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Research Article

Exploring the Relationship between *SLC30A8* Gene Polymorphism and Type 2 Diabetes Susceptibility in District Vehari, Pakistan

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Abstract: Type 2 diabetes mellitus (T2DM) is estimated to afflict 537 million individuals globally and has reached an epidemic scale. These global estimates are to develop innovative preventive and treatment methods and to put these methods into action. To investigate if *SLC30A8* gene polymorphisms can be used to predict the onset of T2DM in residents of Punjab, Pakistan, two groups were established based on prospective follow-up of appropriate population samples. Males made up 29.6% and women 70.4% of the T2DM unit. Deoxyribonucleic acid (DNA) was separated. Polymerase chain reaction or PCR was used for genotyping, and real-time PCR was then conducted. The statistical analysis was performed utilizing the statistical package SPSS 16.0 software program. The *SLC30A8* gene genotype TT rs13266634 was linked to an increased risk of type 2 diabetes mellitus (T2DM) (relative risk — RR 1.51, 95% confidence interval — CI 1.11 – 2.05, p = 0.008). A protective benefit against T2DM was linked to the *SLC30A8* gene's CC genotype, rs13266634 (RR 0.57, 95% CI 0.35 – 0.92, p = 0.026). The T2DM group comprised 442 individuals in the District Vehari. The average age at the time of the initial screening was 56.2 ± 6.7 years. 531 individuals without diabetes were chosen to serve as controls; their average age was 56.1 ± 7.1 years. In the control group, the frequencies of single nucleotide polymorphisms (abbreviated as SNP) match the expected frequencies as per the Hardy–Weinberg equilibrium. The *SLC30A8* gene's rs13266634 polymorphism shows its correlation with the likelihood of developing T2DM and can be a potential candidate for a diabetes risk score.

Keywords: Genotype, rs13266634, *SLC30A8*, Single Nucleotide Polymorphism, Type 2 Diabetes Mellitus.

1. INTRODUCTION

Type 2 diabetes mellitus (T2DM) accounts for 537 million instances of diabetes globally. In 2021 the International Diabetes Federation (IDF) concluded 32,964,500 total cases of adults who had diabetes, indicating a 26.7% prevalence, among 123,526,400 total population of adults in Pakistan [1]. In 2022, the International Diabetes Federation appraised that 26.7% of Pakistani adults had diabetes, accounting for over 33,000,000 diabetic cases [2]. Diabetes has multiple recognized etiological causes, including genetic mutation, physiological changes, societal pressures, and unhealthy lifestyle choices. One of the most fatal diseases is diabetes, also referred to as glucose intolerance. It ranks as the fourth most deadly illness at the moment, and its prevalence is quickly increasing [3].

There are four different types: Insulin production by the pancreas is impaired in people with Type 1 Diabetes Mellitus (Insulin-dependent or IDDM), whereas, in Type 2 (Non-Insulin dependent or NIDDM), in which the body fails to react correctly to the action of produced insulin, Type 3 (gestational), and Type 4 including monogenic diabetes syndrome (Neonatal diabetes or diabetes among individuals below 25), exocrine pancreatic disease (pancreatitis, cystic fibrosis) and drug or chemically induced diabetes [4]. A chronic illness known as type 2 diabetes is brought on by improper insulin activity in the body. It is characterized by tissue resistance to insulin action, which increases levels of blood glucose. Type 2 diabetes is brought on by the pancreatic cells that produce insulin. The body receives a release of the hormone insulin from the pancreas when blood sugar (glucose) levels rise.

