Trends in Medical and Biological Sciences

Journal homepage: https://scholarsacademiaworld.com/index.php/TMBS

Association between Glycated Hemoglobin Level and Sorting Nexin 19 Gene Polymorphism in Individuals with Type 2 Diabetes

Amjad Khan [1], Adil Khan [2], Shakir Ullah [3], Taj Ali Khan [4], Muhammad Nabi [5], Naveed Ahmad [6], Abdul Jabbar [7]

[1] Department of Medical Lab technology, University of Haripur, Haripur, Pakistan, Institute of Pharmaceutical Sciences, Khyber Medical University, Peshawar, Pakistan amjad.ips@kmu.edu.pk

[2] Institute of Pharmaceutical Sciences, Khyber Medical University, Peshawar, Pakistan adil624999@gmail.com

[3] Institute of Pharmaceutical Sciences, Khyber Medical University, Peshawar, Pakistan, Institute of Basic Medical Sciences, Khyber Medical University, Peshawar, Pakistan shakir.ibms@kmu.edu.pk

[4] Institute of Pathology and diagnostic Medicine, Khyber Medical University, Peshawar, Pakistan tajalikhan.ibms@kmu.edu.pk

[5] Institute of Pharmaceutical Sciences, Khyber Medical University, Peshawar, Pakistan muhammadnabi.ips@kmu.edu.pk

[6] Institute of Pharmaceutical Science, Faculty of Life Sciences & Medicine, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London SE1 9NH, United Kingdom naveed.ahmad@kcl.ac.uk

[7] Department of Medical Lab technology, University of Haripur, Haripur, Pakistan abdul.jabbar@uoh.edu.pk

ARTICLE INFO

Received:
January 12, 2025

Revised:
January 25, 2025

Accepted:
February 8, 2025

Available Online:
February 22, 2025

Keywords:
Type 2 diabetes; Sorting nexin 19 gene; polymorphisms; HbA1c

Corresponding Author:
Amjad Khan
amjad.ips@kmu.edu.pk

ABSTRACT

Background: Sorting Nexin 19 gene has an important role in different diseases and controls the transport of different hormones and peptides. But his role in type 2 diabetes is still not explored. The Sorting Nexin 19 gene is associated with insulin signaling. Therefore, the current study was conducted to investigate the association between the Sorting Nexin 19 gene and HbA1c levels in individuals with type 2 diabetes

Methods: This prospective cohort study was conducted at the Institute of Pharmaceutical Sciences, Khyber Medical University, and endocrinology department of Hayatabad Medical Complex, Peshawar. A total of 60 type 2 diabetes individuals were enrolled, and blood samples were collected from each participant. Non-probability purposive sampling was used. Blood was collected at baseline to measure HbA1c levels, DNA extraction, and conduct Sanger sequencing. After three months of follow-up, blood sample was collected to determine HbA1c level.

Results: The mean age of patients was 54.37±11.61±SD. There was no Sorting Nexin 19 rs431825173 gene mutation among the selected patients. Notably, other Sorting Nexin 19 polymorphisms on a flanking region of rs431825173, specifically rs3751036 (C>G), were detected. Out of the total 60 samples, 2 were of the CC wild type, 41 were GG homozygous, and 17 were heterozygous GC. It has been observed that rs431825173 mutation is not deleterious and has no clinical significance in type 2 diabetes.

Conclusion: The study concluded that no association was found between sorting nexin 19 gene polymorphisms and HbA1c levels and clinical outcome in type 2 diabetes patients.

