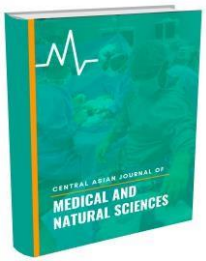

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Article

Effect of SLC30A8 rs13266634 Genetic Polymorphism with Diabetic Nephropathy Patients: A Case-Control Study

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Abstract: Diabetic nephropathy is a pathological disease that is commonly observed in persons with diabetes. The purpose of this research was to compare the effects of the SLC30A8 rs13266634 gene polymorphism on people with and without diabetic nephropathy. The study comprised 170 patients, aged 40 to 75, who were admitted to the dialysis unit at the Al-Diwaniyah Teaching Hospital in Iraq in 2021 and 2022. There is a connection between SLC30A8 gene polymorphisms and diabetic nephropathy, according to the results of a study looking at the interaction between comorbid diseases and patients with the disease. Particularly, we discovered that individuals with the C allele of T/C (OR = 0.509, 95% Cl = 0.307- 0.843, p-value = 0.008 < 0.05), the TC genotype of T/C (OR = 0.280, 95% Cl = 0.118- 0.665, p-value = 0.003 < 0.05), and the TC & CC genotype had higher odds of infection with DN compared to the TT genotype (OR = 0.391, 95% Cl = 0.203- 0.756, p-value = 0.005 < 0.05).

Keywords: diabetic nephropathy, DN, Zinc transport ZnT8, SLC30A8, diabetic kidney disease, DKD

1. Introduction

Diabetes is known to be the primary cause of diabetic nephropathy, a chronic microvascular condition that is extremely worrying and can lead to the development of end-stage renal disease (ESRD) [1]. Diabetic nephropathy (DN) is commonly characterised by hyperfiltration and albuminuria as early signs, followed by a progressive deterioration in renal function. Diabetic kidney disease (DKD) can appear in a variety of ways, especially in people with type 2 diabetes mellitus (T2DM) who also have severe peripheral vascular disease and concurrent glomerular/tubular diseases [2]. Compared to diabetics without nephropathy, those with diabetic kidney disease (DKD) have an overall mortality rate that is thirty times higher. The majority of DKD patients will pass away from cardiovascular illness before developing end-stage renal disease (ESRD) [3]. Papadopoulou-Marketou et al. (2017) [4] state that the disease can be effectively prevented or delayed by managing metabolic and hemodynamic imbalances. Addressing metabolic and hemodynamic imbalances is essential to effectively prevent and delay the progression of diabetic kidney disease (DKD) [5]. Diabetes mellitus (DKD) is a widespread condition with substantial social and economic repercussions that affects people all around the world [6]. Diabetes-related nephropathy complications can appear gradually over several months or even years. A new study's results show that compared to people without the disease, those with type 2 diabetes have a higher frequency of a non-synonymous polymorphism in the SLC30A8 (solute carrier family 30 (zinc transporter), member 8) gene. In order to keep the body's zinc balance intact, the zinc transporter ZnT8 is essential [7]. The deterioration of ZnT8

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levels affects β cell function and Zn(++) homeostasis maintenance, which contributes to the onset of diabetes mellitus (DM) [8]. ZnT8 expression has been found in the retinal pigment epithelium (RPE) by [9]. Additionally, a study by Daniels et al. (2020) [10] found a strong correlation between decreased insulin production in non-diabetic relatives of those with type 2 diabetes and the dominant allele of the SLC30A8 single nucleotide polymorphism (SNP), rs13266634. When the participants received intravenous glucose stimulation, this connection was evident. Pancreatic alpha- and beta-cells express the zinc transporter protein (ZnT8), which is encoded by the SLC30A8 gene. The protein in issue is found in the membrane of insulin secretory granules, where it is essential for supplying zinc needed for the processes of insulin maturation and/or storage. It also makes zinc easier to accumulate from the cytoplasm in intracellular vesicles containing insulin. because of its part in how beta cells function [11].

