Evaluation of Polymorphisms in miRNAs; miR-196a2 and miR-146a and Type 1 Diabetes Mellitus Children

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Abstract

Background: Type 1 diabetes mellitus (T1DM) results from a cellular mediated autoimmune destruction of pancreatic β-cells, leaving patients insulin-dependent for life . The triggering of autoimmunity against β-cells arises from a multifaceted interaction between multiple genetic and environmental risk factors. More than 50 genes have been identified to influence the risk of T1DM, with HLA class II genes having the greatest impact on susceptibility. Other loci have minor impact on the risk for T1DM; however, the combination of HLA haplotypes and non-HLA polymorphisms has been shown to aid disease prediction. Hence, the discovery of new polymorphisms associated with T1DM may improve the prediction of this disease. miRNAs are key regulators of gene expression and, like other genes, their coding sequences are subject to genetic variation. Polymorphisms in miRNA genes can have marked effects on miRNA functionality at all levels, including miRNA transcription, maturation and target specificity and, consequently, they may contribute to T1DM pathogenesis. Recent studies have reported that miRNAs are critical posttranscriptional regulators of 30–50% of human gene expression and thus they may contribute in different pathological conditions. The SNPs in the sequences of miRNAs genes are relatively rare and highly conserved, which indicates the functional importance of these SNPs. The *miR-196a2* rs11614913 C > T polymorphism is located on chromosome 12 (12q13.13). To date, *miR-196a* gene polymorphism of rs11614913 is one of the most investigated SNPs in case control studies concerning cancer and cardiac diseases but no previous study has reported its association with T1DM. The association of miR-196a rs11614913 with the previous diseases could be explained that miR-196a2 expression was upregulated 9-fold in cells transfected with miR-196a2-C allele but increased only by 4.5-fold with miR-196a2-T allele. Other reporter demonstrated increased expression of miR-196a2 was associated with the C allele.

Keywords: Type 1 Diabetes Mellitus, Genes, miR-196a2 and miR-146a

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Introduction

Diabetes mellitus is the collective term for heterogeneous metabolic disorders whose main finding is chronic hyperglycaemia. The cause is either a disturbed insulin secretion or a disturbed insulin effect or usually both **(1).**

Diabetes Mellitus (DM) is a syndrome of disturbed metabolism involving carbohydrate, protein, and fat which results from the degree of insulin deficiency (absolute or relative) and tissue sensitivity to its actions. The combination(s) of insulin deficiency and sensitivity to its actions bring about distinct clinical phenotypes with varying severity of disturbed metabolism, most conveniently monitored by the degree of hyperglycemia.

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels **(2).**

The distinction between the two types has historically been based on age at onset, degree of loss of β cell function, degree of insulin resistance, presence of diabetes-associated autoantibodies, and requirement for insulin treatment for survival.However, none of these characteristics unequivocally distinguishes one type of diabetes from the other, nor accounts for the entire spectrum of diabetes phenotypes **(3).**

Diabetes mellitus (DM) is a metabolic disease, involving inappropriately elevated blood glucose levels. DM has several categories, including type 1, type 2, maturity-onset diabetes of the young (MODY), gestational diabetes, neonatal diabetes, and secondary causes due to endocrinopathies, steroid use, etc. The main subtypes of DM are T1DM and Type 2 diabetes mellitus (T2DM), which classically result from defective insulin secretion T1DM and/or action T2DM. T1DM presents in children or adolescents, while T2DM is thought to affect middle-aged and older adults who have prolonged hyperglycemia due to poor lifestyle and dietary choices. The pathogenesis for T1DM and T2DM is drastically different, and therefore each type has various etiologies presentations, and treatments **(4).**

Diabetes Mellitus Type 1 Childhood

T1DM is an autoimmune condition that leads to the destruction of pancreatic beta cells which in turn causes insufficient insulin production, resulting in hyperglycemia. T1DM is a chronic disease requiring insulin replacement and intensive effort by the patient **(5).**

