Research Article

Genetic Variants Flanking the FGF21 Gene Were Associated with Renal Function in Chinese Patients with Type 2 Diabetes

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Received 10 January 2019; Accepted 7 April 2019; Published 7 May 2019

Academic Editor: Akira Sugawara

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Aims. Fibroblast growth factor 21 (FGF21) is closely linked with metabolic disorders including diabetes. Evidences suggested that FGF21 may play a protective role against diabetic kidney disease (DKD). However, the relationship between genetic variants in the FGF21 gene region and DKD remains unknown. In our study, we aimed to investigate the association of genetic variations in this gene region with DKD as well as DKD-related quantitative traits.

Materials and Methods. We recruited 1340 Han Chinese participants with type 2 diabetes, including 596 DKD patients and 744 patients who was diagnosed with diabetes for more than 5 years but did not progress to DKD. Three single-nucleotide polymorphisms were selected (rs2071699, rs838136, and rs499765) and genotyped. The association between these SNPs and DKD as well as DKD-related quantitative traits was analyzed.

Results. We did not find any significant association between these SNPs and susceptibility to DKD in the present study. However, a significant association with estimated glomerular filtration rate (eGFR) was detected: in the non-DKD group, rs838136 was significantly associated with eGFR under an additive model ($\beta = 0.013 \pm 0.006$, $P = 0.0295$, $\beta$ was calculated for log10eGFR) as well as a recessive model ($P = 0.0385$) and rs499765 was associated with eGFR under a dominant model ($P = 0.0411$) and in the DKD group, rs499765 showed a trend toward association with eGFR under an additive model ($\beta = -0.022 \pm 0.012$, $P = 0.0820$, $\beta$ was calculated for log10eGFR) and showed a significant association with eGFR under a dominant model ($P = 0.0182$).

Conclusions. Our findings indicated that genetic variations adjacent to FGF21 were associated with eGFR in Chinese diabetic patients.

1. Introduction

Diabetes has become one of the most common chronic diseases in the world, and the prevalence of the disease has experienced an exploding increase in recent years, especially in the Chinese population [1]. As an important microvascular complication of diabetes, diabetic kidney disease (DKD) is the most frequent cause of end-stage renal disease (EASD) in different countries [2]. A genetic basis has been suggested for this disease according to the evidence of familial clustering of DKD [3] and higher risks of DKD in certain racial groups [4]. Thus, investigation of the genetic profile of DKD could reveal the underlying mechanism of the disease.

FGF21 is an endocrine member of the fibroblast growth factor (FGF) gene family, predominantly expressed in the liver. It exerts beneficial metabolic effects in animal models, such as reducing blood glucose and triglyceride levels, increasing insulin sensitivity, suppressing hepatic glucose production, increasing expenditure, and losing weight [5–8]. So it is recognized as a novel metabolic regulator, and its therapeutic potential has also been suggested for the treatment of metabolic disorders including diabetes and obesity.

Recently, studies have suggested that FGF21 may play a role in the pathogenesis of DKD. Kim et al. revealed that daily administration of FGF21 in diabetic db/db mice markedly decreased urinary albumin excretion and...
mesangial expansion, suppressed profibrotic molecule synthesis, and improved renal lipid metabolism and oxidative stress injury [9]. Cheng et al. found that fenofibrate prevents the development of diabetic nephropathy in mice with type 1 diabetes via upregulating FGF21 and stimulating PI3K/Akt/GSK-3β/Fyn-mediated activation of the Nrf2 pathway [10]. Furthermore, a recent study compared the therapeutic effect of rhFGF21 and PEG-rhFGF21 on diabetic nephropathy in diet-induced obesity (DIO) mice, and the results showed that PEG-rhFGF21 was more efficacious in ameliorating functional and morphological abnormalities induced by diabetic nephropathy in db/db and DIO mice [11]. However, whether genetic variants in the FGF21 gene region relate to DKD is still unknown. So we aimed to evaluate the association of genetic variants in the FGF21 gene region with DKD and its related quantitative traits in this study.