This study aims to investigate the effects of genotypes and SLC30A8 alleles in patients with diabetic nephropathy and in healthy individuals (Control). Furthermore, the research endeavours to identify the particular alleles and genotypes, in addition to their corresponding quantities, that are associated with an increased risk of developing diabetic nephropathy.

2. Materials and Methods

2.1. Study population

A total of 170 individuals, comprising both males and females, with ages ranging from 40 to 75 years, were surveyed at the Dialysis Department at Al-Diwaniyah Teaching Hospital in Iraq. The study sample comprised 80 individuals diagnosed with Diabetic nephropathy (G2) and 90 individuals without any health conditions (G1).

2.2. PCR amplification

T-ARMS primers are used to amplify isolated DNA, as indicated in Table 1.

Table 1. The SLC30A8 (T/C) gene polymorphism's name, sequence, and melting point

Gene Name	*Sequence(5′–>3′)*	*Peak*	*Tm (ºC)*
SLC30A 8	FO 5′- CTG CTG ATA GCA TTT GGG ACA GG-3′	826	64
	RO 5′- CCA ATT GAT TGA TGG ATC TCA GTG C -3′	520	
	FI 5′- GCT TCT TTA TCA ACA GCA GCC AGC T -3′ RI 5′-CGA ACC ACT TGG CTG TCC CG -3′	350	

F: Forward, R: Reverse, I: Inner, O: Outer

Table 1 displays the prefixes produced by Addbio, a Korean company, for every gene used in this investigation. After drying and being treated with high purity H2O, each sample was divided and kept at a temperature of -20°C in accordance with the manufacturer's instructions. The PCR products were electrophoresed on a 2% agarose gel.

3. Results and Discussion

3.1. Amplification of SLC30A8 T/C Gene Polymorphism

Humans' SLC30A8 gene codes for the zinc transporter 8 protein [12], which is made up of 369 amino acids. Human insulin release has been connected to the zinc transporter ZNT8 [13]. Although some SLC30A8 alleles seem to increase the risk of type 2 diabetes, a

mutation that disrupts the gene's function appears to significantly lower the risk [14]. Insulin stability, storage, and release are all dependent on the preservation of zinc homeostasis within pancreatic beta cells, which is facilitated by ZnT-8. ZnT-8 controls zinc ion outflow from the cytoplasm into insulin secretory vesicles, according to [15]. Together with the insulin molecule, these vesicles are in charge of storing and releasing zinc ions. Hojs et al. (2020) [16] claim that zinc ions stabilise insulin hexamers, which helps to control how much insulin is broken down. Primers FI, RI, FO, and RO make up the gene SLC30A8. The Gel evidence from a PCR experiment with eight DNA samples revealed that the primers (TT) produced identical bands at 826,520 bp, while the primers (CC) produced bands at (826,350 bp). The gel results showed that the primers (TC) produced bands at 826 bp, 520 bp, and 350 bp, respectively.

