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The polymorphism in the *let-7* targeted region of the *Lin28* gene is associated with increased risk of type 2 diabetes mellitus

Jie Zhang [1], Longyi Zhang [1], Runping Fan [1], Nengneng Guo, Chunhui Xiong, Lin Wang, Sheng Jin, Wei Li, Jianxin Lu *

Key Laboratory of Laboratory Medicine, Ministry of Education, Zhejiang Provincial Key Laboratory of Medical Genetics, Wenzhou Medical College, Wenzhou, Zhejiang 325035, China

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ABSTRACT

Genetic polymorphisms in the miRNAs pathway of the pathogenesis of disease might contribute to the risk of disease. However, it is unclear whether these polymorphisms about miRNAs are associated with the risk of type 2 diabetes mellitus (T2DM). We performed a case-control study to investigate two polymorphisms in the *let-7*/*Lin28* pathway based on 588 T2DM patients and 588 age and sex matched controls. The results showed that the rs3811463 polymorphism was associated with increased risk of T2DM (odds ratio (OR) = 1.47, 95% confidence inference (95%CI) = 1.13–1.93, $P = 0.005$), while the rs3811464 not (OR = 1.04, 95%CI = 0.79–1.36, $P = 0.78$). For the rs3811463 polymorphism, the variant genotypes were associated with increased risk of disease in females; statistically differences were observed in the clinical features of age at diagnosis, hypertension and peripheral neuropathy for the variant and wild genotype of the rs3811463 in T2DM. In summary, the results indicated that the rs3811463 polymorphism in the *let-7*/*Lin28* pathway could significantly increase the risk of T2DM.

1. Introduction

T2DM is the most highly prevalent metabolic disorder characterized by chronic hyperglycaemia resulted from pancreatic dysfunction and insulin resistance (Sun et al., 2010; Tahrani et al., 2011; Touskova et al., 2012). It could increase the risks of the cardiovascular diseases, kidney failure, blindness, neuropathy and peripheral circulatory diseases (Stumvoll et al., 2005). Diabetes is a global healthy problem with devastating human, social and economic impacts (Papazoglou et al., 2011; Stumvoll et al., 2005; Tahrani et al., 2011). The number of people with diabetes is increasing due to the population growth, aging, obesity, urbanization, and physical inactivity. It estimated that almost 250 million people worldwide are living with diabetes, and by 2025 this total is expected to increase to over 380 million (Rauseo et al., 2010). It is well-established that T2DM is a complex disease which is influenced by both genetic factors and environmental factors (Patel et al., 2010; Stolerman and Florez, 2009; Sun et al., 2011). Results from different populations have indicated that T2DM has been a multiple gene disease (Stolerman and Florez, 2009), and new genes have been uncovered gradually.

miRNAs are a class of approximately 19–24 nucleotides-long noncoding RNA molecules that are involved in the regulation of gene expression (El-Khairi et al., 2012; Greco et al., 2011). They play important roles in multiple physiological processes, including developmental timing, cellular proliferation, differentiation, and apoptosis (Cochrane et al., 2012; Esquela-Kerscher and Slack, 2006). miRNAs might play critical roles in many diseases (Chitwood and Timmermans, 2010), including diabetes. Previously studies indicated that miRNAs-targeted genes was important for pancreas development, beta-cell proliferation, insulin secretion and exocytosis, such as miR-375, miR-21, miR-34a (Guay et al., 2011). In recently, Zhu et al. reported that the *Lin28*/*let-7* microRNA pathway might play important roles in regulating metabolism, and *Lin28a* and *Lin28b* promoted an insulin-sensitized state that resisted high-fat-diet induced diabetes (Zhu et al., 2011). In addition, Frost and Olson (2011) showed that the let-7 family of microRNAs contributed to the control of glucose homeostasis and insulin sensitivity, and knockdown of the let-7 could be taken as a potential treatment for type 2 diabetes. However, the clear mechanism of the *Lin28*/*let-7* for diabetes is still unknown and it is needed to be studied.

In recently years, several studies have further highlighted that the miRNA-related SNPs, especially those located within miRNA complementary sites, could remarkable alter the biogenesis and the function of the corresponding miRNA (Slaby et al., 2012). Thus, a large number of miRNAs-realted SNPs have been identified as diseases risk factors, such as rs2910164 of the miR-146a (Yue et al., 2011), rs3811463 of the *Lin28*/*let-7* (Chen et al., 2011). Chen reported the genetic variants that associated with the *let-7*/*Lin28*

* Corresponding author. Tel.: +86 577 86689805; fax: +86 577 86689717.
E-mail address: jxlu@163.com (J. Lu).
[1] Contributed equally to this work.

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could alter breast cancer risk (Chen et al., 2011). They demonstrated that the variant allele of Lin28 leads to increased expression of the Lin28 protein, likely because let-7 has a diminished capacity to suppress its translation. This in turn would be expected to decrease let-7 expression. In addition, Zhu et al. (2011) have shown that let-7 targets a number of proteins required for efficient insulin activity, including the insulin receptor, IRS2, and the IGF-I receptor. Hence, increased expression of LIN28, by suppressing let-7, might tend to boost insulin sensitivity. Considering the *Lin28*/*let-7* might play important roles in the pathogenesis of T2DM, these findings prompted us to investigate whether there were any polymorphisms in the *let-7*/*Lin28* pathways could alter the risk of T2DM, and to investigate whether miRNA associated polymorphisms could be risk factors for T2DM. To our knowledge, this is the first study that conducted with respect to the association between miRNA associated polymorphisms and T2DM risk in Chinese population.

