RESEARCH

Relationship of microRNA locus with type 2 diabetes mellitus: a case–control study

Qiuyu Huang[1,2,*], Hanshen Chen[3,*], Fan Xu[1,2], Chao Liu[4], Yafeng Wang[5], Weifeng Tang[6] and **Liangwan Chen[1,2]**

[1]Department of Cardiovascular Surgery, Union Hospital, Fujian Medical University, Fuzhou, Fujian Province, China
[2]Key Laboratory of Cardio-Thoracic Surgery (Fujian Medical University), Fujian Province University, Fuzhou, Fujian Province, China
[3]Department of Anesthesiology, The First Affiliated Hospital of Fujian Medical University, Fuzhou, Fujian Province, China
[4]Department of Cardiothoracic Surgery, Affiliated People's Hospital of Jiangsu University, Zhenjiang, Jiangsu Province, China
[5]Department of Cardiology, The People's Hospital of Xishuangbanna Dai Autonomous Prefecture, Jinghong, Yunnan Province, China
[6]Department of Cardiothoracic Surgery, Nanjing Drum Tower Hospital, Nanjing University Medical School, Jiangsu Province, China

Correspondence should be addressed to W Tang or L Chen: twf001001@126.com or chenliangwan@tom.com

*(Q Huang and H Chen contributed equally to this work)

Abstract

Type 2 diabetes mellitus (T2DM) is considered as a metabolic disease with hyperglycemia. Accumulating investigations have explored the important role of hereditary factors for T2DM occurrence. Some functional microRNA (miR) polymorphisms may affect their interactions with target mRNAs and result in an aberrant expression. Thus, miR variants might be considered as a biomarker of the susceptibility of T2DM. In this study, we recruited 502 T2DM cases and 782 healthy subjects. We selected miR-146a rs2910164 C>G, miR-196a2 rs11614913 T>C and miR-499 rs3746444 A>G loci and carried out an investigation to identify whether these miR loci could influence T2DM occurrence. In this investigation, a Bonferroni correction was harnessed. After adjustment, we found that rs2910164 SNP was a protective factor for T2DM (GG vs CC/CG: adjusted $P = 0.010$), especially in never drinking (GG vs CC/CG: adjusted $P = 0.001$) and BMI ≥24 kg/m^2 (GG vs CC/CG: adjusted $P = 0.002$) subgroups. We also identified that rs11614913 SNP was a protective factor for T2DM in smoking subjects (CC/TC vs TT: adjusted $P = 0.002$). When we analyzed an interaction of SNP–SNP with the susceptibility tof T2DM, rs11614913/rs3746444, rs2910164/rs3746444 and rs11614913/rs2910164 combinations were not associated with the risk of T2DM. In summary, this study highlights that rs2910164 SNP decreases the susceptibility of T2DM, especially in BMI ≥24 kg/m^2 and never drinking subgroups. In addition, we also identify that rs11614913 C allele decreases the susceptibility of T2DM significantly in smoking subgroup.

Key Words
- polymorphism
- type 2 diabetes mellitus
- risk
- microRNA

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Introduction

Type 2 diabetes mellitus (T2DM) is considered as a metabolic disease with hyperglycemia and vascular and/or nervous complications. In China, the reported prevalence of T2DM is 11.6% (1), which appears to have increased in the past decades (2). Latest investigation suggested that healthy lifestyle in terms of reasonably restricted calorie intake and adequate physical exercise was associated with lower incidence rate and older age of onset in T2DM (3). However, the etiology of T2DM was not fully understood. The important risk factors, calorie abundant diet, inadequate physical activities, overweight and obesity, may lead to this phenomenon (4, 5). Recently, some investigations also suggested genetic components could be implicated in development of T2DM (3, 6). Accumulating case–control studies have focused on the important role of hereditary factors in the occurrence of T2DM, and individual's genotype variants deserve a close look.

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MicroRNA (miR) is a kind of non-coding RNA, which is composed of about 22 nucleotides (7, 8). On the post-translational process, miRs could regulate the expression of target genes (9). miRs are involved in a lot of complex diseases, such as T2DM, which seem to play a role in the development of inflammation (10), fat deposition (11), pancreatic β-cell apoptosis (12) and so on. In addition, more and more studies focused on functional importance of miRs in the process of proliferation of smooth muscle cells, oxidative stress and energy metabolism (13, 14, 15, 16, 17, 18), which are correlated with T2DM and its complications.

