Original Article

Affection of Single-Nucleotide Polymorphisms in miR-27a, miR-124a, and miR-146a on Susceptibility to Type 2 Diabetes Mellitus in Chinese Han People

Tong-Tong Wang[1], Yong-Jie Chen[2], Lu-Lu Sun[1], Si-Jia Zhang[1], Zhong-Yu Zhou[1], Hong Qiao[1]

[1]Department of Endemic Disease, The Second Affiliated Hospital, Harbin Medical University, Harbin, Heilongjiang 150081, China
[2]Department of Biostatistics, Public Health College, Harbin Medical University, Harbin, Heilongjiang 150081, China

Abstract

Background: Polymorphisms of microRNA (miRNA), as a novel mechanism, are closely associated with disease states by interfering with miRNA function. Direct correlations have been identified between single-nucleotide polymorphisms (SNPs) in miRNA, but the effect on type 2 diabetes mellitus (T2DM) onset among Chinese population remains unclear. Therefore, the aim of this study was to identify correlations between common SNPs in miR-27a, miR-146a, and miR-124a with T2DM among a Chinese population, as well as to explore diabetic pathological mechanisms and the impact of environmental factors.

Methods: SNPscan technology was used to genotype 995 patients newly diagnosed with T2DM and 967 controls. Logistic regression analysis was performed to compare mutation frequencies between cases and controls.

Results: We found no significant correlations between all genotypes of these miRNAs and T2DM in our research. However, stratification analysis identified a lower risk of T2DM associated with the rs531564GC genotype among younger subjects (age < 45 years) (adjusted $P = 0.043$; odds ratio [OR] = 0.73; 95% confidence interval [CI] = 0.54–0.99). Furthermore, the rs895819CC genotype in overweight people (24 ≤ body mass index [BMI] < 28) was significantly associated with an increased risk of T2DM (adjusted $P = 0.042$; OR = 1.73; 95% CI = 1.02–2.94), while the rs2910164 genotype in miR-146a was not significantly correlated with T2DM. The genetic risk score was calculated based on the number of risk alleles of the three SNPs and was found to be correlated to total cholesterol (adjusted $P = 0.021$).

Conclusions: The rs531564GC genotype acted as a protective factor to decrease the risk of T2DM in younger subjects (age < 45 years), while the presence of the rs895819CC genotype increased the risk of illness among overweight subjects (24 ≤ BMI < 28 kg/m^2). The presence of SNPs in miRNA might promote disease by affecting miRNA expression and gene function. Thus, miRNA mimics or inhibitors that directly regulate miRNA expression present novel and promising therapeutic targets.

Key words: Epigenetics; Genetic Variation; MicroRNA; Single-Nucleotide Polymorphism; Type 2 Diabetes Mellitus

Introduction

Type 2 diabetes mellitus (T2DM) is the most common endocrine and metabolic disease among western and more recently, Asian countries, and is characterized by chronically elevated blood glucose levels. Current projections predict that the global prevalence of T2DM, estimated at 366 million cases in 2011, will increase to 552 million by the year 2030. Equally alarming is the predicted increase in diabetes cases in China from 90 to 129.7 million over the same period.[1] T2DM increases the risk of onset of all reported diabetes-associated comorbidities, including cardiovascular disease, kidney disease, blindness, amputation, etc., by years to decades. However, such problems can be imminently addressed by consideration of the root causes of T2DM. Although T2DM is universally known to be associated with a genetic predisposition and is increasingly diagnosed in younger patients, the understanding of the etiology and pathogenesis of this disease remains insufficient.

Single-nucleotide polymorphisms (SNPs) are the most common type of DNA sequence deviations and reportedly account for approximately 90% of genetic variations in the human genome.[2] SNPs theoretically can occur within any region of the human genome and those disrupting the coding region promote transcription of coding SNPs, which alter the amino acid sequences of transcribed proteins, thereby contributing to a state of disease.[3] Although genome-wide association studies have become a cutting-edge method to explore risk loci for T2DM,[4-6] SNPs only explain part of the heritability

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.4103/0366-6999.151112

Address for correspondence: Prof. Hong Qiao, Department of Endemic Disease, The Second Affiliated Hospital, Harbin Medical University, Harbin, Heilongjiang 150081, China
E-Mail: qiaoh0823@sina.com

of T2DM, while the use of microRNA (miRNA), as only one aspect of epigenetic approaches, may be more appropriate to establish intimate correlations between genetic backgrounds and environmental factors. Therefore, elucidation of the interface between SNPs and miRNAs may be a reasonable approach to interpret the mechanism as well.