3.1.1. The experimental group (G1) exhibited the genotype of the SLC30A8 gene

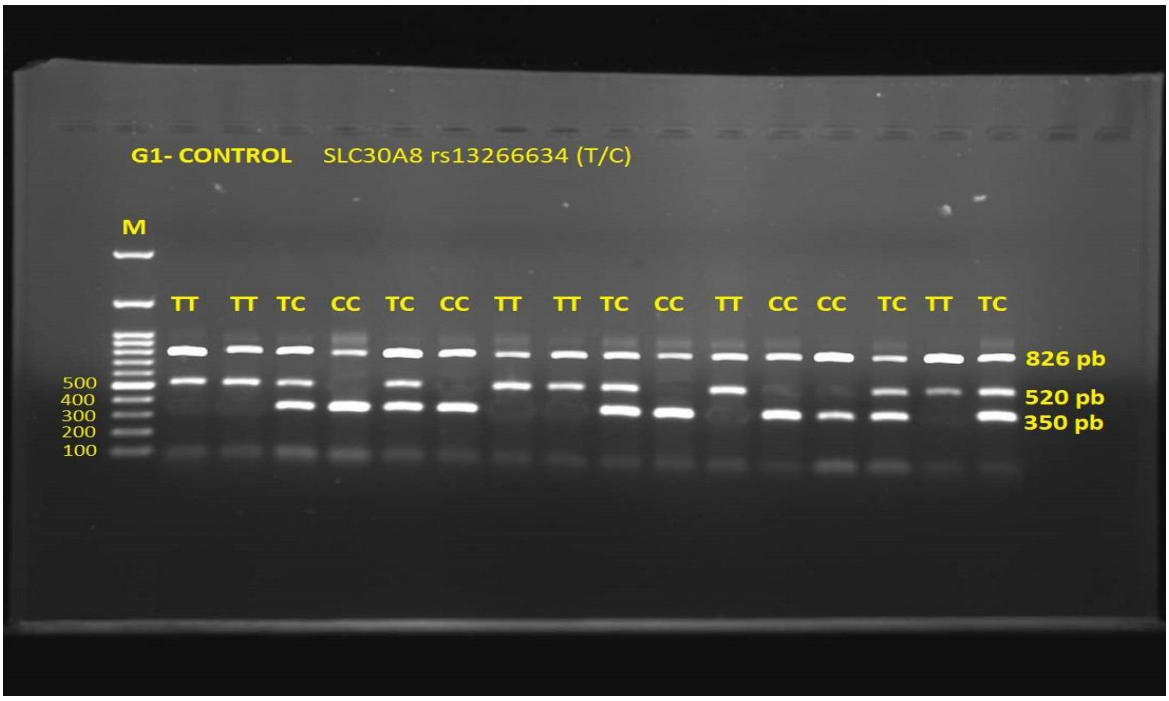

Figure 1. The SLC30A8 (T/C) gene polymorphism was amplified by conventional PCR using a particular pair of primers, as demonstrated by gel electrophoresis in the control group (G1). DNA ladder of 100–3000 base pairs, M. The PCR products were stained with a secure stain dye. It has been determined that there are three genotypes: homozygous TT (826,520 base pairs), heterozygous TC (826,520,350 base pairs), and homozygous CC (826 and 350 base pairs)