SNPs are located at pre-miR genes. Some functional miR-SNPs may affect the interaction with mRNA of their target genes and result in an aberrant expression (19). Thus, miR-SNP might be considered as a useful biomarker of the susceptibility of T2DM. A previous study suggested that *miR-146a* influenced the development of cancer cell by facilitating migration and invasion (20), while *miR-146a* rs2910164 C>G SNP could promote the expression of *miR-146a* and results in immune suppression (21, 22, 23). Liu *et al.* reported that mimics of *miR-146a* could decrease the peripheral neuropathy of T2DM mice (24). This *miR-146a* SNP might be implicated in the susceptibility and development of T2DM. There are several studies on the correlation of rs2910164 SNP with risk of T2DM (25, 26, 27, 28). Recently, a meta-analysis has suggested that rs2910164 G allele might be associated with risk of T2DM. However, only 4 case–control studies with 2069 cases and 1950 controls were included (29), and the observations might be underpowered. Additionally, the relationship of miR-196a2 rs11614913 T>C and miR-499 rs3746444 A>G SNPs with the risk of T2DM was also explored. The included participants were more limited, and the findings were more conflicting. In 2020, Gholami *et al.* systematically reviewed the SNPs associated with T2DM (30). In view of the potential effect of miR-SNPs in the occurrence of T2DM, we selected rs11614913, rs2910164 and rs3746444 loci and carried out an investigation to identify whether these miR-SNPs could influence the risk of T2DM.

Materials and methods

Study population and ethical approval

In this study, 502 T2DM cases who had presented to the Zhenjiang No. 1 hospital (Zhenjiang City, China) and Union Hospital of Fujian province (Fuzhou City, China) between October 2014 and May 2016 consecutively were recruited. And at the same time, 782 healthy subjects were enrolled as controls. Consent has been obtained from each patient or subject after full explanation of the purpose and nature of all procedures used. The criterion of the included T2DM patients was diagnosed according to the criterion of WHO 1999 guidelines (31). The criteria of healthy subjects were (1) normoglycemia (fasting plasma glucose (FPG) <6.1 mmol/L), (2) postprandial plasma glucose <7.8 mmol/L and (3) no history of diabetes mellitus (32). Two authors visited each subject and collected risk factors and demographic variables. We summarized the information in Table 1. Weight, height and blood pressure were measured. In Chinese adults, a BMI ≥24 kg/m^2 was regarded as the criterion for overweight and obesity (33, 34). Serum HDL-C, FPG, total cholesterol, LDL-C and triglycerides were measured. The protocol was approved by Committee of Ethics at Fujian Medical University.

SNP selection

To determine the potential relationship between miR-SNPs and T2DM, we selected the miR-146a rs2910164 C>G, miR-196a2 rs11614913 T>C and miR-499 rs3746444 A>G loci polymorphism according to the literature, which was significantly associated with cancer (20, 21, 22, 23), coronary artery disease (35, 36) and type 2 diabetes (25, 26, 27, 28, 30), in some studies.

DNA extraction and genotyping

EDTA anticoagulant vacutainer tube (BD, Franklin Lakes NJ, USA) was used to collect the blood sample. Genomic DNA was extracted by using the DNA Purification Kit (Promega). SNPscan™ genotyping assay was carried out to identify the genotypes of rs3746444, rs11614913 and rs2910164. As summarized in Table 2, more than 99% sample was successfully genotyped. The genotypes of rs3746444, rs11614913 and rs2910164 SNP were confirmed with DNA sequence method in 4% DNA samples randomly.

Statistical analysis

SAS 9.4 software (SAS Institute, Cary, NC, USA) was harnessed to perform statistical analyses. Mean ± S.D. was used to express continuous variables. The difference of continuous variables between two groups was measured by Student's *t* test. The differencces categorical variables (smoking status, alcohol consumption, BMI, genotypes, sex and age) were measured using chi-square test (χ^2). Using the genotype number in controls of rs3746444, rs11614913 and rs2910164, Hardy–Weinberg equilibrium (HWE)

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Table 1 Distribution of selected demographic variables and risk factors in T2DM cases and controls.

Variable	**Cases** (*n* =502)		**Controls** (*n* =782)		*P*[a]
	n	%	*n*	%	
Age (years)	65.20 (±9.51)		64.67 (±9.80)		0.347
Age (years)					0.113
<65	227	45.22	389	49.74	
≥65	275	54.78	393	50.26	
Sex					0.819
Male	332	66.14	522	66.75	
Female	170	33.86	260	33.25	
Alcohol use					0.263
Never	453	90.24	690	88.24	
Ever	49	9.76	92	11.76	
Smoking status					0.264
Never	333	66.33	542	69.31	
Ever	169	33.67	240	30.69	
Height (m)	1.68 (±0.08)		1.66 (±0.07)		**0.015**
Weight (kg)	67.63 (±11.42)		64.62 (±9.96)		**<0.001**
BMI (kg/m^2)	24.95 (±3.64)		23.51 (±2.94)		**<0.001**
BMI (kg/m^2)					**<0.001**
<24	210		436		
≥24	292		346		
FPG (mmol/L)	8.08 (±2.76)		5.13 (±0.49)		**<0.001**
Total cholesterol (mmol/L)	4.61 (±1.24)		4.88 (±1.02)		**<0.001**
Triglyceride (mmol/L)	1.74 (±1.14)		1.55 (±0.96)		0.001
HDL-C (mmol/L)	1.13 (±0.37)		1.30 (±0.37)		**<0.001**
LDL-C (mmol/L)	3.00 (±1.07)		3.14 (±0.82)		0.010
Systolic pressure (mmHg)	135 (±18)		134 (±18)		0.297
Diastolic pressure (mmHg)	80 (±10)		80 (±10)		0.649

[a]Two-sided χ^2 test and Student's *t* test; bold values are statistically significant ($P < 0.05$).
FPG, fasting plasma glucose.

was evaluated by an internet-based calculator (37, 38, 39, 40, 41). The correlations of rs3746444, rs11614913 and rs2910164 polymorphisms with occurrence of T2DM were determined by odds ratios (ORs) and CIs. A $P < 0.05$ (two-tailed) was regarded as the statistical significance. In this case–control study, a Bonferroni correction was harnessed to confirm our findings (42, 43).

