Genetic association between miR-27a and miR-449b polymorphisms and susceptibility to diabetes mellitus

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Abstract. Various mutations in microRNAs (miRs) are associated with the pathogenesis of several diseases including cancers and vascular diseases. The present study aimed to investigate the potential association between miR-27a A>G (rs895819) and miR-449b A>G (rs10061133) polymorphisms with the prevalence of type 2 diabetes mellitus (T2DM), and its associated risk factors in the Korean population. Genotype analysis was performed using PCR-restriction fragment length polymorphism analysis to assess the frequency of miR-27a and miR-449b gene polymorphisms in patients diagnosed with T2DM (n=238) and healthy controls (n=247). The miR-27a GG genotype, recessive model, and G allele were significantly associated with a decreased risk of T2DM [adjusted odds ratio (AOR)=0.378, 95% confidence interval (CI): 0.208-0.686, P=0.001; AOR=0.425, 95% CI: 0.246-0.734, P=0.002; AOR=0.640, 95% CI: 0.493-0.831, P=0.001, respectively). Although the miR-449b polymorphism was not associated with the prevalence of T2DM, the genotype and allele combination analyses for miR-27a and miR-449b polymorphisms showed associations with T2DM prevalence. Furthermore, stratification analysis revealed that the miR-27a polymorphism was associated with DM risk factors including body mass index (<28.12 kg/m^2, P=0.031), waist circumference (<93.03 cm, P=0.036), systolic blood pressure (<132.67 mmHg, P=0.017), fasting blood glucose levels (<106.26 mg/dl, P=0.015), glycosylated hemoglobin, type A1C (≤125.5 mg/dl, P=0.001), total cholesterol (≤240 mg/dl, P=0.010) and low-density lipoprotein levels (≤130 mg/dl, P=0.028). The present study revealed an association between miR-27a A>G and miR-449b A>G polymorphisms and the risk of DM in Koreans, which suggests that these gene polymorphisms could represent potential markers for predicting T2DM risk.

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Introduction

Diabetes mellitus (DM) is a complex and heterogeneous disease that can affect individuals at different stages of their life. Medical experts mostly agree on the significant risk factors such as sex, age, total cholesterol level, systolic blood pressure (SBP), physical activity, body mass index (BMI) and high-density lipoprotein cholesterol (HDL-C) levels in patients with type 2 DM (T2DM) (1). The heterogeneity of diabetes is not only appreciated as a product of the deeper understanding of the genetics, risk factors and pathophysiology, but is also influenced by a constellation of changing lifestyles, technology and societal development (2,3). Lifestyle changes over a couple of decades have contributed to the increase in prevalence of diabetes in Western countries, and are predicted to do so in developing countries in the coming decades (4). Research efforts to counteract the expected diabetes epidemic by understanding the molecular networks regulating glucose absorption and metabolism are underway (4-6).

MicroRNAs (miRs) play a chief role in regulating the hematopoietic gene expression networks (7). They also regulate the expression of target genes through mechanisms similar to those of other RNAs, such as transcriptional activation or inhibition, epigenetic repression, and controlled degradation rates (8-10). Upstream signaling initiates the transcription of miR genes and creates feedback loops by targeting their transcription factors (10). As miRs are involved in various cellular processes including proliferation, differentiation, apoptosis and development, polymorphisms within miRs and the dysregulation of their expression are associated with metabolic diseases such as cancer, T2DM, cardiovascular disease and gestational diabetes mellitus (GDM) (11,12). In miR polymorphism genotypes, miR-27a is involved in synergizing Akt kinase by targeting the FoxO1 transcription factor (miR-27) repressed by Akt phosphorylation (7). miR-27a targets the peroxisome proliferator-activated receptor γ (PPARγ) gene and negatively regulates adipogenesis, whose processes are involved in adipocyte differentiation from preadipocytes, insulin resistance and T2DM (11). miR-449b has been reported to alter the expression of certain molecules associated with adhesion and invasion (13), and to be downregulated and inactivated in a variety of human malignancies (8,14). Several studies also suggested that the effects of variant alleles of miR-27a and miR-449b need to be examined using combination and haplotype-based analyses (9).

