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Association of Sirtuin 1 rs7069102 Gene Polymorphism with The Risk of Diabetic Nephropathy in Egyptian Patients

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Abstract

Background: *Diabetes is a heterogeneous complex metabolic disease marked by raised concentration of blood glucose due to either insulin resistance, inadequate production of insulin, or both.*

Aim: *To examine the association between SIRT1 rs7069102 polymorphism and diabetic nephropathy (DN) in Egyptian cases who have type 2 diabetes mellitus (T2DM).*

Patients and methods: *This case–control research has been performed on 90 individuals: 30 cases with DM type II with diabetic nephropathy [albumin-to-creatinine ratio (ACR) above 30 milligrams per gram], 30 patients with DM type II without DN (ACR under 30 milligrams per gram), and 30 apparently healthy individuals of matched sex and age selected as a control group. Cases were selected from the clinics of the internal medicine department, Beni-Suef University Hospital.*

Results: *The risk of acquisition of diabetic nephropathy (compared with diabetes without nephropathy) was increased by the presence of CC 3.14 times, and the probability of risk of DN decreased by the presence of CG 0.23 times. The GG genotype expression didn't differ significantly between DM and DN. A statistically significant variance has been observed between the three groups with regard to the SIRT1 rs7069102 polymorphism.*

Conclusion: *The current research illustrated that a significant correlation has been observed between Sirtuin 1 rs7069102 polymorphism and the possibility of developing diabetic nephropathy in Egyptian cases. CC genotype and C allele have been related to an elevated possibility of developing DN.*

Keywords: *Sirtuin 1 rs7069102 gene, polymorphism, diabetic nephropathy*

1. INTRODUCTION

Diabetes is a heterogeneous metabolic disease marked by raised concentration of blood glucose due to either resistance to insulin, inadequate production of insulin, or both. The primary clinical manifestation of diabetes is hyperglycemia [1]. Insulin deficiency and/or insulin resistance are linked to anomalies in lipid and protein metabolism, as well as disruptions in minerals and electrolytes [2].

The majority of diabetic patients are categorized into two main types: type 1 diabetes mellitus, which results from a complete or near-complete deficiency of insulin, & type 2 diabetes mellitus, which is marked by insulin resistance coupled with an inadequate compensatory rise in insulin secretion. Furthermore, females who develop diabetes throughout their pregnancy are categorized as having gestational diabetes. Lastly, there are a variety of rare and different types of diabetes, which can be caused by drugs, infections, pancreatic destruction, endocrinopathies, and genetic defects [3].

DN is the most serious and frequent microvascular complication resulting from diabetes. The main pathogenic alteration is the glomerular basement membrane thickness and hyperplasia of many basement membranes in the mesangial area. The rising prevalence of diabetes mellitus (DM) has led to an annual rise in the morbidity associated with diabetic nephropathy, which has been identified as the 1ry etiology of end-stage renal disease (ESRD) [4].

Diabetic nephropathy has been the primary etiology of chronic kidney disease. Diabetic nephropathy not only brings the body's disease and the life pressure to cases; additionally, it causes a serious economic burden to cases' families. A variety of research has demonstrated the influence of genetic factors on the susceptibility

to diabetic nephropathy [5].

Sirtuin 1 (SIRT1) is an NAD+-dependent deacetylase that is part of a class of enzymes recognized as silent information regulators, which are expressed in several organs, as the adipose tissue, kidney, liver, pancreas, and muscle. The SIRT1 gene is on chromosome 10. It alters proteins included in the repair of DNA, stress & inflammatory responses, and the control of energy metabolism. Sirtuin 1 stimulates lipolysis in white adipose tissue, protects against excess accumulation of lipids in the liver and the skeletal muscle, and enhances secretion of insulin [6].

The role of inflammation in complications associated with diabetes is well known. Sirtuin 1 inhibits inflammatory pathways inside the cell, the production of pro-inflammatory genes, and the release of IL-1β, TNF-α, p53, IL-6, FoxO, and other pro-inflammatory molecules [7].

