is involved in the regulation of islet cell function and glucose homeostasis *in vivo* by binding to the glucagon receptor (GLP-1R) [27]. *GLP1R* gene is located on chromosome 6p21.2, and *GLP1R* gene polymorphisms have been found to be associated with obesity, cardiovascular risk factors, adipokine levels, and the efficacy of GLP-1R agonists [28,29]. It has been found that T2DM patients with the AA genotype of the *GLP1R* rs3765467 locus have a higher prevalence of response to DPP-4 inhibitors and a greater decrease in HbA1c, suggesting that genetic polymorphisms in *GLP1R* can influence the biological function of GLP-1R [30,31]. The GLP-1R is massively expressed not only in the pancreas but also in many tissues and organs such as kidney, nerve, gastrointestinal tract, heart, and vascular smooth muscle [32]. The mRNA of GLP-1R is expressed in both glomeruli and proximal tubules in kidney, and GLP-1R can exert diuretic and natriuretic effects after binding to ligands in physiological state [33]. *In vivo* studies have shown that treatment with exenatide, a GLP-1R agonist, can improve glomerular hypertrophy and nephropathy by inhibiting oxidative stress, reducing cytokine production, and decreasing type IV collagen deposition in the kidneys of rats through multiple pathways, thereby markedly reducing the 24-h urinary protein excretion rate in diabetic rats and bringing to bear a nephroprotective effect independent of the hypoglycemic effect [34]. Fujita et al. found that GLP-1R was expressed on the capillaries of mouse kidney glomeruli, and that knocking out GLP-1R resulted in higher urinary albumin levels than controls, while administration of liraglutide, a GLP-1R agonist, significantly decreased urinary protein levels [35]. It is suggested in previous studies that GLP-1R is closely related to the development of DKD, that GLP-1R agonists may exert nephroprotective effects through a specific mechanism, and that changes in GLP-1R functional activity may influence DKD susceptibility. Zhang et al. enrolled 31 patients with T2DM accompanying microalbuminuria in a study divided into glimepiride and exenatide groups, and after 16 weeks of treatment, urinary albumin decreased significantly in the exenatide group compared with the glimepiride group, which demonstrated that agonistic *GLP1R* had a nephroprotective effect independent of glycemic improvement at the clinical level [36]. The present study identified the relationship between the *GLP1R* rs3765467 locus gene polymorphism and the susceptibility to DKD in patients with T2DM, providing a theoretical basis for the exploration of the pathogenesis, intervention and precise treatment of DKD. The results of this study showed that TC levels were higher in carriers of the *GLP1R* rs3765467 A allele, and a possible mechanism for this phenomenon is that the genetic polymorphism at the rs3765467 locus leads to differences in the functional status of GLP-1R, which in turn affects cholesterol levels. GLP-1R agonist treatment was able to decrease both cholesterol and triglycerides in patients with lipid metabolism disorders, suggesting that the functional capacity of GLP-1R can influence cholesterol and triglyceride levels, which is a theory also consistent with our findings [37,38].

Among populations of different ethnic backgrounds, which includes Chinese, Korean, and Mexican, frequent SNPs of *PPARD* are correlated with an elevated risk of dysglycemia and insulin resistance [39–42]. In the current research, we also concerned the genetic variation of *PPARD* and revealed that the frequency of the G allele of rs3777744 was more frequent in patients with T2DM (42.67 %) compared to the healthy subjects (33.50 %) and that the level of PPG and HbA1c was significantly higher in G allele carriers. In contrast, the genetic variation of the *PPARD* rs2016520 locus in our study did not exhibit a significant correlation with susceptibility to T2DM or DKD. The results of this study also showed that patients with T2DM who have *PPARD* rs2016520 TC and CC genotypes have an elevated BMI and TG levels, and this suggests a role of genetic variants that could potentially contribute to the prevalence of overweight, obesity and dyslipidemia in T2DM patients. PPARδ is involved in regulating lipid metabolism in skeletal muscle and adipose tissue, and this may be the mechanism by which *PPARD* gene polymorphisms have been associated with obesity [43,44].

