

Sirtuin 1 rs7069102 polymorphism is associated with diabetic nephropathy in patients with type 2 diabetes mellitus

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ABSTRACT

The global prevalence for diabetes mellitus nearly doubled from 4.7% in 1980 to 8.5% in 2014. Sirtuin 1 (SIRT1) is an NAD⁺-dependent deacetylase that is expressed in a variety of tissues. It modifies proteins that participate in DNA repair, stress, and inflammatory response. The aim of the study was to investigate the relationship between SIRT1 rs7069102 polymorphism and diabetic nephropathy (DN) in patients with type 2 diabetes mellitus (T2DM). In our retrospective association study, we included 724 Slovene (Caucasian) patients who have had T2DM for at least 10 years. We classified the participants into two groups, the first group was comprised of 301 patients with DN, and the second (control) group was comprised of 423 patients without DN. We analyzed the rs7069102 polymorphism using StepOne real-time polymerase chain reaction (PCR) System and TaqMan SNP Genotyping Assay. We found a statistically significant difference in the distribution of rs7069102 genotypes and alleles between the two groups. We used logistic regression analysis and adjusted for systolic pressure, arterial hypertension (AH), duration of AH, triglycerides, the value of HbA_{1c}, carotid disease, diabetic foot, and diabetic retinopathy. Furthermore, we discovered that patients with the CC genotype are significantly more likely to develop DN according to both the codominant (odds ratio [OR] = 1.94; 95% confidence interval [CI] = 1.09-3.45; $p = 0.02$) and recessive (OR = 2.39; 95% CI = 1.12-5.08; $p = 0.02$) models of inheritance. We found a significant association between the SIRT1 rs7069102 polymorphism and DN in T2DM. We speculate that SIRT1 rs7069102 might be an interesting marker of DN.

KEYWORDS: SIRT1; rs7069102; diabetic nephropathy; type 2 diabetes mellitus; association study

INTRODUCTION

The global prevalence for diabetes mellitus nearly doubled from 4.7% in 1980 to 8.5% in 2014. Most of this increase comes from low- and middle-income countries, and the majority of these patients suffer from type 2 diabetes mellitus (T2DM). T2DM is a disorder characterized by chronic hyperglycemia caused by resistance of body tissues to the effects of insulin [1]. Hyperglycemia causes macrovascular and microvascular complications, which can also affect kidneys. This condition is called diabetic nephropathy (DN) and is present in 20-40%

of diabetic patients. DN is defined by decreased glomerular filtration rate and/or elevated levels of urine albumin excretion [2]. Patients with diabetes who suffer from DN are more prone to cardiovascular diseases and have a higher mortality rate than those without DN [3].

Familial clustering of DN can be explained by both genetic and environmental factors, and most likely the combination of the two. Several association studies have identified a strong genetic component to both albuminuria and impaired glomerular filtration rate [4]. Identifying genes that are involved in DN could lead to novel forms of treatment and maybe even prevention of this life-threatening condition.

Sirtuin 1 (SIRT1) is a NAD⁺-dependent deacetylase, belongs to a group of enzymes called silent information regulators that are expressed in a variety of tissues including kidney, adipose, muscle, liver, and pancreas. It modifies proteins that participate in DNA repair, stress and inflammatory response, and regulation of energy metabolism. SIRT1 promotes lipolysis in white adipose tissue, protects from excessive lipid accumulation in skeletal muscle and the liver, and supports insulin secretion [5]. Animal studies show that SIRT1 improves insulin sensitivity and secretion of insulin, making it a strong anti-diabetic agent [6]. It is induced during periods of calorie restriction and indirectly inhibits stress-induced apoptosis [7].

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The role of inflammation in diabetes related complications is very well established. SIRT1 suppresses inflammatory pathways in the cell, the expression of pro-inflammatory genes and the secretion of TNF- α , IL-1 β , IL-6, p53, FoxO, and other pro-inflammatory molecules [8,9]. A study has specifically linked SIRT1 and chronic renal injury caused by inflammation [10]. SIRT1 also has renoprotective effects, as it offers resistance to inflammation, apoptosis of tubular and glomerular cells and reduces interstitial fibrosis just to name a few [11].

A number of studies showed that SIRT1 and its polymorphisms are involved in visceral obesity, which can lead to type 2 diabetes [5,12-15].