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Approximately 90% of instances of diabetes are type 2, which is significantly more frequent than type 1. Adolescence is when it typically happens. The body generally reacts poorly to insulin, which is why the pancreas here does not produce enough of it to maintain normal blood glucose levels [5, 6]. In recent years, there has been a notable increase in the prevalence of type 2 diabetes mellitus (T2DM), a condition that has substantial socioeconomic repercussions. According to the "International Diabetes Federation", there are 537 million adult cases of diabetes worldwide among people aged 20 to 79, and by 2045, there should be 783 million cases (IDF Diabetes Atlas 10th Edition). This disorder is the cause of high mortality due to repercussions such as cerebrovascular abnormalities, amputations, cardiovascular diseases, and renal failure. The disease's complicated etiology encompasses a number of variables that affect a person's risk and prognosis, including family history, ethnicity, a poor diet, a sedentary lifestyle, obesity, and dyslipidemia [7, 8]. In terms of genetics, extensive global research has demonstrated the correlation between this illness and many genotypic variations of almost 80 potential genes. The majority of the variations found in these genes have an impact on insulin secretion, however, it's still unclear exactly how they work at the molecular level [9].

In addition to having high blood sugar, people with T2DM have the possibility of developing multiple other conditions, including heart attacks, strokes, renal failure, blindness, and amputation of lower limbs. The prevalence of diabetes is predicted to increase further, affecting about one in three people by the year 2050 [10]. One of the consistently reported risk factor SNPs to T2DM is the rs13266634 SNP found in the *SLC30A8* gene. Located on chromosome 8q24.11, the *SLC30A8* gene is made up of eight 37kb exons that encode a 369 amino acid protein, zinc transporter protein member-8 (ZnT-8) [11]. The rs13266634 is a missense SNP in the last exon of *SLC30A8* gene, where a change between the two nucleotides C/T occurs, leading to alteration between the two amino acids arginine (R) and tryptophan (W) at the position 325 (R325W). The risk C allele that encodes for arginine has shown an association with lower early insulin response to glucose and increased susceptibility to developing T2DM disease [12, 13]. While the T allele acted as a protective polymorphism against the onset of T2DM the rs13266634 C allele raised the chance of developing T2DM [14, 15]. Nonetheless, our aim in this research project is the diagnosis of T2DM patients by investigating the genotypic frequencies of rs13266634 SNP of gene *SLC30A8*, among Pakistani individuals in the District Vehari.

2. MATERIALS AND METHODS

This one-year study was conducted in 2023 among 1000 Individuals; out of these, only 442 individuals were selected for further research purposes, while the remaining 558 were excluded. Individuals were selected from DHQ Hospital of District Vehari, in the 30–79 age range, who were T2DM affected and those who were solely taking T2DM medication, and further experimental lab work was conducted in labs of the Institute of Microbiology and Molecular Genetics of Punjab University, Lahore. Patients who were smokers or consumers of nicotine, alcoholic, expected women, who were taking steroid medications, like prednisolone, and had other types of diabetes were excluded. The salting out method for the extraction was used to separate DNA from blood. The known information about the polymorphisms of the candidate genes and their correlation with T2DM guided the selection process. Additionally, potential mechanisms of their application in the pathophysiology of T2DM were considered throughout the gene selection process. The *SLC30A8* gene's rs13266634 polymorphism was detected using a polymerase chain reaction. The restriction fragment length polymorphism was then analyzed. Primer 5'-GTCAGAGCAGTCGCCCAT-3' (Forward primer, binds to the sense strand of target DNA, having 60% GC content and its melting temperature is approximately 58-60 °C) and 5'-CCTGGTCAACTGGAGATTCCA-3' (Reverse primer, binds to the antisense strand of DNA, 55% GC content and its Tm is approximately 56-58 °C) were employed to bind specifically to a target sequence with a high degree of complementarity with genotype of *SLC30A8* gene for rs13266634. 33 cycles of denaturation at 95 °C for 30 s, primer annealing at 56°C for 30 s, and elongation at 72 °C for 30 s comprised the temperature regime used for amplification. Restrictase MspI enzyme was used at 37 °C for 16 hours, which have recognition sites C↑CGG GGC↓C. Electrophoresis in 1% agarose gel was used to identify amplification and restriction products, which were then stained with ethidium

bromide. The amplification product measured 171 bp in size (Figure 1). Following restriction, products corresponding to the TT genotype, the CC genotype, and the heterozygous CT genotype were found.

