Effects of Genetic Variants of Nuclear Receptor Y on the Risk of Type 2 Diabetes Mellitus

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Received 20 December 2018; Accepted 4 March 2019; Published 7 May 2019

Abstract

Nuclear factor-Y (NF-Y) also named CBF that consists of three evolutionary conserved subunits including NF-YA, NF-YB, and NF-YC; it is a critical transcriptional regulator of lipid and glucose metabolism and adipokine biosynthesis that are associated with type 2 diabetes mellitus (T2DM) occurrence, while the impacts of genetic variants in the NF-Y gene on the risk of T2DM remain to be investigated. In the present study, we screened five single-nucleotide polymorphisms (SNPs) with the SNaPshot method in 427 patients with T2DM and 408 healthy individuals. Subsequently, we analyzed the relationships between genotypes and haplotypes constructed from these SNPs with T2DM under diverse genetic models. Furthermore, we investigated the allele effects on the quantitative metabolic traits. Of the five tagSNPs, we found that three SNPs (rs2268188, rs6918969, and rs28869187) exhibited nominal significant differences in allelic or genotypic frequency between patients with T2DM and healthy individuals. The minor alleles G, C, and C at rs2268188, rs6918969, and rs28869187, respectively, conferred a higher T2DM risk under a dominant genetic model, and the carriers of these risk alleles (either homozygotes of the minor allele or heterozygotes) had statistically higher levels of fasting plasma glucose, cholesterol, and triglycerides. Haplotype analysis showed that SNPs rs2268188, rs6918969, rs28869187, and rs35105472 formed a haplotype block, and haplotype TTAC was protective against T2DM (OR = 0.76, 95% CI = 0.33-0.82, P = 0.004), while haplotype GCCG was associated with an elevated susceptibility to T2DM (OR = 2.33, 95% CI = 1.43-3.57, P = 0.001). This study is the first ever observation to our knowledge that indicates the genetic variants of NF-YA might influence a Chinese Han individual’s occurrence of T2DM.

1. Introduction

Type 2 diabetes mellitus (T2DM), accounting for more than 90% of all cases of diabetes, is increasing rapidly and becoming a major public health threat throughout the world. During the last few decades, the number of people with T2DM has risen to 360 million worldwide, and this is expected to increase up to 592 million by 2035 [1], and this figure is anticipated to increase by 20% in developed countries and by 70% in developing countries in the next 20 years [2]. Many risk factors have been identified to influence the prevalence or incidence of T2DM. In addition to environmental parameters: obesity, dietary habits, physical activity, psychosocial stress, smoking, and so on, the evidence derived from familial studies including those in twins suggests that T2DM has a strong genetic basis [3]. Indeed, numerous studies through either candidate gene approach or the genome-wide association strategies have associated specific genetic variants with T2DM risk. Up to now, more than 88 loci have been identified to confer susceptibility to T2DM [4]. Their effects however are small, which are not enough to explain the heritability of T2DM.

Nuclear factor-Y (NF-Y) is also named CBF that consists of three evolutionary conserved subunits including NF-YA,
NF-YB, and NF-YC (also known as CBP-B, CBP-A, and CBP-C, respectively). NF-Y is a ubiquitously expressed protein with CCAAT-binding activity [5]. The CCAAT motif is widely present in promoters of diverse classes of mammalian genes, so its binding partner NF-Y is involved in the regulation of numerous biological processes, such as cell cycle progression [6], embryonic development [7], neurodevelopment [8], cholesterol and fatty acid metabolism [9, 10], and muscle cell differentiation [11]. Recent studies indicate that the altered NF-Y activity is associated with diabetes. G6PC2 (also termed IGRP gene) encodes for the glucose-6-phosphatase catalytic subunit (G6Pase) that is a key gluconeogenic enzyme in hepatocytes; a genetic variant of G6PC2 is found to affect the NF-Y DNA interaction and enhance G6PC2 expression, resulting in an increased hepatic gluconeogenesis and glucose production [12]. We recently found that the NF-YA liver-specific knockout mice showed significantly reduced blood glucose levels; further evidences demonstrated that NF-YA controls glucose production mainly through upregulating the gluconeogenic enzyme expression, such as phosphoenolpyruvate carboxykinase (PEPCK) and G6Pase [13]. Moreover, NF-YA is closely near the T2DM susceptibility locus identified in European and Pakistani descendents and Chinese [14]. On the basis of these observations, NF-YA is considered a convincing candidate gene for the predisposition to T2DM.