A series of studies reported associations between miR polymorphisms and DM (2,12,15-18). One study revealed that the expression of miR-210 in T2DM patients was related to decreases in the number and function of peripheral endothelial progenitor cells (EPCs) (16). Another study suggested miR-20a-5p as a potential biomarker for GDM in South African women (12). Several miRs, such as miR-126, miR-222-3p, miR-182, let-7b-5p and miR 1-3p were identified as downregulated in a range of diverse patients with T2DM, whereas others including miR-21, miR-30d, miR-148a-3p, miR-146b and miR-486 showed the opposite results (2).

However, the previous studies did not fully consider the genetic association of miR-27a and miR-449b with reductions in the susceptibility to T2DM. The present study aimed to determine the susceptibility to T2DM associated with miR-27a A>G (rs895819) and miR-449b A>G (rs10061133) polymorphisms bound to the 3'untranslated region (UTR) of the target mRNAs in a Korean cohort.

Materials and methods

Study subjects. A total of 485 subjects (238 T2DM patients, including 203 males and 35 females, with a mean (± SD) age of 49.69±8.12 years, and an age range of 30-65; and 247 unrelated healthy controls, including 132 males and 115 females, with a mean age of 48.93±9.98 years, and an age range of 32-80) were recruited for this study. T2DM was diagnosed in accordance with the World Health Organization (WHO) criteria (19). The fasting blood glucose (FBG) and glycosylated hemoglobin-A1C (HbA1C) levels were determined based on WHO regulations (20) for the confirmation of T2DM. T2DM patients were selected who were receiving diet and exercise therapy, but no medications that could affect blood glucose levels. Patients with cardiovascular disease, renal failure, pancreatitis, anemia or liver failure were excluded from the study. The control group was selected following health screening to exclude those with a history of chest pain, diabetes, hypertension or general illnesses. As this was a retrospective analysis, a sample size of >200 individuals each in the patient and the control group was determined sufficient for the study, assuming 80% power at the 5% significance level.

All enrolled subjects provided written consent to participate in the study. This study was approved by the Institutional Review Board (approval no. JEJUNUH 2020-07-005) of Jeju National University Hospital (Jeju, Republic of Korea). The biospecimen and data used in this study were provided by the Biobank of Jeju National University Hospital, a member of the South Korea Biobank Network supported by the Ministry of Health and Welfare.

Genetic analysis. Genomic DNA was extracted from leukocytes using a G-DEX blood extraction kit (Intron, Seongnam) according to the manufacturer's instructions. Genotyping of miR-27a A>G (rs895819) and miR-449b A>G (rs10061133) polymorphisms was performed by PCR and the restriction fragment length polymorphism technique as described in a previous paper (9). Restriction digestions were performed at 37˚C for 17 h with *Dra*III and *Bsm*AI enzymes from New England BioLabs. The primer sequences, PCR conditions, restriction enzymes and genotype fragments are summarized in Table I.

Phenotypic measurements. BMI was calculated based on the height and weight. Waist circumference (WC) was determined using a non-stretchable fiber measuring tape. Blood pressure (BP) was measured in the seated position after 10 min of rest, and the average of three measurements was used. For the biochemical measurements, the levels of plasma FBG, triglycerides, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and HDL-C levels were measured with commercially available enzymatic colorimetric tests using an automated analyzer (TBA 200FR NEO, Toshiba Medical Systems). The HbA1C values were measured using an ion-exchange high-performance liquid chromatography system (HLC-723 G11, Tosoh Corporation) automated glycohemoglobin analyzer, and the results were reported in eAg units (mg/dl).