T1DM makes up an estimated 5–10% of all diabetes cases. The number of people affected globally is unknown, although it is estimated that about 80,000 children develop the disease each year.Within the United States the number of people affected is estimated at one to three million.Rates of disease vary widely, with approximately one new case per 100,000 per year in East Asia and Latin America and around 30 new cases per 100,000 per year

in Scandinavia and Arab countries. It typically begins in children and young adults **(6).**

***Immune-mediated diabetes*:**

This form of diabetes, which accounts for only 5–10% of those with diabetes, previously encompassed by the terms insulin-dependent diabetes, T1DM, or juvenile-onset diabetes, results from a cellular-mediated autoimmune destruction of the β-cells of the pancreas **(7).**

***Idiopathic diabetes*:**

Some forms of T1DM have no known etiologies. Some of these patients have permanent insulinopenia and are prone to ketoacidosis but have no evidence of autoimmunity. Although only a minority of patients with T1DM fall into this category, of those who do, most are of African or Asian ancestry.Individuals with this form of diabetes suffer from episodic ketoacidosis and exhibit varying degrees of insulin deficiency between episodes. This form of diabetes is strongly inherited, lacks immunological evidence for β-cell autoimmunity, and is not HLA associated. An absolute requirement for insulin replacement therapy in affected patients may come and go **(8).**

Etiology

T1DM comprises several diseases of the pancreatic ß cells which lead to an absolute insulin deficiency. This is usually considered to be the result of an autoimmune destruction of the pancreatic ß cells (type 1A). Some patients with T1DM with no evidence of ß cell autoimmunity have underlying defects in insulin secretion often from inherited defects in pancreatic ß cell glucose sensing and from other genetic or acquired diseases **(9).**

Genetics

T1DM is partially caused by genetics, and family members of T1DM have a higher risk of developing the disease themselves. In the general population, the risk of developing T1DM is around 1 in 250. For someone whose parent has T1DM, the risk rises to 1-9%. If a sibling has T1DM, the risk is 6-7%. If someone's identical twin has T1DM, they have a 30-70% of developing it themselves **(10).**

Environmental Factors

Besides the familial predispositions, much evidence points to a major role of environmental factors in the disease pathogenesis. More than 60% of identical twins affected by T1DM are discordant for the disease and most of the non-diabetic twins lack islet cell autoantibodies **(11).**

Epidemiology

T1DM is one of the most common endocrine and metabolic conditions in childhood. Data on incidence of childhood onset T1DM are very limited. Data from large epidemiological studies worldwide indicate that on an annual basis, the overall increase in the incidence of T1DM is around 3% and about 78 000 children under age 15 years develop T1DM worldwide **(12).**

Egypt is located on the northeastern corner of Africa and has the largest settled population among the Arab countries. The Nile Delta is one of the

most densely populated and cultivated regions in Egypt. Egypt is divided into 28 governorates, nine of them located in the Nile Delta including, Dakahlia, Damietta, Kafr el-Sheikh and Gharbia; each is subdivided into urban and rural areas **(13).**

Type 1 Diabetes Mellitus Predisposition Genes

The possibility of targeting the causal genes along with the mechanisms of pathogenically complex diseases has led to numerous studies on the genetic etiology of some diseases. In particular, studies have added more genes to the list of T1DM suspect genes, necessitating an update for the interest of all stakeholders. Therefore, this review articulates T1DM suspect genes and their pathophysiology **(13).**

In most cases, T1DM occurs when the immune system, which normally attacks only foreign antigens, faultly destroys the β-cells. Consequently, the pancreas stops making insulin, leading to the retention of glucose in the blood.). Some people develop a condition known as secondary DM, which is like T1DM, but the immune system does not destroy the β-cells. Instead, the cells are removed by certain stimuli in the pancreas, as indicated by histopathological changes **(5).**