Background

Diabetes mellitus is a metabolic disorder characterized by persistent hyperglycemia, resulting from irregularities in insulin activity or secretion (Petersen & Shulman, 2018). The American Diabetes Association (ADA) introduced a widely recognized classification scheme for diabetes in 1997, which categorizes the disease into four main types: Type 1, Type 2, Other Specific Types, and Gestational Diabetes Mellitus (GDM). Type 2 diabetes (T2D) accounts for 90-95% of diabetic patients, primarily affecting adults (Kharroubi & Darwish, 2015). Obesity increases the risk of T2D development, especially in individuals with a family history of the disease (Lyssenko et al., 2008). The global prevalence of T2D is expected to reach 587 million by 2045 (Al-Rifai et al., 2019). In Pakistan, the prevalence of T2D is 11.77%, with varying rates across different provinces. Province-wise, the prevalence rates in Pakistan are Sindh (16.2% in males, 11.7% in females), Punjab (12.14% in males, 9.83% in females), Baluchistan (13.3% in males, 8.9% in females), and Khyber Pakhtunkhwa (9.2% in males, 11.6% in females) represented in figure S1 (Meo, Zia, Bukhari, & Arain, 2016). Genetic studies have identified 75 susceptibility loci linked to T2D (Saraiva, Vieira, & O'garra, 2019). It has been observed that the prevalence and incidence of type 2 diabetes is quite high in Pashtun population. Among the different causes consanguineous marriage is high in Pashtun population and increase the risk of familial type 2 diabetes. Over the past 25 years, various approaches to investigating the genetics of type 2 diabetes (T2D) have produced evidence supporting the idea that insulin resistance alone does not cause the disease in humans (Raciti et al., 2015). Different factors are involved in T2D, in which genetic factors play crucial role (Jensen et al., 2020). The subfamily of sorting nexins (SNX-PXA-RGS-PXC) belongs to the superfamily of SNX proteins, that perform a variety of functions in membrane trafficking and cellular signaling (Amatya et al., 2021). A member of this family with 992 amino acids, SNX19 is connected to insulin signaling; specifically, its decreasing lowers the insulin secretion (Harashima et al., 2012). SNX19 gene has a role in insulin production and insulin regulates blood glucose levels; in the absence of this, blood glucose binds to hemoglobin, raising glycated hemoglobin levels (Norton, Shannon, Gastaldelli, & DeFronzo, 2022). While SNX19's role in type 1 diabetes is studied, its impact on T2D, especially glycated hemoglobin levels, remains unexplored (Hu, Zhang, Cai, Harashima, & Notkins, 2005). It has been discovered that the transmembrane protein (insulinoma-associated protein 2 (IA-2) which is present on dense core vesicles (DCVs) and SNX19 interact with each other. About 70 to 80 percent of newly diagnosed individuals have auto antibodies against IA-2, a key autoantigen in T1D (Kawasaki, 2023). SNX19 has a significant role in the development of types 1 diabetes but its role in type 2 diabetes has not been explored yet, especially its impact on the level of HbA1c. The aim of this study is to determine the association of SNX19 gene polymorphism with HbA1c level and clinical outcome in T2D patients.

Methods

Study Setting

Research was conducted in the Endocrinology Department of Hayatabad Medical Complex, Peshawar, and the Institute of Pharmaceutical Sciences at Khyber Medical University (IPS-KMU). A total of 60 whole blood samples were collected from patients with type 2 diabetes using consent form and sample size was calculated using Open Epi (https://www.openepi.com/SampleSize/SSCohort.htm). It was a prospective cohort study and its duration was 1 year. The study was approved by the Advanced Study and Research Board (ASRB) and Ethical Board (UOH/DASR/2024/2242).

Inclusion and Exclusion Criteria

The inclusion criteria for study were type 2 diabetic patients for metformin, willing to participate in the study, HbA1c level >6.5%, age group 30-80 and exclusion criteria was type 1 diabetic patients, gestational diabetic patients, other co-morbidities and drug used which enhance sugar level.

All patients were enrolled on written consent form, which outlined the purpose, risks, and benefits of the study, was provided to each participant.

Blood Sampling and Analysis

Blood sample was collected at baseline and at 3rd month of the therapy. The blood was used to measure the HbA1c level and extract DNA for genetic analysis. The HbA1c test was performed on freshly collected samples, which were then stored at -20°C for future DNA extraction. HbA1c level was measured using Enzyme Linked Immunosorbent Assay (ELISA) on cobas c 503, lot No. 08056668 190.

Genetic Analysis

DNA extraction was carried out using the commercially available Thermo Fisher Scientific extraction kit, following the instructions provided in the kit's literature. The sequence of Sorting Nexin 19 rs431825173 was retrieved from the dbSNP database on the NCBI website. Primers for rs431825173were designed using the Primer3 tool. The target sequence is shown below Figure 1.

