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Association Between Mirna-196a-2 (Rs11614913) Gene Polymorphism And Type 2 Diabetes Mellitus In Indian Population

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Abstract

Background: 1 in 7 individuals diagnosed with diabetes comes from India. The number of diabetes cases in India is said to grow exponentially by 69% by the year 2024 as shown in reports by International Diabetes Federation. India accounts for over 74 million people living with diabetes and majority of which are Type 2 diabetes mellitus (T2DM). Studies show that genetic variations in various genes involved in energy metabolism leads to T2DM. [1,2]

Materials and Methods: To investigate the effect of microRNA-196a-2 (miRNA-196a-2) gene polymorphism (rs11614913) in T2DM patient, subjects were genotyped for miRNA-196a-2 Single Nucleotide Polymorphism (SNP) by allele discrimination assay. The downstream targets of the miRNA were analysed to determine the pathways involved in T2DM.

Results: To investigate the effect of microRNA-196a-2 (miRNA-196a-2) gene polymorphism (rs11614913) in T2DM patient, subjects were genotyped for miRNA-196a-2 Single Nucleotide Polymorphism (SNP) by allele discrimination assay. The frequency of the T allele was higher in patients than in controls. The odds ratio 3.16 suggests an association of the T allele with increased risk of T2DM. The T allele and CT genotype were significantly more frequent in patients with T2DM compared to controls. The potential targets for miR-196a-3p and miR-196a-5p were SFRP1, FGF2, DHFR and HOXC8, BACH1, CDKN1B respectively.

Conclusion: This study findings suggest that miRNA-196a-2 T/C polymorphism (rs11614913) is associated with an increased risk of insulin resistance and decreased beta-cell function in T2DM patients. There is also an increased susceptibility of CT genotype patients towards development of T2DM. This provides further insights on the pathogenesis of insulin resistant T2DM patients.

Keywords: Type 2 diabetes mellitus; microRNAs; Single nucleotide polymorphism; Genetics; Insulin resistance; India.

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic disorders that share the phenotype of hyperglycemia and are caused by a complex interaction of genetic and environmental factors. Type 2 diabetes mellitus (T2DM) is characterized by impaired insulin secretion, variable degrees of insulin resistance, excessive hepatic glucose production and abnormal fat metabolism. [3]

MicroRNAs (miRNAs) belongs to a class of small non-coding regulatory RNA (19-24 nucleotides long) that act through binding to the 3′-UTR of target mRNA. This leads to transcriptional repression or degradation of the target mRNA at the post-transcriptional level. [4] It has also been reported that miRNAs can increase expression of the target genes. [5] MiRNAs have emerged to play important roles in many physiological and pathophysiological processes such as embryonic development, organogenesis, tumorigenesis and other human diseases such as arrhythmia, ischaemic heart disease, cardiac hypertrophy, viral hepatitis and diabetes. [6]

Recent advances in genomics and proteomics have generated many new candidate gene markers for DM. [2] Recent studies have unveiled many candidate miRNAs which play important roles in diverse aspects of development and functions. [7] miRNAs are known to be involved in critical and diverse cellular processes, such as proliferation, differentiation, cellular migration and apoptosis. [8] miRNA-196a-2, by decreasing the expression of its target gene, may increase insulin-secreting cell mass and function. [9] Thereby, leads to increase in insulin sensitivity of cellular tissues. Thus, pharmacological formulations encompassing "miRNA-196a activators" can be used to treat DM. The present study aims to investigate the effect of miRNA-196a-2 gene polymorphism on T2DM patients.

According to recent data, miR-196a-2 T/C polymorphism (rs11614913) contributes to pathogenesis of variety of diseases and is a possible genetic predisposing factor for DM. It is closely associated with the regulation of annexin A1(ANXA1) which is linked to decreased TNF-α levels. This miRNA target is involved in thrombosis and inflammation pathway in the circulation system. [10] Thus we investigated the effect of miRNA-196a-2 gene polymorphism in patients with Type 2DM.