SIRT1 possesses renoprotective influence, including resistance to apoptosis, inflammation of tubular and glomerular cells, and mitigating interstitial fibrosis, among other beneficial effects [8].

Numerous investigations conducted across numerous populations have demonstrated a strong correlation among DN and DR. Sirtuin 1 has a protective role in the development of diabetic retinopathy; nevertheless, there is now no published research examining the association among Sirtuin 1 polymorphisms and diabetic retinopathy [9].

The goal of this research is to examine the association between the SIRT1 rs7069102 polymorphism and diabetic nephropathy in Egyptian individuals who have type 2 diabetes mellitus.

2.PATIENTS AND METHODS

This case–control research had ninety participants: thirty cases who have type II diabetes mellitus with diabetic nephropathy (albumin-to-creatinine ratio [ACR] above 30 milligrams per gram), thirty cases who have type II diabetes mellitus without diabetic nephropathy (ACR under 30 milligrams per gram), and thirty age- & sex-matched healthy individuals serving as the control group. Cases have been chosen from the internal medicine department clinics at Beni-Suef University Hospital.

Inclusion criteria: Cases with Type II diabetes diagnosed five to ten years ago, depending on the criteria established by the American Diabetes Association in 2021, are as follows: Fasting plasma glucose (FPG) > 126 milligrams per deciliter (7.0 millimoles per liter) Fasting is referred to as no caloric intake for not less than eight hours. Two-hour plasma glucose (2-hr PG) ≥200 milligrams per deciliter (11.1 mmol per liter). Hemoglobin A1C is equal to or more than 6.5% (48 millimoles per liter) Random plasma glucose (PG) is greater than 200 milligrams per deciliter (11.1 millimoles per liter) In people who have symptoms of hyperglycemia.

Cases with type 2 diabetes mellitus were then categorized according to urine albumin-creatinine ratio (UACR) and fundus examination.

Exclusion criteria: Cases with the following conditions have been excluded from this research: Poor glycemic control, the existence of other probable etiologies of kidney illness: Active infection, Alcoholism, Significant heart failure (New York Heart Association [NYHA] Classification II-IV), hematuria, & chronic hepatic disease

Ethical consideration: Informed consent has been attained from all cases before the study, following ethical committee approval at Beni-Suef Faculty of Medicine."

All participants have been subjected to the following:

Careful history taking with particular stress on diabetic complications, manifestations, & management & family history, Clinical examination, routine laboratory investigations.

DNA extraction:

Extraction of DNA has been performed from EDTA-anticoagulated whole blood utilizing the QIAamp DNA Mini Kit.

Principle:

The extraction of DNA is crucial for the analysis of deoxyribonucleic acid. The polymerase chain reaction (PCR) demands isolated and purified DNA, which may be extracted from many sample sources, including

cultured cells, whole blood, formalin-fixed paraffin-embedded tissue sections, yeast, and bacteria. Within the nucleus, DNA is tightly related to several proteins, forming a structure recognized as chromatin. It is essential to eliminate these proteins, along with other cellular proteins, to extract the DNA. This is accomplished by the utilization of proteinase K salt. Proteinase K is synthesized by the fungus Tritirachium album and demonstrates extensive cleavage specificity, making it useful for digestion of protein in biological systems. The quantity and quality of DNA will fluctuate based on the sample's age, size, and white blood cell count. Utilized Biosystems Inc., Foster City, CA, United States of America. Clini, QUIAGEN, 51104 Spruce St., St. Louis, MO, United States.

Procedure:

Cells have been lysed throughout a short incubation with proteinase K and a particular lysis buffer in the existence of guanidine-HCl, which instantly stop all nucleases. Cellular nucleic acids (NA) bind selectively to particular glass fibers pre-packed in the high-purity purification filter tube. A series of quick "wash-and-spin" steps are applied, the importance of which is to get rid of PCR inhibitory contaminants and to purify NA from salts, proteins, and other cellular impurities. The previous step involves the use of a special inhibitor removal buffer and a wash buffer; then, an elution buffer was utilized to release nucleic acids from the glass fiber.

