Association of Zinc Transport Znt8 SLC30A8 Rs13266634 Gene Polymorphism with T2DM Patients: A Case-Control Study

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Abstract
The zinc transport ZnT8 Solute Carrier Family 30 Member 8 (SLC30A8) is a Protein Coding gene. Diseases associated with SLC30A8 include Type 2 Diabetes Mellitus. It has been suggested to have a role in the progress of blood suger complications. This paper aims to reveal the SLC30A8 T/C gene polymorphism and its role in the pathophysiology of DM in Al-Diwaniyah city in Iraq. Our study involved (170) people aged (40-75) years who were distributed into two groups, the control group (G1) involved (90) healthy, and the second group (G2) included (80) T2DM patients. The purpose of the current study was to reveal the SLC30A8 polymorphism and its role in the pathophysiology of type 2 diabetes mellitus. The overall genotype of the SLC30A8 rs13266634 gene was significantly different between the type 2 diabetes mellitus patients (G2) and control(G1) was significantly different between the T2DM patients (G2) as compared with control (G1) for the genotype TT ($\chi 2$ = 7.197, p-value = 0.027), C allele ($\chi 2$ = 9.067, p-value = 0.003), and CC & TC compared to the TT genotype ($\chi 2$ = 7.109, p-value = 0.008). Genotype TT, C allele, and CC & TC levels in the blood were substantially higher in T2DM patients, with a clear correlation with the increase in blood suger. In conclusion, the results of the genotype and allele distribution of SLC30A8 rs13266634 gene in type 2 diabetes patients group showed that there is an association between SLC30A8 rs13266634 gene and type 2 diabetes mellitus.
Keywords: Diabetes mellitus, T2DM, Zinc transport ZnT8, SLC30A8

1. Introduction

High blood suger (HBS) is a serious disease with persistent elevation in arterial Diabetes mellitus[1]. It is considered a multifactorial condition with multiple genetic and environmental causes. The metabolic disease is a long-term condition that affects the body's Diabetes mellitus is a rapidly expanding global problem with significant social, health, and economic implications. In 2010, it was projected that 285 million people (about 6.4 percent of the adult population) were affected by the disease worldwide. In the absence of better control or cure, this number is projected to rise to 430 million[2]. The increase is due to two factors: an aging population and obesity. Furthermore, nearly half of all presumed diabetics are not identified for another ten years after the commencement of the disease, implying that the true global diabetes prevalence must be enormous. A multitude of systems and pathways work together in the human body to [3]achieve and maintain a healthy physiological state. The ability of the organism to maintain a consistent stable condition, or homeostasis, is at the heart of these activities. The development of an injury or a diseased state in numerous organs is caused by a disruption of homeostasis. DM impairs a person's ability to control the amount of glucose in their blood, resulting in a variety of major and small consequences. Regulation of the levels of glucose in the blood is based on a negative feedback loop and acts via the release of insulin and glucagon. When glucose levels in blood are high, the B cells of the islet of langerhans in the pancreas are triggered to release insulin, a 51-amino acid polypeptide that is composed of two chains (A and B) connected by disulphide bridges[4]. Insulin is synthesized from pro-insulin by the pro-hormone convertases (PC1 and PC2), and exo-protease carboxypeptidase.' The action of these enzymes generates insulin and C-peptide." Insulin binds to the tyrosine kinase insulin receptor which is made up of two α subunits (extracellular) and two β subunits (intramembrane) linked by disulfide bonds[5]. The binding of insulin to the B subunit of tyrosine kinase insulin receptor promotes autophosphorylation of the B subunit. Insulin signals the liver to convert the excess glucose to glycogen for storage;[6] it also triggers other cells in the body (adipose/ skeletal muscle cells) to take up more glucose by the translocation of glucose transporter (GLUT4) to the cell surface. This helps to bring the circulating glucose concentrations to normal levels.[7] When the glucose concentration in the blood is low, the cells of the pancreas are stimulated to release glucagon. Glucagon signals the liver to convert stored glycogen into glucose which is released into the blood to achieve homeostasis. In diabetes, there is an aberration either in the synthesis or secretion of insulin as seen in Type I diabetes mellitus (TIDM) and stenosis in the pancreatic duct, or the development of