MicroRNAs are a class of endogenous small noncoding, but functional, RNA molecules composed of 18–24 nucleotides. The generally accepted view of miRNA biogenesis is that primary miRNA transcripts are cleaved to stem-loop intermediates, known as pre-miRNAs, in the nucleus by the RNase III enzyme Drosha. These pre-miRNAs are then subsequently transported to the cytoplasm and lysed by the cytoplasmic RNase III enzyme dicer to generate mature miRNAs, although expression of these molecules might be affected by any of several aspects of this intermediate process. Nonetheless, miRNAs typically degrade or directly suppress translation of target genes by specific complementary base-pairing to the 3′ untranslated region (UTR) of the target mRNA to regulate multiple pathways, such as cell death, cell proliferation, embryonic development, and metabolic processes.[7-9] It has been suggested that miRNAs may regulate translation of more than one-third of human genomic mRNAs. Recent studies have shown that gain or loss of miRNA function contributes to the onset of T2DM. For example, miR-27a expression was found to be up-regulated in adipose tissue of a spontaneous rat model of T2DM and circulating levels were found to be increased in patients diagnosed with metabolic syndrome and T2DM.[10,11] Moreover, serum miR-9, miR-29a, miR-30d, miR-34a, miR-124a, miR-146a, and miR-375 levels in T2DM were found to be significantly elevated compared to a normal glucose tolerance group and up-regulation five compared to a prediabetes group.[12] Compared with healthy controls, circulating miRNA-146a levels were significantly up-regulated in patients newly diagnosed with T2DM.[13] However, a previous study reported a negative correlation between downregulation of miR-146a expression and that of the target gene tumor necrosis factor (TNF) receptor-associated factor 6, glycated hemoglobin (HbA1c), insulin resistance, poor glycemic control, several proinflammatory cytokine genes, and circulatory levels of TNF-α and interleukin-6.[14]

Single nucleotide polymorphisms in miRNAs are emerging as an innovative method to alter functional polymorphisms present in the human genome to interfere with the function of miRNAs. For example, the presence of rs2910164 in pre-miR-146a affects miRNA expression levels and leads to a genetic predisposition to papillary thyroid carcinoma.[15] A recent study verified correlations among 13 candidate loci derived from the literature and computational data regarding T2DM susceptibility that identified the mutated allele of rs895819 in miR-27a and rs531564 in miR-124a, but not rs2910164 in miR-146a, were relevant to T2DM onset in Italian nationals.[16] However, these three SNPs have not been reported in Chinese diabetic patients, and their study did not address the correlations between gene mutations and clinical phenotypes. Therefore, in this case–control study, we replicated the effects of these three polymorphisms on T2DM susceptibility in a larger Han population from Northern China, which suggest the diagnostic and therapeutic potential of these SNPs of miRNA.

Methods

Patients and healthy subjects

We recruited 995 newly diagnosed T2DM patients and 967 controls aged 20–79 years from September 2009 to September 2013. The patients were from the endocrine inpatient and outpatient clinic of the Second Affiliated Hospital at Harbin Medical University (Harbin, China) and controls were recruited from the physical examination center in the same hospital. A standard diagnosis of T2DM was in accordance with fasting plasma glucose (FPG) ≥ 7.0 mmol/L (126 mg/dl) or a 2-hour postglucose level ≥ 11.1 mmol/L (200 mg/dl) by either a random elevated fasting glucose value on more than one occasion or an oral glucose tolerance test. The duration of newly diagnosed T2DM was shorter than 6 months, and patients who used oral medications or insulin injections to achieve adequate glucose control were excluded from this study. All patients routinely underwent arterial Doppler ultrasound electrocardiography, as well as nerve conduction velocity and ophthalmic examinations, to identify complications of T2DM. For elimination of prediabetes (impaired fasting glucose and impaired glucose tolerance), we recruited controls with an FPG level of ≤ 5.1 mmol/L (91.8 mg/dl) at the same time as a HbA1c level of <6.0%. Exclusion criteria were the presence of malignancies, nephropathy, severe liver disease, and autoimmune diseases. To exclude type 1 diabetes patients, we tested for glutamic acid decarboxylase autoantibody and insulin autoantibody, otherwise, we attempted to eliminate gestational and type-specific diabetes.

Information collection

The age and sex of the study subjects were recorded and blood pressure, circumference of the waist and hips, height, and weight were measured by trained medical staff. Approximately 2 ml of venous blood was collected from all subjects via venipuncture into tubes containing sodium ethylenediaminetetraacetic acid and then stored at −20°C. Levels of lipids, FPG, fasting insulin (FINS), C-peptide, and HbA1c were determined by physicians from two affiliated hospitals. Body mass index (BMI) was calculated as weight/height^2 (kg/m^2); homeostasis model assessment of β cell function (HOMA-β) was calculated as 20 × FINS (mIU/L)/FPG (mmol/L) - 3.5(%); homeostasis model assessment of insulin sensitivity (HOMA-IS) was calculated as 1/(FPG (mmol/L) × FINS (mIU/L); and homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as FPG (mmol/L) × FINS (mIU/L)/22.5. The aim of the study was explained to each of the participants and

all consented to donate blood samples and the use of the collected demographic and physiologic data.

Single-nucleotide polymorphism selection

We obtained miR-27a (rs895819), miR-124a (rs531564), and miR-146a (rs2910164) as previously described.[16] Our previous analysis of 13 miRNAs as candidate genes showed that there were no correlations between SNPs in 11 miRNAs associated with T2DM, with the exception of a protective effect of the mutated GG genotype of rs895819 in miR-27a and a risk effect of the mutated GG genotype of rs531564 in miR-124a. We chose miR-146a as a target in our investigation and found that the C allele in miR-146a (rs2910164) was associated with a lower risk of diabetic polyneuropathy.[17] We also considered racial and ethnic differences in miRNA expression profiles and gene polymorphic genetic variants.[18,19]

