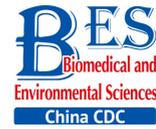


## Research Highlight



# A Tiny RNA Molecule with a Big Impact on Type 2 Diabetes Mellitus Susceptibility\*

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Biological molecules could be used as risk assessment tools for predicting incident Type 2 Diabetes Mellitus (T2DM), such as microRNAs (miRNAs). Numerous studies have shown association between miRNA and susceptibility of T2DM, suggesting that miRNAs might be common biological factors for T2DM. Interestingly, miRNAs could also serve as novel biomarkers for T2DM epidemiology and therapeutic targets<sup>[1]</sup>. As one class of small non-coding RNA molecules, microRNAs (miRNAs) negatively regulate post transcription through directing mRNA degradation and/or translational inhibition<sup>[2]</sup>. miRNAs act as regulators of gene expression either in physiology homeostasis or pathological processes<sup>[3]</sup>. In the following sections, we will focus on the updated review of the association of miRNAs genetic susceptibility with T2DM as well as biomarkers in this chronic disease.

*Overview of miRNA Biogenesis* Lin-4 was discovered as the first miRNA in 1993<sup>[4]</sup>. Since then, miRNAs have been widely identified in different organisms<sup>[2]</sup>. As a class of powerful gene expression regulators, miRNAs are involved in multiple physiological and pathobiological processes<sup>[2]</sup>.

In nucleus, cellular genomic sequences are transcribed to primary miRNA (pri-miRNA) mainly guided by RNA Polymerase II<sup>[2]</sup>. Then, the RNase III enzyme Drosha in concert with the RNA-binding protein DGCR8 excises pri-miRNA to ~60 nt stem-loop precursor miRNA (pre-miRNA). After that, the Exportin-5 protein transfers the pre-miRNA from the nucleus to the cytoplasm. In the cytoplasm, the pre-miRNA is cleaved by another RNase enzyme, Dicer, into a duplexed of mature miRNA. One of the strands (guide sequence) combines with RNA-induced silencing complex (RISC) to interfere with mRNA degradation and/or translational inhibition<sup>[2]</sup>. The other strand, known as the

passenger or star miRNA (miRNA\*) is generally degraded; however, both guide and passenger strands are biofunctional under certain circumstances<sup>[6-7]</sup>.

RISC consists primarily of the mature miRNA strand and one of the 4 Argonaut (AGO) proteins 1-4<sup>[8]</sup>. miRNA guides and binds to a target mRNA, whereas the AGO proteins serve as translational inhibitory effectors<sup>[5]</sup>. At the 5' end of miRNA, 2-7 nucleotide sequences are crucial for mRNA target recognition and miRNA-mediated repression function. This region has been named the 'seed region' and it usually matches the 3'UTR of the target mRNA. Evolutionary conservation of miRNAs and their 3'UTR binding sites facilitates computational prediction and experimental identification of authentic miRNA targets<sup>[9]</sup>. By computational analysis, one gene may be regulated by multiple miRNAs, and a single miRNA may target hundreds of candidate genes. At least one conserved miRNA-binding site is predicted to exist in over 60% of human related protein-coding genes<sup>[10]</sup>, suggesting important regulatory roles of miRNAs in gene expression.

*Association between miRNA Polymorphism and T2DM* T2DM is a complex, multifactorial disease fueled by interactions of multiple susceptible genetic loci and various environmental and behavioral factors<sup>[11-13]</sup>. Disparity in the risk of T2DM between different ethnic groups after controlling for diverse environmental attributes indicates a genetic predisposition in the development of T2DM<sup>[14-16]</sup>. Previous reports demonstrated that genetic polymorphisms in miRNA genes associated with T2DM affect the expression of target genes and contribute to the susceptibility to T2DM<sup>[17]</sup>. Single-nucleotide polymorphisms (SNPs) in miRNA genes may affect target genes' function through either pri-/pre-miRNA processing or miRNA-mRNA

doi: 10.3967/bes2017.116

\*This work was supported by grants from National Natural Science Foundation of China [81370083, 81673247]; and Australian-China Collaborative Grant [NH&MRC-APP1112767-NSFC 81561128020].

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interactions<sup>[17]</sup>. For example, miR-27a and miR-124a are associated with T2DM susceptibility, with the variant allele of rs895819 in miR-27a serving a protective function for the development of T2DM, while the variant allele of rs531564 in miR-124a seems to increase the T2DM risk. Both of the SNPs are located out of the miRNA seed region, and specifically the rs895819 is in the pre-miRNA region, while the rs531564 is in the pri-miRNA region<sup>[18]</sup>. Some of the T2DM-related genetic polymorphisms in miRNAs and miRNA target sites have been reviewed<sup>[17]</sup>. Since then, there has been much more progress in this research field. Here we will update the review with the latest progress related to association between miRNA genetic polymorphisms and susceptibility of T2DM.