T1DM is the most frequent chronic illness among children, accounting for 5–10% of all DM cases. It is the most burdensome form of DM, with a decreased life expectancy. About 30 million people live with T1DM worldwide, and the prevalence is predicted to increase three-fold in the 2040s. Uncontrolled T1DM may cause chronic complications, including retinopathy, nephropathy, neuropathy, and vascular diseases. T1DM has a strong genetic etiology modulated by some environmental triggers that may prevent the disease if avoided before autoimmunity **(14).**

The treatment involves constant insulin injections, which is expensive; thus, a better approach is necessary to stem the burden of the disease. As autoimmunity is the hallmark of T1DM, an intervention that re-programs the immune system may be a potential way to treat the disease **(15).**

T1DM Suspect Genes

At least 18 regions in the human genome were identified as hotspots for T1DM using genome-wide linkage analysis. These regions host many genes and are labeled IDDM1-IDDM18 because T1DM was formerly called insulin-dependent DM (IDDM). However, improvement in biological techniques has led to a greater understanding of the genetic basis of T1DM. More precise studies have reaffirmed the roles of some previously suspected genes in T1DM pathogenesis and new ones have been discovered. Many genes have been reportedly linked with T1DM **(16).**

Most frequently suspected T1DM predisposing genes

Mutations in the major histocompatibility complex (MHC), insulin gene (INS), cytotoxic T-lymphocyte-associated protein 4 (CTLA4), protein tyrosine phosphatase, non-receptor type 2 (PTPN2), and protein tyrosine phosphatase, non-receptor type 22 (PTPN22) genes

account for most of the T1DM cases. Detailed information on the diabetogenic activities of these genes is given below **(16).**

MHC

MHC is about a 4 megabases genetic section on chromosome 6 (6p21) containing immune recognition genes. Many versions of MHC occur among animal species. In humans, it is called the HLA complex and contains over 200 genes grouped into class I, II, and III. Class I has three major genes, named HLA-A, HLA-B, and HLA-C.The proteins encoded by these genes are expressed on the surface of most nucleated cells, where they bind to protein fragments (peptides) exported from within the cell **(17).**

Class I proteins present these peptides to the immune system and apoptosis is induced if the peptides are recognized as foreign, such as viral or bacterial peptides. Human MHC class II has six main genes, including HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQB1, HLA-DRA, and HLA-DRB1. The proteins produced by these genes are expressed mainly on the surface of certain immune system cells, including B lymphocytes, dendritic cells, macrophages, and activated T lymphocytes. Class II proteins display peptides to the immune system **(18).**

HLA class I and II genes are highly polymorphic, and inheritance of certain variants may predispose the affected individual to an autoimmune disorder. Certain HLA class II alleles or combinations of alleles (haplotypes) significantly increase the risk of T1DM, while others confer reduced or protective effects **(19).**

For example, most individuals with T1DM are carriers of either HLA-DR3, DQB1*0201 (also called DR3-DQ2), or DR4-DQB1*0302 (otherwise known as DR4-DQ8). Also, inheriting the HLA haplotype DRB1*0302-DQA1*0301, particularly when combined with DRB1*0201-DQA1*0501, increases genetic predisposition to T1DM as much as 20-fold **(19).** In contrast, the haplotype DRB1*0602-DQA1*0102 rarely predisposes to T1DM, while the haplotype HLA-DQ6 (HLA-DQA1*0102-DQB1*0602) is protective **(20).**

***INS* gene**

INS gene was the second gene linked with T1DM and accounts for about 10% of T1DM cases. It encodes the precursor to insulin, which assists the body to store energy for later use. For example, insulin helps the body store glucose as glycogen or fat rather than metabolizing it. Insulin has two separate polypeptide chains, chains A and B, and are connected by disulfide bonds **(21).**