Owing to the importance of the CCAAT motif in gene transcription and the critical roles of NF-Y in numerous biological processes, there is a significant number of investigations developing agents that alter NF-Y DNA-binding pattern or its activity. Indeed, various compounds have been shown to affect NF-Y activity. Pyrrolobenzodiazepine conjugates are sequence-specific DNA-binding agents affecting NF-Y DNA interactions [15]. The synthetic antitumor agent HNM-176 can suppress the expression of multidrug resistance gene (MDR1) by inhibiting NF-Y activity in cancer cell lines [16]. Histone deacetylase inhibitors, such as trichostatin A, vorinostat, and valproic acid, clearly alter NF-Y activity via the impact on its acetylation [17, 18]. Thus, it is therapeutically feasible to modulate NF-Y activity through designing proper compounds. This strengthens the importance of assessing whether NF-Y may be a risk gene of T2DM. Therefore, this aim of the present study was to examine the relationship between genetic variants in NF-YA gene with a T2DM risk in a Chinese Han population.

2. Materials and Methods

2.1. Subjects. As described previously [19], a total of 835 unrelated Chinese Han subjects including 427 patients with T2DM and 408 sex- and age-matched healthy individuals were recruited from the West China Hospital of Sichuan University. The anthropometric parameters such as height, weight, and blood pressure were collected. T2DM was diagnosed according to the criteria of 1999 World Health Organization and defining as fasting glucose level ≥ 7.0 mmol/L and/or 2 h postprandial plasma glucose ≥ 11.1 mmol/L. Patients with a history of other forms of diabetes were excluded. The age-matched healthy individuals with normal fasting glucose levels and without family history of diabetes were from physical examination. This study was approved by the ethics review board of West China Hospital, Sichuan University. Written informed consent was obtained from each participant.

2.2. Biochemical Measurements. Overnight fasting venous blood was collected using vacutainers and transferred to ethylene diamine tetraacetic acid (EDTA) tubes. After centrifuging at 4000 g for 5 min, the plasma was collected for measuring the biochemical parameters. The plasma levels of glucose and lipid profiles (total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglyceride) were determined with commercially enzymatic kits (Boehringer Mannheim, USA).

2.3. SNP Selection and Genotyping. Genotype data of the Chinese population for the NF-YA region were obtained from the 1000 genome project resources (http://www.1000genomes.org/). Five tagSNPs with an r² threshold of 0.8 and a minor allele frequency of 0.05 were selected using Tagger software implemented in Haploview software (Table S1), which are rs2268188, rs6918969, rs28869187, rs35105472, and rs76109475. The genotype of each SNP was determined by the ABI SNAPshot method from the ABI 3130 Genetic Analyzer (Applied Biosystems, Carlsbad; CA, USA). The data were analyzed by using GeneMapper 4.0 software (Applied Biosystems).

2.4. Statistical Analyses. Statistical analyses were performed with SPSS software (SPSS Inc., Chicago, IL, USA). We compared the demographic and biochemical data between cases and controls with Student’s t-test. We examined the Hardy-Weinberg equilibrium (HWE) of each SNP using two-sided chi-square analysis. The frequencies of genotypes or alleles between the T2DM patients and the controls were compared under different genetic models including dominant, recessive, and additive models; Akaike’s information criterion (AIC) were used to select the best one with the smallest AIC value. We compared the biochemical parameters between participants with different genotypes at each SNP with a chi-square test. A two-sided significance level of P < 0.05 was used for significant tests. Odds ratios (ORs) and its 95% confidence intervals (CIs) were estimated using unconditional logistic regression analyses [21, 22]. Haplotype reconstruction and pairwise linkage disequilibrium (LD) estimation were performed using Haplovie 4.2 (https://www.broadinstitute.org/haplovie/haplovie).