2.1. Statistical analysis

The statistical software program SPSS 16.0 was used for statistical processing. The X^2 test was used to determine how well genotype frequencies matched the Hardy–Weinberg equilibrium. Using Fisher's exact two-tailed test and Pearson's Chi-Square or X^2 test, the significance of the genotype frequency differences between the T2DM group and the control group was determined. The threshold of significance was set at $p < 0.05$.

A binary logistic regression approach with a feature sequential inclusion function was utilized to create statistical risk assessment models. The genotype and allele frequencies of the two investigated polymorphisms in genes (*SLC30A8*) among the T2DM unit and the untreated or control group were analyzed. Furthermore, these frequencies were assessed independently in males and females under the age of 55 and in those over the age of 55.

3. RESULTS

With a verified diagnosis of a new case of T2DM, the T2DM group comprised 443 individuals (70.4% women and 29.6% males). The average age at the time of the initial screening was 56.2 ± 6.7 years. A total of 532 individuals (32.7% males and 67.3% females) with no history of diabetes were chosen as controls; their average age was 56.1 ± 7.1 years. The Hardy–Weinberg equilibrium predicts that the observed frequencies of SNP genotypes rs13266634 of the *SLC30A8* gene in the control group correspond to the predicted frequencies (X^2 = 0.52), see Table 1 and Figure 2.

The T2DM group's proportion of homozygotes with CC showed a substantial decrease (odds ratio (OR) 0.575; 95% confidence interval (95% CI) 0.36 – 0.93; $p < 0.026$). Therefore, it is likely that the

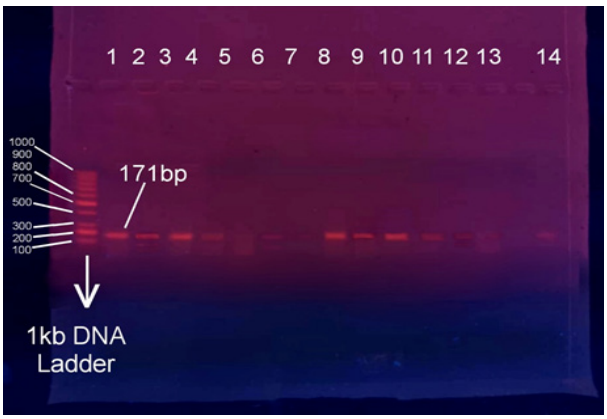

Fig. 1. Pictorial representation of gel electrophoretic results in which a 1kbp DNA Ladder was used. The product measured 171 bp in size. This image was taken under a UV spectrophotometer, making Ethidium bromide with DNA, glow and make DNA visible to the human eye.

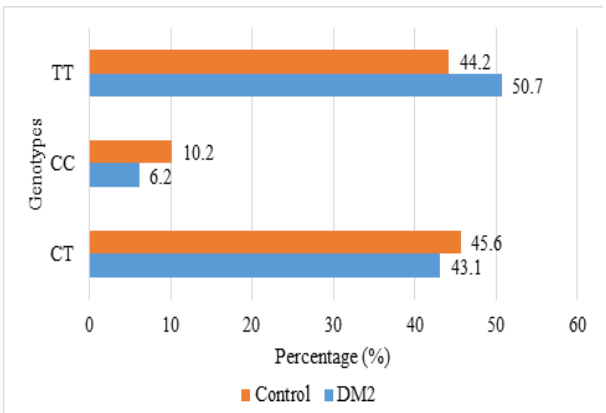

Fig. 2. Bar Chart representation of genotype frequencies of the *SLC30A8* gene's rs13266634 polymorphisms are compared between those with type 2 diabetes mellitus unit and the control group.

Table 1. Groups with and without disease of type 2 diabetes mellitus for the investigated polymorphisms' genotype frequencies.