DNA preparation and genotyping assay

Genomic DNA was extracted from all blood samples using the TIANamp Blood DNA Kit (Tiangen Biotech Co., Ltd., Beijing, China). The quality and concentration of DNA were assessed by agarose gel electrophoresis (Invitrogen Corporation, Waltham, MA, USA). We used SNPscan minisequencing to identify SNPs. SNP genotyping was performed using a custom-by-design 48-Plex SNPscan Kit (catalog no.: G0104K; Genesky Biotechnologies Inc., Shanghai, China) according to methods described by Chen *et al*.[20] This kit was an SNP genotyping technology patented by Genesky Biotechnologies, Inc. based on the ligase and multiplex fluorescence polymerase chain reactions.[20]

Statistical analysis

For quality control, each SNP in the control population was assessed for conformity to the Hardy–Weinberg equilibrium using the Pearson's Chi-squared test. SNPs with a $P < 0.05$ were excluded from further analysis. Categorical data are presented as frequencies (percentages) and continuous variables as mean ± standard deviation (SD). Student's *t*-test was used to identify differences among quantitative variables and the Chi-squared test was used for comparisons of categorical variables of basic characteristics and genotype frequencies in cases and controls. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to assess correlations between genotypes and risks of T2DM by logistic regression analysis adjusted for age, gender, and BMI. All analyses were performed using SPSS version 17.0 software (SPSS, Inc., Chicago, IL, USA). A $P < 0.05$ was considered statistically significant.

Results

Basic characteristics

The characteristics of T2DM cases and controls are shown in Table 1. There were no obvious differences in gender and low-density lipoprotein cholesterol (LDL-C) levels between the two groups ($P > 0.05$). Of the studied basic characteristics, significant differences existed for age, BMI, systolic blood pressure, diastolic blood pressure, FPG, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), FINS, HbAlc, HOMA-β, HOMA-IS, and HOMA-IR (all, $P < 0.05$). In contrast, there were no significant differences in gender and LDL-C between cases and controls (all, $P > 0.05$).

Table 1: Baseline characteristics of individuals in the case and control group

Variables	Case (n=995)	Control (n=967)	P
Gender (male:female)	612:383	568:399	0.210
Age (years)	46.11 ± 12.56	42.93 ± 11.70	<0.001
BMI (kg/m^2)	25.78 ± 3.58	23.33 ± 3.35	<0.001
SBP (mm Hg)	130.16 ± 17.51	121.30 ± 15.09	<0.001
DBP (mm Hg)	84.63 ± 11.17	79.24 ± 9.64	<0.001
FPG (mmol/L)	10.04 ± 3.40	4.84 ± 0.29	<0.001
TC (mmol/L)	5.00 ± 1.29	4.88 ± 1.01	<0.001
TG (mmol/L)	2.38 ± 2.25	1.42 ± 0.95	0.021
HDL-C (mmol/L)	1.21 ± 0.32	1.47 ± 0.35	<0.001
LDL-C (mmol/L)	2.91 ± 0.96	2.92 ± 0.86	0.815
FINS (μU/ml)	12.90 ± 7.58	7.87 ± 4.43	<0.001
HbAlc	9.30 ± 2.36	5.12 ± 0.47	<0.001
HOMA-β	$5.93\times10^{1} \pm 1.69\times10^{2}$	$1.31\times10^{2} \pm 1.50\times10^{2}$	<0.001
HOMA-IS	$1.29\times10^{-2} \pm 1.32\times10^{-2}$	$3.74\times10^{-2} \pm 3.23\times10^{-2}$	<0.001
HOMA-IR	$5.76\times10^{0} \pm 4.04\times10^{0}$	$1.70\times10^{0} \pm 9.74\times10^{-1}$	<0.001

Data are presented as mean ± SD. BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; FPG: Fasting plasma glucose; TC: Total cholesterol; TG: Triglyceride; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; FINS: Fasting insulin; HbAlc: Glycated haemoglobin; HOMA-β: Homeostasis model assessment of β cell function; HOMA-IS: Homeostasis model assessment of insulin sensitivity; HOMA-IR: Homeostasis model assessment of insulin resistance; SD: Standard deviation.

Associations between alleles and genotypes of single-nucleotide polymorphisms in microRNA and type 2 diabetes mellitus

The results of this study showed that there were no correlations between alleles and genotypes of miR-146a rs2910164, miR-124a rs531564, and miR-27a rs895819 with the risk for T2DM (all, $P > 0.05$) after adjustment for age, gender, and BMI. Furthermore, there were no significant correlations between genetic risk score (GRS), which was calculated by the accumulative effect of risk alleles of the three SNPs, and disease [Tables 2 and 3]. We also compared genotypes of all gene loci in dominant and recessive models of T2DM, but there were still no statistically significant differences (data not shown).

Assessment of the association between single-nucleotide polymorphisms and glucose-lipid metabolism targets

The risk alleles at rs2910164 and rs531564 had no effect on glucose and lipid metabolism, whereas the risk allele at rs895819 had a primary effect on FINS (adjusted, $P = 0.024$). However, we considered that the sample size in this study might has been insufficient to identify correlations between SNPs and the incidence of T2DM, thus we calculated the GRS by accumulation of three risk alleles as described elsewhere.[21] Strikingly, the GRS values derived from the three SNPs were statistically associated with TC (adjusted, $P = 0.021$) [Table 4].