Results

Baseline characteristics

Table 1 summarizes the demographics and risk factors, anthropometric data, biochemistry characteristics. Gender, age, smoking status and alcohol consumption were well matched. Compared with controls, the mean level of BMI was higher in the T2DM group ($P < 0.05$). In this study, the mean level of diastolic pressure and systolic pressure was similar. Other information, HDL-C, FPG, total cholesterol, LDL-C and triglycerides, were also summarized in Table 1. Table 2 shows the primary SNPs information of rs3746444, rs11614913 and rs2910164. Minor allele frequency is summarized in Table 2.

Association of rs3746444, rs11614913 and rs2910164 loci with T2DM

The rs3746444, rs11614913 and rs2910164 genotype distributions are shown in Table 3. The distributions of the rs3746444 and rs2910164 genotypes were suggested to be in HWE. In an analysis of *miR-146a* rs2910164 SNP, compared with *miR-146a* rs2910164 CC or CC/CG genotype, the frequency distribution of *miR-146a* rs2910164 GG genotype was different between healthy controls and T2DM cases (GG vs CC: crude $P = 0.046$ and GG vs CG/CC: crude $P = 0.006$). After a logistic regression analysis, it is also suggested that *miR-146a* rs2910164 SNP decreased the susceptibility of T2DM (GG vs CC: adjusted $P = 0.036$ and GG vs CG/CC: adjusted $P = 0.004$). However, miR-196a2 rs11614913 and miR-499 rs3746444 SNPs were not correlated with the occurrence of T2DM.

Subgroup analysis of the association of miR-SNPs with T2DM

miR-196a2 rs11614913 genotypes in the stratified analysis are shown in Table 4. The subgroup analysis was conducted according to the included risk factors. In ≥65 years

Table 2 Primary information for miR-146a rs2910164 C>G, miR-196a2 rs11614913 T>C and miR-499 rs3746444 A>G polymorphisms.

Genotyped SNPs	miR-146a rs2910164 C>G	miR-196a2 rs11614913 T>C	miR-499 rs3746444 A>G
Chromosome	5	12	20
Function	nc-transcript-variant	nc-transcript-variant	nc-transcript-variant
Chr Pos (NCBI Build 38)	160485411	53991815	3499048
MAF for Chinese in database	0.35	0.34	0.15
MAF in our controls ($n = 1109$)	0.38	0.44	0.15
P value for HWE test in our controls	0.119	0.034	0.702
Genotyping method	SNPscan	SNPscan	SNPscan
% Genotyping value	99.61%	99.61%	99.61%

HWE, Hardy–Weinberg equilibrium; MAF, minor allele frequency.

subgroup, after adjustment for smoking status, gender, alcohol use and BMI, rs11614913 TC and CC/TC genotypes might decrease T2DM susceptibility compared with rs11614913 TT genotype (TC vs TT: adjusted $P = 0.013$ and CC/TC vs TT: adjusted $P = 0.011$). Compared with rs11614913 TT genotype, rs11614913 TC, CC and CC/TC genotypes decreased the susceptibility of T2DM significantly in smoking subgroup (TC vs TT: adjusted $P = 0.005$, CC vs TT: adjusted $P = 0.018$ and CC/TC vs TT: adjusted $P = 0.002$). In drinking subgroup, compared with rs11614913 TT genotype, rs11614913 TC and CC/TC genotypes decreased the susceptibility of T2DM significantly (TC vs TT: adjusted $P = 0.007$ and CC/TC vs TT: adjusted $P = 0.020$). In BMI <24 kg/m^2 subgroup, compared with rs11614913 TT genotype, rs11614913 CC and CC/TC genotypes decreased the susceptibility of T2DM significantly (CC vs TT: adjusted $P = 0.013$ and CC/TC vs TT: adjusted $P = 0.030$).

In the stratified analysis, *miR-146a* rs2910164 genotypes are shown in Table 5. In female subgroup, compared with rs2910164 CC and CC/CG genotypes, rs2910164 GG genotypes might decrease T2DM susceptibility (GG vs CC: adjusted $P = 0.019$ and GG vs CC/CG: adjusted $P = 0.010$). In ≥65 years subgroup, rs2910164 GG genotypes might decrease T2DM

Table 3 The relationship of miRNA polymorphisms with T2DM.