Gene	Genotype	Group T2DM		Control group	
		n	%	n	%
SLC30A8	TT	224	50.7	235	44.2
	CC	27	6.2	54	10.2
	CT	191	43.1	242	45.6

***Note:** n is the number of individuals. Here TT genotype of the *SLC30A8* gene depicts higher frequencies in the type 2 diabetes mellitus group as compared to the genotypic frequencies of the control group for rs13266634 polymorphism. In other words, CC and CT genotypic frequencies are higher in the control group than in T2DM patients.

homozygous CC genotype of the *SLC30A8* gene's rs13266634 polymorphism confers conditional protection against T2DM. The frequency of genotypes of the *SLC30A8* gene's rs13266634 polymorphism showed statistically significant differences solely in women when groups divided by sex were compared. Compared to carriers of the other two genotypes, women carrying the TT genotype have a 1.5-fold increased risk of having T2DM (OR 1.51; 95% CI 1.11 – 2.05; p = 0.008), see Table 2 and Figure 3. There were no statistically significant differences between the T2DM and control groups when comparing the genotype frequencies of the *SLC30A8* gene's rs13266634 polymorphism, segregated by age (p > 0.05 in both groups). But when the groups were split out according to age and sex, women 55 years of age and above showed statistically significant differences (p = 0.032), as shown in Table 3 and Figure 4.

The findings of this study indicate that there were no statistically significant variations in the percentage of women aged 55 and above who were TT homozygotes or CT heterozygotes of the *SLC30A8* gene's rs13266634 polymorphism in the T2DM group as opposed to the control group (p = 0.055 and p = 0.455, respectively). In the T2DM

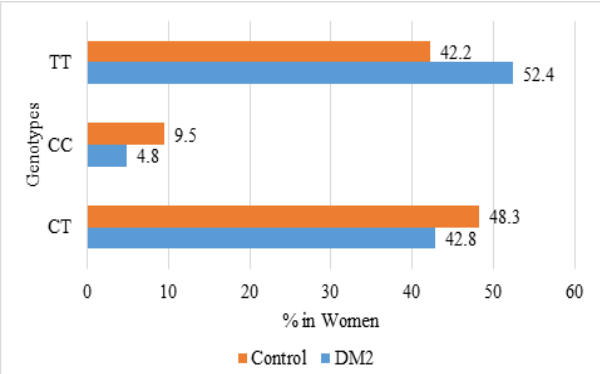

Fig. 3. This Bar chart represents a comparison of the *SLC30A8* genotype frequencies (rs13266634) in women with type 2 diabetes mellitus disease and the control group.

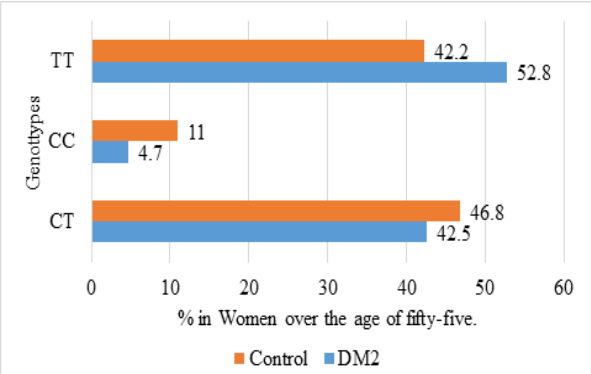

Fig. 4. This Bar chart represents a comparison of the *SLC30A8* genotype frequencies (rs13266634) in women over the age of fifty-five with type 2 diabetes mellitus disease and the control group.

Table 2. Genotype frequency of the *SLC30A8* gene's rs13266634 polymorphism, broken down by sex, in the groups with diabetes type 2 mellitus and the control group.

Genotype	**T2DM**		**Control**	
In Men				
	n	%	n	%
TT	61	46.6	84	48.5
CC	12	9.1	20	11.6
CT	58	44.3	69	39.9
Total	131	100	173	100
			p = 0.666	
In Women				
	n	%	n	%
TT	163	52.4	151	42.2
CC	15	4.8	34	9.5
CT	133	42.8	173	48.3
Total	311	100	358	100
			p = 0.008	

***Note:** Here, p denotes the significance, and n represents the number of persons. Men with type 2 diabetes mellitus disease and the control group's *SLC30A8* genotype frequencies (rs13266634) are shown in the first half of the table, which shows a significant value of p = 0.666 among men. The table's second half shows a significant value of p = 0.008 for women, allowing us to compare the genotype frequencies of *SLC30A8* (rs13266634) between women with type 2 diabetes and the women in the control group. Overall, TT genotypic frequencies are greater in both situations.