Table 2: Association of rs2910164, rs531564 and rs895819 SNPs with type 2 diabetes in the Chinese Han people

SNP	Gene	Allele	*n* (%)		*P*	OR (95% CI)	*P**	OR* (95% CI)
			Cases	Controls				
rs2910164	miR-146a	C	1132 (56.9)	1121 (58.0)	0.495	0.96 (0.84–1.81)	0.497	0.95 (0.83–1.09)
		G	858 (43.1)	813 (42.0)				
rs531564	miR-124a	G	1653 (83.1)	1635 (84.5)	0.210	0.90 (0.76–1.06)	0.149	0.88 (0.73–1.05)
		C	337 (16.9)	299 (15.5)				
rs895819	miR-27a	T	1469 (73.8)	1415 (73.2)	0.642	1.03 (0.90–1.19)	0.368	1.07 (0.92–1.25)
		C	521 (26.2)	519 (26.8)				
GRS					0.156	1.07 (0.98–1.17)	0.074	1.09 (0.99–1.20)

The results of logistic regression analysis were shown. *Adjusted for age, gender, and BMI. rs2910164 C, rs531564 G and rs895819 C were considered as risk alleles. The GRS was calculated according to the number of risk alleles of the 3 SNPs. CI: Confidence interval; OR: Odds ratio; BMI: Body mass index; GRS: Genetic risk score; SNPs: Single-nucleotide polymorphisms.

Table 3: Genotype distributions of 3 SNPs in case and control groups

dbSNP	Genotypes	*n* (%)		*P*	OR (95% CI)	OR* (95% CI)	*P**
		Cases	Controls				
rs2910164	CC	313 (31.5)	322 (33.3)	N/A	1	N/A	1
	GC	506 (50.9)	477 (49.3)	0.391	0.92 (0.75–1.12)	0.90 (0.72–1.1)	0.317
	GG	176 (17.7)	168 (17.4)	0.576	0.93 (0.71–1.21)	0.93 (0.70–1.23)	0.603
rs531564	GG	681 (68.4)	689 (71.3)	N/A	1	N/A	1
	GC	291 (29.2)	257 (26.6)	0.179	0.87 (0.72–1.06)	0.84 (0.68–1.04)	0.107
	CC	23 (2.3)	21 (2.2)	0.738	0.90 (0.50–1.65)	0.91 (0.48–1.72)	0.733
rs895819	TT	554 (55.7)	526 (54.4)	N/A	1	N/A	1
	CT	361 (36.3)	363 (37.5)	0.550	1.06 (0.88–1.30)	1.12 (0.92–1.37)	0.262
	CC	80 (8.0)	78 (8.1)	0.876	1.03 (0.74–1.43)	1.06 (0.74–1.52)	0.742

The results of logistic regression analysis were shown. *Adjusted for age, gender, and BMI. CI: Confidence interval; OR: Odds ratio; N/A: Not applicable; BMI: Body mass index; SNPs: Single-nucleotide polymorphisms; dbSNP: Database of single-nucleotide polymorphisms.

Stratified analysis between respective single-nucleotide polymorphism genotypes and type 2 diabetes mellitus

Analysis of stratification was dependent on gender, mean age, and BMI. The BMI of Chinese adults was classified according to the guidelines for the prevention and control of overweight (BMI = 24–28) and obesity (BMI ≥ 28).[22] Our results suggested a significant impact of the rs531564 GC genotype in younger patients (age < 45 years) on the onset of T2DM (adjusted, P = 0.043; OR = 0.73; 95% CI = 0.54–0.99). Furthermore, females with the GC genotype of rs531564 had a lower risk of T2DM (P = 0.014; OR = 0.67; 95% CI = 0.49–0.92), but we observed that this result changed after correction for age, gender, and BMI (adjusted, P = 0.060; OR = 0.72; 95% CI = 0.51–1.02). Furthermore, the rs895819CC genotype in overweight patients (24 ≤ BMI < 28) was significantly associated with T2DM risk (adjusted, P = 0.042; OR = 1.73; 95% CI = 1.02–2.94). According to gender, age, and BMI, rs2910164 in miR-146a was not correlated with an increased risk for onset of T2DM [Table 5].

Table 4: The effects of risk alleles of 3 SNPs on glucose and lipid metabolism of participants (*P* values)

Items	rs2910164	rs531564	rs895819	GRS
FPG	0.433	0.099	0.577	0.079
TC	0.374	0.080	0.213	0.021
TG	0.140	0.352	0.710	0.080
HDL-C	0.677	0.521	0.822	0.923
LDL-C	0.848	0.692	0.454	0.207
FINS	0.304	0.842	0.024	0.559
HbAlc	0.787	0.362	0.214	0.150
HOMA-β	0.410	0.992	0.405	0.951
HOMA-IS	0.996	0.313	0.601	0.392
HOMA-IR	0.540	0.520	0.061	0.282

The results of linear regression analysis after adjusting for age, gender, and BMI were shown. The GRS is calculated according to the number of risk alleles of the 3 SNPs. rs2910164 C, rs531564 G, and rs895819 C were considered as risk alleles. SNPs: Single-nucleotide polymorphisms; BMI: Body mass index; GRS: Genetic risk score; FBG: Fasting plasma glucose; TC: Total cholesterol; TG: Triglyceride; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; FINS: Fasting insulin; HbA1c: Glycated hemoglobin; HOMA-β: Homeostasis model assessment of β cell function; HOMA-IS: Homeostasis model assessment of insulin sensitivity; HOMA-IR: Homeostasis model assessment of insulin resistance.