To produce insulin, the INS gene secretes an inactive insulin precursor called preproinsulin, which is converted to another inactive substance called proinsulin by removal of a signaling peptide.Insulin is finally produced from proinsulin by removal of the C-peptide that binds chains A and B together. However, mutations in the INS gene may disrupt the insulin biosynthetic network, causing some diseases. A point mutation in the INS gene known as C96Y synthesizes mutant proinsulin, resulting in endoplasmic reticulum (ER) stress, which progressively causes death of β-cells and T1DM. Several other point mutations have also been reported in

individuals and animals with T1DM **(22).**

The promoter region of the INS gene is highly polymorphic and contains several variable numbers of tandem repeats (VNTRs), grouped into classes I, II, and III. VNTR, I contain 26–63 repeats, VNTR II has 80 repeats, and VNTR III has 140–210 repeats **(21).**

VNTR I homozygotes often develop T1DM than VNTR III, while VNTR II confers a protective effect. A polymorphism in the promoter region of the INS gene determines the regulatory activities of the transcription factor autoimmune regulator on thymic expression of insulin **(18).**

The VNTR I allele reduces tolerance to insulin and its precursors, repressing insulin transcription and predisposing the individual to T1DM. The VNTR II allele promotes the expression of insulin mRNA in the thymus **(21).**

CTLA4 gene

CTLA4 gene is expressed on the surface of activated T cells, where it attenuates the immune response by binding to ligands CD80 or CD86 expressed on the surface of antigen-presenting cells. The CTLA4-CD80/CD86 complex represses the IL-2 receptor (CD25), reducing IL-2 synthesis or triggering cell death in already activated cells. CTLA4 protein also mediates the suppressive activity of CD4+ CD25+ T regulatory cells. The expression of CTLA4 in activated T lymphocytes shows that it maintains immune function by preventing the inflammatory response and autoimmunity **(23).**

Thus, CTLA4 plays a negative regulatory role in immune function by preventing the overexpression of T cells. Functional impairment in the CTLA4 gene may cause overexpression of T cells, causing it to attack self-antigens. Deletion of the CTLA4 gene in mice leads to massive proliferation of lymphocytes, resulting in autoimmunity and death **(24).**

Several autoimmune disorders have been associated with functional loss or impairment of the CTLA4 gene. A SNP +6230G>A characterized by splicing of the gene or altered mRNA is linked with an increased risk for T1DM. The promoter region of the CTLA4 gene is polymorphic and a particular SNP −319C>T, which reduces the transcription of the gene, is related to a high risk of T1DM **(25).**

SNP involving an A-to-G substitution at nucleotide 49 in exon 1 (G49A), causing an amino acid substitution (Thr17Ala) has also been reported in patients with T1DM. The predisposing Ala17 allele is partially glycosylated in the ER, resulting in retrograde transport of some molecules into the cytoplasm for lysis, leading to less CTLA4 (Ala17) at the cell surface, which may be responsible for loss of function of the CTLA4 gene expressed by individuals with the +49G allele. Thus, the G49A allele reduces the negative regulatory role of CTLA4 and predisposes to TIDM, compared with the 49 G/G alleles, which confers protection **(26).**

PTPN22 and *PTPN2* genes

PTPN22 and PTPN2 genes both code for protein tyrosine phosphatase (PTP) signaling molecules that modulate and regulate several biological processes, including cell growth, survival, and differentiation **(27).**

PTPs are important regulators of signaling transduction, as they relay signals from the cell into the nucleus. Besides cell growth and differentiation, these molecules initiate cell signaling for T cell immune regulatory activities.PTPs are so important in immune regulation that they are more expressed on immune cells than other body tissues. A deficiency in these signaling molecules because of inactivation or loss of PTPN2 or PTPN22 leads to decreased suppression of the inflammatory response resulting from reduced negative regulation **(28).**