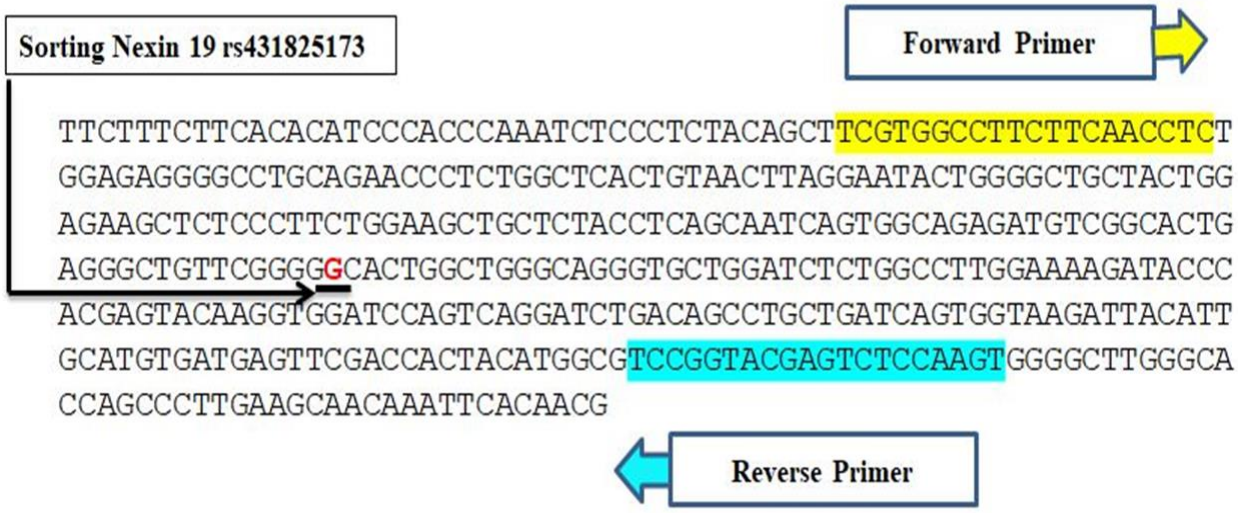

Figure 1: Primers for target sequence of sorting nexin 19 gene

SNX19 (rs431825173) was sequenced using Sanger sequencing. Approximately 20 µL of each PCR product sample was individually dispensed into separate sterilized 0.2 µL PCR tubes. The tubes were then sent to the Genome Sequencing Laboratory at Khyber Medical University via a proper cold chain. The sequencing of the samples was carried out using the SeqStudio™ Genetic Analyzer from Life Technologies show in figure 2. Following sequencing, the samples were analyzed using Finch TV, through chromatogram analysis and nucleotide blast in NCBI.

Figure 2: Seqstudio™ Genetic analyzer used for sequencing

Statistical Analysis

Data was represented in Office Excel 2010 and analyzed using the Statistical Package for Social Sciences (SPSS) version 27. Dependent continuous variables, specifically HbA1c levels, were analyzed using T-tests and ANOVA. Categorical variables were defined as responders if the decrease in HbA1c was ≥0.8% and non-responders if the decrease was less than 0.8%. The association between the SNX19 gene and the decrease in HbA1c in type 2 diabetes was analyzed using the One-way ANOVA and Fisher's exact test. All results were presented in graphs, figures, and chart.

Results

Demographic and Clinical Characteristics

The current study included 31 (52%) female and 29 (48%) male participants with type 2 diabetes. A significant proportion of participants, 47, reported a positive family history and 13 had negative family history of diabetes Shown in Figure 3.

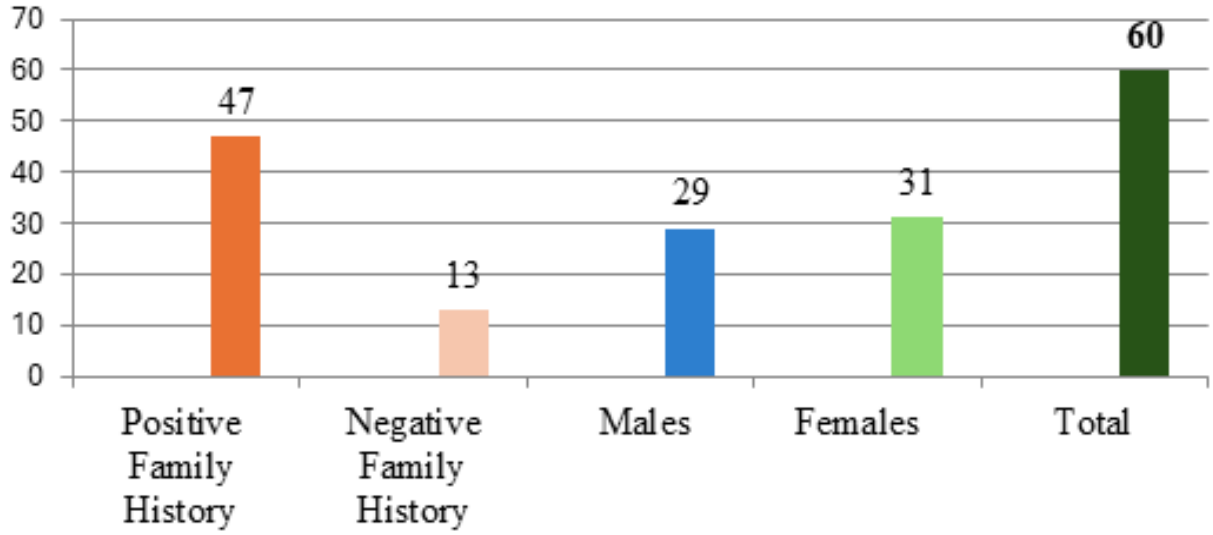

Figure 3: Distribution of gender and family history (N=60). Bar chart is used to show the demographic data