Discussion

Type 2 diabetes mellitus is characterized by insulin resistance and decreased insulin secretion, which can aggravate the injury to vital organs in humans. Until recently, causative factors of T2DM have remained elusive. It is now widely recognized that a combination of environmental and genetic factors contribute to the onset and progression of T2DM. Therefore, analysis of miRNAs and SNPs could explain the associations between genetic variations and clinical

Table 5: Stratified analysis of 3 SNPs with T2DM susceptibility

Items	rs2910164 cases/controls			GC		GG		GC		GG	
	CC	GC	GG	*P*	OR (95% CI)	*P*	OR (95% CI)	*P**	OR* (95% CI)	*P**	OR* (95% CI)
Gender											
Male	195/198	309/262	108/108	0.170	0.84 (0.65–1.08)	0.928	0.99 (0.71–1.37)	0.262	0.86 (0.65–1.12)	0.946	1.01 (0.71–1.44)
Female	118/124	197/215	68/60	0.815	1.04 (0.76–1.43)	0.425	0.84 (0.55–1.29)	0.697	0.93 (0.66–1.32)	0.283	0.77 (0.48–1.24)
Age (years)											
<45	154/187	216/276	79/95	0.671	1.06 (0.80–1.40)	0.959	0.99 (0.69–1.43)	0.995	1.00 (0.74–1.36)	0.974	1.01 (0.68–1.50)
≥45	159/135	292/201	97/73	0.158	0.81 (0.61–1.09)	0.534	0.89 (0.61–1.30)	0.170	0.81 (0.60–1.10)	0.476	0.87 (0.58–1.29)
BMI (kg/m^2)											
<24	91/186	171/281	40/100	0.174	0.80 (0.59–1.10)	0.374	1.22 (0.78–1.91)	0.204	0.80 (0.57–1.10)	0.219	1.33 (0.84–2.11)
24≤BMI<28	131/112	201/151	94/59	0.441	0.88 (0.63–1.22)	0.141	0.73 (0.49–1.11)	0.657	0.94 (0.67–1.32)	0.137	0.73 (0.48–1.12)
≥28	91/24	134/45	42/9	0.400	1.27 (0.73–2.24)	0.632	0.81 (0.35–1.90)	0.400	1.27 (0.73–2.24)	0.646	0.82 (0.35–1.92)
Items	**rs531564 cases/controls**			**GC**		**CC**		**GC**		**CC**	
	GG	GC	CC	*P*	OR (95% CI)	*P*	OR (95% CI)	*P**	OR* (95% CI)	*P**	OR* (95% CI)
Gender											
Male	431/395	172/163	9/10	0.796	1.03 (0.80–1.33)	0.679	1.21 (0.49–3.01)	0.641	0.94 (0.72–1.23)	0.845	1.10 (0.43–2.79)
Female	250/294	119/94	14/11	0.014	0.67 (0.49–0.92)	0.328	0.67 (0.30–1.50)	0.060	0.72 (0.51–1.02)	0.577	0.78 (0.33–1.87)
Age (years)											
<45	304/408	131/140	12/10	0.112	0.80 (0.60–1.05)	0.273	0.62 (0.27–1.46)	0.043	0.73 (0.54–0.99)	0.343	0.64 (0.25–1.61)
≥45	377/281	160/117	11/11	0.895	0.98 (0.74–1.30)	0.498	1.34 (0.57–3.14)	0.778	0.96 (0.71–1.29)	0.524	1.33 (0.55–3.18)
BMI (kg/m^2)											
<24	213/402	80/152	9/13	0.967	1.01 (0.73–1.38)	0.545	0.77 (0.32–1.82)	0.905	1.02 (0.74–1.42)	0.691	0.90 (0.37–2.21)
24≤BMI<28	269/224	149/91	8/7	0.054	0.73 (0.54–1.01)	0.925	1.05 (0.38–2.94)	0.075	0.73 (0.53–1.01)	0.727	1.26 (0.44–3.63)
≥28	199/63	62/14	6/1	0.305	0.71 (0.37–1.36)	0.556	0.53 (0.06–4.46)	0.321	0.72 (0.38–1.38)	0.542	0.51 (0.06–4.46)
Items	**rs895819 cases/controls**			**CT**		**CC**		**CT**		**CC**	
	TT	CT	CC	*P*	OR (95% CI)	*P*	OR (95% CI)	*P**	OR* (95% CI)	*P**	OR* (95% CI)
Gender											
Male	339/308	219/209	54/51	0.854	1.05 (0.82–1.34)	0.962	1.04 (0.69–1.57)	0.871	1.01 (0.78–1.30)	0.897	1.04 (0.67–1.61)
Female	215/218	142/154	26/27	0.656	1.07 (0.80–1.44)	0.935	1.02 (0.58–1.81)	0.054	1.38 (0.99–1.92)	0.714	1.12 (0.60–2.10)
Age (years)											
<45	242/299	161/211	44/48	0.664	1.06 (0.81–1.38)	0.582	0.88 (0.57–1.38)	0.476	1.11 (0.83–1.49)	0.832	0.95 (0.58–1.55)
≥45	312/227	200/152	36/30	0.753	1.05 (0.80–1.37)	0.605	1.15 (0.69–1.92)	0.405	1.13 (0.85–1.50)	0.531	1.19 (0.70–2.01)
BMI (kg/m^2)											
<24	177/314	100/216	25/37	0.199	1.22 (0.90–1.64)	0.522	0.83 (0.49–1.43)	0.274	1.22 (0.89–1.66)	0.432	0.79 (0.45–1.38)
24≤BMI<28	235/166	61/119	30/37	0.774	1.05 (0.77–1.43)	0.036	1.75 (1.04–2.94)	0.725	1.07 (0.78–1.46)	0.042	1.73 (1.02–2.94)
≥28	142/46	100/28	25/4	0.593	0.86 (0.51–1.48)	0.212	0.49 (0.16–1.49)	0.640	0.88 (0.51–1.51)	0.205	0.49 (0.16–1.48)