Genotype	Cases (n =502)		Controls (n =782)		Crude OR (95% CI)	*P*	Adjusted OR[a] (95% CI)	*P*
	n	%	*n*	%				
miR-499 rs3746444								
AA	363	73.04	565	72.25	1.00		1.00	
AG	125	25.15	201	25.70	0.97 (0.75–1.25)	0.806	0.95 (0.73–1.23)	0.689
GG	9	1.81	16	2.05	0.88 (0.38–2.00)	0.753	0.89 (0.39–2.06)	0.788
AG+GG	134	26.96	217	27.75	0.96 (0.75–1.24)	0.759	0.94 (0.73–1.22)	0.657
AA+AG	488	98.19	766	97.95	1.00		1.00	
GG	9	1.81	16	2.05	0.88 (0.39–2.01)	0.767	0.90 (0.39–2.08)	0.813
G allele	143	14.39	233	14.90				
miR-146a rs2910164								
CC	187	37.63	308	39.39	1.00		1.00	
CG	258	51.91	349	44.63	1.22 (0.96–1.55)	0.112	1.22 (0.96–1.56)	0.110
GG	52	10.46	125	15.98	**0.69 (0.47–0.99)**	**0.046**	**0.67 (0.46–0.97)**	**0.036**
CG+GG	310	62.37	474	60.61	1.07 (0.86–1.36)	0.530	1.07 (0.85–1.36)	0.548
CC+CG	445	89.54	657	84.01	1.00		1.00	
GG	52	10.46	125	15.98	**0.61 (0.44–0.87)**	**0.006**	**0.60 (0.42–0.85)**	**0.004**
G allele	362	36.42	599	38.30				
miR-196a2 rs11614913								
TT	165	33.20	229	29.28	1.00		1.00	
TC	251	50.50	415	53.07	0.84 (0.65–1.08)	0.177	0.83 (0.64–1.07)	0.151
CC	81	16.30	138	17.65	0.82 (0.58–1.14)	0.237	0.83 (0.59–1.18)	0.301
TC+CC	332	66.80	553	70.72	0.83 (0.65–1.06)	0.140	0.83 (0.65–1.06)	0.135
TT+TC	416	83.70	644	82.35	1.00		1.00	
CC	81	16.30	138	17.65	0.91 (0.67–1.23)	0.533	0.94 (0.69–1.27)	0.682
C allele	413	41.55	691	44.18				

Bold values are statistically significant ($P < 0.05$).
[a]Adjusted for age, sex, smoking, status of BMI and drinking.

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Table 4 Stratified analyses between miR-196a2 rs11614913 T>C polymorphism and T2DM risk by sex, age, BMI, smoking status and alcohol consumption.

Variable	miR-196a2 rs11614913 T>C (case/control)[a]			Adjusted OR[b] (95% CI); *P*			
	TT	TC	CC	TC vs TT	CC vs TT	CC/TC vs TT	CC vs TT/TC
Sex							
Male	115/161	158/275	56/86	0.78 (0.57–1.07); $P = 0.117$	0.92 (0.60–1.39); $P = 0.678$	0.81 (0.60–1.09); $P = 0.165$	1.07 (0.73–1.55); $P = 0.742$
Female	50/68	93/140	25/52	0.90 (0.57–1.42); $P = 0.650$	0.70 (0.38–1.29); $P = 0.254$	0.85 (0.55–1.32); $P = 0.461$	0.75 (0.44–1.28); $P = 0.292$
Age							
<65	66/121	118/197	40/71	1.12 (0.76–1.64); $P = 0.571$	1.12 (0.68–1.84); $P = 0.661$	1.12 (0.78–1.61); $P = 0.551$	1.04 (0.67–1.61); $P = 0.851$
≥65	99/108	133/218	41/67	**0.64 (0.45–0.91); $P = 0.013$**	0.66 (0.41–1.07); $P = 0.091$	**0.65 (0.46–0.90); $P = 0.011$**	0.87 (0.57–1.34); $P = 0.528$
Smoking status							
Never	99/166	173/280	59/96	1.03 (0.75–1.41); $P = 0.855$	1.07 (0.71–1.62); $P = 0.743$	1.04 (0.77–1.41); $P = 0.797$	1.05 (0.73–1.51); $P = 0.784$
Ever	66/63	78/135	22/42	**0.52 (0.33–0.81); $P = 0.005$**	**0.46 (0.24–0.88); $P = 0.018$**	**0.50 (0.32–0.78); $P = 0.002$**	0.70 (0.39–1.24); $P = 0.216$
Alcohol consumption							
Never	145/207	234/361	70/122	0.91 (0.69–1.19); $P = 0.489$	0.83 (0.57–1.19); $P = 0.310$	0.89 (0.68–1.15); $P = 0.371$	0.88 (0.63–1.22); $P = 0.434$
Ever	20/22	17/54	11/16	**0.32 (0.14–0.73); $P = 0.007$**	0.70 (0.26–1.90); $P = 0.481$	**0.40 (0.19–0.87); $P = 0.020$**	1.39 (0.58–3.35); $P = 0.465$
BMI (kg/m^2)							
<24	75/123	104/225	29/88	0.73 (0.50–1.06); $P = 0.101$	**0.52 (0.31–0.87); $P = 0.013$**	**0.67 (0.47–0.96); $P = 0.030$**	0.63 (0.40–1.00); $P = 0.052$
≥24	90/106	147/190	52/50	0.89 (0.63–1.28); $P = 0.534$	1.20 (0.74–1.94); $P = 0.467$	0.96 (0.68–1.35); $P = 0.797$	1.29 (0.84–1.97); $P = 0.250$