MATERIALS AND METHODS

Data Collection

This study comprises of 90 subjects, including 60 patients diagnosed with T2DM from the Department of Medicine, Victoria Hospital, Bowring and Lady Curzon Hospital, Bangalore and 30 healthy age and gender matched individuals as controls in a study period of May 2017 to June 2017. Genetic analysis was done in Department of Human Genetics, NIMHANS. Outpatients and Inpatients at the Department of Medicine diagnosed with T2DM according to the American Diabetes Association criteria and fulfilling the inclusion and exclusion criteria was taken for the study. After obtaining written informed consent from the cases and controls patients, 5 ml of fasting venous blood was collected by venipuncture under aseptic conditions and divided into 2 parts, first part of blood was taken in a sterile EDTA tube and used for genetic studies and HbA1C measurement. Second part was taken in a centrifuge tube, centrifuged and separated serum was used for measuring serum insulin, and other parameters. 2 ml of blood was collected in a centrifuge tube after 2 hour of food which was then centrifuged and separated serum was used for post prandial glucose estimation.

Inclusion criteria

Indian patients clinically diagnosed with T2DM according to American Diabetes Association criteria [11] and between 30-80 years of age were taken as T2DM patient samples. Age and sex matched healthy individuals were taken as controls.

Exclusion criteria

Obese patients with Body Mass Index > 30kg/m^2, pregnant and lactating mothers and individuals with any type of genetic disorders including cancer were excluded from the study.

Genotyping

Genomic DNA from blood was isolated using NucleoSpin® Blood kit (Macherey-Nagel), according to manufacturer's protocol. Nanodrop ND2000c spectrophotometer was used for quantitation and purity checking. DNA with A260/280 ratio 1.75–1.85 was used for genotyping. Genotyping of the rs11614913 was performed using TaqMan® SNP Genotyping Assay for miR-196a2 T (Applied Biosystems, Foster City, CA) with a readymade primer probe set (assay ID C_31185852_10). Genotyping was performed

in Applied Biosystems 7500 fast real time machine. The presence of two primer/probe pairs in each reaction allows genotyping of the two possible variants at the single-nucleic polymorphism (SNP) site in a target template sequence. The primer sequence is given in Supplementary Table 1.

miRNA target and pathway analysis

The genetic sequence of miRNA 196a-2 from miRbase database and the clinical implications of rs11614913 SNP on miRNA expression from miRNASNP database(http://bioinfo.life.hust.edu.cn/miRNASNP/) was analysed to elucidate the effect of the rs11614913 SNP on miRNA expression pattern. miRTarBase was used to identify experimentally verified target genes of miR-196a-3p and miR-196a-5p. DIANA miRPath online tool was used to identify pathways associated with the target genes of both the miRNAs.

Statistical analysis

Student's t-test was used to compare the characteristics of T2DM patients and the control group. The Chi-square ($\chi 2$) test was used to analyse the genotype and allele frequencies and to assess the compatibility of the genotype frequencies with Hardy-Weinberg equilibrium. 95% confidence intervals were used to determine the relative risks, odds ratios. Associations of genotypes with Type 2DM were analysed by the one-way analysis of variance (ANOVA) test followed by Tukey's test for multiple comparisons. Analysis was performed using SPSS, version 17.0. Data are presented as means ± standard deviations (SD). A value of $P < 0.05$ was considered statistically significant.

RESULTS

The anthropometric and glycaemic parameters are summarised in Table 1. The T2DM patients had higher BMI, systolic and diastolic BP than in the control group. T2DM patients also showed significant increase in FBG, fasting serum insulin levels, and insulin resistance, represented by higher HOMA-IR and lower HOMA-β values. Both groups were age matched. Allele frequencies and genotype distribution of miRNA-196a-2 (rs11614913) polymorphism in the control group and T2DM patients are tabulated in Table 2. The minor T allele of the miRNA-196a-2 (rs11614913) polymorphism had a significantly higher frequency in T2DM patients than in the control subjects with OR = 3.16 (95 % CI 1.53-6.55). The frequencies of CT genotypes were significantly higher than the frequency of the CC genotype in Type 2 DM patients. The genotype distribution was compatible with Hardy-Weinberg equilibrium in the whole study sample ($\chi 2 = 0.07$, $P = 0.892$), in subjects with type 2 DM patients ($\chi 2 = 0.83$, $P = 0.459$) and control subjects ($\chi 2 = 0.17$, $P = 0.784$). The T allele and CT genotype of the miRNA-196a2 T/C polymorphism (rs11614913) were significantly more frequent in type 2 diabetes patients compared to controls. ($p = 0.012$, p 0.014; respectively). The T allele was associated with a 3.16 -fold increased risk in T2DM patients. The association of factors like BMI, Fasting Blood Sugar, HbA1c, Serum Insulin, Insulin Resistance, Beta cell function and different genotypes is shown in Table 3 and Table 4. There was a significant association between Serum Insulin, Insulin Resistance, Beta cell function and different genotypes.