PTPN22 is the fourth gene linked with T1DM in which the rs2476601 SNP disrupts PTP intracellular signaling, leading to loss of negative immune regulation. The SNP causes a single substitution of arginine for tryptophan in the encoded protein (R620W), leading to a decrease in T cell and B cell receptor signaling. This may disrupt tolerance in both T and B cells, ultimately resulting in diabetes-specific autoimmunity.The autoimmunity induced by this SNP is characterized by a preponderance of autoreactive B cells and autoantibodies, both of which are biomarkers for the onset and progression of T1DM (**27**). The mechanism for the role of the PTPN2 gene in the pathogenesis of T1DM is complex, consequent to expression in many cells, but is suspected to involve destruction of pancreatic β-cells **(29).**

Functional loss of the PTPN2 gene may also hamper negative regulation of the apoptotic pathway, leading to overexpression of T cells. Repression of the PTPN2 gene impairs insulin production by β-cells in diabetic mice. In normal individuals, PTPN2 gene blocks insulin signaling by dephosphorylation of its β-chain receptor with the assistance of PTP1B phosphatase. This, in turn, controls gluconeogenesis in the liver by suppressing STAT3 signaling and decreasing glucose production. Mutant PTPN2 induces mitochondrial apoptotic pathways, resulting in β-cell apoptosis and unbalanced glucose metabolism **(29).**

A version of PTPN2 (rs1893217) has been reported to upregulate T cell receptor signaling in T1DM, causing impaired self-antigen recognition and β-cells destruction consequent to the loss of negative regulation **(30)**

Application of autoantibodies and proteins of T1DM suspect genes as biomarkers

No guidelines have been established for T1DM genetic testing as it is done in DM with monogenetic etiology. This is partly because several genes may interact to cause T1DM and testing for individual genes may not be cost-effective.Also, genetic susceptibility alone may not fully explain the etiology of the disease as environmental triggers, such as diet, infection, and pollutants may play a role in onset of the disease .Nevertheless, the Immunology of Diabetes Society has suggested that certain autoantibodies in individuals with a family history of T1DM may predict the likelihood of the disease. These autoantibodies include

islet cell antibodies, insulin autoantibodies, the GAD autoantibody, and the protein tyrosine phosphatase IA-2/ICA512 **(31).**

The levels of the proteins produced by genes predisposing to enteroviruses, such as B coxsackieviruses, are also a good biomarker of the disease. *Notable* among these genes are the HLA, PPTN22, PPTN2, and CTLA4 genes discussed earlier **(28).**

Abnormal levels of the MHC and CTLA4 proteins could indicate enteroviral induced autoimmunity in T1DM, prompting a therapeutic measure. Viral infection may also raise the concentration of interferons in islet β-cells, which overexpress MHC I, resulting in increased susceptibility to cytotoxic CD8+ T cell recognition and destruction **(32).**

MicroRNA Expression

Definition

miRNAs are a class of non-coding RNAs that play important roles in regulating gene expression. The majority of miRNAs are transcribed from DNA sequences into primary miRNAs and processed into precursor miRNAs, and finally mature miRNAs. In most cases, miRNAs interact with the 3′ untranslated region (3′ UTR) of target mRNAs to induce mRNA degradation and translational repression. However, interaction of miRNAs with other regions, including the 5′ UTR, coding sequence, and gene promoters, have also been reported (**33**).

Under certain conditions, miRNAs can also activate translation or regulate transcription. The interaction of miRNAs with their target genes is dynamic and dependent on many factors, such as subcellular location of miRNAs, the abundancy of miRNAs and target mRNAs, and the affinity of miRNA-mRNA interactions. miRNAs can be secreted into extracellular fluids and transported to target cells via vesicles, such as exosomes, or by binding to proteins, including Argonautes. Extracellular miRNAs function as chemical messengers to mediate cell-cell communication.

Both Ambros and Ruvkun continued to study *lin-4* and *lin-14* after leaving the Horvitz's lab, only to discover later that *lin-4* was not a protein-coding RNA but indeed a small non-coding RNA. They also found that *lin-14* was post-transcriptionally downregulated through its 3′ untranslated region (UTR) and that *lin-4* had a complementary sequence to that of the 3′ UTR of *lin-14*. Therefore, they proposed that *lin-4* regulates *lin-14* at the post-transcriptional level (**33**).