**Polymorphisms in miRNA Processing and Mature miRNA** Informatics analysis indicates that the human pre-miRNAs have relatively low levels of SNPs (~10%), and even lower in the functional seed region of mature miRNAs (< 1%)<sup>[19]</sup>. Polymorphisms in pre-miRNA or seed region may have influence on the efficiency of miRNA biogenesis or the regulatory role of mature miRNA. As a result, these genetic polymorphisms may be associated with diseases, especially T2DM related diseases in different ethnic groups. Previous study has shown that SNPs located in miRNA genetic regions are associated with T2DM in Caucasian populations<sup>[18]</sup>. Ciccacci et al. found that common polymorphisms in miR-146a (rs2910164), miR-128a (rs11888095) and miR-27a (rs895819) contribute to neuropathy susceptibility in T2DM<sup>[20]</sup>. Further analysis indicated that miR-146a (rs2910164) is significantly associated with diabetic nephropathy in T1DM and diabetic macular oedema in T2DM of Caucasian patients<sup>[21]</sup>. These studies strongly indicate that a single polymorphism could be associated with different DM related diseases. In the Han Chinese population, the associations between 5 SNPs (rs895819 in miR-27a, rs531564 in miR-124a, rs11888095 in miR-128a, rs3820455 in miR-194a and rs2910164 in miR-146a) and T2DM or individual metabolic traits were evaluated. The results show that the C allele of rs531564 in miR-124a may protect against T2DM. In contrast, the C allele of rs2910164 in miR-146a may increase the risk of developing T2DM. However, those 5 SNPs exhibited no significant associations with individual metabolic traits in both of the T2DM and non-diabetic groups. This report indicated that the association between genetic variations of miRNAs and T2DM susceptibility in a Han Chinese population must be

explored for disease prevention/treatment<sup>[22]</sup>. Interestingly, the miR-146a rs2910164 is associated with carotid atherosclerosis in Chinese patients with T2DM, suggesting that one polymorphism of a single miRNA may contribute to different T2DM related diseases<sup>[23]</sup>. Polymorphism of miR-125 (rs12976445) is associated with risk of diabetic nephropathy through alternating miR-125 and Interleukin-6R expression<sup>[24]</sup>. In an Iranian population, the miR-146a rs2910164 but not miR-149 rs2292832 variants is also associated with the susceptibility of T2DM and its related metabolic traits<sup>[25]</sup>. In another study, the SNP of miR-27a (rs895819) showed association with T2DM susceptibility of Iranian cohort<sup>[26]</sup>, which is consistent with previous studies that have been carried out on different ethnic groups<sup>[18]</sup>. In a study in Poland, SPN of miR-196a was shown to contribute to the risk of cardiovascular disease in T2DM patients<sup>[27]</sup>. Polymorphism of miR-196a2 may also contribute to T2DM development through regulating fat distribution<sup>[28]</sup>. As shown in Table 1, these reports above suggest the common feature of association between miRNA genetic variations and the susceptibility of T2DM associated diseases in different ethnic groups.

**Polymorphisms in miRNA Target Binding Sites** Genetic polymorphisms in miRNA target binding sites may induce abnormal gene expression, which ultimately contributes to potential disease process<sup>[29]</sup>. The miRNA-related SNP database (miRNASNP v2.0; <http://www.bioguo.org/miRNASNP/index.php>, accessed 10 March 2016) provides a resource for prediction of miRNA-related SNPs<sup>[30]</sup>. miRNASNP (v2) could be used to predict SNPs on miRNA binding sites, which is associated with T2DM. For example, SNPs on protein binding sites of CCCTC-binding factor (CTCF), E1A binding protein p300 (EP300), forkhead box A1 (FOXA1), and A2 (FOX A2) were identified using this method, which altered expression in a way that may contribute to T2DM development<sup>[31]</sup>. Wolfram encoding gene (*WFS1*) is mapped to the short arm of Chromosome 4 (4p16.1). The variation of *WFS1* causes Wolfram syndrome, which is characterized by neurological disorder, diabetes mellitus with optic atrophy, and deafness. SNPs on miRNA binding sites of *WFS1* gene are risk factors for T2DM<sup>[32-33]</sup>. In another study, genotypes of 11 3'-untranslated region (3'UTR) SNPs in 7 susceptibility genes for T2DM were determined in 353 T2DM patients and 448 control subjects. An allele in 3'UTR SNP (rs2229295) of the HNF1 homeobox B (*HNF1B*) gene