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resistance to insulin or its subnormal production as in the case of Type 2 diabetes (T2DM) and certain secondary diabetes[8]. A non-synonymous polymorphism in the SLC30A8 (solute carrier family 30 (zinc transporter), member 8) gene was recently reported to be more frequent in subjects with type 2 diabetes than in healthy controls [9]. Furthermore, it was shown that the major allele of the SLC30A8 SNP, rs13266634, was associated with reduced insulin secretion after stimulation with intravenous glucose in non-diabetic relatives of subjects with type 2 diabetes[10] . The SLC30A8 gene encodes a zinc transporter protein (ZnT8) which is expressed in pancreatic alpha- and beta-cells. It is localized in the membrane of the insulin secretory granules, facilitates the accumulation of zinc from the cytoplasm in intracellular insulin-containing vesicles and plays a major role in providing zinc for insulin maturation and/or storage processes. Because of its role in beta-cell function.[11]

2. Material and Methods

Study population

The study population consisted of (170) people of both genders (males and females), their ages ranged between (40-75) years, who attended Al-Diwaniyah Teaching Hospital in Al-Diwaniyah, Iraq. The study population consisted of 80 patients with T2DM(G2), and 90 healthy controls (G1).

Extraction of dna

Genomic DNA was extracted from whole blood using a DNA Extraction Kit (AddBio/Korea), were equal to or less than 20 µg/ml. The concentration of extracted DNA and its purity were estimated by measuring the absorbance at A 260 nm and A 280 nm by the Nanodrop device. The concentration of the DNA samples was (50 ng/µL), and the purity of the DNA samples (1.8 µg/ml).

Pcr amplification

Isolated DNA is amplified with T-ARMS primers, as shown in Table 1.

Table 1- The name and sequence and melting point of prepared ACE (I/D) gene polymorphism.

Gene Name	SNP name	Sequence (5'–>3')	Peak	Tm(°C)
		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				

The prefixes were designed by the Korean company Pioneer for all genes used in this study as shown in Table 1. All were dried and prepared with high purity H2O (AddBio, Korea) according to the manufacturer's instructions, and all were split and kept at - 20 °C. The PCR products were run on 2% agarose gel electrophoresis. The different fragments obtained were homozygous (TT) genotypes (826,520 bp); heterozygous (TC) genotypes (826,520, 350 bp); and homozygous (CC) genotypes (826,350 bp).

3. Results and Discussion

Investigation between the Association of SLC30A8 T/C Gene Polymorphisms and Risk of Diabetes mellitus as Compared with Control

These studies examined the association between SLC30A8 T/C gene polymorphism with the risk of T2DM as compared with control. The study was based on the results obtained from the genotyping. The statistical analysis between the allele frequencies and genotype distributions of SLC30A8 gene Polymorphisms in the two groups (Control G1, and Type 2 diabetes mellitus patients G2) was confirmed by the descriptive statistical at (p-value < 0.05).