The mean age of enrolled patients was 54.37 ± SD years. The patients had a mean weight of 68.52 ± SD kg and a mean height of 5.44 ± SD feet. Their average Body Mass Index (BMI) was 25.29 ± SD. They received metformin in doses ranging from 500 to 1000 mg/day, averaging 840 ± SD mg/day. The mean Glycated Hemoglobin (HbA1c) levels were 9.3% at the baseline and 8.4% at the follow-up visit (Shown in Table 1). There were no statistically significant differences between males (52.41 ± SD years) and females (56.19 ± SD years) in age ($p = 0.81$), weight (71.62 ± SD kg vs. 65.61 ± SD kg; $p = 0.45$), BMI ($p = 0.49$), or administered dose (836.21 ± SD vs. 846.77 ± SD; $p = 0.85$). HbA1c levels at the first and second visits also showed no significant gender-based differences ($p = 0.81$ and 0.19, respectively). However, males had significantly greater height (5.63 ± 0.39 vs. 5.28 ± 0.32; $p = 0.0001$), as shown in Table 2.

Table 1: Demographic and clinical characteristics of study participants (N=60)

Variable	Range	Mean ± SD
Age (year)	35-75	54.37±11.61
Weight/kg	50-85	68.52±8.19
Height/feet	4.8-6.5	5.44±0.39
BMI	15.40-35.97	25.29±4.54
Metformin Dose (mg/day)	500-1000	840±210.76
HbA1c (1st visit)	6.60-12.50	9.3 ± 1.7
HbA1c (2nd visit)	4.78-11.40	8.4 ± 1.9

Table 2: Clinical and demographic characteristics based on gender (N=60). To compare the mean of male and female group Independent Sample T-Test was used and P valve was determined

Variable	Male	Female	P value
Age (year)	52.41± 11.30	56.19± 11.78	0.81
Weight (kg)	71.62 ± 7.13	65.61± 8.16	0.45
Height (feet)	5.63 ± 0.39	5.28 ± 0.32	0.0001
BMI	24.89±4.37	25.66±4.73	0.49
Metformin Dose (mg/day)	836.21 ± 214.18	846.77 ± 210.92	0.85
HbA1c(1st visit)	8.8 ± 1.6	9.7 ± 1.6	0.81
HbA1c(2nd visit)	7.9 ± 1.9	8.8 ± 1.8	0.19

Genotypes SNX19

Gel Electrophoresis was employed to assess the quality of the extracted DNA. Due to the large size of the DNA, a 1% Agarose gel was utilized. A 100-base pair (BP) ladder was run alongside the DNA samples to estimate the size of the extracted DNA. Figure 4 to 7 displays all 60 DNA samples on the gel, which were examined under a gel documentation system.