Statistical analysis. To compare the clinical characteristics between DM cases and the control group, a Student's t-test and a χ^2 test were used for continuous variable and categorical variable analyses, respectively. Logistic regression analysis was used for comparison regarding the frequencies of genotype, genotype combination, and stratified analysis between DM cases and the control group. To determine the strength of the association between miR-27a and miR-449b gene polymorphisms and the DM group, the polymorphisms with DM incidence were calculated using adjusted odds ratios (AORs) and 95% confidence intervals (CIs) from the logistic regression analysis after adjusting for age and sex. It was also used to identify deviations from Hardy-Weinberg equilibrium in comparison analyses of genotype frequencies. A χ^2 test and Fisher's exact test were used for comparison analysis of allele combination frequencies. $P<0.05$ was considered to indicate a statistically significant difference. All statistical analyses were performed using GraphPad Prism version 4.0 (GraphPad Software Inc.) and MedCalc version 12.7.1.0 (Medcalc Software).

Results

The demographic characteristics of the Korean T2DM patients and control subjects are shown in Table II. The patient group had significantly increased BMI, WC, SBP, BP, FBG, HbA1C, TG and LDL-C values compared to the control group (all $P<0.001$), whereas the HDL-C levels of the patients were lower than that of the control group ($P<0.001$). However, age and TC levels were not significantly different between the two groups ($P=0.321$ and $P=0.152$, respectively).

The genotype and allele frequencies of the miR-27a and miR-449b polymorphisms in the T2DM and control groups are shown in Table III. The genotype distributions of the miR-27a and miR-449b polymorphisms were in accordance with the Hardy-Weinberg equilibrium in both groups. The miR-27a GG genotype and the recessive model were significantly associated with a decreased risk of DM (BB vs. AA: AOR=0.378; 95% CI, 0.208-0.686; P=0.001; and BB+AB vs. AA: AOR=0.425; 95% CI, 0.246-0.734; P=0.002). However, there was no association between the miR-27a AG genotype

Table I. Primer sequences used for PCR amplification.

SNP	Primer sequence, 5'-3'	PCR annealing temperature	Product size, bp	Genotype, bp	Restriction enzyme
miR-27a A>G		58	182	AA: 155, 27; AG: 182, 155, 27; GG: 182	*Dra*III
Forward	5'-GAACTTAGCCACTGTGAACACCACTTGG-3'				
Reverse	5'-TTGCTTCCTGTCACAAATCACATTG-3'				
miR-449b A>G		56	119	AA: 119; AG: 119, 97, 22; GG: 97, 22	*Bsm*AI
Forward	5'-GGTATCCAGAGCACTTCATTGACA-3'				
Reverse	5'-ACCTGAATCAGGTAGGCAGTGTCT-3',				

miR, microRNA.

and the dominant model. A significant difference was detected between the cases and controls in the allelic distribution of the miR-27a A>G polymorphism (A allele vs. G allele: AOR=0.640; 95% CI, 0.493-0.831; P=0.001). There was no statistically significant difference in the miR-449b A>G polymorphism between the two groups.

Using genotype combination analyses of miR-27a A>G and miR-449b A>G polymorphisms, the AG/AG (AOR=0.536; 95% CI, 0.290-0.991; P=0.047), GG/AA (AOR=0.364; 95% CI, 0.152-0.875; P=0.024) and GG/AG (AOR=0.314; 95% CI, 0.135-0.731; P=0.007) combined genotypes were associated with a lower risk of DM (Table IV).

The results of allele combination analysis of miR-27a A>G and miR-449b A>G polymorphisms to ascertain their synergistic effect revealed that the G-A and A-G allele combination frequencies of miR-27a G>A and miR-449b G>A were significantly higher in the control group than in the DM case group (OR=0.598; 95% CI, 0.439-0.814; P=0.001; OR=0.652; 95% CI, 0.432-0.985; P=0.042, respectively; Table V).