The results of logistic regression analyses were shown. SNPs: Single-nucleotide polymorphisms; BMI: Body mass index; CI: Confidence interval; OR: Odds ratio. *Adjusted for age, gender, and BMI.

phenotypes of disease to improve the accuracy of diagnosis and the efficacy of targeted therapies.

To the best of our knowledge, this study is the first to report correlations of miR-27a, miR-124a, and miR-146a polymorphisms with T2DM among the Han population of northern China. Our observations of 995 Chinese T2DM patients and 967 controls were in contrast with the results of a study of 163 Italian cases and 185 healthy controls. In our study, there were no visible differences in association analysis of mutational alleles and T2DM, while the Italian study found that the G allele of rs895819 conveyed a significant protective effect, while the G allele of rs531564 was associated with an increased risk of T2DM, but rs2910164 in miR-146a was not significantly correlation with disease onset, as indicated by our results.[16] One possible reason for these contradictory findings may be due to differences in sample size, ethnicity of subjects, geographic locations, and T2DM-associated miRNAs and SNPs. A recent report suggested that up-regulated miR-144 expression was closely correlated to T2D onset among Swedes (OR = 2.43, $P = 0.035$), but not Iraqis.[18] Frequencies of mutant alleles in Mendelian disorders are influenced by race and ethnic backgrounds, and genetic polymorphisms have been strongly correlated with race and ethnic backgrounds.[18] Previous studies reported differences in associations between the G allele of rs11196218 and the incidence of T2DM between southern and northern Chinese population,[23,24] similar to those between Italian and Chinese populations.

Although our findings revealed that the mutated alleles were not significantly associated with T2DM, the GRS was calculated to assess the effect of multiple candidate loci on disease onset. However, the GRS was not significantly

associated with T2DM, but apparently had an effect on TC, which exists in the blood in the form of lipoproteins. Evidence clearly indicates that cholesterol regulates the function and survival of β-cells, and HDL-C regulates insulin sensitization, which are generally recognized as important pathogenic mechanisms in T2DM.[25-27] We also found that TC in cases was distinctly higher than in controls. Therefore, we reasoned that these three gene loci were important susceptibility candidates for T2DM in the Chinese population.

Moreover, our stratified data showed that the genotype GC of rs531564 in miR-124a alleviated the risk of T2DM among younger persons (age < 45 years) and the genotype CC of rs895819 in miR-27a elevated the risk of T2DM in an overweight group (24 ≤ BMI < 28 kg/m^2). This evidence indicates that the miR-27a might suppress adipocyte differentiation by blockade of the expression of peroxisome proliferator-activated receptor-γ (PPAR-γ) and CCAAT/enhancer-binding protein alpha, which are extremely important regulators of adipogenesis.[28] In the latter experiment, PPAR-γ was shown to be a direct target gene of miR-27a.[29] Moreover, miR-27a expression was strongly and positively correlated with fasting glucose level.[11] Moreover, overexpression of miR-124a$_2$ was involved in the regulation of pancreatic development and reduced insulin secretion by directly targeting forkhead box a$_2$ mRNA.[30] In addition, up-regulation of miR-124a decreased glucose-stimulated insulin secretion by directly targeting Rab GTPase family 27a and rabphilin-3A-like.[31] Current evidence indicates that the mutated allele of rs895819 in miR-27a decreased the risk of gastric cancer via mature miR-27a and its target gene zinc finger and BTB domain containing 10.[32] Although there was no correlation between rs531564 in miR-124 and Alzheimer's disease in a Mongolian population, this SNP site also changed expression levels of mature miR-124.[33] Therefore, we reasoned that SNPs in rs895819 or rs531564 also influenced expression levels of mature miR-27a or miR-124a and their targets in the dysregulation of adipocyte differentiation, pancreatic development, and insulin secretion. However, there was no pronounced association between obese patients who carried genotype CC of rs895819 and T2DM. Based on the observed variations in circulating miRNA with altered insulin sensitization,[34] we speculated that miRNA profiles might change with weight gain from the status of overweight to obese. However, further studies are needed to validate this hypothesis. Therefore, our next study will explore correlations between SNPs in human miRNA and expression levels of mature miRNAs and functions of these miRNAs in the onset of T2DM among the Chinese Han population.