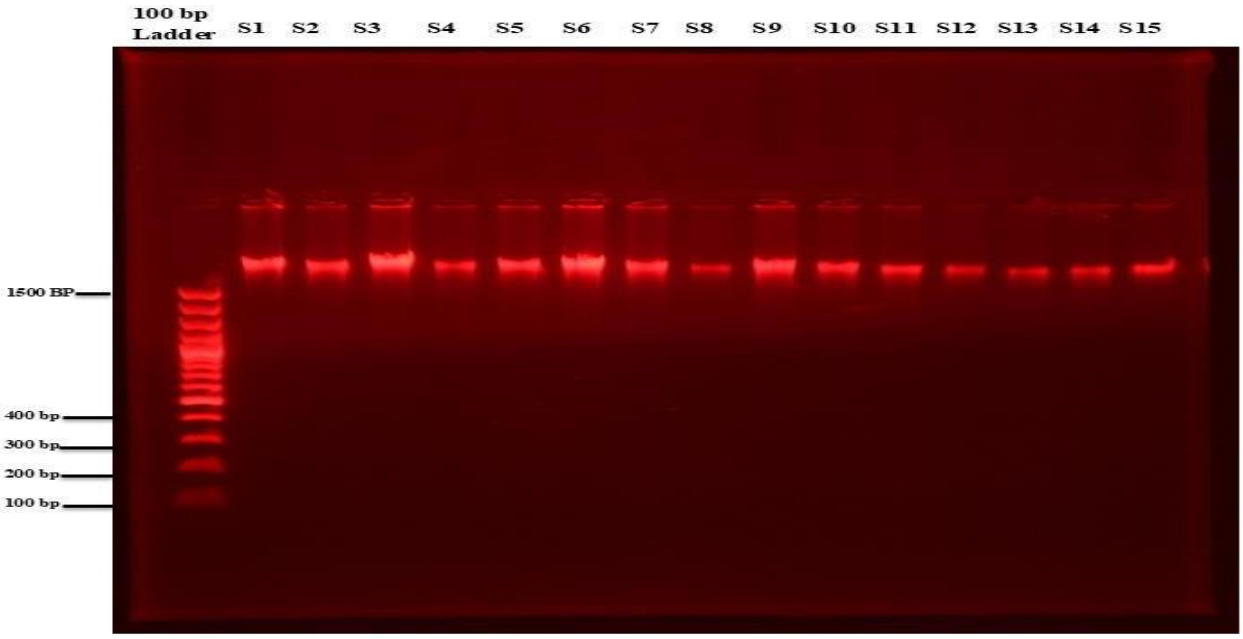

Figure 4: Confirmation of genomic DNA on 1% (w/v) agarose gel. L1: 100bp ladder.

S1- S15: Extracted DNA samples

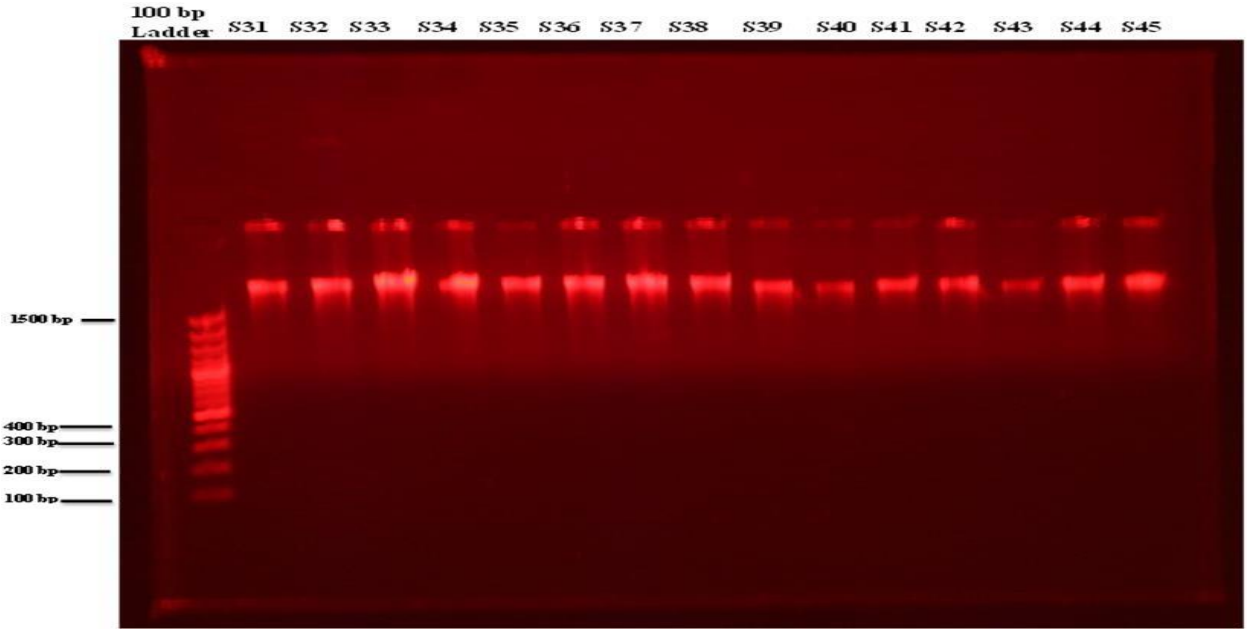

Figure 5: Confirmation of genomic DNA on 1% (w/v) agarose gel. L1: 100bp ladder.