[a]For miR-196a2 rs11614913 T>C, the genotyping was successful in 497 (99.00%) T2DM cases and 782 (100.00%) controls; [b]Adjusted for age, sex, smoking, drinking, BMI, hypertension, T2DM and hyperlipidemia (besides stratified factors accordingly) in a multiple logistic regression model.
Bold values are statistically significant ($P < 0.05$).

susceptibility compared with rs2910164 CC and CC/CG (GG vs CC: adjusted $P = 0.049$ and GG vs CC/CG: adjusted $P = 0.020$). Compared with rs2910164 CC and CC/CG genotypes, rs2910164 GG genotypes decreased the susceptibility of T2DM significantly in individuals 7,without tobacco consumption (GG vs CC: adjusted $P = 0.024$ and GG vs CC/CG: adjusted $P = 0.004$). In non-drinking subgroup, compared with rs2910164 CC and CC/CG genotypes, rs2910164 GG genotype decreased the susceptibility of T2DM significantly (GG vs CC: adjusted $P = 0.014$ and GG vs CC/CG: adjusted $P = 0.001$). In obesity/overweight subgroup, compared with rs2910164 CC and CC/CG genotypes, rs2910164 GG genotype decreased the susceptibility of T2DM significantly (GG vs CC: adjusted $P = 0.045$ and GG vs CC/CG: adjusted $P = 0.002$). However, compared with rs2910164 CC genotype, rs2910164 CG genotype might increase the susceptibility of T2DM significantly in obesity/overweight subgroup (CG vs CC: adjusted $P = 0.028$).

When we focused on the association of the miR-499 rs3746444 SNP with the risk of T2DM, null association was found (Table 6).

Bonferroni correction

A Bonferroni correction was harnessed to confirm our findings in this study. After adjustment, we found that *miR-146a* rs2910164 SNP was a protective factor for T2DM, especially in non-drinking and BMI ≥24 kg/m^2 subgroups. We also identified that *miR-196a2* rs11614913 SNP was a protective factor for T2DM in smoking subjects.

Combination analysis of miRNA polymorphisms

miR-SNPs combined analyses were summarized in Table 7. When we analyzed the interactions of SNP–SNP with the susceptibility of T2DM, rs11614913/rs3746444, rs2910164/rs3746444, rs11614913/rs2910164 and rs11614913/rs2910164/rs3746444 combinations were used. However, null association was found.

Power calculation

Using $\alpha = 0.05$ as the criterion of the Type I error probability (two-sided), we calculated the power value of this study. For *miR-146a* rs2910164 SNP, the power value was 0.838 in overall comparison, 0.916 in non-drinker subgroup and 0.877 in BMI ≥24 kg/m^2 subgroup among the GG vs CG/CC genetic model. For *miR-196a2* rs11614913 SNP, the power value was 0.898 in drinker subgroup among the

Table 5 Stratified analyses between miR-146a rs2910164 C>G polymorphism and T2DM risk by sex, age, BMI, smoking status and alcohol consumption.

Variable	miR-146a rs2910164 C>G (case/control)[a]			Adjusted OR[b] (95% CI); *P*			
	CC	CG	GG	CG vs CC	GG vs CC	GG/CG vs CC	GG vs CC/CG
Sex							
Male	118/208	174/235	37/79	1.34 (0.99–1.82); *P* = 0.057	0.84 (0.53–1.33); *P* = 0.466	1.22 (0.91–1.63); *P* = 0.185	0.71 (0.47–1.09); *P* = 0.119
Female	69/100	84/114	15/46	1.04 (0.68–1.59); *P* = 0.874	**0.45 (0.23–0.88); *P* = 0.019**	0.87 (0.58–1.30); *P* = 0.484	**0.44 (0.23–0.82); *P* = 0.010**
Age							
<65	83/164	118/166	23/59	1.35 (0.94–1.94); *P* = 0.104	0.76 (0.44–1.33); *P* = 0.339	1.20 (0.85–1.69); *P* = 0.307	0.65 (0.38–1.09); *P* = 0.099
≥65	104/144	140/183	29/66	1.09 (0.77–1.53); *P* = 0.631	**0.60 (0.36–1.00); *P* = 0.049**	0.95 (0.69–1.32); *P* = 0.775	**0.57 (0.36–0.92); *P* = 0.020**
Smoking status							
Never	127/213	171/238	33/91	1.18 (0.88–1.59); *P* = 0.273	**0.59 (0.37–0.93); *P* = 0.024**	1.02 (0.77–1.35); *P* = 0.909	**0.54 (0.35–0.82); *P* = 0.004**
Ever	60/95	87/111	19/34	1.38 (0.88–2.14); *P* = 0.159	0.94 (0.48–1.83); *P* = 0.856	1.27 (0.83–1.94); *P* = 0.267	0.79 (0.43–1.45); *P* = 0.441
Alcohol consumption							
Never	172/277	233/300	44/113	1.23 (0.95–1.60); *P* = 0.114	**0.60 (0.40–0.90); *P* = 0.014**	1.06 (0.83–1.36); *P* = 0.650	**0.54 (0.37–0.78); *P* = 0.001**
Ever	15/31	25/49	8/12	1.01 (0.45–2.24); *P* = 0.985	1.32 (0.44–3.95); *P* = 0.619	1.07 (0.50–2.29); *P* = 0.859	1.31 (0.49–3.50); *P* = 0.585
BMI (kg/m^2)							
<24	82/170	102/205	24/61	1.01 (0.70–1.44); *P* = 0.969	0.81 (0.47–1.40); *P* = 0.452	0.96 (0.68–1.35); *P* = 0.825	0.81 (0.49–1.34); *P* = 0.411
≥24	105/138	156/144	28/64	**1.47 (1.04–2.08); *P* = 0.028**	**0.59 (0.35–0.99); *P* = 0.045**	1.20 (0.87–1.66); *P* = 0.273	**0.48 (0.30–0.77); *P* = 0.002**