MicroRNAs are attractive drug targets because they regulate expression levels of many important proteins and multiple cellular pathways. MiR-122 is highly abundant in the liver, and abnormal expression levels are closely related to many liver-related diseases. Besides, a recent study showed that miRNA inhibition acted to suppress hepatitis C viremia for extended periods.[35-37] In addition, a SNP 829C-T near the miR-24 binding site in the 3′ UTR of dihydrofolate reductase was closely associated with methotrexate resistance by interfering with miR-24 function,[38] while SNPs in miRNAs lead to altered expression levels and functions of miRNA, resulting in an increased risk of illness.[15,32] Thus, miRNA mimics or inhibitors are extensively used in a clinical setting.

In conclusion, the work presented here demonstrated that rs895819 in miR-27a and rs531564 in miR-124a were significantly associated with T2DM in overweight (24 ≤ BMI < 28 kg/m^2) and younger (age <45 years) groups of the Han population in northern China. The genotype CC of rs895819 in miR-27a was associated with an increased risk of T2DM, while genotype GC of rs531564 in miR-124a conveyed a protective effect, and miR-146a (rs2910164) was not associated with these traits in any stratification analysis. We speculated that SNPs in miRNAs possibly participate in the regulation of mature miRNA and mRNA targets. These observations provide evidence of the genetic mechanisms of miRNA polymorphisms in T2DM.

References

1. Whiting DR, Guariguata L, Weil C, Shaw J. IDF diabetes atlas: Global estimates of the prevalence of diabetes for 2011 and 2030. Diabetes Res Clin Pract 2011;94:311-21.
2. Collins FS, Brooks LD, Chakravarti A. A DNA polymorphism discovery resource for research on human genetic variation. Genome Res 1998;8:1229-31.
3. Cargill M, Altshuler D, Ireland J, Sklar P, Ardlie K, Patil N, *et al.* Characterization of single-nucleotide polymorphisms in coding regions of human genes. Nat Genet 1999;22:231-8.
4. Zeggini E, Scott LJ, Saxena R, Voight BF, Marchini JL, Hu T, *et al.* Meta-analysis of genome-wide association data and large-scale replication identifies additional susceptibility loci for type 2 diabetes. Nat Genet 2008;40:638-45.
5. Shu XO, Long J, Cai Q, Qi L, Xiang YB, Cho YS, *et al.* Identification of new genetic risk variants for type 2 diabetes. PLoS Genet 2010;6:e1001127.
6. Voight BF, Scott LJ, Steinthorsdottir V, Morris AP, Dina C, Welch RP, *et al.* Twelve type 2 diabetes susceptibility loci identified through large-scale association analysis. Nat Genet 2010;42:579-89.
7. Hwang HW, Mendell JT. MicroRNAs in cell proliferation, cell death, and tumorigenesis. Br J Cancer 2006;94:776-80.
8. Laurent LC. MicroRNAs in embryonic stem cells and early embryonic development. J Cell Mol Med 2008;12:2181-8.
9. Moore KJ, Rayner KJ, Suárez Y, Fernández-Hernando C. microRNAs and cholesterol metabolism. Trends Endocrinol Metab 2010;21:699-706.
10. Herrera BM, Lockstone HE, Taylor JM, Ria M, Barrett A, Collins S, *et al.* Global microRNA expression profiles in insulin target tissues in a spontaneous rat model of type 2 diabetes. Diabetologia 2010;53:1099-109.
11. Karolina DS, Tavintharan S, Armugam A, Sepramaniam S, Pek SL, Wong MT, *et al.* Circulating miRNA profiles in patients with metabolic syndrome. J Clin Endocrinol Metab 2012;97:E2271-6.
12. Kong L, Zhu J, Han W, Jiang X, Xu M, Zhao Y, *et al.* Significance of serum microRNAs in pre-diabetes and newly diagnosed type 2 diabetes: A clinical study. Acta Diabetol 2011;48:61-9.
13. Rong Y, Bao W, Shan Z, Liu J, Yu X, Xia S, *et al.* Increased microRNA-146a levels in plasma of patients with newly diagnosed type 2 diabetes mellitus. PLoS One 2013;8:e73272.
14. Balasubramanyam M, Aravind S, Gokulakrishnan K, Prabu P,