Amplification of the target genes by RT-PCR:

The SIRT1 rs7069102 area encompassing the SNP has been amplified utilizing TaqMan® 5-nuclease chemistry to amplify & identify particular polymorphisms in pure genomic DNA samples. PCR includes synthesizing two oligonucleotide primers of about twenty base pairs in length complementary to the DNA flanking a specific sequence of DNA of interest. The primers facilitated the amplification of the sequence through successive cycles of denaturation, wherein the double-stranded DNA was heated, followed by the annealing of the primer to the resultant single-stranded DNA template & the extension of the primer on this template utilizing DNA polymerase in the presence of deoxyribonucleoside triphosphates (dNTPs) to generate double-stranded copies of the target DNA sequence. The TaqMan® MGB probes/extension primers were (VIC AGAAGAAAGAAAGGCATAATCTCTG) for detecting allele 1 sequence & (FAM AGAAAAGCCATTATTTCTGCAGATA) for detecting allele 2 sequence. The successive cycles of deoxyribonucleic acid synthesis yield an exponential enhancement of the target deoxyribonucleic acid sequence, resulting in a 10^5 to 10^6-fold rise in the quantity of the deoxyribonucleic acid sequence of interest [10].

The total volume of the polymerase chain reaction was twenty microliters, including forty nanograms per microliter of gDNA, ten microliters of 2x universal TaqMan master mix II, 0.5 microliters of 20x SNP assay mix, & adjusted to a final volume of twenty microliters with nuclease-free water.

Real-time quantitative PCR is a dependable method for finding & quantifying products produced throughout each cycle of the process of PCR, which are directly proportional to the initial amount of template. This method has illustrated that the thermostable enzyme Thermus aquaticus (Taq) deoxyribonucleic acid polymerase possesses 5' to 3' exonuclease activity. This group demonstrated that the cleavage of a target probe throughout PCR, facilitated by the 5' nuclease activity of Taq polymerase, may be utilized to identify the amplification of the target- particular product, an oligonucleotide. The probe, intended for hybridization inside the target sequence, has been included in the PCR test. The annealing of the probe to one strand of the polymerase chain reaction product throughout amplification produced a substrate conducive to exonuclease activity. Throughout amplification, the 5' to 3' exonuclease activity of Taq DNA polymerase degraded the probe into smaller fragments as the enzyme expanded from an upstream primer into the probe area, allowing for differentiation from the undegraded probe. The dependence on polymerization guaranteed that probe cleavage transpired just when the target sequence had been amplified. Following PCR, the cleavage of the probe has been assessed by thin-layer chromatography to differentiate cleavage fragments from the intact probe. The introduction of dual-labeled oligonucleotide fluorogenic probes facilitated the removal of post- polymerase chain reaction processing for the assessment of degradation of the probe [12].

The probe has a reporter fluorescent dye at the 5' end & a quencher dye at the 3' end. Although the probe

remains intact, the presence of the quencher markedly diminishes the fluorescence generated by the reporter dye. A fluorescence signal is released solely upon the cleavage of the probe, according to the principle of fluorescence resonance energy transfer (FRET). The real-time quantitative TaqMan® test utilized a fluorogenic nonextendable probe known as the "TaqMan" probe [13]. The probe possesses a fluorescent reporter dye at its 5' end & a quencher dye at its 3' terminus. If the target sequence exists, the fluorogenic probe anneals downstream from one of the primer locations and is cleaved by the 5' nuclease activity of the Taq polymerase enzyme throughout the extension phase of the polymerase chain reaction. When the probe remains intact, FRET transpires, resulting in the fluorescence emission of the reporter dye being absorbed by the quenching dye. The cleavage of the probe by Taq polymerase throughout PCR separates the quencher and reporter dyes, resulting in an increase in fluorescence from the former. Furthermore, cleavage eliminates the probe from the target strand, permitting primer extension to proceed to the terminus of the template strand, thus not obstructing the exponential amplification of PCR product. Each cycle results in the cleavage of additional reporter dye molecules from their respective probes, causing a fluorescence intensity rise that correlates with the amount of amplicon generated.

Interpretation of data has been performed utilizing an allelic discrimination plot.