3. Results

3.1. Characteristics of the Participants. The individual’s demographic data and biochemical parameters (Suppl. Table 2) have been reported previously [19]. Among the 427
participants with T2DM, 219 (51.28%) were male, and 208 (48.72%) were female. Out of 408 control subjects, 209 (51.22%) were male and 199 (48.78%) were female. The mean age of case and control groups was about 58 years. The average BMIs were $24.16 \pm 2.25$ and $23.28 \pm 2.13$ in diabetic patients and control individuals, respectively. There is no significant difference regarding gender, age, and BMI between the two groups ($P > 0.05$). Compared to healthy controls, the patients with diabetes had significantly lower plasma HDL-C concentrations ($1.22 \pm 0.33 \text{ mmol/L}$ vs. $1.38 \pm 0.37 \text{ mmol/L}$, $P = 0.008$) and statistically higher levels of glucose ($9.77 \pm 1.15 \text{ mmol/L}$ vs. $4.86 \pm 0.58 \text{ mmol/L}$, $P = 0.001$), cholesterol ($5.11 \pm 0.92 \text{ mmol/L}$ vs. $4.87 \pm 0.88 \text{ mmol/L}$, $P = 0.009$), and triglycerides ($1.72 \pm 0.53 \text{ mmol/L}$ vs. $1.21 \pm 0.34 \text{ mmol/L}$, $P = 0.003$), while the LDL-C levels are approximately equal between the two groups ($P > 0.05$). Meanwhile, the systolic and diastolic blood pressures were significantly higher in diabetic patients than those in healthy controls ($P < 0.01$).

3.2. Distribution of the NF-YA SNPs between T2DM Patients and Healthy Individuals. Figure 1 illustrates the physical location of five tagSNPs genotyped. The frequencies of the genotype and allele of each SNP are summarized in Table 1; they were in accordance with the HWE in both groups ($P > 0.05$). We examined the associations of these five tagSNPs with T2DM. Three SNPs (rs2268188, rs6918969, and rs28869187) exhibited nominal significant differences in allelic or genotypic frequencies between patients with T2DM and healthy individuals, whereas others did not. Compared to the allele T at rs2268188, T at rs6918969, and A at rs28869187, the allele G at rs2268188 ($OR = 1.31$, 95% CI: $1.06–1.61$, $P = 0.011$), C at rs6918969 ($OR = 1.34$, 95% CI: $1.11–1.63$, $P = 0.011$), and C at rs28869187 ($OR = 1.33$, 95% CI: $1.08–1.64$, $P = 0.011$) are associated with a higher risk of T2DM, respectively. The genotypes of these three SNPs were distributed statistically differently between T2DM patients and control individuals ($P < 0.05$) (Table 1).