The rs11614913 SNP is located on the miR-196a-3p mature miRNA region opposite to the miR-196a-5p mature sequence as shown in Figure 1. Studies show that rs11614913 show variations in expression of mature miRNA 196a-2 which in turn may lead to changes in expression of miR-196a-3p and miR-196a-5p. [12] Experimentally validated miRNA targets of miR-196a-3p and miR-196a-5p were retrieved from miRTarBase database and given in Supplementary Table 2. The target analysis showed that HOX genes, HMGA genes were direct targets of miR-196a-5p and the targets of miR-196a-3p consisted of SFRP1, FGF2, DHFR, PI4K2B. These target genes were used to identify the associated pathways. The associated pathways within the target genes were pathways in cancer, focal adhesion and regulation of actin cytoskeleton as shown in Supplementary Table 3.

DISCUSSION

T2DM being a metabolic disorder is influenced by both genetic and lifestyle factors. [13] The disruption in insulin signalling mechanism plays the central role in diabetic disease development. [14] microRNAs are known to be involved in different cellular processes which contribute to various diseases like

diabetes and cancer. [13] Studying the role of microRNAs (miRNAs) in the development and progression of diabetes mellitus can provide valuable insights into the molecular mechanism of the disease. [15]

Studies show that miRNAs play a crucial role in altering cellular pathways involved in β-cell survival, function, and insulin signalling which are all contributing factors in the onset and progression of T2DM. [16] Gene polymorphisms play a crucial role in determining susceptibility to metabolic diseases like T2DM. By analysing these variations, we can develop specific treatment plans that target the genetic factors leading to the disease and further aid in disease prevention. Early detection and intervention can potentially delay or prevent the onset of T2DM in high-risk individuals. Understanding these genetic variations can lead to discovery of new biomarkers for the disease leading to improved accuracy of T2DM diagnosis and more effective treatment. [17]

A study found positive association between rs11614913 in miR-196a2 with expression levels of its two target genes, SFMBT1 and HOXC8 and experimentally showed that these two genes are direct targets of miR-196a2. [18] The target analysis also revealed HOX genes as targets of miR-196a-5p. Recent studies have identified few blood-borne miRNAs, such as miR-15a, miR-29b, miR-126, and miR-28-3p, that show correlation with the T2DM disease progression. [19]

miRNAs comprise a key regulatory layer that intersect with transcriptional and translational control mechanisms to maintain the metabolic homeostasis. [20] The correlation of glycaemic parameters with the SNP genotypes also revealed an association of Insulin Resistance, Beta cell function with T2DM genotypes. Deregulation of miRNA activities is implicated in cardiovascular diseases. [21] Accordingly, numerous studies have provided strong evidences for a crucial role of miRNAs in fat cell development and obesity. [22] Recent report show circulating miRNAs associated with the pathogenesis of T2DM, leading to altered glucose metabolism. [23] Together these findings show possibility of using miRNA genetic profiles in various samples to identify critical miRNA regulators in diabetes patients and identify the underlying mechanisms of the disease. Our present study give evidence for the involvement of miRNA-196a-2 T/C polymorphism (rs11614913) in the pathogenesis of T2DM and shows a correlation with insulin resistance and beta cell function. Further investigation is needed to fully understand the role of miRNA in Type 2 Diabetes Mellitus, which can lead to the discovery of new therapeutic strategies and personalized medicine approaches.

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Table 1. General and glycemic characteristics of the study population

Variables	Control (N=30)	Cases (N=60)	p
Age (years)	54.18±8.63	55.23±4.22	0.457
BMI (kg/m2)	22.73±1.74	22.90±3.97	0.345
Systolic BP (mm Hg)	110.15±9.25	141.0±15.47	0.067
Diastolic BP (mmHg)	70.45±6.98	90.35±8.35	0.064
Glycemic parameters			
FBG (mg/dl)	99.83±11.04	165.71±78.08	<0.0001*
HbA1c (%)	5.11±0.98	9.71±2.60	<0.0001*
Fasting Serum Insulin (μIU/L)	10.53±5.31	13.75±8.68	0.03*
HOMA-IR	2.61±1.43	5.59±4.58	0.00438*
Beta cell function index	120.66±102.38	72.93±62.95	0.00439*

Data is represented as Mean±SD. Comparisons are performed by students t test.
*Significantly different from normal control at 95% confidence limits.