S16-S30: Extracted DNA samples

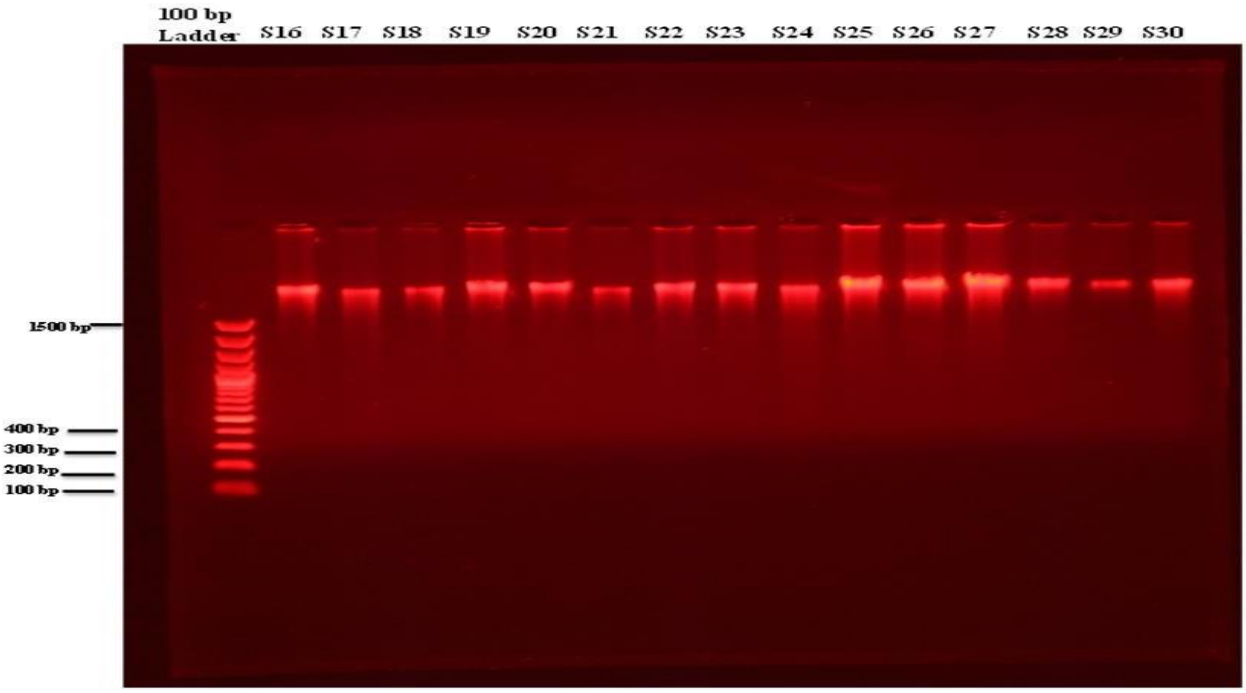

Figure 6: Confirmation of genomic DNA on 1% (w/v) agarose gel. L1: 100bp ladder.

S31- S45: Extracted DNA samples

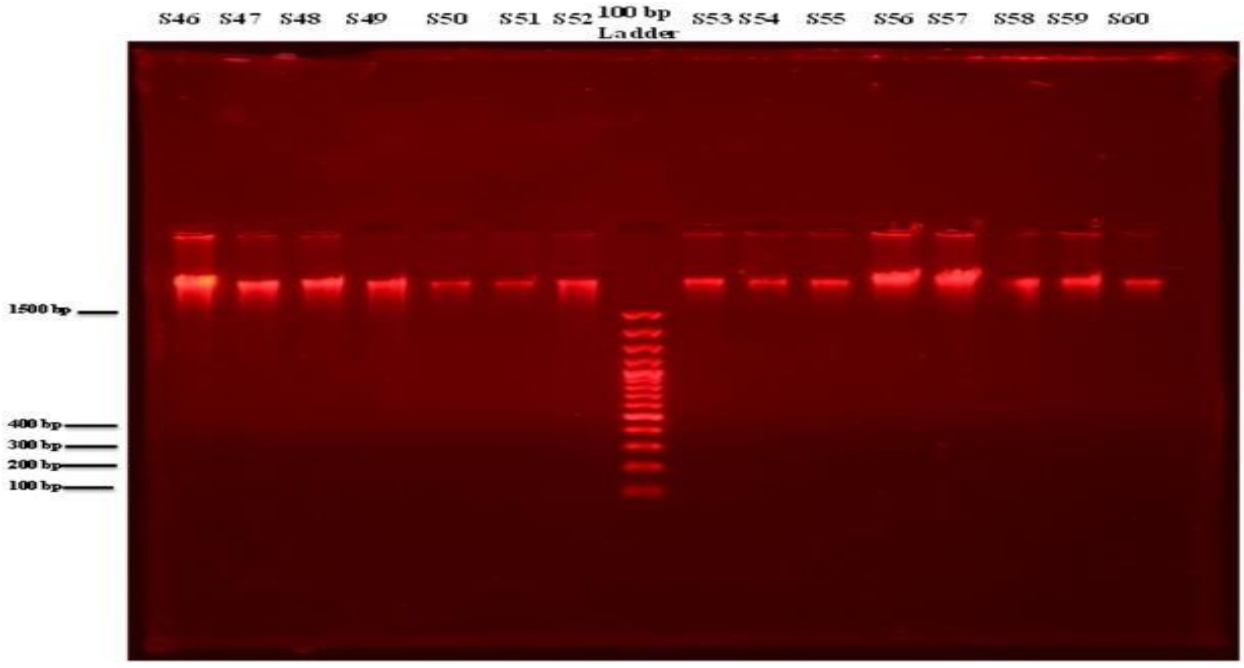

Figure 7: Confirmation of genomic DNA on 1% (w/v) agarose gel. L1: 100bp ladder.