3.3. Association of SNPs with T2DM in Diverse Genetic Models. To reach the maximum power in genetic association studies, the genetic model (dominant, recessive, or additive) used in the analysis is required to be concordant with the "true" inheritant model of disease susceptibility loci. By comparing the frequency distributions of different genotypes at every SNP between groups in diverse models, the best genetic model was determined on the basis of Akaike’s information criteria. For each SNP, the allele with relatively lower frequency compared to another one is recognized as the minor allele (Table 1). We found that the minor alleles G, C, and C.
important roles in the regulation of glucose metabolism and
the complexity of the NF-YA gene in T2DM development.
rs3510542 were associated with the risk of T2DM, implying
lotypes derived from rs2268188, rs6918969, rs28869187, and
genetic variants contribute to the T2DM susceptibility. Hap-
 haplotype analysis also strongly supports that the NF-YA
T2DM risk under a dominant genetic model. Moreover, the
risk, and their minor alleles could confer an increased
rs6918969, and rs28869187) are associated with the T2DM
acid triggers pancreatic beta cell apoptosis and insulin resist-
tance in peripheral tissues [26]. Secondly, NF-Y is recently
demonstrated to control the expressions of adipokines
including adiponectin and leptin in adipose tissue [27, 28].
Over the past decades, emerging evidence has indicated that
the content of adipose tissue is associated with insulin resist-
tance and T2DM [29]. Adiponectin and leptin can act as
linkers between obesity and insulin resistance by performing
special roles in the regulation of body metabolism [30].
Moreover, the adipose tissue-specific NF-Y knockout ani-
imals exhibit hyperglycemia and hyperinsulinemia [27].
Finally, NF-Y is identified to control hepatic gluconeogene-
sis, which is the primary source of glucose production in
patients with T2DM. In line with its function in gluconeogene-
ics, liver-specific NF-YA knockout mice showed significa-
cantly decreased fasting blood glucose levels [13]. These
observations imply that NF-YA genetic variants might influence
the risk of T2DM by combining multiple pathways.
T2DM has been associated with abnormal triglyceride
and cholesterol concentrations [31]. In this study, we
observed that the diabetic risk alleles were associated with
the elevated plasma levels of triglyceride and cholesterol,
suggesting the possible participation of genetic variants
of NF-YA in T2DM etiology. Indeed, NF-Y is ascertained
to regulate the expression of genes encoding the key
enzymes responsible for the metabolism of cholesterol
and fatty acids, such as 3-hydroxy-3-methylglutaryl-CoA
synthase and reductase, squalene synthase, fatty acid syn-
 thase, and acetyl-CoA carboxylase [9, 10, 32]. In addition,
NF-Y regulates the transcription of SREBP-1 [33] that is a
master transcription factor of cholesterol and fatty acid bio-
synthesis [34]. Moreover, the DNA binding of NF-Y predis-
poses a positive chromatin environment for transcriptional
induction by SREBP [35]. Therefore, NF-Y may affect
the development of T2DM by regulating the lipid metabolism.
The statistically significantly associated variants are
within the NF-YA intron regions, which are considered to
have no apparent biological function. However, intronic
SNPs might impact gene expression [36]. It is also likely that
the diabetic risk variants of NF-YA are only surrogate
markers for the causal functional SNPs elsewhere. We only

### Table 2: Association between NF-YA SNPs and the risk of T2DM under different genetic models.

| SNP          | Genetic model*                     | P value | OR [95% CI]** |
|--------------|------------------------------------|---------|---------------|
| rs2268188    | Dominant (TG+GG) vs. TT            | 0.026   | 1.36 [1.04–1.79] |
| rs6918969    | Dominant (TC+CC) vs. TT            | 0.003   | 1.54 [1.16–2.03] |
| rs28869187   | Dominant (AC+CC) vs. AA            | 0.016   | 1.40 [1.07–1.84] |
| rs35105472   | Recessive GG vs. (CG+CC)           | 0.074   | 1.62 [0.95–2.77] |
| rs76109475   | Dominant (AG+AA) vs. GG            | 0.586   | 1.08 [0.82–1.42] |

*For each SNP, only the best genetic model determined by Akaike’s information criterion (AIC) is provided. **The ORs and CIs that are statistically significant are bolded, along with the rs number of the corresponding SNP.

at rs2268188, rs6918969, and rs28869187, respectively, con-
ferred a significant risk of T2DM under a dominant model (Table 2).

### 3.4. Effects of NF-YA Variants on Metabolic Parameters

We further examined the effects of rs2268188, rs6918969,
and rs28869187 on the metabolic variables in participants
with normal glucose regulation under a dominant model.
Compared to the individuals with genotypes composed of
homozygous wild-type alleles (T, T, and A at rs2268188,
rs6918969, and rs28869187, respectively) and the carriers
of the risk alleles (either homozygotes of the minor allele
or heterozygotes) exhibited statistically higher levels of fast-
ing plasma glucose, cholesterol, and triglycerides (all P <
0.05), while no such association was found for HDL-C levels (Figure 2).

### 3.5. NF-YA Haplotypes and T2DM. Linkage disequilibrium

(LD) in pairwise combinations of alleles in different SNPs
was assessed by using maximum likelihood from the geno-
type frequency in all participants. As shown in Figure 3, four
SNPs (rs2268188, rs6918969, rs28869187, and rs35105472)
were in moderate LD and formed a haplotype block.

We subsequently compared the frequency distributions
of haplotypes constructed from these four SNPs between
T2DM patients and controls. As shown in Table 3, haplotype
TTAC was associated with a lower risk of T2DM (OR =
0.76, 95% CI = 0.33–0.82, P = 0.004), and haplotype GCCG
was associated with an elevated susceptibility of T2DM
(OR = 2.33, 95% CI = 1.43–3.57, P = 0.001). None of the other
haplotypes was found to have significant effects.