Secretion and Uptake of microRNAs

Although some extracellular miRNAs are regarded as by-products of cellular activities, such as cell injury or death, increasing evidence suggests that the release of extracellular miRNAs is a regulated process. It has been shown that the secretion of exosomal miRNAs is mediated by a ceramide-dependent pathway and the secreted miRNAs exert growth regulatory effects in target cells (**34**).

Previously, it was demonstrated that atheroprotective laminar shear stress induced the release of vesicle-free miR-

126-3p and other miRNAs, as well as AGO2, from endothelial cells by activating vesicle-associated membrane protein 3 (VAMP3) and synaptosomal-associated protein 23 (SNAP23). This study also showed that miRNAs secreted from endothelium can regulate the activity of smooth muscle cells.

In neuroendocrine cells, miRNAs in large dense-core vesicles (LDCVs) are released by exocytosis through vesicle fusion, and this process is mediated by the SNARE complex and accelerated by Ca^{2+} stimulus (**34**).

Secretion of miRNAs via exosomes has also been reported to be regulated by signaling molecules, such as interleukin-4 (IL4) and Docosa-hexaenoic acid (DHA). IL4-activated macrophages were found to secrete exosomes carrying oncogenic miRNAs to promote invasiveness of breast cancer cells.

On the other hand, DHA, which has anticancer and anti-angiogenic activities, induced the secretion of miRNA-containing exosomes that exert inhibitory effects on tumor angiogenesis (**34**).

Many studies have also demonstrated that extracellular miRNAs can exert biological functions in recipient cells to regulate their activity, thereby acting as intercellular signaling molecules. For example, exosome mediated transfer of miR-105 from metastatic breast cancer cells to endothelial cells directly targeted a tight junction protein, zonula occludens 1 (ZO-1), and this led to the destruction of the barrier function of endothelium and promoted metastasis. Moreover, exosomes from umbilical cord blood were found to be enriched in miR-21-3p, which promoted the proliferation and migration of fibroblasts, and induced the angiogenic activities of endothelial cells, leading to accelerated wound healing (**35**).

miRNAs, specifically miR-342–3p and miR-1246, secreted from a highly metastatic human oral cancer cell line, were found to induce metastasis in a poorly metastatic cancer cell line (**35**).

Extracellular miRNAs have also been reported to bind to Toll-like receptors, activate downstream signaling events, and eventually lead to biological responses, such as tumor growth and metastasis, and neurodegeneration. Thus, miRNAs may act as chemical messengers to regulate cell-cell communications.

The mechanisms of extracellular miRNA uptake are not well understood. It has been proposed that vesicle-associated extracellular miRNAs may enter cells by endocytosis, phagocytosis, or direct fusion with the plasma membranes, while vesicle-free secreted miRNAs may be taken up by specific receptors on the cell surface. Indeed, several studies have shown that miRNAs enter recipient cells by endocytosis and micropinocytosis. This process has been reported to be dependent on clathrin, but not on caveolae or lipid rafts in PC12 cells (**36**).

However, another study conducted in A549-P cells showed that endocytosis of exosomal miRNAs is mediated by caveolae- and lipid raft-dependent pathways. Furthermore, vesicle-free miRNAs associated with HDL are taken up by HDL receptor and scavenger

receptor BI (SR-BI) receptor in the plasma membrane of the target cells. miRNAs have also been shown to transfer between co-cultured cells via direct cell-cell contact and gap junctions. While these studies suggest that extracellular miRNAs can interact with recipient cells via multiple mechanisms, the factors that determine the specificity of such interactions need to be investigated (**37**).

miR-196a2 genotype polymorphism

MiR-196a2 predicted target genes were identified from multiple databases, including miRDB (http://mirdb.org/miRDB/) and Target Scan Human v7.2 database (http://www.targetscan.org/vert_72/) . The structure and relationship between SNP and miRNA for the target SNP/miRNA are shown in Figure 1 (miRNASNP database http://bioinfo. life.hust.edu.cn/miRNASNP2/ index.php) (**38**).