was predicted to be associated with decreased risk of T2DM and confirmed by luciferase reporter assays. Through altering the binding efficiency of 2 miRNAs (miR-214-5p and miR-550a-5p), rs2229295 altered expression of the *HNF1B* associated with susceptibility of T2DM<sup>[34]</sup>. The miRNA-binding site polymorphisms of *CDKN2A/B* genes were found to be associated with gestational diabetes mellitus (GDM) susceptibility<sup>[35]</sup>. Further investigation indicated that two more miR-binding SNPs SLC30A8 (rs2466293) and INSR (rs1366600) could increase GDM susceptibility in Chinese pregnant women<sup>[36]</sup>. Genome-wide association scans (GWAS) have advanced the study of identifying robustly replicating susceptibility of T2DM loci. Using bioinformatics analyses, a variant located in the *IRS1* gene (rs13306464) could impair miR-210-3p, miR-146a-5p, and miR-146b-5p binding sites<sup>[37]</sup>. The altered expression of *IRS1* in patients may have an important role in T2DM as previously described<sup>[38]</sup>. The polymorphisms in miRNA target binding sites above are shown in Table 2.

#### *miRNA as a Biomarker in Type 2 Diabetes*

Biological markers (biomarkers) are measurable in biological media such as human tissues, cells, or fluids, and are indicative of exposure to environmental chemicals. Recently, biomarkers are used to predict the incidence of diseases, which could be measured in the body or its products<sup>[39-41]</sup>. Numerous biomarkers have been shown to have potential roles in diagnosis, prevention and treatment of human disease, including metabolic disease, infections, and cancer<sup>[40]</sup>. The recent discovery of notable characteristics of circulating miRNAs highlighted the possibility of them being ideal biomarkers<sup>[42]</sup>. Circulating miRNAs could be obtained from blood or other easily accessible biologic fluids, such as asurine, saliva, amniotic fluid, and breast milk. Besides their intracellular regulatory roles, miRNAs combined with functional proteins and secreted by cells in exosomes could also be used as biomarkers<sup>[41]</sup>. They are relatively stable and could be easily detected by highly sensitive and specific quantitative real-time PCR<sup>[42-44]</sup>.

Circulating miRNA as a biomarker in T2DM has been demonstrated in a characteristic expression profiling of miRNAs in the blood of patients with T2DM. Multiple miRNAs displayed a characteristic of deregulated expression, suggesting a potential role as novel biomarkers for disease estimation and classification<sup>[43-44]</sup>. For example, plasma miR-126 has the potential to be a biomarker for early prediction

of T2DM in susceptible individuals<sup>[45-47]</sup>. Another study showed that peripheral blood miR-15a expression level was significantly lower in patients with T2DM and pre-diabetes compared to healthy individuals<sup>[48]</sup>. Overexpression of miR-199a in the plasma of patients, may also serve as a biomarker for T2DM<sup>[49]</sup>. The expression level changes of miR-130b in serum may serve as a biomarker that correlates with the severity of diabetic nephropathy<sup>[50]</sup>. MiR-224 could be detected in urine of DM patients as a potential indicator of Beta-cell demise<sup>[51]</sup>. Circulating miRNAs could be innovational biomarkers in T2DM related specific disease, such as miR-106b, miR-26a and miR-29b in T2DM with Diarrhea-Predominant Irritable Bowel Syndrome<sup>[52]</sup> and miR-33b in T2DM with Dyslipidemia<sup>[53]</sup>. In another study, miR-93 is associated with high risk of T2DM with retinopathy<sup>[54]</sup>. The expression of miR-18a and miR-34c could be as novel biomarkers in circulating monocytes associated with vulnerability to T2DM and insulin resistance<sup>[55]</sup>. These studies indicate the potential of circulating miRNAs as novel biomarkers in T2DM diagnosis as well as potential therapeutic targets in treatment (Table 3). To obtain reliable miRNA as a biomarker, however, there must be large prospective epidemiologic studies in diverse ethnic populations.