Gene polymorphism can only in part explain inheritance, whereas SNP-SNP interactions are usually identified as the essential contributor to such compound genetic disorders [22]. In this study, we investigated the influence of SNP-SNP interactions on the susceptibility to DKD. Accordingly, the interaction effect of rs3765467 and rs3777744 was identified as statistically significant, and obviously increased the susceptibility to DKD. Explicitely, despite that the polymorphism of rs3777744 had no apparent influence on DKD, the interaction with the genetic variants of rs3765467 was revealed to have a greater influence. The G allele of rs3777744

potentiated the harmful effect of DKD in the rs3765467 A allele carriers. We found convincing support for an outgrowth connection between G allele of rs3777744 and rs3765467, which might increase susceptibility to DKD in the Chinese Han populations. In our previous study, we found that PPARδ can bind to the promoter region of *GLP1R* and thus regulate the expression of GLP-1R [22], which may be the rationale for this SNP-SNP interaction of different genes.

However, there are some underlying limitations of this study that should be noted. First, we only examined genetic variants in *PPARD* and *GLP1R*, and with a relatively small sample size; however, this is only a preliminary study and larger studies may provide more information, especially considering that no such study has been conducted in Chinese Han patients with T2DM. Another possible limitation of our study is that tissue samples from DKD patients were not obtained and the results of SNP-protein expression correlation studies are missing. Further studies to determine whether rs3765467 SNP is associated with protein expression changes of *GLP1R* gene in DKD are more compelling. Meanwhile, other susceptible genetic variations that are potentially involved in variations of DKD susceptibility should be examined in future studies. Finally, more detailed studies are needed to elucidate the molecular mechanisms by which the *GLP1R* rs3765467 polymorphism affects DKD susceptibility.

Indeed, the current results need to be combined with additional studies before they can be applied to the practice, but they are nonetheless undeniably clinically implications. With the continuous exploration of DKD susceptibility genes, it will be conducive to the early warning of people at high risk of progressing to DKD, targeted therapy for DKD patients and the discovery of novel DKD therapeutic targets. It is worth noting that patients with different genetic profiles often exhibit varied responsiveness to the use of therapeutic drugs, and the findings of disease susceptibility genes are also important for guiding the proceeding of pharmacogenomics research. Given that the current study included only the Chinese Han population and the overall sample size was limited, it is necessary to conduct more extensive clinical studies in different racial and ethnic populations to determine the possible role of *PPARD-GLP1R* variant in the development of DKD. Similarly, it is essential to raise that the molecular mechanism of the genetic variant of *PPARD-GLP1R* involved in the development of DKD still needs to be further investigated due to the complexity of gene action. With in-depth studies about genetic contributors to DKD susceptibility, it is more likely that the management of the risk of progression to DKD in patients with T2DM will be at the forefront of translating exploratory research into clinical practice in some situations.

5. Conclusions

In conclusion, the findings of our study revealed that the A allele of rs3765467 is correlated with an increased susceptibility to DKD in the Chinese Han population. The present research also revealed a marked SNP-SNP interaction between rs3765467 and rs3777744, which can significantly increase the risk of DKD. Altogether, our findings strongly suggest that SNPs are important factors that influence the susceptibility to DKD, and the effect of *GLP1R* and *PPARD* gene polymorphisms on DKD susceptibility deserves attention.

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Additional information

No additional information is available for this paper.

Ethical approval

This study was carried out in accordance with the recommendations of the Ethics Committee of the Affiliated Hospital of Xuzhou Medical University (reference No. XYFY2021-KL094-01) and the Ethics Committee of the Affiliated Hospital of Jiangnan University (reference No.LS2021091). All information obtained in the study was kept confidential.

CRediT authorship contribution statement

Jinfang Song: Writing – review & editing, Writing – original draft, Funding acquisition, Formal analysis, Data curation. **Yongru Zhuang:** Formal analysis, Data curation. **Xiaojun Pan:** Writing – review & editing, Formal analysis. **Ya Chen:** Writing – review & editing, Data curation. **Feng Xie:** Writing – review & editing, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Jinfang SONG reports financial support was provided by National Natural Science Foundation of China. Jinfang SONG reports financial support was provided by Jiangsu Research Hospital Association for Precision Medication. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e35289.

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