The general process for analyzing information for allelic genotyping or discrimination includes Creating & setting up a post-PCR plate read document. Conducting a post-PCR plate analysis using a real-time PCR apparatus. Analyzing the experiment. Making manual or automatic allele calls. Confirming types of alleles. The software of Life Technologies' real-time equipment demonstrates the outcomes of allelic discrimination information as a scatter plot of Allele 1 (VIC® dye) against. Allele 2 (FAM dye). Each well of the 96-well plate is depicted as a distinct point on the plot.

3.RESULTS

Table 1 illustrated that a statistically insignificant variance has been observed among the examined groups with regard to their age and sex.

Table 2 illustrated that there was a statistically significantly higher A/C ratio level in diabetic nephropathy than in the control group and diabetes mellitus groups, but there was an insignificant difference between the diabetes mellitus and control groups.

Table 3 illustrated that statistically significant variance has been observed between the three groups regarding the SIRT1 rs7069102 polymorphism. The CC sequence was significantly more prevalent in the diabetic nephropathy group than the other 2 groups, but there was an insignificant difference between DM and control. The CG sequence was significantly higher in diabetes mellitus than the other 2 groups, but the difference between DN and control was not significant. The GG sequence was significantly more prevalent in the control group in comparison with the other 2 groups, but the variance among DN and DM was statistically insignificant. The C allele was significantly prevalent in the DN and the DM groups in comparison with the control group, but it didn't vary significantly among the DM and the DN groups.

Table 4 showed that the risk of acquisition of diabetic nephropathy (compared with diabetes without nephropathy) was increased by the presence of CC 3.14 times, and the probability of risk of DN decreased by the presence of CG 0.23 times. The GG genotype expression didn't differ significantly between DM and DN.

Table 5 illustrated that there was a statistically insignificant correlation between SIRT1 rs7069102 genotypes and their laboratory parameters in diabetic nephropathy patients, but the age of patients with GC was significantly older than CC but didn't differ from GG, but without clinical significance, as all groups were more than 50 years old.

Table 6 illustrated that there was a significant correlation between the DR & CC genotypes (P-value = 0.009).

Table (1) Age and sex distribution of the examined groups

Items	Control (no=30)	Diabetic Nephropathy (no=30)	Diabetes Mellitus (no=30)	P-value
Age (mean±SD)	53.4±3.6	55.1±7.5	51.0±10.3	0.125
Sex				>0.999
Females	15(50.0%)	15(50.0%)	15(50.0%)	
Males	15(50.0%)	15(50.0%)	15(50.0%)	

*P-value is significant

Table (2) Comparative analysis between the examined groups regarding their A/C ratio:

Items	Control (no=30)	Diabetic Nephropathy (no=30)	Diabetes Mellitus (no=30)	P-value
A/C ratio(mean±SD)	15.3±7.4	1904.3±278.7	22.1±7.3	<0.001*
P1 (controls vs DN) <0.001*				
P2 (controls vs DM) >0.999				
P3 (DN vs DM) <0.001*				

Table (3) Comparative analysis between the examined groups with regard to their SIRT1 rs7069102 polymorphism:

Items	Control (no=30)	Diabetic Nephropathy (no=30)	Diabetes Mellitus (no=30)	P-value
Alleles				
C	16(26.7%) a	39(65%) b	33(55%) b	**<0.001***
G	44(73.3%) a	21(35%) b	27(45%) c	
Gene				
CC (pathogenic)	4(13.3%) a	**16(53.3%) b**	8(26.7%) a	0.003*
CG	8(26.7%) a	**7(23.3%) a**	**17(56.7%) b**	0.016*
GG	**18(60.0%) a**	7(23.3%) b	5(16.7%) b	<0.001*

*P-value is significant different letters denote significant variances among groups.