[a]For miR-146a rs2910164 C>G, the genotyping was successful in 497 (99.00%) T2DM cases, and 782 (100.00%) controls; [b]Adjusted for age, sex, smoking, drinking and BMI (besides stratified factors accordingly) in a multiple logistic regression model.
Bold values are statistically significant ($P < 0.05$).

Table 6 Stratified analyses between miR-499 rs3746444 A>G polymorphism and T2DM risk by sex, age, BMI, smoking status and alcohol consumption.

Variable	miR-499 rs3746444 A>G (case/control)[a]			Adjusted OR[b] (95% CI); *P*			
	AA	AG	GG	AG vs AA	GG vs AA	GG/AG vs AA	GG vs AA/AG
Sex							
Male	245/376	78/134	6/12	0.88 (0.63–1.22); *P* = 0.427	0.77 (0.28–2.10); *P* = 0.605	0.87 (0.63–1.19); *P* = 0.378	0.79 (0.29–2.16); *P* = 0.650
Female	118/189	47/67	3/4	1.03 (0.66–1.62); *P* = 0.891	1.19 (0.26–5.50); *P* = 0.825	1.04 (0.67–1.61); *P* = 0.860	1.18 (0.26–5.43); *P* = 0.833
Age							
<65	167/290	53/93	4/6	0.92 (0.62–1.37); *P* = 0.682	1.10 (0.30–4.05); *P* = 0.888	0.93 (0.63–1.37); *P* = 0.718	1.12 (0.31–4.12); *P* = 0.864
≥65	196/275	72/108	5/10	0.94 (0.66–1.34); *P* = 0.726	0.79 (0.26–2.36); *P* = 0.669	0.93 (0.66–1.31); *P* = 0.664	0.80 (0.27–2.39); *P* = 0.691
Smoking status							
Never	240/398	85/132	6/12	1.03 (0.75–1.41); *P* = 0.875	0.83 (0.31–2.26); *P* = 0.716	1.01 (0.74–1.38); *P* = 0.949	0.83 (0.30–2.24); *P* = 0.706
Ever	123/167	40/69	3/4	0.75 (0.47–1.20); *P* = 0.233	0.88 (0.19–4.15); *P* = 0.866	0.76 (0.48–1.20); *P* = 0.238	0.94 (0.20–4.44); *P* = 0.940
Alcohol consumption							
Never	325/504	116/171	8/15	1.03 (0.78–1.36); *P* = 0.857	0.87 (0.36–2.09); *P* = 0.752	1.01 (0.77–1.33); *P* = 0.922	0.86 (0.36–2.07); *P* = 0.740
Ever	38/61	9/30	1/1	0.47 (0.20–1.11); *P* = 0.084	2.17 (0.12–40.78); *P* = 0.606	0.51 (0.22–1.17); *P* = 0.113	2.60 (0.14–48.65); *P* = 0.523
BMI (kg/m^2)							
<24	153/317	53/107	2/12	0.99 (0.67–1.45); *P* = 0.948	0.33 (0.07–1.50); *P* = 0.152	0.92 (0.63–1.34); *P* = 0.669	0.33 (0.07–1.50); *P* = 0.152
≥24	210/248	72/94	7/4	0.91 (0.63–1.30); *P* = 0.592	2.18 (0.62–7.73); *P* = 0.227	0.96 (0.67–1.36); *P* = 0.801	2.24 (0.63–7.91); *P* = 0.211

[a]For miR-499 rs3746444 A>G, the genotyping was successful in 497 (99.00%) T2DM cases and 782 (100.00%) controls; [b]Adjusted for age, sex, smoking, drinking and BMI (besides stratified factors accordingly) in a multiple logistic regression model.