**Conclusion and Perspective** The prevalence of T2DM has become a big concern in the world following global lifestyle and environmental changes. The recent discoveries of miRNAs genetic susceptibility might hold the potential to reshape gene expression profiling for the T2DM associated diseases. As a significant genetic factor, the polymorphisms occurring in the miRNA biogenesis pathway eventually induce the phenotypic variations via changes in gene expression. Numerous studies have shown the association of miRNA single-nucleotide polymorphisms (miRSNPs) with T2DM and related diseases; however, most of the studies have a small number of samples in each group. This limitation needs to be resolved before the practical implementation. On the other hand, the target genes of miRNA and associated signaling pathways also need to be discovered for the T2DM prevention or clinical treatment.

Glucose, insulin or lipid levels in serum could be used to diagnose T2DM. But these measurements are not sufficient for the early pre-diabetes determination for the prevention and treatment of T2DM and related diseases. Thus, health management should be improved by the acquisition

**Table 1.** The Summary of Polymorphisms in miRNA Processing and Mature miRNA

Author, Year (Ref.)	Country	Sample	Sample Size	Age*	Gender (M/F)	SNPs in miRNA	Allele	Effect	OR	Associated Diseases
Ciccacci et al., 2014 <sup>[18]</sup>	Italy	Blood	154	64.7 ± 8.6	88/66	miR-146a (rs2910164)	G > C	P	0.280	Neuropathy susceptibility in T2DM
Ciccacci et al., 2014 <sup>[18]</sup>	Italy	Blood	154	64.7 ± 8.7	88/67	miR-128a (rs11888095)	C > T	R	8.780	Neuropathy susceptibility in T2DM
Ciccacci et al., 2014 <sup>[18]</sup>	Italy	Blood	154	64.7 ± 8.8	88/68	miR-27a (rs895819)	A > G	R	2.660	Neuropathy susceptibility in T2DM
Kaidonis G et al., 2016 <sup>[21]</sup>	Australia; UK	Blood	2,230	66.2	1,208/1,022	miR-146a (rs2910164)	G > C	R	1.250	Diabetic macular oedema in T2DM
Li Y et al., 2015 <sup>[23]</sup>	China	PL	738	50.26 ± 11.5	467/271	miR-124a (rs531564)	C > G	P	0.758	T2DM
Shen J et al., 2015 <sup>[23]</sup>	China	PB	975	62.26 ± 9.87	540/435	miR-146a (rs2910164)	G > C	R	1.602	Carotid atherosclerosis with T2DM
Li C et al., 2015 <sup>[24]</sup>	China	PB	594	52 ± 8.95	476/118	miR-125 (rs12976445)	T > C	R	1.450	Diabetic nephropathy
Alipoor B et al., 2016 <sup>[25]</sup>	Iran	Blood	375	54.5 ± 7.85	197/178	miR-146a (rs2910164)	G > C	R	1.160	Susceptibility of T2DM and its related metabolic traits
Alipoor B et al., 2016 <sup>[25]</sup>	Iran	Blood	375	54.5 ± 7.85	197/178	miR-149 (rs2292832)	C > T	None	0.970	Susceptibility of T2DM and its related metabolic traits
Ghaedi H et al., 2016 <sup>[26]</sup>	Iran	Blood	413	53.58 ± 9.17	146/267	miR-27a (rs895819)	T > C	P	0.720	T2DM susceptibility
Buraczynska M et al., 2014 <sup>[27]</sup>	Poland	PL	920	63 ± 15.8	432/488	miRNA-196a2 (rs11614913)	T > C	R	1.750	Cardiovascular disease in T2DM

**Note.** PL: Peripheral lymphocytes; PB: Peripheral blood; SNP: Single nucleotide polymorphism; OR: Odds ratio; P: Protective; R: Risk; T2DM: Type 2 diabetes. \* Represented as mean ± standard deviation.

**Table 2.** The Summary of Polymorphisms in miRNA Target Binding Sites

Author, Year (Ref.)	Country	Sample	Sample Size	Age*	Target Gene	Chr. Position	Putative Binding miRNA	Assay Method	Effect of Allele	OR	Associated Diseases
Elek Z et al., 2015 <sup>[33]</sup>	Hungary	BEC	380	48 ± 12.7	WFS1 (rs9457)	N.R	miR-185	TaqMan assay	Risk	1.42	T2DM
Goda N et al., 2015 <sup>[34]</sup>	Japan	PL	801	54.1 ± 5.8	HNF1B (rs2229295)	N.R	miR-214-5p; miR-550a-5p	PCR	Risk	1.00	T2DM
Wang X et al., 2015 <sup>[35]</sup>	China	Blood	1,739	31.5	CDKN2B (rs1063192)	9:22003368	miR-323b-5p	TaqMan assay	Risk	1.48	GDM
Wang X et al., 2017 <sup>[36]</sup>	China	PL	1,739	31.5	SLC30A8 (rs2466293)	8:117173699	miR-181a-2-3p	TaqMan assay	Risk	1.46	GDM
Wang X et al., 2017 <sup>[36]</sup>	China	PL	1,739	31.5	INSR (rs1366600)	19:7112870	miR-106	TaqMan assay	Risk	2.19	GDM