Table 3. Frequencies of genotypes and alleles of the rs13266634 polymorphism of the *SLC30A8* gene in the group of type 2 diabetes mellitus and the control group in women aged 55 years and older.

Genotype	T2DM		Control	
	n	%	n	%
TT	88	52.8	79	42.2
CC	8	4.7	21	11.0
CT	71	42.5	88	46.8
Total	167	100	188	100
			p = 0.032	

***Note:** n is the number of women over the age of fifty-five. This table depicts the *SLC30A8* genotype frequencies (rs13266634) in women over the age of fifty-five with type 2 diabetes mellitus disease and the control group.

group of women 55 years of age and above, there was a noteworthy decline in the percentage of CC homozygotes (OR 0.4; 95% CI 0.17 – 0.93; p = 0.033). Therefore, it may be concluded that the homozygous genotype CC is conditionally protective against T2DM in women 55 years of age and older and that the differences between it and the genotype at TT, while statistically insignificant, are close to threshold values.

4. DISCUSSION

To study or to overcome diabetes among the Pakistani population we explored the baseline characteristics like the genotypic frequencies of the *SLC30A8* gene's SNP rs13266634 among diabetic and non-diabetic (control) groups. Between 2021 and 2045, middle-income nations are predicted to experience the largest relative rise in the overall incidence of diabetes (21.1%), in contrast to high-income-(12.2%) and low-income (11.9%) countries. The predicted cost of diabetes-related medical expenses worldwide was 966 million dollars in 2021 and is expected to rise to 1,054 billion USD by 2045. Approximately 463 million individuals globally have diabetes, which translates to more than 10.5% of adult adults worldwide living with this illness [16]. Medical professionals also think that Pakistan's diabetes epidemic is partly caused by poor eating habits, inactivity, and an increase in obesity [17]. Over a year, the combination drug linagliptin and metformin preserved the comparable safety profile and clinically substantial improvements for glycemic control [18]. Zinc transporter ZnT-8 promotes the collection of zinc across the cytoplasm towards intracellular vesicles; zinc transporting zinc to insulin synthesis and/or storage mechanisms within insulin-secreting pancreatic β-cells may be mediated by ZnT-8. Insulin synthesis & storage depend on zinc. In terms of pathological conditions, zinc and both types of diabetes seem to have intricate relationships [19, 20]. A significant amount of illness risk can be explained by a polymorphism within the zinc transporter *SLC30A8*, which is only expressed in β-cells that produce insulin. This polymorphism also serves as proof of concept for a genome-wide strategy for the elucidation of complicated hereditary features [21]. Growing older and a longer period are linked to the prevalence of diabetes [22]. ZnT8 trafficking to the cell surface is facilitated by glucose-stimulated insulin secretion (GSIS) [23]. Zinc transporter 8 (ZnT8) serves as a prominent autoantigen, that is widely distributed on the surface of β-cells. In type 1 diabetes, this particular molecular target may protect β-cells from autoimmune assaults [24].

Since there are significant variations among human and rodent models, novel strategies include lower-frequency variants for a tool for clarifying gene functions, enabling greater comprehension of the illness and offering potential therapeutic targets. Most studies regarding the role of ZnT8 in T2DM have focused on animal models and frequent high-risk variants [25, 26]. In-depth research on the interactions between all of the underlying elements, such as gene polymorphism, is necessary to have a better knowledge of this disease [27]. The information gathered regarding the *SLC30A8* gene's rs13266634 polymorphism is in line with the findings of prior investigations. It has previously been demonstrated that variations in this gene are linked to the onset of T2DM in several populations [28 - 30]. Specifically, research conducted by Russian scientists revealed that having the T allele raises the likelihood of having T2DM (OR = 1.36), but having the C allele lowers this likelihood (OR = 0.74) [31]. There are conflicting findings on the relationship between T2DM and the *SLC30A8* gene's rs13266634 polymorphism. This could be