Table (4) Risk of SIRT1 rs7069102 gene of acquisition of diabetic nephropathy compared with diabetic patients without nephropathy:

Items	Diabetic nephropathy (no=30)	Diabetes Mellitus (no=30)	P-value	Odds ratio (95%CI)
Gene				
CC (pathogenic)	**16(53.3%)**	8(26.7%)	0.035*	3.14(1.06,9.26)
CG	**7(23.3%)**	**17(56.7%)**	0.008*	0.23(0.08,0.71)
GG	7(23.3%)	5(16.7%)	0.520	1.52(0.42,5.47)
Alleles			0.265	1.52(0.73,3.17)
C	39(65%)	33(55%)		
G	21(35%)	27(45%)		

*P-value is significant

Table (5) Association between the different SIRT1 rs7069102 genotypes and patients age and laboratory parameters in diabetic nephropathy patients (n=30):

Variables	Genotype	Number of patients	Mean	SD	P-value
Age	CC	16	51.7500	6.64831	.009*
	CG	7	61.5714	5.06153	
	GG	7	56.1429	7.40335	
HB	CC	16	9.8813	1.97997	.748
	CG	7	10.2286	1.89887	
	GG	7	9.4000	2.30000	
NA	CC	16	136.3750	3.66742	.446
	CG	7	137.7143	3.14718	
	GG	7	135.2857	3.54562	
K	CC	16	4.5625	.40311	.146
	CG	7	4.4429	.76126	
	GG	7	4.1000	.38730	
UREA	CC	16	77.6875	62.70962	.209
	CG	7	120.0000	96.23929	
	GG	7	54.7143	45.24273	
Creatinine	CC	16	2.9850	2.96659	.283
	CG	7	4.7429	5.20316	
	GG	7	1.7286	2.41848	
HBA1C	CC	16	8.2875	1.39421	.424
	CG	7	7.5571	1.21361	
	GG	7	8.0000	.56862	
A/C Ratio	CC	16	2499.500	3506.81439	.232
	CG	7	2107.000	1691.6692	
	GG	7	341.0000	178.83698	
FBG	CC	16	132.9375	37.57919	.992
	CG	7	135.2857	48.95479	
	GG	7	134.2857	42.20077	
2hpp	CC	16	204.6250	57.13595	.972
	CG	7	198.2857	61.02927	
	GG	7	203.7143	65.84506	
Plt	CC	16	326.8125	149.50907	.395
	CG	7	287.1429	134.81769	
	GG	7	403.7143	207.69907	
TLC	CC	16	8.3467	2.42925	.134
	CG	7	11.4571	6.12314	
	GG	7	8.0857	1.73631	
Albumin	CC	16	3.4250	.35684	.832
	CG	7	3.4857	.59562	
	GG	7	3.5429	.43534	

Table (6) Association between the different SIRT1 rs7069102 genotypes and patient's fundus examination in all patients (n=60):

Diabetic nephropathy		Gene			Total
		CC	CG	GG	
Fundus Examination	DR	11	3	1	15
		45.8%	12.5%	8.3%	25.0%
	Normal	13	21	11	45
		54.2%	87.5%	91.7%	75.0%
Total		24	24	12	60
		100.0%	100.0%	100.0%	100.0%
P-value		0.009*			

*P-value is significant

4.DISCUSSION

There was a statistically insignificant variance among the examined groups with regard to their age and gender. There was a statistically significantly higher A/C ratio level in diabetic nephropathy than in the control group and diabetes mellitus groups, but there was insignificant variance between the diabetes mellitus and control groups.

A statistically significant variance has been observed between the three groups regarding the SIRT1 rs7069102 polymorphism. The CC sequence was significantly more prevalent in the diabetic nephropathy group than in the other two groups, but there was an insignificant difference between DM and control. The CG sequence was significantly higher in diabetes mellitus than in the other 2 groups, but the variance between DN and control was insignificant. The GG sequence was significantly more prevalent in the control group in comparison with the other 2 groups, but the variance between DN and DM was statistically insignificant. The C allele was significantly prevalent in the DN and the DM groups in comparison with the control group, but it didn't differ significantly between the DM and the DN groups. The risk of acquisition of diabetic nephropathy (compared with diabetes without nephropathy) was increased by the presence of CC 3.14 times, and the probability of risk of DN decreased by the presence of CG 0.23 times. The GG genotype expression didn't differ significantly between DM and DN.