As shown in Table VI, when the DM patients were stratified according to possible risk variables (BMI, WM, SBP, DBP, FBS, HbA1C, TC, TG, LDL-C and HDL-C), the miR-27a AG+GG vs. AA was protective against four components of DM susceptibility (BMI <28.12 kg/m^2, P=0.031; WC <93.03 cm, P=0.036; SBP <132.67 mmHg, P=0.017; FBG <106.26 mg/dl, P=0.015; HbA1C eAg≤125.5 mg/dl, P=0.001; TC ≤240 mg/dl, P=0.010; and LDL ≤130 mg/dl, P=0.028). However, miR-449b A>B polymorphism were not associated with increased or decreased DM risk.

Discussion

Several studies have reported that miR polymorphisms may influence an individual's susceptibility to a specific health risk (9,12,15,18,21). Previous studies on the effects of miR polymorphisms on diabetes investigated the role of miRs that are involved in gene translation and the regulation of biological processes (12,16,17). The effects of miR-27a and miR-449b polymorphisms on DM have been reported in a limited number of studies (22,23). Since more evidence in support of the functional importance of miRs in lipid metabolism has been attained, whether miR-27a and miR-449b polymorphisms were associated with DM susceptibility in a Korean cohort was assessed in the present study.

The results showed an association between the miR-27a GG genotype, recessive model, and miR-27a variant G allele with a lower DM risk. DM is known to be associated with an increased risk of metabolic or vascular disorders such as metabolic syndrome, atherosclerosis and cardiovascular events (17,24). The findings suggest that these factors might be significantly related to elevated miR-27a levels, and thus, this may be targeted to reduce the risk factors of lipid metabolism disorders or increase other body chemical concentrations to treat T2DM. Several studies have reported that T2DM patients frequently had EPC dysfunction, involving decreased cell numbers and impaired proliferation, adhesion and angiogenesis (16,25). As other studies mentioned, miRs seem to be promising therapeutic targets according to their size, conservation and ability to manipulate gene expression. Therefore,

Table II. Baseline characteristics of the recruited cohort.

Characteristic	Control, n=247[c]	Type 2 diabetes mellitus, n=238[c]	P-value[b]
Age, year	48.93±9.98	49.69±8.12	0.321
Body mass index, kg/m^2	23.94±3.45	26.74±3.75	<0.0001[a]
Waist circumference, cm	81.33±8.65	91.25±11.10	<0.0001[a]
Systolic blood pressure, mmHg	118.51±11.24	128.44±14.99	<0.0001[a]
Diastolic blood pressure, mmHg	71.36±8.63	81.97±10.36	<0.0001[a]
Fasting blood glucose, mg/dl	88.18±8.91	147.29±40.98	<0.0001[a]
Glycosylated hemoglobin, type A1C eAg, mg/dl	112.50±9.05	178.34±88.96	<0.0001[a]
Total cholesterol, mg/dl	193.35±30.18	188.21±40.27	0.152
Triglyceride, mg/dl	92.15±57.38	178.70±150.51	<0.0001[a]
Low-density lipoprotein-cholesterol, mg/dl	126.24±32.17	114.74±34.45	0.0002[a]
High-density lipoprotein-cholesterol, mg/dl	57.45±14.25	47.06±13.56	<0.0001[a]

[a]P<0.001. [b]Student's t-test for continuous variables or a χ^2 for categorical variables. [c]Mean ± SEM.

Table III. Comparison of genotype and allele frequencies between T2DM patients.