S46- S60: Extracted DNA samples

The amplified product for the sanger sequence is shown in Figure 8 to 11. Gel Electrophoresis was used to evaluate DNA quality. A 2% Agarose gel and a 100-base pair (BP) ladder were employed to estimate the DNA size. The gel ran for 60 minutes at 80 volts. Clear, solid bands indicated sample purity, while smears or glowing bands suggested impurities.

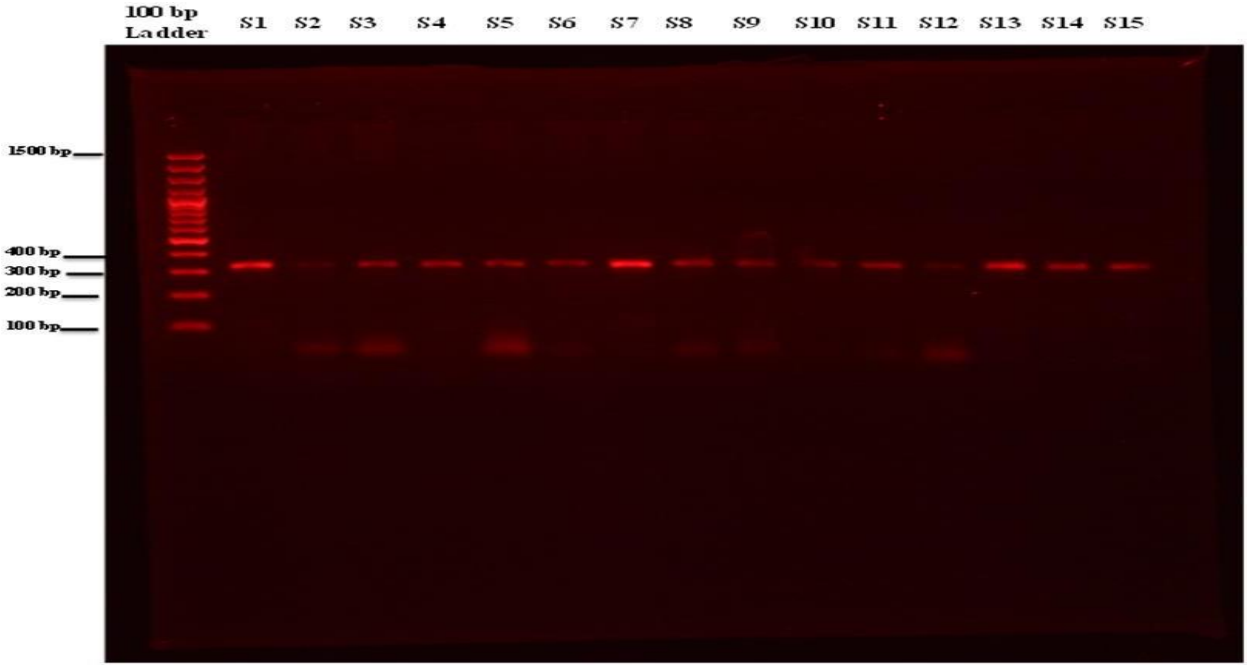

Figure 8: Representative 2 % (w/v) agarose gel of SNX19 amplified product. L1: 100bp ladder,