The SIRT1 gene is located on chromosome 10. A study on *SIRT1* polymorphism rs7069102 found that the C allele reduced the risk of visceral obesity in Caucasian males [15]. Studies have linked rs7069102 with genetic susceptibility to chronic obstructive pulmonary disease (COPD) [16], myocardial infarction [17,18], left ventricular myocardial hypertrophy [19], hypertension [20], and early-onset psoriasis [21].

In this study, we investigated the relationship between the *SIRT1* rs7069102 polymorphism and DN in patients with T2DM.

MATERIALS AND METHODS

Patients

In our retrospective association study, we included 724 Slovene patients who have had T2DM for at least 10 years. The diagnosis of T2DM and DN was made according to the World Health Organization criteria. We classified the participants in two groups, according to their nephrological diagnosis. The first group was comprised of 301 patients with DN, and the second (control) group was comprised of 423 patients without DN. The exclusion criteria for our study were overt nephropathy, active infection, poor glycemic control (glycated hemoglobin [HbA1c] above 10), significant heart failure (New York Heart Association [NYHA] Classification II-IV), alcoholism, and the presence of other possible causes of renal disease. We used a questionnaire to obtain information about age, sex, duration of T2DM, blood pressure, systolic pressure, diastolic pressure, duration of arterial hypertension (AH), smoking status, and other microvascular complications of T2DM (diabetic retinopathy [DR] and its duration, diabetic foot) and carotid disease. We measured the participants height, weight, and waist circumference. We took blood samples and measured the total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides, and HbA1c.

All participants provided informed consent before their blood samples were taken and analyzed. Our study was

conducted according to the Helsinki declaration and approved by the Slovenian commission for medical ethics. The ethics number for our study was 105/12/2011.

Biochemical analyses

HbA1c, total cholesterol, HDL, LDL, and triglycerides were determined by standard biochemical methods.

Genotyping

We extracted genomic DNA from leukocytes in 100 μ l of peripheral blood using QIAamp DNA Blood Mini Kit (Qiagen GmbH, Hilden, Germany). We analyzed the rs7069102 polymorphism using StepOne™ (48-well) real-time polymerase chain reaction (PCR) systems (Applied Biosystems by Life Technologies, USA) and TaqMan SNP Genotyping Assay (Applied Biosystems, Foster City, California, USA) according to the instructions of the manufacturers. The volume of the reaction mix was 5 μ l. The reaction mix was comprised of TaqMan Universal Master Mix, oligonucleotide primers labeled with VIC/FAM fluorescent dyes, 1.88 μ l of purified H₂O, and 0.5 μ l of DNA. The following protocol was used for the amplification of DNA: Step 1, 30 second read of starting fluorescence at 60°C (pre-PCR read); step 2, 10 minutes of starting denaturation at 95°C, followed by 35 cycles of amplification. One cycle consisted of 15 second denaturation at 95°C, followed by 60 second of extension at 60°C; step 3, 30 second read of final fluorescence at 60°C (post-PCR read).

Statistical analysis

Allele discrimination plots were visualized using StepOne Software version 2.2 (Applied Biosystems, Foster City, California, USA). We used IBM SPSS Statistics for Windows version 21.0 (IBM Corp., Armonk, New York, USA) for statistical analysis. Continuous variables were compared using Student's t-test or Mann-Whitney's U test. Chi-square test was used to compare discrete variables. We used logistic regression analysis to analyze the relationship between rs7069102 polymorphism and DN after adjusting for systolic pressure, triglycerides, HbA1c, AH, duration of AH, DR, carotid disease, and diabetic foot. The value of $p < 0.05$ was considered statistically significant. Fisher's exact test was used to determine the deviation from the Hardy-Weinberg equilibrium (HWE) (<http://ihg.gsf.de/>).

RESULTS

Demographic, clinical, and laboratory characteristics of patients are presented in Tables 1 and 2. The groups differed significantly in the following variables: Systolic pressure, AH,

TABLE 1. Demographic and clinical characteristics of patients with T2DM with DN and control(patients with T2DM without DN)