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Table 7 Combination analysis of miRNA loci (rs3746444, rs2910164 and rs11614913) in T2DM cases and controls.

Genotype	Case		Control		OR (95% CI)	*P* value
	n	%	*n*	%		
rs11614913/rs2910164						
TT/CC	51	10.26	87	11.13	1.00	
TT/CG	93	18.71	106	13.55	1.50 (0.96–2.33)	0.074
TT/GG	21	4.23	36	4.60	1.00 (0.52–1.89)	0.988
TC/CC	105	21.13	165	21.10	1.09 (0.71–1.66)	0.704
TC/CG	125	25.15	185	23.66	1.15 (0.76–1.74)	0.501
TC/GG	21	4.23	65	8.31	0.55 (0.30–1.01)	0.051
CC/CC	31	6.24	56	7.16	0.94 (0.54–1.65)	0.841
CC/CG	40	8.05	58	7.42	1.18 (0.69–2.00)	0.548
CC/GG	10	2.01	24	3.07	0.71 (0.31–1.61)	0.410
rs11614913/rs3746444						
TT/AA	119	23.94	159	20.33	1.00	
TT/AG	43	8.65	67	8.57	0.86 (0.55–1.35)	0.504
TT/GG	3	0.60	3	0.38	1.34 (0.26–6.74)	1.000
TC/AA	189	38.03	307	39.26	0.82 (0.61–1.11)	0.200
TC/AG	57	11.47	98	12.53	0.78 (0.52–1.16)	0.221
TC/GG	5	1.01	10	1.28	0.67 (0.22–2.01)	0.470
CC/AA	55	11.07	99	12.66	0.74 (0.49–1.11)	0.150
CC/AG	25	5.03	36	4.60	0.93 (0.53–1.63)	0.794
CC/GG	1	0.20	3	0.38	0.45 (0.05–4.34)	0.639
rs2910164/rs3746444						
CC/AA	133	26.76	220	28.13	1.00	
CC/AG	48	9.66	82	10.49	0.97 (0.64–1.47)	0.879
CC/GG	6	1.21	6	0.77	1.65 (0.52–5.24)	0.387
CG/AA	190	38.23	247	31.59	1.27 (0.96–1.69)	0.099
CG/AG	65	13.08	98	12.53	1.10 (0.75–1.60)	0.633
CG/GG	3	0.60	4	0.51	1.24 (0.27–5.63)	1.000
GG/AA	40	8.05	98	12.53	0.68 (0.44–1.03)	0.070
GG/AG	12	2.41	21	2.69	0.95 (0.45–1.98)	0.882
GG/GG	0	0.00	6	0.77	0.13 (0.01–2.28)	0.089
rs11614913/rs2910164/rs3746444						
TT/CC/AA	35	7.04	58	7.42	1.00	
TT/CC/AG	14	2.82	29	3.71	0.80 (0.37–1.72)	0.566
TT/CC/GG	2	0.40	0	0.00	8.24 (0.38–176.72)	0.149
TT/CG/AA	70	14.08	76	9.72	1.53 (0.90–2.59)	0.117
TT/CG/AG	22	4.43	29	3.71	1.26 (0.63–2.52)	0.518
TT/CG/GG	1	0.20	1	0.13	1.65 (0.10–27.36)	1.000
TT/GG/AA	14	2.82	25	3.20	0.93 (0.43–2.02)	0.851
TT/GG/AG	7	1.41	9	1.15	1.29 (0.44–3.77)	0.642
TT/GG/GG	0	0.00	2	0.26	0.33 (0.02–7.07)	0.530
TC/CC/AA	78	15.69	125	15.98	1.03 (0.62–1.72)	0.897
TC/CC/AG	24	4.83	36	4.60	1.10 (0.57–2.15)	0.769
TC/CC/GG	3	0.60	4	0.51	1.24 (0.26–5.89)	1.000
TC/CG/AA	93	18.71	128	16.37	1.20 (0.73–1.98)	0.464
TC/CG/AG	30	6.04	55	7.03	0.90 (0.49–1.67)	0.746
TC/CG/GG	2	0.40	2	0.26	1.66 (0.22–12.30)	0.635
TC/GG/AA	18	3.62	54	6.91	0.55 (0.28–1.09)	0.085
TC/GG/AG	3	0.60	7	0.90	0.71 (0.17–2.93)	0.742
TC/GG/GG	0	0.00	4	0.51	0.18 (0.01–3.51)	0.293
CC/CC/AA	20	4.02	37	4.73	0.90 (0.45–1.78)	0.753
CC/CC/AG	10	2.01	17	2.17	0.97 (0.40–2.37)	0.955
CC/CC/GG	1	0.20	2	0.26	0.83 (0.07–9.48)	1.000
CC/CG/AA	27	5.43	43	5.50	1.04 (0.55–1.97)	0.903
CC/CG/AG	13	2.62	14	1.79	1.54 (0.65–3.65)	0.326
CC/CG/GG	0	0.00	1	0.13	0.55 (0.02–13.86)	1.000
CC/GG/AA	8	1.61	19	2.43	0.70 (0.28–1.76)	0.445
CC/GG/AG	2	0.40	5	0.64	0.66 (0.12–3.60)	1.000
CC/GG/GG	0	0.00	0	0.00	-	-

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dominant genetic model. These findings suggested that *miR-146a* rs2910164 and *miR-196a2* rs11614913 SNPs could be protective factors of T2DM occurrence.