brought about by variations in the racial, ethnic, and regional makeup of the populations under study, as well as in the methods used for sampling and analysis. In the Chinese population, the C allele of the rs13266634 polymorphism is linked to the dysregulation of glucose and T2DM [32]. The rs13266634 polymorphism of the *SLC30A8* gene has a moderate effect on the susceptibility to type 2 diabetes, according to research on its relationship with T2DM in India [33]. rs13266634 may be a significant genetic risk factor for T2DM in Asian and European populations, but not in African people, according to many meta-analyses [34]. The protective impact of SNP rs13266634 of the *SLC30A8* genome on T2DM disease in Pakistani women 55 years of age and older is validated and supported by the results of the prospective investigation of the study [35]. Diabetes mortality can be decreased by closely monitoring blood glucose levels because the consequences of the disease are growing increasingly severe. Diabetes mellitus affects women more frequently. If we consider how long the disease has lasted, we can determine that women who have diabetes mellitus have doubled in number. Nonetheless, based on the comparison of men's and women's adherence to medication, it may be inferred that males are marginally more likely than women to take medication [36]. When it comes to following a diet, it is evident that men are more likely than women to break their diets. The incidence of diabetes is correlated with age and duration of time as aging is associated with a decline in insulin sensitivity and beta-cell function, which is crucial for regulating blood sugar levels so, that when people grow older the chances of diabetes increase. The longer the person is exposed to risk factors such as poor diet, sedentary lifestyle, obesity, and genetic predisposition, the higher the likelihood of developing diabetes as, over time, these factors can lead to insulin resistance and beta-cell dysfunction, culminating in diabetes [37]. Nonetheless, in this research project, we explored the relationship among T2DM patients and investigated the genotypic frequencies of rs13266634 SNP of gene *SLC30A8*, among Pakistani individuals.

Clinical significance of the results The SNPs that were shown to be likely T2DM markers had results of linkage with T2DM, were distinct from those of the Pakistani population, and were also examined for the inaugural time. It is thought to be possible to include the examined gene variations in the disease's risk scale model. Thus, the *SLC30A8* gene's rs13266634 polymorphism, which has been linked to the occurrence of T2DM, can now be included in the T2DM risk meter.

5. CONCLUSIONS

The *SLC30A8* gene's rs13266634 polymorphism has demonstrated its correlation with the likelihood of developing T2DM and thus is a viable option for inclusion into the T2DM genetic risk meter. The *SLC30A8* gene's genotype TT of rs13266634 was linked to a 1.5-fold increased risk of T2DM (RR 1.51, 95% CI 1.11 – 2.05, $p = 0.008$) in Men, Women, and even in specific groups of women over the age of fifty-five. The T2DM group's proportion of homozygotes with CC showed a substantial decrease (odds ratio (OR) 0.575; 95% confidence interval 0.36 – 0.93; $p < 0.026$). Therefore, it is likely that the homozygous CC genotype of the *SLC30A8* gene's rs13266634 polymorphism confers conditional protection against T2DM. To completely describe the underlying, causative variant, and frequently the causal gene itself, a lot of obstacles still need to be overcome. The future appears promising for the creation of innovative treatments and diagnostics for diabetes of the type 2 variety and its associated characteristics, nevertheless, if progress is made on these fronts.

6. ETHICAL APPROVAL

Ethical approval was obtained from the Microbiology and Molecular Genetics, Institutional Research Ethics and Biosafety Committee under reference number D/227/MMG.

7. CONFLICT OF INTEREST

The authors declare no conflict of interest.

8. ACKNOWLEDGMENT

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9. AUTHOR'S CONTRIBUTION

FG performed research work and did data analysis with the writeup, SR did proofread and helped in data analysis.

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