Amplification of SLC30A8 T/C Gene Polymorphism

Zinc transporter8 is a protein encoded by the SLC30A8 gene in humans[12] consisting of 369 amino acids. n humans, ZNT8 is a zinc transporter linked to insulin secretion. Although certain alleles of the SLC30A8 gene tend to raise the risk of type 2 diabetes, a loss-of-function mutation appears to dramatically reduce the risk[13]. The SLC30A8 gene is found at 8q24.11 on the long (q) arm of human chromosome. Zinc transporter protein member-8 (ZnT-8) is encoded by this gene, which has 369 amino acids. ZnT-8 regulates zinc homeostasis in pancreatic beta cells (cells) and is essential for insulin stability, storage, and release. ZnT-8 also controls the outflow of zinc ions from the cytoplasm into insulin secretory vesicles, where zinc ions are reported to be stored and released alongside insulin molecules[14]. Zinc ions also limit insulin breakdown by stabilizing insulin hexamers. The SLC30A8 gene consists of the primers (T, C, and TC), and when performing a PCR assay for these primers and for a set of eight DNA samples, Gel documentation showed that the primers (TT) give the same bands that appeared at (826,520 bp), also Gel documentation showed that the primers (CC) give the bands that appeared at (826,350 bp). While Gel documentation showed that the primers (TC) give the bands that appeared at (826 bp, 520 and 350 bp). Amplification of SLC30A8 T/C gene polymorphism is shown in Figures (1, and 2).

The Genotype of the SLC30A8 Gene in the Control Group (G1)

The genotype frequencies and allele distributions of

T/C polymorphisms of SLC30A8 gene for the Control (G1) group that are shown in Table 2 are calculated from Figure 1.

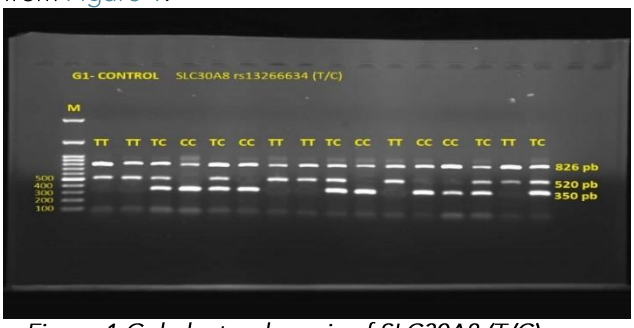

Figure 1-Gel electrophoresis of SLC30A8 (T/C) gene polymorphism amplified with a specific pair of primers using conventional PCR of control group (G1).

The Genotype of the SLC30A8 Gene in the T2DM Group (G2)

The genotype frequencies and allele distributions of T/C polymorphisms of SLC30A8 gene for the Diabetes mellitus (G2) group that are shown in Table 2 are calculated from Figure 2.

Figure 2- Gel electrophoresis of SLC30A8 (T/C) gene polymorphism amplified with a specific pair of primers using conventional PCR of Diabetes mellitus group (G2).

The amplification product of the SLC30A8 gene polymorphism (T/C) is three alleles, the values of whose bands were calculated from the Figures (1, and 2), and those values are shown in Table 2.

Table 2- Size of bands of 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

There are three alleles in the SLC30A8 (T/C) gene, and they are homozygous TT, heterozygous TC, and homozygous CC. When the bands appear at 826,520 bp, (826,520,350 bp) and 826,350 bp, respectively. Based on the research from these alleles, the percentage of all alleles was calculated for control (G1), Diabetes mellitus patients (G2), as shown in Figures (1, and 2), respectively.

Polymorphisms SLC30A8 (T/C)

When studying the Figures of comparison of genotype distribution and allele frequencies that appeared of SLC30A8 gene, it was found that this gene has two types of alleles: T and C, where the percentage of allele T was greater than the percentage of allele C in all groups (Control, and Diabetes mellitus patients), it was found that the percentage of allele T was 147 (81.6%), and 108 (67.5%) in Control, and T2DM patients, respectively. While the percentage of allele C was 33 (18.3%), and 52 (32.5%), in control, and T2DM patients, respectively, as shown in Figure 3.

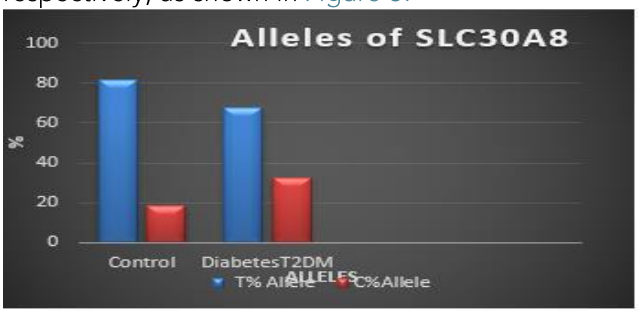

Figure 3-The percentage of alleles T/C of SLC30A8 in blood samples of Control, and Diabetes mellitus patients.