### 4. Discussion

In this present study, we examined the relationships between
five tagSNPs in the NF-YA region and their susceptibilities to
T2DM. Our results suggest that the three SNPs (rs2268188,
rs6918969, and rs28869187) are associated with the T2DM
risk, and their minor alleles could confer an increased
T2DM risk under a dominant genetic model. Moreover, the
haplotype analysis also strongly supports that the NF-YA
genetic variants contribute to the T2DM susceptibility. Hap-
lotypes derived from rs2268188, rs6918969, rs28869187, and
rs3510542 were associated with the risk of T2DM, implying
the complexity of the NF-YA gene in T2DM development.

Indeed, numerous evidence has shown that NF-Y plays
important roles in the regulation of glucose metabolism and
Figure 3: Linkage disequilibrium (LD) plots for SNPs genotyped in the NF-YA region, which were generated by Haploview 4.2 software. SNPs are identified by their rs number in dbSNP. Red shades (left) show the strength of the pairwise LD based on $D'$ expressed as a percentile within squares. Black shades (right) demonstrate the strength of the pairwise LD based on $r^2$, and the number is the $r^2$ value expressed as a percentage.
selected SNPs with minor allele frequency of 5% in the present study; thus, those rare disease-causing variants might be missed. However, the rare variants in genetic architecture are demonstrated to be critically important in disease etiology in a recent study [37]. Thus, the investigation of rare variants of NF-YA may lead to a better understanding of their roles in susceptibility to the development of T2DM. In addition, NF-Y is a trimeric protein complex composed by the NF-YA, NF-YB, and NF-YC subunits, which are all necessary for the formation of NF-Y complexes and binding to target gene [5]. The absence of any of the NF-Y subunits results in loss of binding of the NF-Y complex to DNA- and NF-Y-directed transcription [5]. Therefore, it is necessary to establish the association of all three subunits of NF-Y with the diabetes in the future study.

In conclusion, this study is the first ever observation to our knowledge that demonstrates genetic variants of NF-YA are associated with T2DM. It is necessary to determine if the associated alleles influence gene expression, which will clarify the molecular mechanisms underlying the association of NF-Y and T2DM.

Data Availability

Data are included in this published article and its supplementary information files. We can provide detailed data upon reasonable request.

Conflicts of Interest

There is no duality of interest associated with this manuscript.

Authors’ Contributions

WY, GQY, and YYM are responsible for collecting, processing, and interpreting the data and recruiting the patients. YSS, CJL, ZYW, and ZXP carried out the molecular genetics. SZG is responsible for funding acquisition and analyzing and writing the manuscript. Ying Wang, Shanshan Yang, and Quyue Guan contributed equally to this work.

Table 3: Frequencies of pairwise haplotype constructed by SNPs in NF-YA.

| Haplotype* | Frequency Case | Frequency Control | P value | OR [95% CI]** |
|------------|----------------|-------------------|---------|---------------|
| TTAC       | 0.337          | 0.348             | 0.004   | 0.76 [0.33–0.82] |
| GCCG       | 0.344          | 0.319             | 0.001   | 2.33 [1.43–3.57] |
| GCCC       | 0.122          | 0.123             | 0.954   |               |
| TTAG       | 0.109          | 0.113             | 0.443   |               |
| CGTG       | 0.085          | 0.079             | 0.217   |               |

*The order of SNPs from left to right is rs2268188, rs6918969, rs28869187, and rs35105472. Only haplotypes with a frequency > 3% in at least one group were listed. ** Only haplotypes distributed significantly differently (P < 0.05) were calculated.

Acknowledgments

The study was supported by grants from the National Natural Science Foundation of China (No. 81770814) and Key R&D Program of Sichuan Province (No. 2018SZ0171).

Supplementary Materials

Supplemental Table 1. Five SNPs in the NF-YA gene in the study. Supplemental Table 2. Characteristics of T2DM patients and control subjects. (Supplementary Materials)

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