3.1.2. The genotype of the SLC30A8 gene in the group of individuals with diabetic nephropathy (G2)

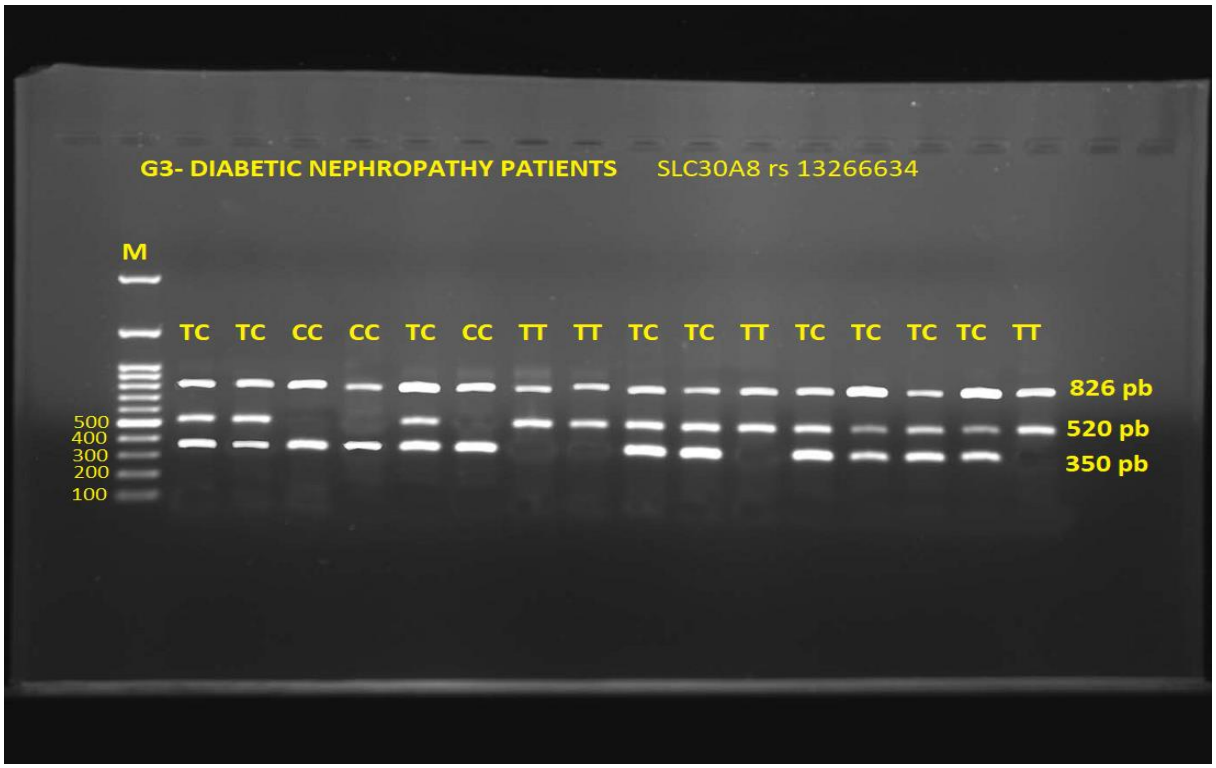

Figure 2. In the diabetic nephropathy group (G2), gel electrophoresis was employed to detect the amplification of the SLC30A8 (T/C) gene polymorphism using a specific pair of primers, employing conventional PCR procedures. Subject: DNA ladder (100-3000 base pairs). The PCR products were treatment with a non-toxic staining agent. The three genotypes are as follows: homozygous TT (826,520 bp), heterozygous TC (826,520,350 bp), and homozygous CC (826 and 350 bp)

Table 2 displays the values of the bands, which were derived from Figure 1 and 2.

Table 2. Dimensions of bands associated with the SLC30A8 (T/C) gene polymorphism

Genotype		**No. of bands**	**Size of bands (bp)**
Homozygous	TT	2	826,520
Heterozygous	TC	3	826,520,350
Homozygous	CC	2	826,350

Figures 1 and 2 illustrate the percentages that correspond to the frequencies of alleles for (G1) and (G2) based on the results from these alleles. We were able to determine that this gene has two distinct alleles, T and C, after examining the Figures. Allele T was more prevalent than allele C in both the diabetic nephropathy patient group and the control group. The percentages of alleles T in Control and DN patients were 111 (69.3%) and 147 (81.6%), respectively. Likewise, the proportions of allele C were 33 (18.3%) and 49 (30.6%).

