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

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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 \( \geq 24 \text{ kg/m}^2 \) (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 to 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 \( \geq 24 \text{ kg/m}^2 \) and never drinking subgroups. In addition, we also identify that rs11614913 C allele decreases the susceptibility of T2DM significantly in smoking subgroup.

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.
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/m² 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 differences categorical variables (smoking status, alcohol consumption, BMI, genotypes, sex and age) were measured using chi-square test (χ²). Using the genotype number in controls of rs3746444, rs11614913 and rs2910164, Hardy-Weinberg equilibrium (HWE)
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
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, \textit{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 susceptibility compared with rs2910164 TT genotype (adjusted \( P = 0.020 \)).

| 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%               |

**Table 3** The relationship of miRNA polymorphisms with T2DM.

| Genotype | Cases (n = 502) | Controls (n = 782) | Crude OR (95% CI) | \( P \) | Adjusted OR* (95% CI) | \( P \) |
|----------|----------------|-------------------|------------------|--------|----------------------|--------|
| miR-499 rs3746444 | | | | | | |
| AA       | 363            | 565               | 1.00             | 1.00   | 0.97 (0.75–1.25)     | 0.806  | 0.95 (0.73–1.23)     | 0.689  |
| AG       | 125            | 201               | 0.97 (0.75–1.25) | 0.806  | 0.97 (0.75–1.25)     | 0.806  | 0.95 (0.73–1.23)     | 0.689  |
| GG       | 9              | 16                | 0.88 (0.65–1.18) | 0.753  | 0.89 (0.65–1.18)     | 0.753  | 0.89 (0.65–1.18)     | 0.788  |
| AG+GG    | 134            | 217               | 0.96 (0.75–1.24) | 0.759  | 0.94 (0.73–1.22)     | 0.759  | 0.94 (0.73–1.22)     | 0.657  |
| AA+AG    | 488            | 766               | 1.00             | 1.00   | 0.94 (0.73–1.24)     | 0.759  | 0.94 (0.73–1.22)     | 0.657  |
| GG       | 9              | 16                | 0.88 (0.65–1.18) | 0.759  | 0.94 (0.73–1.24)     | 0.759  | 0.94 (0.73–1.22)     | 0.657  |
| G allele | 143            | 233               | 1.00             | 1.00   | 0.94 (0.73–1.24)     | 0.759  | 0.94 (0.73–1.22)     | 0.657  |

| miR-146a rs2910164 | | | | | | |
| CC       | 187            | 308               | 1.00             | 1.00   | 0.94 (0.73–1.24)     | 0.759  | 0.94 (0.73–1.22)     | 0.657  |
| CG       | 258            | 434               | 1.22 (0.96–1.55) | 0.112  | 1.22 (0.96–1.55)     | 0.112  | 1.22 (0.96–1.55)     | 0.110  |
| GG       | 52             | 125               | 0.69 (0.47–0.99) | 0.046  | 0.67 (0.46–0.97)     | 0.046  | 0.67 (0.46–0.97)     | 0.036  |
| GC+GG    | 310            | 474               | 1.07 (0.85–1.36) | 0.530  | 1.07 (0.85–1.36)     | 0.530  | 1.07 (0.85–1.36)     | 0.548  |
| CC+CG    | 445            | 657               | 1.00             | 1.00   | 1.07 (0.85–1.36)     | 0.530  | 1.07 (0.85–1.36)     | 0.548  |
| GG       | 52             | 125               | 0.61 (0.44–0.87) | 0.006  | 0.60 (0.42–0.85)     | 0.006  | 0.60 (0.42–0.85)     | 0.004  |
| G allele | 362            | 599               | 1.00             | 1.00   | 0.60 (0.42–0.85)     | 0.006  | 0.60 (0.42–0.85)     | 0.004  |

| miR-196a2 rs11614913 | | | | | | |
| TT       | 165            | 229               | 1.00             | 1.00   | 0.83 (0.64–1.07)     | 0.83 (0.64–1.07) | 0.83 (0.64–1.07) | 0.151  |
| TC       | 251            | 415               | 0.84 (0.65–1.08) | 0.177  | 0.83 (0.65–1.08)     | 0.177  | 0.83 (0.65–1.08)     | 0.151  |
| CC       | 81             | 138               | 0.82 (0.58–1.14) | 0.237  | 0.83 (0.59–1.18)     | 0.237  | 0.83 (0.59–1.18)     | 0.301  |
| T+TC     | 332            | 553               | 0.83 (0.65–1.06) | 0.140  | 0.83 (0.65–1.06)     | 0.140  | 0.83 (0.65–1.06)     | 0.135  |
| CC       | 81             | 138               | 0.91 (0.67–1.23) | 0.533  | 0.94 (0.69–1.27)     | 0.533  | 0.94 (0.69–1.27)     | 0.682  |
| C allele | 413            | 691               | 1.00             | 1.00   | 0.94 (0.69–1.27)     | 0.533  | 0.94 (0.69–1.27)     | 0.682  |

Bold values are statistically significant \( (P < 0.05) \).