Genotypes	Control, n=247 (%)	T2DM, n=238 (%)	Adjusted odds ratio (95% confidence interval)	P-value
miR-27a A>G				
AA	84 (34.0)	106 (44.5)	1..000 (reference)	
AG	109 (44.1)	105 (44.1)	0.817 (0.537-1.242)	0.343
GG	54 (21.9)	27 (11.4)	0.378 (0.208-0.686)	0.001[b]
Dominant			0.674 (0.453-1.003)	0.052
Recessive			0.425 (0.246-0.734)	0.002[a]
A allele	277 (56.1)	317 (66.6)	1..000 (reference)	
G allele	217 (43.9)	159 (33.4)	0.640 (0.493-0.831)	0.001[b]
miR-449b A>G				
AA	124 (50.2)	134 (56.3)	1..000 (reference)	
AG	107 (43.3)	85 (35.7)	0.668 (0.444-1.006)	0.054
GG	16 (6.5)	19 (8.0)	1.554 (0.687-3.514)	0.290
Dominant			0.754 (0.511-1.114)	0.156
Recessive			1.771 (0.816-3.845)	0.148
A allele	355 (71.9)	353 (74.2)	1..000 (reference)	
G allele	139 (28.1)	123 (25.8)	0.903 (0.679-1.200)	0.482

[a]P≤0.01, [b]P≤0.001. T2DM, Type 2 diabetes mellitus; miR, microRNA.

understanding their role will be critical in preventing, diagnosing, and treating human diseases (7,10,15).

Similarly, several studies have supported the potential association between miR-27a and T2DM (11,15,26). A study by Ciccacci *et al* (26) suggested that the G allele in miR-27a showed a protective effect in T2DM, as overexpression of miR-27a in pre-adipocytes suppressed the expression of PPARγ and the differentiation of adipocytes. Gong *et al* (15) also showed that miR-27a was downregulated in EPCs from T2DM patients by blocking the transcriptional induction of PPARγ and CCAAT/enhancer-binding protein. The activation of PPARγ, an anti-inflammatory factor, reduced hyperglycemia by enhancing sensitivity to peripheral insulin and diminishing hepatic glucose levels (27). Insulin resistance, a major pathogenic factor in T2DM, is involved in the reduced sensitivity of tissues to insulin-mediated biological function and thereby, can increase obesity, hypertension and dyslipidemia in DM patients (17). Ghaedi *et al* (11) found that pre-miR-27a polymorphisms rs895819 (T/C) may contribute to T2DM susceptibility in an Iranian cohort where the C allele exerted a protective role against the disease. In contrast, Wang *et al* (22) reported that there were no differences in the association analysis of mutational alleles of miR polymorphisms and T2DM based on various sample sizes, ethnicities and geographic locations.

Table IV. Combined genotype analysis for SNPs in T2DM patients and controls.

Genotype combination					
SNP 1	SNP 2	Control, n=247 (%)	T2DM, n=238 (%)	Adjusted odds ratio (95% confidence interval)	P-value
miR-27a	miR-449b				
AA	AA	44 (17.8)	63 (26.5)	1..000 (reference)	
	AG	36 (14.6)	37 (15.5)	0.823 (0.436-1.554)	0.548
	GG	4 (1.6)	6 (2.5)	1.049 (0.253-4.352)	0.948
AG	AA	54 (21.9)	60 (25.2)	0.915 (0.515-1.627)	0.762
	AG	47 (19.0)	35 (14.7)	0.536 (0.290-0.991)	0.047
	GG	7 (2.8)	10 (4.2)	1.391 (0.446-4.333)	0.57
GG	AA	27 (11.0)	11 (4.6)	0.364 (0.152-0.875)	0.024[a]
	AG	24 (9.7)	13 (5.5)	0.314 (0.135-0.731)	0.007[b]
	GG	4 (1.6)	3 (1.3)	0.681 (0.126-3.664)	0.654

[a]P<0.05, [b]P<0.01. T2DM, Type 2 diabetes mellitus; miR, microRNA.

Table V. Comparison of allele combination between T2DM patients and controls.

Haplotype	Overall, 2n=970	Control, 2n=494	T2DM, 2n=476	Odds ratio, 95% confidence interval	P-value
miR-27a A>G/miR-449b A>G					
A-A	0.418	0.4142	0.5132	1.000 (reference)	
A-G	0.1321	0.1465	0.1527	0.841 (0.578-1.224)	0.366
G-A	0.3294	0.3084	0.2284	0.598 (0.439-0.814)	0.001[a]
G-G	0.1205	0.1308	0.1057	0.652 (0.432-0.985)	0.042

[a]P≤0.001. T2DM, Type 2 diabetes mellitus; miR, microRNA.