2. Materials and Methods

2.1. Subjects. 1340 unrelated Han Chinese participants were recruited from the Shanghai Diabetes Institute Inpatient Database of Shanghai Jiao Tong University Affiliated Sixth People’s Hospital, including 596 DKD patients (DKD group) and 744 patients who was diagnosed with diabetes for more than 5 years but did not progress to DKD (non-DKD group). All subjects were diagnosed with type 2 diabetes according to the 1999 WHO criteria (https://www.who.int/entity/diabetes/currentpublications/en). Patients with estimated glomerular filtration rate (eGFR) < 90 mL/min per 1.73 m², albuminuria ≥ 30 mg/24 h, or ACR (albumin-to-creatinine ratio) ≥ 30 μg/mg were diagnosed with DKD.

2.2. Clinical Measurements. Anthropometric and biochemical traits related to diabetes were collected in detail for each participant, including height, weight, blood pressure, fasting glucose, 2-hour glucose, glycated hemoglobin A1c (HbA1c), serum lipid profile, liver function, and renal function. The albuminuria level was measured using the samples from 24-hour urine collection, which was repeated in three consecutive days, and then the mean values were used for further analysis. eGFR was calculated by using a Chinese population-specific formula derived from the Modification of Diet in Renal Disease (MDRD) equation [12].

2.3. SNP Selection, Genotyping, and Quality Control Analysis. We chose three SNPs flanking the FGF21 gene on chromosome 19 (all within 8 kb) including rs2071699, rs838136, and rs499765. All these SNPs were genotyped using the MassARRAY Compact Analyzer (Sequenom, San Diego, CA, USA), with matrix-assisted laser desorption ionization time-of-flight mass spectroscopy detecting primer extension

| Group        | n   | rs2071699     | P value | rs499765     | P value | rs838136     | P value |
|--------------|-----|---------------|---------|--------------|---------|--------------|---------|
| DKD group    | 596 | 290/239/42    | 0.4479  | 201/263/104  | 0.2716  | 199/282/93   | 0.6791  |
| Non-DKD group| 744 | 357/306/58    | 0.5000  | 244/349/124  | 0.9668  | 282/320/113  | 0.1650  |
of multiplex products. All SNPs met the quality control thresholds with a genotyping call rate over 90%. The minor allele frequencies for rs2071699, rs499765, and rs838136 were 28.9%, 41.6%, and 39.3%, respectively.

2.4. Statistical Analysis. Allele frequencies were determined by gene counting. The Hardy-Weinberg equilibrium tests were then performed for all of the SNPs in the DKD group and the non-DKD group separately. The \( \chi^2 \) test was conducted to compare the allele frequencies of each SNP between the two groups, and multivariable logistic regression analysis was used to examine the differences of genotype distribution between the two groups, with adjustment for confounding factors. A generalized linear model was applied to test the effects of each SNP on quantitative traits, adjusting for the covariates. eGFR and 24-hour urinary albumin excretion rate (AER) were log10 transformed before linear regression because of their skewed distribution. All these analyses were conducted using SAS software (version 8.0; SAS Institute, Cary, NC, USA), with a two-tailed \( P \) value of <0.05 considered statistically significant.

3. Results

All of the SNPs conformed to the Hardy-Weinberg equilibrium (Table 1). The linkage disequilibrium pattern of these SNPs is shown in Figure 1. rs2071699 and rs838136 constructed a haplotype block (block 1) in this region with \( |D'|=0.98 \) and \( r^2 = 0.25 \). The clinical characteristics of both groups are presented in Table 2. Significant differences were detected between the DKD group and non-DKD group with respect to gender, duration of diabetes, BMI, systolic blood pressure (SBP), diastolic blood pressure (DBP), and HbA1c.
Table 5: Associations between the SNPs and eGFR (mL/min/1.73 m²) in the study subjects.