The Figures of genotype distribution observed for SLC30A8 (T/C) rs3266634 (T > C) also showed that there are three types of polymorphisms: TT, TC, and CC. The values of the percentages of the first polymorphism TT were 76.6%, %, 57.5% for control, T2DM patients respectively. The values of the percentages of the second polymorphism TC were 10%, 20% for control, T2DM patients respectively. The values of the percentages of the third polymorphism CC were 13.3%, 22.5% for control, T2DM patients respectively (Figure 4). The TT genotype was the major genotype at control, The TT genotype was the major genotype at T2DM patients in rs 13266634 respectively.

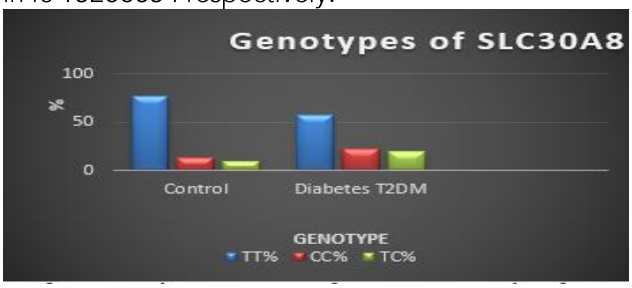

Figure 4- The percentage of TT, TC and CC genotypes of SLC30A8 in samples of Control, and Diabetes mellitus patients.

The values of percentage of T/C alleles, TT, TC, and CC genotypes of SLC30A8 were calculated from the Figures (1, and 2).

Association Between SLC30A8 T/C Gene Polymorphism and Risk of T2DM (G2) as Compared with Control (G1)

The genotype frequencies and allele distributions of T/C polymorphism of SLC30A8 (rs 13266634) for control (G1) and T2DM patients (G2) groups are shown in Table (3). The results revealed a significant association between SLC30A8 (rs13266634) T, C-alleles, andT2DM where$\chi 2$ is 7.197 and (P-value = 0.027 < 0.05), this indicate that there was a significant relationship between this SNP and the risk of T2DM. It means that this gene may be

a cause of diabetes, as there is a discrepancy in the concentration or level of this gene in healthy and diabetic patients. It is known that genes have an effect on disease and this gene is among these genes. Also, the results show that the frequencies were 76.6% for TT, 10% for TC, and 13.3% for CC in G1. The frequencies were 57.5% for TT, 20% for TC, and 22.5% for CC in G2. There was a significant association in rs 13266634 polymorphism of SLC30A8 between G1 and G2 (P-value < 0.05) as shown in Table (3), therefore CC allele was a significant (P-value = 0.049 < 0.05). This means that the genotype CC has an effect on the disease as well, the genotype TC has an effect on diabetes, p-value appeared when comparing the genotype TC with the reference TT equal to 0.029, less than 0.05. This means that this genotype means that this genotype has an effect on the incidence of infection disease if other conditions are met, such as obesity, family history, trauma, and others. In G1, the T allele frequency was 147 (81.6%) and the C allele was 33 (18.3%) and in G2 the frequencies were 108 (67.5%) and 52 (32.5%) for T and C alleles, respectively There was also a statistically significant relationship between the C allele and the T allele (P-value = 0.003 < 0.05). We note from the comparison between the C and T allele in both G1 and G2 that there is a clear difference in the level of alleles in the two groups, which led to the emergence of a p-value = 0.003, which is a highly significant value, as we note that the T allele is predominant and dominant, but its ratio differs, where in G1 it was 81.6 %, that is, more than three quarters of the total percentage, while the T level was equal to 67.5%, that is, it is much lower than its percentage in G1, which led to a discrepancy in the levels. This applies to the C level, where the level of the C allele in G2 is almost twice as high as in G1. Descriptive statistics analyses revealed that the rs 13266634 polymorphism, in dominant model TC and CC genotypes were compared with the TT genotype there was a significant difference (P-value = 0.008 < 0.05), in recessive model when compared between TC and TT genotypes with CC genotype we found there was a non-significant difference (P-value = 0.118 > 0.05).