There was a statistically insignificant correlation between SIRT1 rs7069102 genotypes and their laboratory parameters in diabetic nephropathy cases, but the age of patients with GC was significantly older than CC but didn't differ from GG, but without clinical significance, as all groups were more than 50 years old. There was a significant correlation among the DR and CC genotypes (P-value = 0.009).

The results were consistent with research performed by **Khalil et al. [14]** on Egyptian patients**.** They found a statistically significant rise in the CC genotype in individuals with diabetic nephropathy (DN, group I) compared to those who have type II diabetes mellitus (group II) & healthy subjects in the control group (group III). The results indicated that the presence of the CC genotype raises the possibility of developing DN (odds ratio: 2.64 and 95% confidence interval: 1.21–5.74).

With regard to the research carried out by **Jernej Letonja1 et al. [15]** on Slovene (Caucasian) cases, they found that cases with the CC genotype are more probable to progress to diabetic nephropathy in comparison with cases with other genotypes. Utilizing a codominant inheritance model, cases with the CC genotype exhibited a 1.94-fold raised likelihood of developing DN (p-value equal to 0.02) compared to individuals with alternative genotypes. Furthermore, the application of a recessive inheritance model indicated that T2DM cases possessing the CC genotype are 2.39 times more predisposed to developing diabetic nephropathy compared to those with the GG or CG genotype (p-value equal to0.02; OR: 2.39; ninety-five percent confidence interval (CI): 1.12-5.08).

Maeda et al. [16] investigated other polymorphisms of the Sirtuin 1 gene and their correlation with diabetic

nephropathy in Japanese cases with type 2 diabetes mellitus. The researchers examined the polymorphism (rs4746720), which showed a significant correlation with DN. Furthermore, an association was identified between rs4746720 and three other polymorphisms (rs2236319, rs10823108, rs3818292) concerning the combined phenotypes of proteinuria and ESRD.

Two case-control investigations examined the association between Sirtuin 1 polymorphisms & diabetic nephropathy in the Chinese population. Both investigations identified an association between the rs10823108 polymorphism & diabetic nephropathy **[17]. Yue et al**. additionally clarified the protective role of the Sirtuin 1 polymorphism rs3818292 in association with diabetic nephropathy [17].

In another research, the PCR-RFLP technique has been utilized to ascertain the distribution of single nucleotide polymorphisms genotype frequencies of Sirtuin 1 in both the control and observation groups. The outcome demonstrated that cases with mutations of homozygous type GG in rs10823108 had a greater possibility of diabetic kidney disease in comparison with those with wild homozygous type AA & that GG + GA carriers had a greater probability for illness in comparison with AA carriers. The findings demonstrated that cases with the GG genotype at the rs10823108 locus had an elevated possibility of proteinuria compared to those with the AA genotype. Cases with the GG genotype at the rs3818292 locus exhibited a reduced possibility of illness compared to those who have the AA genotype, & G allele carriers demonstrated a reduced possibility of illness in comparison with A allele carriers, suggesting that the mutation genotype at the rs3818292 locus serves as a protective genetic susceptibility factor in the progression of diabetic kidney disease **[17].**

Proof indicates that certain polymorphisms of SIRT1 contribute to the vulnerability of T2DM cases to DN, particularly among Caucasian, Japanese, and Chinese populations. Additional investigation is required to recognize more of them, as they might be utilized as susceptibility indicators for diabetic nephropathy in T2DM cases. **Jernej Letonja1 et al., [15]**

5.CONCLUSION

The current research illustrated that there was a significant correlation among Sirtuin 1 rs7069102 polymorphism and the possibility of developing diabetic nephropathy in Egyptian cases. The CC genotype and C allele have been related to a greater possibility of developing diabetic nephropathy.