| Group     | SNP     | Major/minor allele | AA† | Aa‡ | aa§ | Additive model β (SE)‡ | P value | Dominant model β (SE) | P value | Recessive model β (SE) | P value |
|-----------|---------|--------------------|-----|-----|-----|------------------------|---------|----------------------|---------|------------------------|---------|
| DKD group | rs2071699 C/T | 109.6 (82.3, 136.3) | 106.0 (81.3, 134.0) | 120.6 (86.5, 144.5) | -0.006 (0.014) | 0.6515 | -0.001 (0.018) | 0.9337 | -0.032 (0.035) | 0.3517 |
|           | rs499765 G/C  | 105.6 (70.8, 134.7) | 112.0 (85.8, 137.4) | 109.9 (81.3, 135.2) | -0.022 (0.012) | 0.0820 | -0.044 (0.019) | 0.0182 | -0.007 (0.023) | 0.7539 |
|           | rs838136 A/G  | 106.5 (81.5, 138.6) | 109.1 (81.5, 133.1) | 113.1 (89.4, 144.5) | -0.018 (0.013) | 0.1758 | -0.016 (0.019) | 0.3937 | -0.035 (0.024) | 0.1534 |
| Non-DKD group | rs2071699 C/T | 120.3 (101.5, 141.5) | 119.3 (102.9, 138.9) | 122.5 (109.5, 147.6) | -0.006 (0.007) | 0.3575 | -0.009 (0.009) | 0.3047 | -0.004 (0.016) | 0.7964 |
|           | rs499765 G/C  | 117.5 (102.1, 136.9) | 123.4 (103.5, 148.0) | 116.8 (103.5, 135.6) | -0.010 (0.006) | 0.1159 | -0.019 (0.009) | 0.0411 | -0.004 (0.012) | 0.7560 |
|           | rs838136 A/G  | 121.7 (104.3, 146.4) | 120.7 (102.2, 141.1) | 114.9 (96.8, 136.6) | 0.013 (0.006) | 0.0295 | 0.014 (0.009) | 0.1108 | 0.024 (0.012) | 0.0385 |

Data are shown as geometric means (SE). P values were adjusted for age, gender, BMI, duration of diabetes, systolic blood pressure, diastolic blood pressure, and HbA1c. P values < 0.05 are shown in bold. †AA = major allele homozygous; Aa = heterozygous; aa = minor allele homozygous. ‡β (SE) was calculated for log_{10}eGFR and refers to major alleles. eGFR: estimated glomerular filtration rate.
AER, eGFR, uric acid, creatinine, blood urea nitrogen, total cholesterol, and total triglycerides ($P < 0.05$, Table 2).

We first evaluated the association between genetic variants and DKD susceptibility. As shown in Table 3, the allele frequencies of the three SNPs did not differ between the DKD group and the control group ($P > 0.05$). We also compared the genotype distribution of these SNPs between the two groups using logistic regression analysis with adjustment for age, gender, BMI, duration of diabetes, HbA$_1c$, SBP, and DBP. However, no significant difference was found either ($P > 0.05$). Furthermore, there is no difference of haplotype distribution between the DKD and non-DKD groups (Table 4).

We then investigated the effects of SNPs on the quantitative traits related to DKD, including eGFR and AER. In the non-DKD group, rs838136 was significantly associated with eGFR under an additive model ($\beta = 0.013 \pm 0.006$, $P = 0.0295$, Table 5) as well as a recessive model ($P = 0.0385$, Table 5) and rs499765 was associated with eGFR under a dominant model ($P = 0.0411$, Table 5). In the DKD group, rs499765 showed a trend toward association with eGFR under an additive model ($\beta = -0.022 \pm 0.012$, $P = 0.0820$, Table 5) and showed a significant association with eGFR under a dominant model ($P = 0.0182$, Table 5). As for AER, no evidence of association was found for the SNPs in either group (Table 6).

### 4. Discussion

As a potent metabolic regulator, FGF21 has been closely related to obesity-related disorders, such as hyperglycemia, dyslipidemia, insulin resistance, and hepatosteatosis [5–8]. There were also evidences that suggested that FGF21 may play a protective role against DKD [9–11], through both improvement of systemic metabolic alterations and antifibrotic effects [9]. We attempted to examine the association between genetic variants in the FGF21 gene region and DKD in a Chinese population in the current study. However, we did not detect any significant association with the susceptibility to DKD for this gene region. Haplotype analysis did not show any significant result either. Since our sample size was relatively small, the association between the FGF21 gene region and DKD should be further explored in a Chinese population with a larger sample size.