Table 3- The genotypes and allele distribution of SLC30A8 (T/C) polymorphism in G1 (Control) and G2 (T2DM).

Polymorphisms SLC30A8 (rs 13266634)	G1 (Control) N=90(%)	G2 (T2DM) N=80(%)	x^2	P value	OR (95%CI)	P value
TT CC TC	69(76.6%) 12(13.3%) 9(10%)	46(57.5 %) 18(22.5%) 16(20%)	7.197	0.027*	1.0ref (1.0ref) 0.444 (0.196-1.009) 0.375 (0.153-0.920)	0.049* 0.029*
T allele	147(81.6%)	108 (67.5%)	9.067	0.003*	1.0^{ref} (1.0^{ref})	
C allele	33(18.3%)	52 (32.5%)			0.466(0.282 -0.770)	
TT	69	46	7.109	0.008*	1.0^{ref} (1.0^{ref})	
CC&TC	21	34			0.412 (0.213-0.786)	
CC	12	18	2.449	0.118	1.0^{ref} (1.0^{ref})	
TT&TC	78	62			0.530 (0.237-1.183)	

The first segment contains two alleles C, T and thus contains three genotypes TT, CC, TC. Through the Table (3), we found that there are significant differences between G1, G2, which means that there are differences between healthy people and patients T2DM, meaning that the SLC30A8 gene is related to diabetes. Based on the values in Table (3), found that individuals carrying the TC genotype of rs 13266634 manifested an increased risk of T2DM in comparison with those carrying the TT genotype (OR = 0.375, 95%CI = 0.153-0.920, P-value = 0.029 < 0.05). these results consistent with the finding by[15] . This means that genotype TC is the most influential in developing diabetes. Also, we observed that there is a statistically significant relationship between the C allele and the T allele, where the percentage of T allele in patients is less than T in control. Also, C allele for patients is almost double of C for control, and this led to the P-value= 0.003, and this difference in the ratio of C, T for patient and control led to the variation in alleles TT, TC]] and CC. Also, the CC genotype of rs 13266634 manifested an increased risk of T2DM compared with those carrying the TT genotype (OR = 0.444, 95%CI = 0.196-1.009, P-value = 0.049 < 0.05). these results consistent with the finding by [16] In the dominant model, observed that the TC & CC genotype of rs 13266634 showed an effect on increasing risk of T2DM compared with the TT genotype (OR = 0.412, 95%CI = 0.213-0.786, P-value = 0.008 < 0.05),). these results consistent with the finding by [17] [18] While in the recessive model, there was showed no significant association found when compared TT & TC genotype with CC genotype, where (OR = 0.530, 95%CI = 0.237-1.183, P- value = 0.118 > 0.05).). these results consistent with the finding by [19] [20]

4. Conclusions

In summary, the results showed there is association between SLC30A8(rs 13266634) gene

polymorphisms and T2DM, we found that individuals carrying the TT genotype of T/C had an increased risk of Infection with T2DM compared to the CC genotype (p-value = 0.027 < 0.05), also individuals carrying C allele of T/C had an increased risk of Infection with T2DM (OR = 0.466, 95%CI = 0.282-0.770, p-value = 0.003 < 0.05), and individuals carrying CC&TC genotype of T/C had increased risk of T2DM compared with the TT genotype (OR = 0.412, 95%CI = 0.213- 0.786, p- value = 0.008 < 0.05).

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