**Note.** BEC: Buccal epithelial cells; PL: Peripheral lymphocytes; Chr. Position: Chromosomal position; N.R: Not reported; T2DM: Type 2 diabetes; GDM: Gestational diabetes mellitus. \* Represented as mean ± standard deviation.

**Table 3.** The Summary of Potential Circulating miRNA Biomarkers

Author, Year (Ref.)	Country	Sample	Sample Size	Age *	Gender (M/F)	miRNA	Assay Method	Exp Change	Associated Disease
Zhang T et al., 2013 <sup>[45]</sup>	Chinese	Plasma	90	63.8	41/49	miR-126	qRT-PCR	Down	T2DM
Liu Y et al., 2014 <sup>[46]</sup>	Chinese	Plasma	455	48.3 ± 7.2	227/228	miR-126	qRT-PCR	Down	Pre-diabetes and T2DM
Zhang T et al., 2015 <sup>[47]</sup>	Chinese	Plasma	40	59.2 ± 10.1	22/18	miR-126	qRT-PCR	up	T2DM
Al-Kafaji G et al., 2015 <sup>[48]</sup>	Bahrain	PB	70	50 ± 7.6	33/37	miR-15a	qRT-PCR	Down	Pre-diabetes and T2DM
Yan ST et al., 2014 <sup>[49]</sup>	Chinese	Plasma	192	46-62	N.R	miR-199a	qRT-PCR	up	T2DM
Lv C et al., 2015 <sup>[50]</sup>	Chinese	Serum	458	52.5 ± 10.8	241/217	miR-130b	qRT-PCR	Down	Diabetic nephropathy
Bacon S et al., 2015 <sup>[51]</sup>	Ireland	Urine	144	38.8	N.R	miR-224	qRT-PCR	up	Beta-cell demise
Tao W et al., 2016 <sup>[52]</sup>	Chinese	plasma	421	18-75	165/256	miR-106b	qRT-PCR	up	T2DM with Diarrhea-Predominant Irritable Bowel Syndrome
Tao W et al., 2016 <sup>[52]</sup>	Chinese	plasma	421	18-75	165/256	miR-26a	qRT-PCR	up	T2DM with Diarrhea-Predominant Irritable Bowel Syndrome
Tao W et al., 2016 <sup>[52]</sup>	Chinese	plasma	421	18-75	165/256	miR-29b	qRT-PCR	up	T2DM with Diarrhea-Predominant Irritable Bowel Syndrome
Kimura Y et al., 2016 <sup>[53]</sup>	Japan	plasma	50	N.R	31/19	miR-33b	TaqMan MicroRNA Assay	up	T2DM with Dyslipidemia
Zou HL et al., 2017 <sup>[54]</sup>	Chinese	plasma	267	48.3 ± 8.96	143/124	miR-93	qRT-PCR	up	T2DM with retinopathy
Wang SS et al., 2017 <sup>[55]</sup>	Chinese	CM	296	50.68 ± 8.5	168/128	miR-18a	qRT-PCR	up	T2DM and insulin resistance
Wang SS et al., 2017 <sup>[55]</sup>	Chinese	CM	296	50.68 ± 8.5	168/128	miR-34c	qRT-PCR	Down	T2DM and insulin resistance

**Note.** PB: Peripheral blood; CM: Circulating monocytes; N.R: Not reported; Exp Change: Expression change; Down: Downregulation; Up: Upregulation; T2DM: Type 2 diabetes. \* Represented as mean ± standard deviation.

of novel biomarkers for T2DM predication, such as circulating miRNAs. miRNAs serve as novel biomarkers with significant advantages such as being stable in serum, easily isolated, and easily measured by high-throughput techniques. However, it could be a challenge to have a universal miRNA marker for T2DM since inconsistent of the profiling through different studies. This challenge may be resolved following more and more miRNAs profiling studies in epidemiological and clinical studies. Moreover, further investigations of miRNAs hold the potential to shed light on the avenue for targeted therapy for T2DM.

**Conflict of Interest** No conflict of interest to declare.

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Received: September 22, 2017;

Accepted: October 30, 2017

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