Characteristic	Patients with T2DM with DN	Patients with T2DM without DN	<i>p</i> -value
Number	301	423	
Age(years)	64.63±9.18	63.76±7.98	0.18
Sex, M(%)	178(59)	220(52)	0.076
Duration of T2DM(years)	14.00(10.00–20.00)	13.50(11.00–19.00)	0.66
Systolic blood pressure(mmHg)	155.27±18.92	149.99±19.64	<0.001
Diastolic blood pressure(mmHg)	84.92±11.55	84.15±11.48	0.38
Hypertension(%)	268(89)	351(83)	0.034
Waist circumference(cm)	109±12.15	108.33±12.76	0.55
BMI	31.17±4.64	30.63±4.52	0.12
Duration of AH (years)	1.00(5.00-18.00)	10.00(5.00-15.00)	0.01
Active smokers(%)	21(7)	34(8)	0.450
DR(%)	129(43)	114(27)	<0.001
Duration of DR(years)	5.00(1.00-7.00)	4.50(1.00-10.00)	0.47
Carotid disease(%)	60(20)	51(12)	0.005
Diabetic foot(%)	45(15)	34(8)	0.001

Statistically significant values are written in bold. Legend: T2DM: Type2 diabetes mellitus; DN: Diabetic nephropathy; BMI: Body mass index; AH: Arterial hypertension; DR: Diabetic retinopathy

TABLE 2. Laboratory characteristics of patients with T2DM with DN and control(patients with T2DM without DN)

Characteristic	Patients with T2DM with DN	Patients with T2DM without DN	<i>p</i> -value
Total cholesterol(mmol/l)	4.64±1.22	4.57±0.98	0.48
HDL cholesterol(mmol/l)	1.23±0.36	1.26±0.36	0.28
LDL cholesterol(mmol/l)	2.48(1.98-3.10)	2.58±0.80	0.61
Triglycerides(mmol/l)	1.61(1.14-2.50)	1.50(1.05-2.30)	0.02
HbA1c(%)	7.75(6.90-8.90)	7.65±1.13	0.01

Statistically significant values are written in bold. Legend: T2DM: Type2 diabetes mellitus; DN: Diabetic nephropathy; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; HbA1c: Glycated hemoglobin

duration of AH, triglycerides, value of HbA1c, carotid disease, diabetic foot, and DR. Patients with DN had higher systolic pressure, more frequent and longer lasting AH, higher values of triglycerides and HbA1c, and a higher incidence of carotid disease, diabetic foot, and DR (Tables 1 and 2).

Distribution of rs7069102 alleles and genotypes is shown in Table 3. There was a significant difference between the groups in the distribution of C allele as well as the CC and CG genotypes. The C allele, CC, and CG genotypes were more common in the group with DN (Table 3). Both groups were in accordance with the HWE.

There was no difference in the waist to hip ratios regarding rs7069102 SIRT gene genotypes (*p* = 0.6) (data not shown).

We used logistic regression analysis and adjusted for systolic pressure, AH, duration of AH, triglycerides, value

TABLE 3. Distribution of rs7069102 polymorphism genotypes and alleles in T2DM patients with DN and without DN

RS7069102	Patients with T2DM and DN(301)	Patients with T2DM without DN(423)	<i>p</i> -value
CC(%)	43(14)	38(9)	0.03
CG(%)	134(45)	179(42)	
GG(%)	124(41)	206(49)	
ALEL C(%)	220(37)	255(30)	0.01
ALEL G(%)	382(63)	591(70)	
PHWE†	0.79	1.0	

Statistically significant values are written in bold. Legend: T2DM: type2 diabetes mellitus; DN: diabetic nephropathy, HWE: hardy-weinberg equilibrium

of HbA1c, carotid disease, diabetic foot, and DR to evaluate whether rs7069102 is independently associated with DN (Table 4). We discovered that patients with the CC genotype are significantly more likely to develop DN in comparison with patients with the GG genotype according to the codominant model of inheritance (odds ratio [OR] = 1.94; 95% confidence interval [CI] = 1.09-3.45; *p* = 0.02). Application of the recessive model of inheritance also yielded the same finding, patients with the CC genotype were more likely to develop DN in comparison with the CG and GG genotypes (OR = 2.39; 95% CI = 1.12-5.08; *p* = 0.02). There was no statistically significant correlation between the CG genotype and DN.

DISCUSSION

In this study, we found an association between the C allele of SIRT1 rs7069102 polymorphism and DN in patients with T2DM (*p* = 0.01). We used linear regression to statistically determine the relationship between rs7069102 genotypes and DN. We discovered that patients with the CC genotype are more likely to develop DN than patients with other genotypes. Using a codominant model of inheritance, the patients with the CC genotype were 1.94 times more likely to develop DN (*p* = 0.02) than the other genotypes. Moreover, the application of a recessive model of inheritance showed that T2DM patients with the CC genotype are 2.39 times more likely to develop DN than those with the CG or GG genotype (*p* = 0.02; OR: 2.39; 95% CI: 1.12-5.08). The dominant model of inheritance did not show a significant correlation of the CC and CG genotype with DN when compared to the GG genotype.