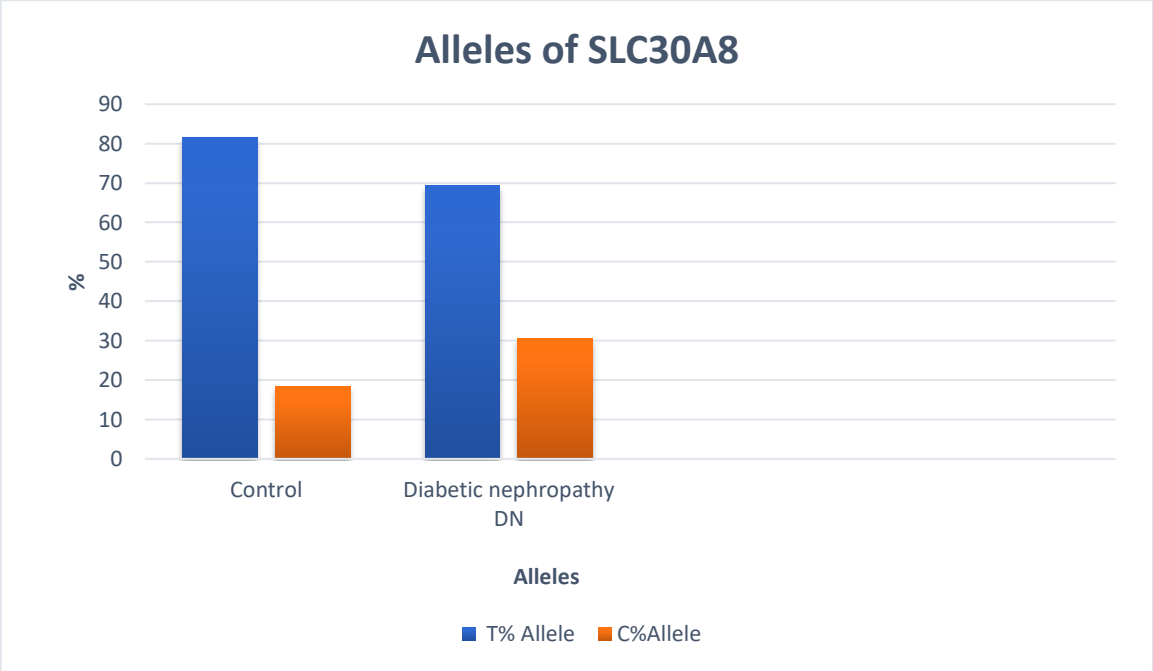

Figure 3. The proportion of T/C alleles of SLC30A8 in blood samples obtained from patients with Control and Diabetic Nephropathy

The genotype distribution of SLC30A8 (T/C) rs13266634 (T > C) revealed the presence of three unique polymorphisms: TT, TC, and CC. It was discovered that the initial polymorphism TT had proportions of 76.6%, %, and 56.3% for patients with diabetic nephropathy and the control group, respectively. For the control group and patients with diabetic nephropathy, the proportions of the second polymorphism TC were determined to be 10% and 26.3%, respectively for people without diabetes nephropathy and those with the condition. The third polymorphism CC was found in 13.3% and 17.5% of the populations, respectively. In the control group, the most common genotype was TT, whereas in DN people, the most common genotype was.

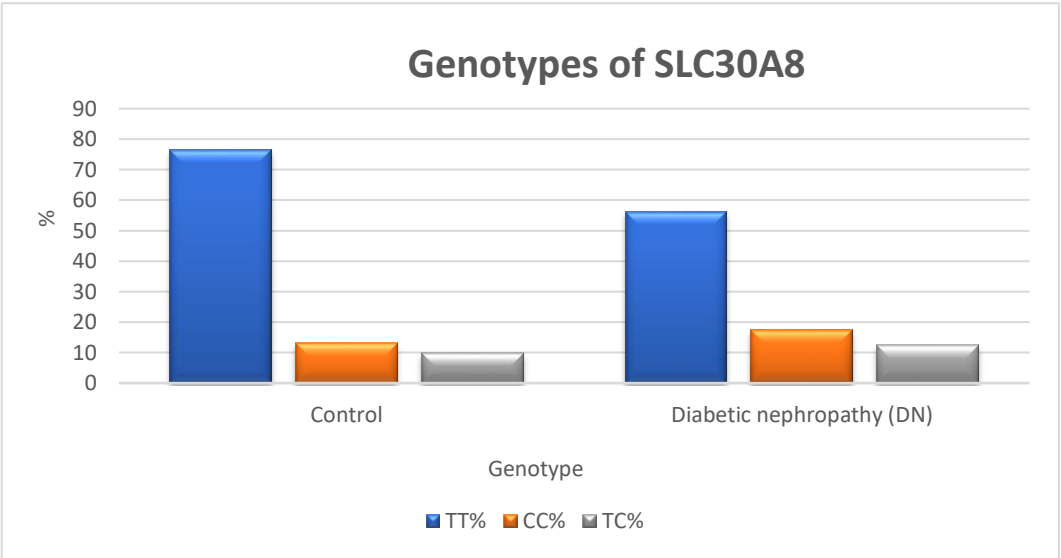

Figure 4. This study examines the prevalence of TT, TC, and CC genotypes of SLC30A8 in samples obtained from Control and DN patients