2. Subjects and methods

2.1. Subjects

This case-control study was carried out among 588 unrelated T2DM patients and 588 healthy controls. The cases and controls were age and sexes matched, and were all Han populations in Zhejiang province. All individuals were subjects of eastern origin of China. T2DM was diagnosed according to the American Diabetes association criteria. They were sequentially recruited from the First Affiliated Hospital of Wenzhou Medical College from February 2009 to October 2011. Patients underwent standardized clinic and laboratory evaluations which were consisted of questionnaire, physical examination, and laboratory tests. Information of height, weight, systolic blood pressure (SBP), diastolic blood pressure (DBP), plasma glucose, total cholesterol (TC), triglyceride (TG), HDL-cholesterol (HDL-c), and LDL-cholesterol (LDL-c) were collected. The body mass index (BMI, kg/m^2) was calculated by using the weight and height. Hypertension was defined as blood pressure levels $\geqslant$140/90 mmHg, and patients being treated with antihypertensive medication whose blood pressure was lower than 140/90 mmHg were also considered hypertensive. A total of 588 age and sex matched healthy controls without family histories of diabetes were obtained from Wenzhou Physical Examination Center and other several cities of Zhejiang province, including Shaoxing. The healthy controls had normal glucose tolerance defined as a fasting plasma glucose level of <6.1 mmol/L and a 2-h 75 g oral glucose tolerance test (OGTT) plasma glucose level of <7.8 mmol/L.

2.2. Selection of polymorphisms and genotyping

It reported that the *let-7* can repress the post-transcriptional translation of L *Lin28*, and the *Lin28* can in turn block the maturation of *let-7*, forming a double-negative feedback loop (Chen et al., 2011). Chen have reported that the C allele of rs3811463, a polymorphism located near the binding site of *let-7* in *Lin28*, could weaken the suppression of *Lin28* by *let-7*, and decreased the level of *let-7* (Chen et al., 2011). Although the rs3811463 polymorphism is outside of the let-7 target site (82 nt downstream of the seed sequence), the functional assays with luciferase reporters had revealed that the T/C variant could lead to differential regulation of the Lin28 mRNA by let-7. So the rs3811463 polymorphism was selected. In addition, the rs3811464 polymorphism which was located in the 5′ flanking region (promoter) of the *Lin28* gene was also analyzed (Chen et al., 2011). The Genomic DNA was extracted from peripheral whole blood leukocytes using standard techniques. The selected polymorphisms were determined with the method of polymerase chain reaction (PCR)–restriction fragment length polymorphism (RFLP). The amplification products were digested by the appropriate restriction enzymes (*Xcm*I, N*la*IV) under the conditions recommended by the manufacturer (MBI). The digested fragments were then separated by electrophoresis in 2% polyacrylamide gels, followed by ethidium bromide staining and direct visualization under ultraviolet light (Figs. S1 and S2). The analyses were preformed by two independent authors blindly without knowing the case and control status. If the results of the same sample could not reach consensus, that sample would be sent to the company to be sequenced for the polymorphism. Additionally, to further ensure the genotyping accuracy, we randomly selected 10% of all samples and genotyped in duplicated, and the results indicated 100% concordant. In addition, relevant PCR products were selected based on RFLP analysis, and sent to BGI-Shanghai for sequencing.

2.3. Statistical analyses

The statistical analyses were performed as previous study (Lee et al., 2010). HWE was tested by Pearson's χ^2 test for each polymorphism. Quantitative data were analyzed by *t*-test, ANOVA, or Wilcoxon rank-sum test, and qualitative data were analyzed by χ^2 test. The association between the alleles/genotypes and T2DM risk were determined by χ^2 test. The crude OR with 95%CI was also determined. All statistical analyses were conducted with SPSS17.0 software.

3. Results

3.1. Comparison of clinical data

A total of 588 T2DM patients and 588 controls were included in this study. The demographic characteristics of the studied subjects are summarized in Table 1. No differences were found between cases and controls with respect to age and gender. However, there was a significant difference in the distribution of BMIs between the T2DM and the control groups. The mean BMI of the T2DM patients was significantly higher than the corresponding controls. The mean duration of diabetes was 7.32 ± 5.84 years. The mean level of HbA1c% is 9.3 ± 2.3. TC, HDL-c, LDL-c, TG, SBP, DBP and fasting plasma glucose were significantly different between the two groups. SBP, DBP, fasting plasma glucose, TC and TG, were relatively higher in the type 2 diabetes patients, where as HDL cholesterol and LDL cholesterol were lower.

3.2. Genotype of the rs3811463 and rs3811464 polymorphisms

There were three genotypes detected at the rs3811463 site: TT, TC and CC (Fig. S1a). There were also 3 genotypes at the rs3811464

Table 1
Clinical characteristic of the study populations.

	Control (n = 588)	T2DM (n = 588)	P value
Age (years)	56.80 ± 10.79	56.80 ± 10.79	1.00
Male/Female	328/260	328/260	1.00
BMI (kg/m^2)	22.65 ± 2.62	24.30 ± 3.29	<0.001
Systolic blood pressure (mmHg)	118.24 ± 14.17	140.80 ± 23.37	<0.001
Diastolic blood pressure (mmHg)	73.67 ± 10.49	82.28 ± 11.47	<0.001
Fasting plasma glucose (mmol/L)	5.11 ± 0.59	9.03 ± 3.93	<0.001
HbA1c%	N.A.	9.33 ± 2.33	N.A.
Total cholesterol (mmol/L)	4.54 ± 1.21	4.72 ± 1.24	0.009
Triglyceride (mmol/L)	1.39 ± 1.23	1.77 ± 1.65	<0.001
HDL-cholesterol (mmol/L)	1.34 ± 0.29	1.27 ± 0.40	<0.001
LDL-cholesterol (mmol/L)	2.72 ± 0.94	2.48 ± 0.50	<0.001

Data are mean ± SD; N.A. not available.