REFERENCES

1. Karalliedde, J., & Gnudi, L. (2016). Diabetes mellitus, a complex and heterogeneous disease, and the role of insulin resistance as a determinant of diabetic kidney disease. Nephrology dialysis transplantation, 31(2), 206-213.
2. Smita, R. M., Shuvo, A. P., Raihan, S., Jahan, R., Simin, F. A., Rahman, A., ... & Sagor, M. A. T. (2022). The role of mineral deficiencies in insulin resistance and obesity. Current diabetes reviews, 18(7), 19-39.
3. Petersmann, A., Müller-Wieland, D., Müller, U. A., Landgraf, R., Nauck, M., Freckmann, G., ... & Schleicher, E. (2019). Definition, classification and diagnosis of diabetes mellitus. Experimental and Clinical Endocrinology & Diabetes, 127(S 01), S1-S7.
4. de Boer, I. H., Rue, T. C., Hall, Y. N., Heagerty, P. J., Weiss, N. S., & Himmelfarb, J. (2011). Temporal trends in the prevalence of diabetic kidney disease in the United States. Jama, 305(24), 2532-2539.
5. Conserva, F., Gesualdo, L., & Papale, M. (2016). A systems biology overview on human diabetic nephropathy: From genetic susceptibility to post-transcriptional and post-translational modifications. Journal of diabetes research, 2016(1), 7934504.
6. Kurylowicz, A. (2016). In search of new therapeutic targets in obesity treatment: sirtuins. International journal of molecular sciences, 17(4), 572.
7. Wang, W., Sun, W., Cheng, Y., Xu, Z., & Cai, L. (2019). Role of sirtuin-1 in diabetic nephropathy. Journal of Molecular Medicine, 97, 291-309.
8. Kitada, M., Kume, S., Takeda-Watanabe, A., Kanasaki, K., & Koya, D. (2013). Sirtuins and renal diseases: relationship with aging and diabetic nephropathy. Clinical science, 124(3), 153-164.
9. Karbasforooshan, H., & Karimi, G. (2018). The role of SIRT1 in diabetic retinopathy. Biomedicine & Pharmacotherapy, 97, 190-194.
10. Pavlov, A. R., Pavlova, N. V., Kozyavkin, S. A., & Slesarev, A. I. (2004). Recent developments in the optimization of thermostable DNA polymerases for efficient applications. Trends in biotechnology, 22(5), 253-260.

11. Holland, P. M., Abramson, R. D., Watson, R., & Gelfand, D. H. (1991). Detection of specific polymerase chain reaction product by utilizing the 5'—3'exonuclease activity of Thermus aquaticus DNA polymerase. Proceedings of the National Academy of Sciences, 88(16), 7276-7280.
12. Lee, L. G., Connell, C. R., & Bloch, W. (1993). Allelic discrimination by nick-translation PCR with fluorgenic probes. Nucleic acids research, 21(16), 3761-3766.
13. Arya, M., Shergill, I. S., Williamson, M., Gommersall, L., Arya, N., & Patel, H. R. (2005). Basic principles of real-time quantitative PCR. Expert review of molecular diagnostics, 5(2), 209-219.
14. Khalil, M. M. I. M., Kasem, H. E., & Genena, S. E. R. (2022). Association of insulin receptor (INR) gene rs2252673 and Sirtuin1 rs7069102 polymorphisms with diabetic nephropathy in patients with type 2 diabetes mellitus. Human Gene, 33, 201078.
15. Letonja, J., Završnik, M., Makuc, J., Šeruga, M., Peterlin, A., Cilenšek, I., & Petrovič, D. (2021). Sirtuin 1 rs7069102 polymorphism is associated with diabetic nephropathy in patients with type 2 diabetes mellitus. Bosnian Journal of Basic Medical Sciences, 21(5), 642.
16. Maeda, S., Koya, D., Araki, S. I., Babazono, T., Umezono, T., Toyoda, M., ... & Nakamura, Y. (2011). Association between single nucleotide polymorphisms within genes encoding sirtuin families and diabetic nephropathy in Japanese subjects with type 2 diabetes. Clinical and experimental nephrology, 15, 381-390.
17. Yue, X. G., Yang, Z. G., Zhang, Y., Qin, G. J., & Liu, F. (2018). Correlations between SIRT1 gene polymorphisms and diabetic kidney disease. Royal Society Open Science, 5(6), 171871.