Table 2. Allele frequencies and genotype distribution of rs11614913 SNP in the control group and type 2 DM patients.

	Control(N=30)	Cases (N=60)	p	OR(95% CI)
T allele	12(21.67)	53 (43.33)		
C allele	48(38.33)	67 (76.67)	0.012*x	3.16(1.53-6.55)
Genotype				
CC	19(11.00)	14(22.0)	0.003*a	0.10(0.012-0.956)
CT	10(16.33)	39(32.67)	0.035 *b	0.189(0.07-0.50)
TT	01(2.67)	07(5.63)	0.014*c	0.557(0.06-5.07)

Comparisons were performed with the $\chi 2$ test; (CI) = confidence interval; OR = odds ratio;
x T vs. C; a CC vs TT; b CT vs TT ;c CT vs. TT; * indicates significant difference at $P < 0.05$.

Table 3. Association of glycemic parameters with different genotypes in T2DM cases

Variables	Category	Genotype codes			Chi square value	p value
		CC	CT	TT		
BMI (kg/m^2)	<20	4(21.1%)	13(68.4%)	2(10.5%)	5.5	0.24
	20-22.5	0 (0.0%)	9(81.8%)	2(18.2%)		
	>22.5	10(33.3%)	18(60.0%)	2(6.7%)		
FBS (mg/dl)	<110	4(28.6%)	9(64.3%)	1(7.1%)	0.9	0.4
	110-150	5(22.7%)	14(63.6%)	3(13.6%)		
	>150	5(20.8%)	17(70.8%)	2(8.4%)		
HbA1c (%)	<6.5	0	4(100%)	0	3.1	0.27
	6.5-10	9(24.3%)	23(62.2%)	5(13.5%)		
	>10	5(26.3%)	13(68.4%)	1(5.3%)		
Serum. Insulin	<12	4(12.9%)	22(71%)	5(16.1%)	5.7	0.01
	12-25	8(33.3%)	15(62.5%)	1(4.2%)		
	>25	2(40%)	4(60%)	0		
Insulin Resistance	<2.7	1(9.1%)	7(63.6%)	3(27.3%)	7.4	0.01
	2.7-5	6(22.2%)	18(66.7%)	3(11.1%)		
	5-10	5(29.4%)	12(70.6%)	0		
	>10	2(40%)	3(60%)	0		
Beta Cell function	<50	4(12.5%)	23(71.9%)	5(15.6%)	6.56	0.008
	50-100	4(30.8%)	8(61.5%)	1(7.7%)		

	>100	6(40%)	9(60%)	0		

p value calculated by Fishers exact test p value<0.05 is significant

Table 4. Association of glycemic parameters with different genotypes in controls

Variables	**Category**	**Genotype codes**			**Chi square value**	**p value**
		CC	**CT**	**TT**		
BMI (kg/m^2)	<20	0(0%)	2 (100%)	0	5.0	0.26
	20-22.5	6(46.2%)	5(38.5%)	2(15.4%)		
	>22.5	8(53.3%)	7(46.7%)	0		
FBS (mg/dl)	<110	14(48.1%)	12(44.4%)	2(7.4%)	0.6	0.72
	110-150	1(33.3%)	2(66.7%)	0		
	>150	0	0	0		
Serum. Insulin	<12	12(46.2%)	13(50%)	1(3.8%)	14.9	0.005
	12-25	2(66.7%)	1(33.3%)	0		
	>25	0	0	1(100%)		
Insulin Resistance	<2.7	7(53.8%)	6(46.2%)	0	15.1	0.005
	2.7-5	7(53.8%)	8(50%)	1(6.2%)		
	5-10	0	0	1(100%)		
	>10	0	0	0		
Beta Cell function	<50	1(100%)	0	0	1.49	0.83
	50-100	9(47.4%)	9(47.4%)	1(5.3%)		
	>100	4(40%)	5(50%)	1(10%)		

p value calculated by Fishers exact test p value<0.05 is significant

Figure 1. The schematic representation of miRNA-196a-2 sequence and polymorphism site.