*Adjusted for age, sex, smoking, status of BMI and drinking.
Combination analysis of miRNA polymorphisms

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

| Variable                  | TT (case/control) | T>C (case/control) | CC (case/control) | TT vs TC | CC vs TT | Adjusted OR (95% CI); P |
|---------------------------|-------------------|--------------------|-------------------|----------|----------|--------------------------|
| **Sex**                   | 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 |
| **Male**                  | 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 |
| **Female**                | 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 |
| **Age**                   | 199/108           | 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 |
| **<65**                   | 0.70 (0.39-1.24); P = 0.39 | 0.78 (1.35-2.16); P = 0.005 | 0.58 (0.32-0.87); P = 0.002 | 1.04 (0.77-1.41); P = 0.797 | 1.05 (0.73-1.51); P = 0.784 | 0.70 (0.39-1.24); P = 0.216 |
| **≥65**                   | 0.32 (0.14-0.73); P = 0.007 | 0.52 (0.33-0.81); P = 0.005 | 1.07 (0.71-1.62); P = 0.743 | 0.50 (0.32-0.78); P = 0.002 | 0.50 (0.32-0.78); P = 0.002 | 0.70 (0.39-1.24); P = 0.216 |
| **Smoking status**        | 99/166            | 173/280            | 59/96             | 0.95 (0.69-1.29); P = 0.489 | 0.95 (0.69-1.29); P = 0.489 | 0.95 (0.69-1.29); P = 0.489 | 0.95 (0.69-1.29); P = 0.489 |
| **Never**                 | 20/22             | 17/54              | 11/16             | 0.32 (0.14-0.73); P = 0.007 | 0.32 (0.14-0.73); P = 0.007 | 0.32 (0.14-0.73); P = 0.007 | 0.32 (0.14-0.73); P = 0.007 |
| **Ever**                  | 145/207           | 72/234             | 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 |
| **Alcohol consumption**   | Never             | 20/22              | 17/54             | 0.32 (0.14-0.73); P = 0.007 | 0.32 (0.14-0.73); P = 0.007 | 0.32 (0.14-0.73); P = 0.007 | 0.32 (0.14-0.73); P = 0.007 |
| **<24**                   | 104/225           | 29/88              | 52/50             | 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 |

*For miR-196a2 rs11614913 T>C, the genotyping was successful in 497 (99.00%) T2DM cases and 782 (100.00%) controls; 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).
## 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)* | Adjusted OR (95% CI); P |
|-----------------------|----------------------------------------|-------------------------|
|                       | CC vs CG                                | GG vs CC                | GG/CG vs CC | GG vs CC/GG |
| Sex                   |                                        |                         |             |             |
| Male                  | 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                | 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                   | 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                   | 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                 | 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                  | 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                 | 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                  | 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/m²)           |                                        |                         |             |             |
| <24                   | 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                   | 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 |

*For miR-146a rs2910164 C>G, the genotyping was successful in 497 (99.00%) T2DM cases and 782 (100.00%) controls; †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)* | Adjusted OR (95% CI); P |
|-----------------------|----------------------------------------|-------------------------|
|                       | AG vs AA                                | GG vs AA                | GG/AG vs AA | GG vs AA/AG |
| Sex                   |                                        |                         |             |             |
| Male                  | 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                | 1.03 (0.66–1.62); P = 0.891             | 1.19 (0.26–5.00); P = 0.825 | 1.04 (0.67–1.61); P = 0.860 | 1.18 (0.26–5.43); P = 0.833 |
| Age                   |                                        |                         |             |             |
| <65                   | 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                   | 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                 | 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                  | 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                 | 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                  | 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 |
| BMI (kg/m²)           |                                        |                         |             |             |
| <24                   | 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                   | 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 |