Sathishkumar C, Ranjani H, *et al.* Impaired miR-146a expression links subclinical inflammation and insulin resistance in type 2 diabetes. Mol Cell Biochem 2011;351:197-205.
15. Jazdzewski K, Murray EL, Franssila K, Jarzab B, Schoenberg DR, de la Chapelle A. Common SNP in pre-miR-146a decreases mature miR expression and predisposes to papillary thyroid carcinoma. Proc Natl Acad Sci U S A 2008;105:7269-74.
16. Ciccacci C, Di Fusco D, Cacciotti L, Morganti R, D'Amato C, Greco C, *et al.* MicroRNA genetic variations: Association with type 2 diabetes. Acta Diabetol 2013;50:867-72.
17. Ciccacci C, Morganti R, Di Fusco D, D'Amato C, Cacciotti L, Greco C, *et al.* Common polymorphisms in MIR146a, MIR128a and MIR27a genes contribute to neuropathy susceptibility in type 2 diabetes. Acta Diabetol 2014;51:663-71.
18. Wang X, Sundquist J, Zöller B, Memon AA, Palmér K, Sundquist K, *et al.* Determination of 14 circulating microRNAs in Swedes and Iraqis with and without diabetes mellitus type 2. PLoS One 2014;9:e86792.
19. Burchard EG, Ziv E, Coyle N, Gomez SL, Tang H, Karter AJ, *et al.* The importance of race and ethnic background in biomedical research and clinical practice. N Engl J Med 2003;348:1170-5.
20. Chen X, Li S, Yang Y, Yang X, Liu Y, Liu Y, *et al.* Genome-wide association study validation identifies novel loci for atherosclerotic cardiovascular disease. J Thromb Haemost 2012;10:1508-14.
21. Sakai K, Imamura M, Tanaka Y, Iwata M, Hirose H, Kaku K, *et al.* Replication study for the association of 9 East Asian GWAS-derived loci with susceptibility to type 2 diabetes in a Japanese population. PLoS One 2013;8:e76317.
22. Chen CM, Kong LZ. Guideline for Prevention and Control of Overweight and Obesity in Adult. Beijing: People's Medical Publishing House; 2006.
23. Ng MC, Tam CH, Lam VK, So WY, Ma RC, Chan JC. Replication and identification of novel variants at TCF7L2 associated with type 2 diabetes in Hong Kong Chinese. J Clin Endocrinol Metab 2007;92:3733-7.
24. Qiao H, Zhang X, Zhao X, Zhao Y, Xu L, Sun H, *et al.* Genetic variants of TCF7L2 are associated with type 2 diabetes in a Northeastern Chinese population. Gene 2012;495:115-9.
25. Rütti S, Ehses JA, Sibler RA, Prazak R, Rohrer L, Georgopoulos S, *et al.* Low- and high-density lipoproteins modulate function, apoptosis, and proliferation of primary human and murine pancreatic beta-cells. Endocrinology 2009;150:4521-30.
26. Fryirs M, Barter PJ, Rye KA. Cholesterol metabolism and pancreatic beta-cell function. Curr Opin Lipidol 2009;20:159-64.
27. Kruit JK, Brunham LR, Verchere CB, Hayden MR. HDL and LDL cholesterol significantly influence beta-cell function in type 2 diabetes mellitus. Curr Opin Lipidol 2010;21:178-85.
28. Lin Q, Gao Z, Alarcon RM, Ye J, Yun Z. A role of miR-27 in the regulation of adipogenesis. FEBS J 2009;276:2348-58.
29. Kim SY, Kim AY, Lee HW, Son YH, Lee GY, Lee JW, *et al.* miR-27a is a negative regulator of adipocyte differentiation via suppressing PPARgamma expression. Biochem Biophys Res Commun 2010;392:323-8.
30. Baroukh N, Ravier MA, Loder MK, Hill EV, Bounacer A, Scharfmann R, *et al.* MicroRNA-124a regulates Foxa2 expression and intracellular signaling in pancreatic beta-cell lines. J Biol Chem 2007;282:19575-88.
31. Lovis P, Gattesco S, Regazzi R. Regulation of the expression of components of the exocytotic machinery of insulin-secreting cells by microRNAs. Biol Chem 2008;389:305-12.
32. Yang Q, Jie Z, Ye S, Li Z, Han Z, Wu J, *et al.* Genetic variations in miR-27a gene decrease mature miR-27a level and reduce gastric cancer susceptibility. Oncogene 2014;33:193-202.
33. Qi L, Hu Y, Zhan Y, Wang J, Wang BB, Xia HF, *et al.* A SNP site in pri-miR-124 changes mature miR-124 expression but no contribution to Alzheimer's disease in a Mongolian population. Neurosci Lett 2012;515:1-6.
34. Ortega FJ, Mercader JM, Moreno-Navarrete JM, Rovira O, Guerra E, Esteve E, *et al.* Profiling of circulating microRNAs reveals common microRNAs linked to type 2 diabetes that change with insulin sensitization. Diabetes Care 2014;37:1375-83.
35. Nassirpour R, Mehta PP, Yin MJ. miR-122 regulates tumorigenesis in hepatocellular carcinoma by targeting AKT3. PLoS One 2013;8:e79655.
36. Wen J, Friedman JR. miR-122 regulates hepatic lipid metabolism and tumor suppression. J Clin Invest 2012;122:2773-6.
37. Lanford RE, Hildebrandt-Eriksen ES, Petri A, Persson R, Lindow M, Munk ME, *et al.* Therapeutic silencing of microRNA-122 in primates with chronic hepatitis C virus infection. Science 2010;327:198-201.
38. Mishra PJ, Humeniuk R, Mishra PJ, Longo-Sorbello GS, Banerjee D, Bertino JR. A miR-24 microRNA binding-site polymorphism in dihydrofolate reductase gene leads to methotrexate resistance. Proc Natl Acad Sci U S A 2007;104:13513-8.

Received: 07-08-2014 **Edited by:** Yuan-Yuan Ji
How to cite this article: Wang TT, Chen YJ, Sun LL, Zhang SJ, Zhou ZY, Qiao H. Affection of Single-Nucleotide Polymorphisms in miR-27a, miR-124a, and miR-146a on Susceptibility to Type 2 Diabetes Mellitus in Chinese Han People. Chin Med J 2015;128:533-9.

Source of Support: This work was funded by the National Natural Science Foundation of China (No. 81172742 and No. 81473053), The Natural Science Foundation of Heilongjiang Province (No. ZD201220).
Conflict of Interest: None declared.