As previously mentioned, SIRT1 plays a role in many cellular mechanisms that are involved in pathogenesis of DN. A decrease in its activity and/or levels would cause an increase in inflammation, stress-induced apoptosis, and renal fibrosis as well as affect glucose regulation. The rs7069102 polymorphism could affect any or all of the above-mentioned mechanisms, however, further research is needed to determine exactly which process or process's it affects.

TABLE 4. Association of rs7069102 and DN in T2DM patients

Model of inheritance	Genotype	Patients with T2DM and DN	Patients with T2DM without DN	Adjusted OR, 95% CI/ <i>p</i>
CODOMINANT	GG	124	206	REFERENCE
	CG	134	179	1.17(0.81-1.69)/0.40
	CC	43	38	1.94(1.09-3.45)/0.02
RECESSIVE	CG+GG	258	385	REFERENCE
	CC	43	38	2.39(1.12-5.08)/0.02

Statistically significant values are written in bold. Legend: †*p*-values were adjusted for systolic pressure, triglycerides, HbA1c, AH, duration of AH, DR, carotid disease, diabetic foot; T2DM: Type2 diabetes mellitus; DN: Diabetic nephropathy, OR: Odds ratio, CI: Confidence interval, HbA1c: Glycated hemoglobin, AH: Arterial hypertension, DR: Diabetic retinopathy

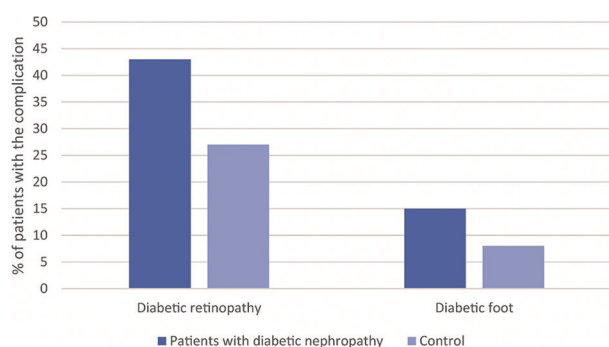


FIGURE 1. Comparison of the prevalence of diabetic retinopathy (DR) and diabetic foot between patients with diabetic nephropathy (DN) and control. In the group of patients with DN 43% of patients also had DR, whereas only 27% of patients without DN had DR. In the group of patients with DN 15% also had diabetic foot, compared to the 8% who had diabetic foot in the control group.

We also discovered a significant association between DR and DN ($p < 0.001$). In the group of patients with DN, a total of 43% of patients also had DR, whereas only 27% of patients without DN had DR (Figure 1). The strong association between DN and DR has been shown by many studies done on different populations [22-25]. SIRT1 has a protective role in the development of DR; however, there are currently no published studies that investigated the relationship between SIRT1 polymorphisms and DR [26,27].

As of now, ours is the only study that researched the influence of rs7069102 polymorphism on DN in patients with T2DM. However, Maeda *et al.* did research other polymorphisms of the SIRT1 gene and their relationship with DN in Japanese patients with T2DM. Of the 11 SIRT1 polymorphisms, they investigated, one (rs4746720) showed a significant association with DN. They also found an association between rs4746720 and three other polymorphisms (rs2236319, rs10823108, rs3818292) and combined phenotypes of proteinuria and end stage renal disease (ESRD) [28]. So far, there are two studies that investigated the correlation between SIRT1 polymorphisms and DN in the Chinese population. Both found a correlation between the rs10823108 polymorphism and DN, thus reinforcing Maeda's findings [29,30]. Yue *et al.* also described the protective role of another SIRT1 polymorphism rs3818292 against DN [30].

CONCLUSION

Based on the evidence available, we can say that some polymorphisms of SIRT1 play a role in the susceptibility of T2DM patients to DN, at least in the Caucasian, Japanese, and Chinese populations. Further research is needed in order to identify more of them, as they could be used as susceptibility markers for DN in T2DM patients. To conclude, we found a significant association between SIRT1 rs7069102 polymorphism and DN in T2DM patients. This implies that SIRT1 rs7069102 could be used as a marker for susceptibility to DN in T2DM patients.

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