Therefore, whether SNPs of miR-27a influenced miR-27a levels and their targets in the dysregulation of insulin secretion was assessed in the present study.

The combined genotype analysis of SNPs indicated that the AG/AG, GG/AA and GG/AG combined genotypes of miR-27a A>G and miR-449b A>G polymorphisms were associated with a decreased risk of T2DM susceptibility. The variant allele of the rs895819 SNP in pre-miR-27a was reported to play a protective role in T2DM susceptibility (15). Song *et al* (28) showed that the expression levels of total pri-miR-27a and pre-miR-27a were significantly higher in the rs895819 AG and GG groups than the AA group, indicating that the AA genotype may be a protective factor for diabetes.

In the haplotype results, the allele combinations of miR-27a A>G and miR-449b A>G in the haplotype of G-A and G-G were significantly different between the control and DM groups. Being a multifactorial disease, development of T2DM involves a complex of interactions between the alleles and confounding factors that regulate or downregulate genetic expression (29). It is expected that haplotype analysis can provide an abundance of useful information above what can be derived from SNP analysis (30). As the present and previous studies also demonstrated, interactive effects in miRs were seen in the SNP-SNP interaction analysis of DM subjects. For example, Goda *et al* (31) found that the binding of two miRs to the 3'UTR of the hepatocyte nuclear factor 1β (HNF1B) gene, which plays important roles in complex transcriptional networks in pancreatic β-cells, may provide protective effects in T2DM. As they described that the dysregulated expression of the HNF1B gene through nucleotide changes within the miR-binding site led to differences in T2DM susceptibility, it was hypothesized that the significant difference between the SNP-SNP combination of T2DM and control groups was attributed to changes *in vivo* within the binding sites of miR-27a and miR-449b. In a genome-wide association study, Cirillo *et al* (5) arranged the various pathways along with the pleiotropic action of genes and found that the gene pathways were related to T2DM pathophysiology.

When the clinical profiles were stratified, the miR-27a AG+GG polymorphism was associated with BMI levels of <28.12 kg/m^2, a WC <93.03 cm, a SBP <132.67 mmHg, FBG levels <106.26 mg/dl, HbA1C eAg levels ≤125.5 mg/dl, TC levels ≤240 mg/dl and LDL levels ≤130 mg/dl. Current evidence indicates that miR-27a expression was significantly positively correlated with fasting glucose levels and BMI, and the mutated miR-27a allele decreased the risk of gastric cancer and recurrent pregnancy loss (9,11). In the Asian population, the results of the stratified analysis performed by Chen *et al* (23) revealed

Table VI. Stratified analysis of miR-27a A>G and miR-449b A>G polymorphisms according to type 2 diabetes mellitus risk factors.