The predicted miRNA target genes were analyzed for gene ontology (GO) terms and the Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment pathway analysis using DIANA-miRPath v3.0 web-server (http://www. microrna.gr/miRPathv3).

Red regions represent mature miRNA fragments. Primary miRNA energy is 51.2 kcal/mol, while SNP-miRNA energy is 46.6 kcal/mol and ΔΔG is 4.6 kcal/mol (miRNASNP database http://bioinfo.life. hust.edu.cn/miRNASNP2/ index.php). (**38**).

MiRNA-196a2-T1DM association was explored using the Disease miRNA Association Networking as Human MicroRNA Disease Databases HMDD v3.0 (http://www.cuilab.cn/hmdd). (**38**).Pathways involved in pathogenesis of T1DM disease were retrieved from the KEGG pathways, followed by intersection of T1DM genes with the miR-196a2 target gene set (**38**)

Single-nucleotide polymorphisms (SNPs) are a common genetic variation in the genome. It is already known that SNPs occur more commonly in the non-coding part of the genome [7]. It has been shown that SNPs in miRNAs may influence both their expression and function, leading to human disease susceptibility. It is therefore not surprising that the distraction of miRNA function leads to many human diseases. SNPs located in the candidate miRNA genes can affect the expression of corresponding targets involved in the pathophysiology of T2DM. MiRNAs seem to play a role in the development of pancreatic islets and the differentiation of insulin-producing cells. For instance, mir-375 is involved in insulin secretion by interaction with the myotrophin gene and the development of islets. Polymorphisms in miRNA genes may influence the miRNA maturation and thereby modulate miRNA expression, leading to dysregulation of target mRNA. The SNP in *MIR146A* (rs2910164 G/C) is a G to C transition at the 60th nucleotide of the gene thereby leading to reduced expression of mature hsa-miR-146a. Previous studies have shown that rs2910164 G/C polymorphism is associated with several diseases. The rs2910164 G/C increased the risk of T2DM in the Chinese and Caucasian populations .It has been shown that hsa-miR-146a reduces the activity of

NF-kappa B. leading to complications of diabetes. The SNP (rs2910164 G/C) is located within the stem-loop of hsa-miR-146a. The polymorphism rs6505162 C/A has been reported to promote the expression of mature mir-423 **(39)**

A recent study suggests that miR-146a plays a crucial role in the prognosis of DM by contributing to the metabolism, proliferation, and death of β-cell. It can be detected in serum, T cells, and α- and β-cells of patients with DM, supposing that the miR-146a is a therapeutic target and potential biomarker **(39)**

Ibrahim et al. findings demonstrated for the first time that miR-196a rs11614913 is associated with T1DM and the decreased expression of miR-196a2 may play a role in the pathogenesis of T1DM. This may open a new window for understanding the pathogenesis of T1DM and finding a new plan for therapies. **(38)**

Polymorphisms in genes encoding miR-155 and miR-146a are associated with protection to type 1 diabetes mellitus

To date, only few studies reported associations between polymorphisms in the miR-146a gene and autoimmune diseases, such as rheumatoid arthritis (RA), multiple sclerosis, asthma, and Crohn's disease No study has evaluated the association between polymorphisms in miR-146a. miR-146a rs2910164 and miR-155 rs767649 polymorphisms are associated with protection for T1DM in Brazilian subjects. These associations are biological plausible considering the involvement of miR-146a and miR-155 in immunity and inflammation, which are key players in T1DM pathogenesis. Further studies are required to confirm these associations in other populations. **(40).**

We demonstrated in our recent study that variants rs11614913 T/C and rs2910164 G/C were linked with the risk of T1DM. The data suggested that rs11614913 T/C and rs2910164 G/C could be considered as novel risk factors in the pathogenesis of T1DM in the Egyptian population. **(41)**

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