Euphorin and albuminuria are the main phenotypes of DKD [13]. A few studies have suggested that serum FGF21 levels were linked with eGFR as well as albuminuria in diabetic patients. A prospective study demonstrated an independent association between elevated serum FGF21 levels and eGFR decline; thus, the circulating FGF21 level was proposed as a predictor for progressive kidney disease in subjects with type 2 diabetes and normoalbuminuria [14]. This is in accordance with the results revealed by our study that rs838136 and rs499765 were significantly associated with eGFR after adjusting for confounding factors. Individuals who carried more G alleles of rs838136 showed lower eGFR, which means that the G allele of rs838136 may be a risk factor for eGFR decline in the non-DKD group. Individuals with the GG genotype of rs499765 showed lower eGFR than those with the CC+CG genotype. rs838136 and rs499765 are both located in the flanking region of the FGF21 gene, and they may affect the transcriptional regulation, resulting in abnormal splicing, or the translational dynamics of FGF21. The mechanism underlying the link between the FGF21 gene region and eGFR is still unknown and should be further investigated by functional studies. Evidences also showed the independent association of FGF21 levels with urinary albumin excretion (UAE) or albuminuria in type 2 diabetic patients [15, 16]. However, no evidence of association with AER was found for variants in the FGF21 gene region in our study.

Several limitations should be noted in the current study. First, the sample size is relatively small and only Southern Chinese population was included, which limits the power of our study. Secondly, we did not measure the circulating FGF21 levels in this study. Further investigations with a larger sample size as well as functional studies are needed to elucidate how FGF21 is involved in renal function in patients with type 2 diabetes.

In conclusion, our data indicated that genetic variants adjacent to FGF21 were associated with eGFR in subjects with type 2 diabetes. Further genetic and functional studies are necessary to replicate the association and illuminate the underlying mechanisms.

### Data Availability

The data used to support the findings of this study are available from the corresponding authors upon request.

#### Table 6: Associations between the SNPs and AER (mg/24 h) in the study subjects.

| SNP       | Major/Minor allele | AER† (mg/24 h) | AER‡ (mg/24 h) | $\beta$ (SE)† | $P$ value |
|-----------|---------------------|---------------|---------------|---------------|-----------|
| rs2071699 | C/T                 | 100.6 (48.3, 318.7) | 78.5 (37.1, 262.0) | 0.030 (0.039) | 0.4389    |
| rs499765  | G/C                 | 90.0 (52.4, 334.3) | 89.8 (44.6, 267.5) | 0.030 (0.034) | 0.3782    |
| rs838136  | A/G                 | 77.3 (43.0, 282.3) | 90.1 (46.1, 227.4) | -0.028 (0.035) | 0.4327    |
| rs2071699 | C/T                 | 9.5 (6.5, 14.7)  | 8.7 (6.0, 13.8)  | 0.014 (0.014) | 0.3311    |
| Non-DKD   | G/C                 | 9.7 (6.1, 14.1)  | 8.5 (6.5, 14.1)  | 0.0006 (0.013) | 0.9632    |
| rs838136  | A/G                 | 8.9 (6.2, 13.8)  | 10.0 (6.9, 14.6) | -0.007 (0.013) | 0.5667    |

Data are shown as geometric means (SE). $P$ values were adjusted for age, gender, BMI, duration of diabetes, systolic blood pressure, diastolic blood pressure, and HbA$_1c$. ¹AA = major allele homozygous; Aa = heterozygous; aa = minor allele homozygous. ²$\beta$ (SE) was calculated for log$_{10}$eGFR and refers to major alleles.

AER: 24-hour urinary albumin excretion rate.
Ethical Approval

The protocol for this research project has been approved by the Institutional Review Board of Shanghai Jiao Tong University Affiliated Sixth People’s Hospital, and it conforms to the provisions of the Declaration of Helsinki.

Consent

All informed consent was obtained from the subjects.

Conflicts of Interest

The authors declared no conflict of interest.

Acknowledgments

This work was funded by the National Natural Science Foundation of China (grant number 81600659), the Open Research Project of Shanghai Key Laboratory of Diabetes Mellitus (grant number SHKLD-KF-1605), and Wenzhou Public Welfare Science and Technology Project (grant numbers Y20140683 and Y20170047). We appreciate the involvement of all the doctors, researchers, and patients in this study.

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