Discussion

Some investigations have found rs11614913, rs2910164 and rs3746444 loci of miR-SNPs may be potential biomarker for increased incidence of T2DM. However, relationship of the mentioned miR-SNPs with development of T2DM was unclear. This case–control study showed that rs2910164 SNP is associated with decreased susceptibility of T2DM, especially in non-drinking and BMI ≥24 kg/m^2 subgroups. In addition, we also found that rs11614913 C allele may be associated with decreased susceptibility of T2DM significantly in smoking subgroup.

Annexin A1 (ANXA1) is found to be an important anti-inflammatory factor. Purvis *et al.* have reported that ANXA1 level significantly increased in people with hyperglycemia (44). In addition, ANXA1 might be a protective factor for T2DM (45) and imply a new treatment strategy for T2DM (44). Huang *et al.* have reported that peptide Ac2-26, an ANXA1 N-terminal peptide, promotes wound healing in diabetes (46). Thus, it is suggested that ANXA1 may be implicated in the development of T2DM. A recent study has suggested that *miR-196a2* regulates the role of ANXA1(47). rs11614913 T→C variant in *miR-196a2* gene locates at the 3p regions in mature *miR* (48). Several investigations found that rs11614913 C allele increased the expression of miR-196a2 (49, 50). Ghanbari *et al.* have found that rs11614913 C allele decreases waist to hip ratio (51). A previous pooled analysis suggested that rs11614913 C allele did not influence the susceptibility of T2DM in China (30). However, in this study, we found that rs11614913 C allele decreased the susceptibility of T2DM in smoking subgroup, which might indicate that the role of rs11614913 C allele could be affected by environmental factor. Yin *et al.* also suggested that an interaction of the rs11614913 genotypes in miR-196a2 gene with environmental factor is associated with an increased susceptibility of lung cancer (52). It might be explained that the correlation of rs11614913 locus with an occurrence of T2DM could be affected by risk factors of environment. Considering a single locus of *miR* could only make low penetrance effects on T2DM, the interaction of gene–environment factors may dilute the role of rs11614913, and more investigations are needed to support our findings in the future.

Considering that an individual SNP contributes little to the susceptibility of T2DM, the current investigation urges a need of sufficient power to get an assessment of rs2910164 with T2DM risk. Recently, the correlation of rs2910164 with T2DM risk was explored by several case–control studies. A meta-analysis suggested an association of rs2910164 in *miR-146a* gene with T2DM risk (29). However, in that meta-analysis, only 2069 cases and 1950 controls were included. The observations may be underpowered. Wang *et al.* suggested that G allele of rs2910164 SNP did not influence the occurrence of T2DM (25), while other investigations reported that G allele of this SNP decreased the risk of T2DM (26, 27, 28). Considering only four publications with moderate sample sizes exploring the correlation of rs2910164 with the occurrence of T2DM in Asians, the observations might be underpowered. In this study, 1284 participants were included to assess a correlation of rs2910164 in *miR-146a* gene with T2DM risk. We identified that rs2910164 in *miR-146a* gene decreased the risk of T2DM. In our study, a Bonferroni correction was harnessed to confirm our findings. After adjustment, the correlation of rs2910164 with the occurrence of T2DM also existed in overall comparison and in BMI ≥24 kg/m^2 and never drinking subgroups, which was similar to the findings of a more recent meta-analysis (30). In the future, more well-matched investigations are needed to confirm or refute our observations.

There are some limitations in our study. First, selective bias might exist. T2DM patients and normal controls came from two different hospitals, and these subjects could not well represent the Chinese populations. Secondly, only three *miR*-SNPs were included and studied in our study, more polymorphisms widely investigated should be included by artificial neural networks database. Thirdly, for temporary limitation of T2DM sample size and type, we couldn't perform genotype-based mRNA expression analysis. However, further investigations with detailed gene–environmental factors and functional exploring are certainly needed (53). Fourthly, in some subgroups, the sample size might be insufficient. Fifth, variant distribution of rs11614913 was suggested to be out of HWE in overall and some subgroups comparisons. The findings should be explained with caution. Finally, this study did not focus on the detailed information on T2DM complications, which might restrict a further evaluation of the mentioned SNPs on prognosis of T2DM.

In summary, this study highlights that *miR-146a* rs2910164 SNP is associated with decreases in susceptibility of T2DM, especially in BMI ≥24 kg/m^2 and non-smoking group. In addition, we also found that *miR-196a2* rs11614913 C allele decreases the susceptibility of T2DM significantly in smoking subgroup.

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Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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