*For miR-499 rs3746444 A>G, the genotyping was successful in 497 (99.00%) T2DM cases and 782 (100.00%) controls; †Adjusted for age, sex, smoking, drinking and BMI (besides stratified factors accordingly) in a multiple logistic regression model.
| Genotype                  | Case   | Control | OR (95% CI) | P value |
|--------------------------|--------|---------|-------------|---------|
|                          | n      | %       | n           | %       |          |
| rs11614913/rs2910164     |        |         |             |         |          |
| TT/CC                    | 51     | 10.26   | 87          | 11.13   | 1.00     |
| TT/GG                    | 108    | 22.59   | 173         | 24.45   | 0.99     |
| TC/CC                    | 107    | 21.98   | 168         | 23.38   | 0.76     |
| TC/GG                    | 125    | 25.71   | 185         | 25.95   | 0.81     |
| CC/CC                    | 31     | 6.42    | 56          | 7.80    | 0.74     |
| CC/GG                    | 40     | 8.05    | 58          | 8.23    | 0.71     |
| CC/AG                    | 10     | 2.00    | 24          | 3.07    | 0.45     |
| rs11614913/rs3746444     |        |         |             |         |          |
| TT/AA                    | 119    | 23.94   | 159         | 20.33   | 1.00     |
| TT/AG                    | 43     | 8.65    | 67          | 8.57    | 0.86     |
| TT/GG                    | 3      | 0.60    | 3           | 0.38    | 1.34     |
| TC/AA                    | 187    | 38.03   | 241         | 33.19   | 0.82     |
| TC/AG                    | 57     | 11.47   | 98          | 13.25   | 0.78     |
| TC/GG                    | 5      | 1.01    | 10          | 1.28    | 0.67     |
| CC/AA                    | 55     | 11.07   | 99          | 12.66   | 0.74     |
| CC/AG                    | 25     | 5.03    | 36          | 4.60    | 0.93     |
| CC/GG                    | 1      | 0.20    | 3           | 0.38    | 0.45     |
| rs2910164/rs3746444      |        |         |             |         |          |
| CC/AA                    | 133    | 26.76   | 220         | 28.13   | 1.00     |
| CC/AG                    | 48     | 9.66    | 82          | 10.49   | 0.97     |
| CC/GG                    | 6      | 1.21    | 6           | 0.77    | 1.65     |
| CG/AA                    | 190    | 38.23   | 247         | 31.59   | 1.27     |
| CG/AG                    | 65     | 13.08   | 98          | 12.53   | 1.10     |
| CG/GG                    | 3      | 0.60    | 4           | 0.51    | 1.24     |
| GG/AA                    | 40     | 8.05    | 98          | 12.53   | 0.68     |
| GG/AG                    | 12     | 2.41    | 21          | 2.69    | 0.95     |
| GG/GG                    | 0      | 0.00    | 6           | 0.77    | 0.13     |
| 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     |
| TT/CC/GG                 | 2      | 0.40    | 0           | 0.00    | 8.24     |
| TT/CG/AA                 | 70     | 14.08   | 78          | 9.72    | 1.53     |
| TT/CG/AG                 | 22     | 4.43    | 29          | 3.71    | 1.26     |
| TT/CG/GG                 | 1      | 0.20    | 1           | 0.13    | 1.65     |
| TT/CG/GG                 | 14     | 2.82    | 25          | 3.20    | 0.93     |
| TT/CC/AA                 | 7      | 1.41    | 9           | 1.15    | 1.29     |
| TT/CC/AG                 | 0      | 0.00    | 2           | 0.26    | 0.33     |
| TT/CC/GG                 | 78     | 15.69   | 125         | 15.98   | 1.03     |
| TT/CG/AA                 | 24     | 4.83    | 36          | 4.60    | 1.10     |
| TT/CG/AG                 | 3      | 0.60    | 4           | 0.51    | 1.24     |
| TT/CG/GG                 | 93     | 18.71   | 128         | 16.37   | 1.20     |
| TT/CG/GG                 | 30     | 6.04    | 55          | 7.03    | 0.90     |
| TT/CC/AA                 | 2      | 0.40    | 2           | 0.26    | 1.66     |
| TT/CC/AG                 | 18     | 3.62    | 54          | 6.91    | 0.55     |
| TT/CC/GG                 | 3      | 0.60    | 7           | 0.90    | 0.71     |
| TT/CG/AA                 | 0      | 0.00    | 4           | 0.51    | 0.18     |
| TT/CC/AA                 | 20     | 4.02    | 37          | 4.73    | 0.90     |
| TT/CC/AG                 | 10     | 2.01    | 17          | 2.17    | 0.97     |
| TT/CC/GG                 | 1      | 0.20    | 2           | 0.26    | 0.83     |
| TT/CG/AA                 | 27     | 5.43    | 43          | 5.50    | 1.04     |
| TT/CG/AG                 | 13     | 2.62    | 14          | 1.79    | 1.54     |
| TT/CG/GG                 | 0      | 0.00    | 1           | 0.13    | 0.55     |
| TT/CG/GG                 | 8      | 1.61    | 19          | 2.43    | 0.70     |
| TT/CG/GG                 | 2      | 0.40    | 5           | 0.64    | 0.66     |
| TT/CC/GG                 | 0      | 0.00    | 0           | 0.00    | -        |
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/m² 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/m² 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/m² and non-smoking group. In addition, we also found that miR-196a2 rs11614913 C allele decreases the susceptibility of T2DM significantly in smoking subgroup.
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.

Funding
The project was supported by the Joint Funds for the Innovation of Science and Technology, Fujian province, China (grant numbers 2017Y9015); the Educational Commission of Yunnan Province, China (grant number 2017ZDX116).

Acknowledgements
The authors appreciate all subjects who participated in this study. We wish to thank Dr Yan Liu (Genesky Biotechnologies Inc., Shanghai, China) for technical support.

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