3.2. Association Between SLC30A8 T/C Gene Polymorphism and Diabetic nephropathy (G2) as Compared with Control (G1)

The study's results showed a substantial connection (x2 = 9.451 and P-value = 0.009 < 0.05) between diabetic nephropathy and the SLC30A8 (rs13266634) T, C-alleles. Additionally, the results imply that the percentages of TT, TC, and CC in G1 (control) were, in that order, 76.6%, 10%, and 13.3%. In G2 patients (those with diabetic nephropathy), the percentages of TT, TC, and CC were 56.3%, 26.3%, and 17.5%, respectively. Table (3) indicates a high association (P-value < 0.05) between G1Control and G2 DN patients regarding the SLC30A8 polymorphism, rs13266634. The T allele was found at 147 (81.6%) frequency in G1, while the C allele was found at 33 (18.3%) frequency. The T and C allele frequencies in G2 were 111 (69.3%) and 49 (30.6%), respectively. Table 3, which was generated using the data shown in Figures 1 and 2, displays the frequencies of genotypes and distributions of alleles for the SLC30A8 rs13266634 polymorphism in the Control (G1) and diabetic nephropathy Patients (G2) groups.

Table 3. In this study, we investigate the genotypes and allele distribution of the SLC30A8 (T/C) polymorphism in two groups: G1 (Control) and G2 (DN)

Polymorphisms SLC30A8 (rs13266634)	G1 (Control) N=90(%)	G2 (DN) N=80(%)	χ^2	P value	OR (95%CI)	P value
TT	69	45			1.0^{ref} (1.0^{ref})	
CC	12	14	9.451	0.009*	0.559 (0.237-1.318)	0.180
TC	9	21			0.280 (0.118-0.665)	0.003
T allele	147 (86.4%)	111 (65.3%)			1.0^{ref} (1.0^{ref})	
C allele	33 (19.4%)	49 (28.3%)	6.993	0.008*	0.509 (0.307-0.843)	
TT	69	45			1.0^{ref} (1.0^{ref})	
CC&TC	21	35	7.992	0.005*	0.391 (0.203-0.756)	
CC	12	14			1.0^{ref} (1.0^{ref})	
TT&TC	78	66	0.568	0.451	0.725 (0.314-1.670)	

Table 3 shows significant differences between G1 and G2. This study raises the possibility that the SLC30A8 gene and diabetic nephropathy are related since it shows differences between those with and without diabetes mellitus (DN). The results shown in Table 3 indicate that, in comparison to individuals with the TT reference genotype, those with the CC genotype of rs13266634 did not show a significant association with the increased risk of diabetic nephropathy (OR = 0.559, 95%Cl = 0.237-1.318, P-value = 0.180 > 0.05). These outcomes support the findings made by [17]. Compared to people with the TT reference genotype, those with the TC genotype of rs13266634 had a notably higher risk of developing diabetic nephropathy (odds ratio [OR] = 0.280, 95% confidence interval [Cl] = 0.118-0.665, p-value = 0.003 < 0.05). These results are consistent with what Chen et al. [18] reported. The C allele and the T allele were also found to be statistically significantly associated, with patients showing a lower proportion of T than the control group. Additionally, a P-value of 0.008 indicates that the prevalence of the C allele among patients is roughly

twice as high as that seen in the control group. Furthermore, variations in the alleles TT, TC, and CC resulted from the difference in the C to T ratio between patients and controls. According to the dominant genetic model, people who had the CC and TC genotypes of rs 13266634 were substantially more likely than people who had the TT genotype to develop diabetic nephropathy (odds ratio [OR] = 0.391, 95% confidence interval [Cl] = 0.203-0.756, p-value = 0.005 < 0.05), These results are consistent with those that Lin et al. (2018) reported [14]. The CC genotype and the TT and TC genotypes showed a statistically insignificant, in the recessive model, suggesting a non-increasing risk of diabetic nephropathy (OR = 0.725, 95%Cl= 0.314-1.670, P-value = 0.451 >0.05), these results are consistent with those that De La Cruz-Cano et al. (2019) reported [19].

4. Conclusion

The study found a link between the onset of diabetic nephropathy and genetic variants in the SLC30A8 (rs13266634) gene. When it comes to DN infection, those with the TC genotype of T/C are more susceptible than people with the TT genotype (odds ratio [OR] = 0.280, 95% confidence interval [Cl] = 0.118-0.665, p-value = 0.003 < 0.05). In a similar vein, people with the T/C C allele also show an increased chance of developing DN infection (OR = 0.509, 95% Cl = 0.307-0.843, p-value = 0.008 < 0.05).

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