	miR-27a AG+GG		miR-449b AG+GG	
Variables	AOR (95% CI)	P-value	AOR (95% CI)	P-value
Body mass index, kg/m^2				
<28.12	0.594 (0.370-0.955)	0.031	0.624 (0.388-1.003)	0.052
≥28.12	0.826 (0.506-1.348)	0.444	0.899 (0.558-1.446)	0.66
Waist circumference, cm				
<93.03	0.607 (0.381-0.967)	0.036	0.779 (0.492-1.235)	0.289
≥93.03	0.803 (0.489-1.320)	0.388	0.697 (0.428-1.135)	0.147
Systolic blood pressure, mmHg				
<132.67	0.558 (0.346-0.902)	0.017	0.661 (0.409-1.066)	0.089
≥132.67	0.850 (0.524-1.377)	0.509	0.824 (0.517-1.315)	0.417
Diastolic blood pressure, mmHg				
<84.21	0.706 (0.438-1.139)	0.154	0.739 (0.461-1.182)	0.207
≥84.21	0.678 (0.419-1.097)	0.114	0.748 (0.465-1.203)	0.23
Fasting blood pressure, mg/dl				
<106.26	0.565 (0.356-0.896)	0.015	0.750 (0.475-1.183)	0.215
≥106.26	0.852 (0.511-1.420)	0.539	0.712 (0.433-1.171)	0.181
Glycosylated hemoglobin, type A1C eAg, mg/dl				
≤125.5	0.229 (0.093-0.561)	0.001	1.319 (0.559-3.115)	0.528
>125.5	1.825 (0.566-5.886)	0.314	1.792 (0.513-6.264)	0.361
Total cholesterol, mg/dl				
≤240	0.573 (0.375-0.876)	0.01	0.831 (0.551-1.252)	0.375
>240	2.114 (0.413-10.817)	0.369	0.393 (0.071-2.184)	0.286
Triglyceride, mg/dl				
<216.26	0.660 (0.425-1.027)	0.065	0.693 (0.448-1.072)	0.1
≥216.26	0.703 (0.405-1.219)	0.209	0.875 (0.510-1.501)	0.627
Low-density lipoprotein-cholesterol, mg/dl				
≤130	0.566 (0.341-0.940)	0.028	0.787 (0.477-1.297)	0.347
>130	0.910 (0.461-1.797)	0.787	0.652 (0.343-1.238)	0.191
High-density lipoprotein-cholesterol, mg/dl				
<44.29	0.717 (0.453-1.135)	0.155	0.702 (0.447-1.103)	0.125
≥44.29	0.623 (0.370-1.049)	0.075	0.844 (0.504-1.412)	0.518

AOR, adjusted odds ratio; CI, confidence interval; miR, microRNA.

that the CC genotype of miR-27a was significantly associated with a decreased risk of DM compared with the TT genotype. Several studies with T2DM subjects suggested that miR-27a expression was associated with an increased risk of T2DM, whereas the results of the present study implied that miR-27a polymorphism could reduce the risk of T2DM. Wang *et al* (22) reported that the genotype CC in miR-27a elevated the risk of T2DM in overweight subjects in a Chinese population. In a stratified analysis, Zhu *et al* (29) reported that accumulated exposure to insulin disturbances caused by miR-27a rs895819 and other variables, such as miR-133a-2 rs13040413, let-7a-1 rs13293512 and a weak immune system could affect elderly patients with T2DM. A study by Karolina *et al* (32) reported that miR-27a and miR-320a in cluster C had a positive correlation with fasting glucose levels, which potentially had an important role in early-phase hyperglycemia and could lead to development of diabetes.

The present study has several limitations. It remains unclear how specifically miR-27a A>G and miR-449b A>G polymorphisms might affect T2DM, although it was demonstrated that these miR polymorphisms exerted a potential synergistic protective effect in T2DM. The study population

was limited to middle-aged Korean individuals. Therefore, the results should be taken with caution, and studies with larger and more diverse cohorts are required.

In conclusion, this study indicated that the polymorphic site in miR-27a A>G, as well as the genotype and allele combinations of miR-27a A>G and miR-449b A>G polymorphisms, were likely to be associated with a lower risk of T2DM in the Korean population. The results suggest that miR-27a itself, at least partially, may be a promising gene to ameliorate insulin resistance and glucose metabolism problems in patients with T2DM.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

SHH collected the medical data and performed the genetic analysis. YC was a major contributor in analyzing and interpreting the data, and in writing the manuscript. All authors read and approved the final manuscript. YC and SHH confirmed the authenticity of all the raw data.

Ethics approval and consent to participate

All enrolled subjects provided written consent to participate in the study. This study was approved by the Institutional Review Board (approval no. JEJUNUH 2020-07-005) of Jeju National University Hospital (Jeju, Republic of Korea).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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