Lane S1- S15: amplified products

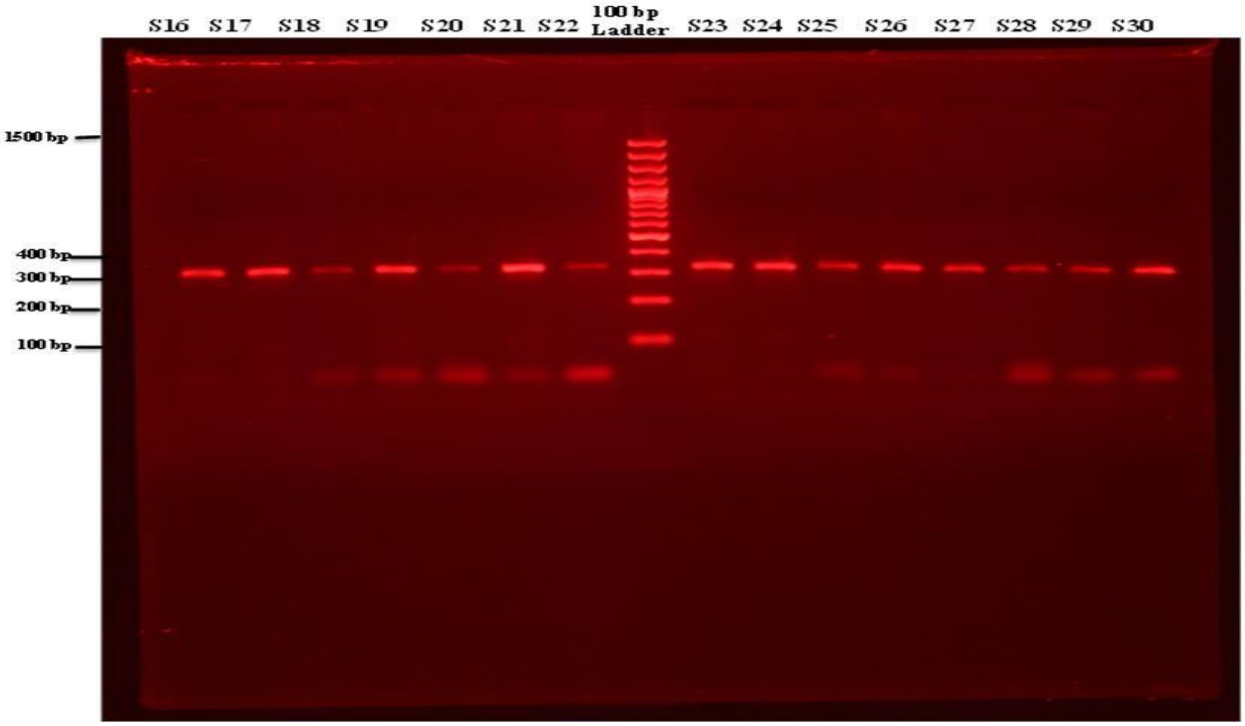

Figure 9: Representative 2 % (w/v) agarose gel of SNX19 amplified product. L1: 100bp ladder,

Lane S16- S30: amplified products

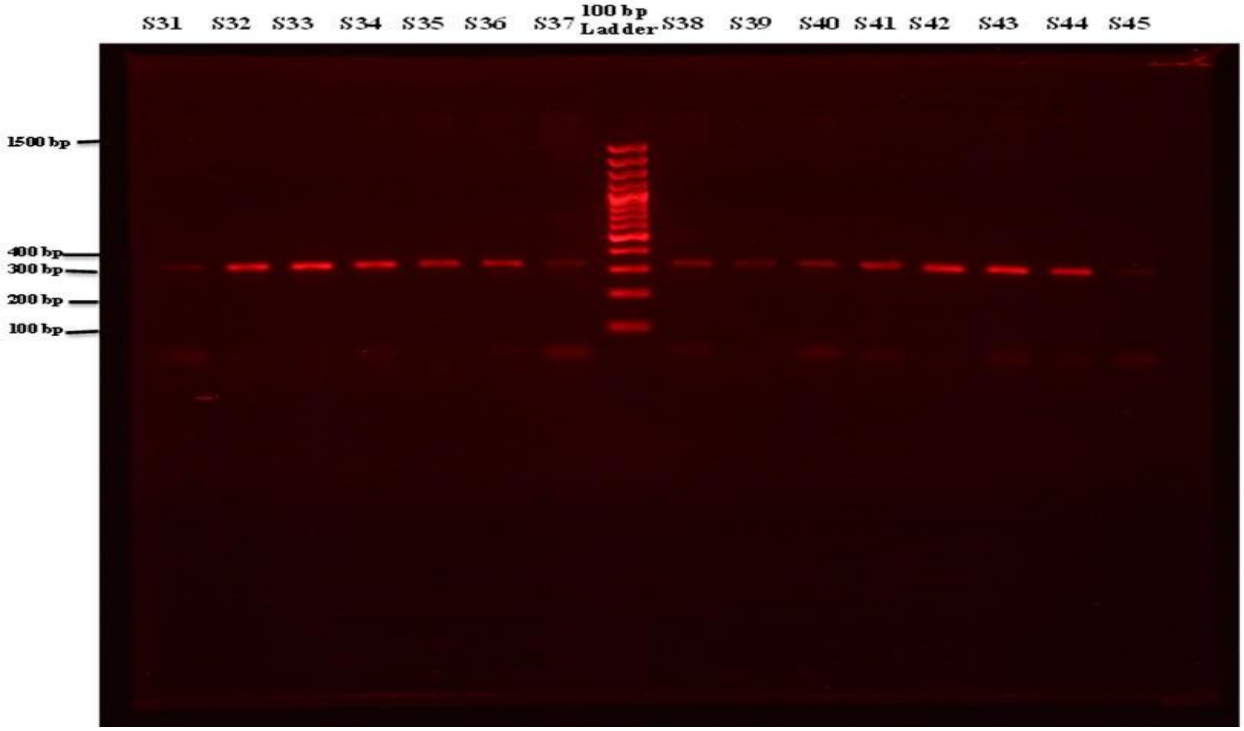

Figure 10: Representative 2 % (w/v) agarose gel of SNX19 amplified product. L1: 100bp ladder, Lane S31- S45: amplified products

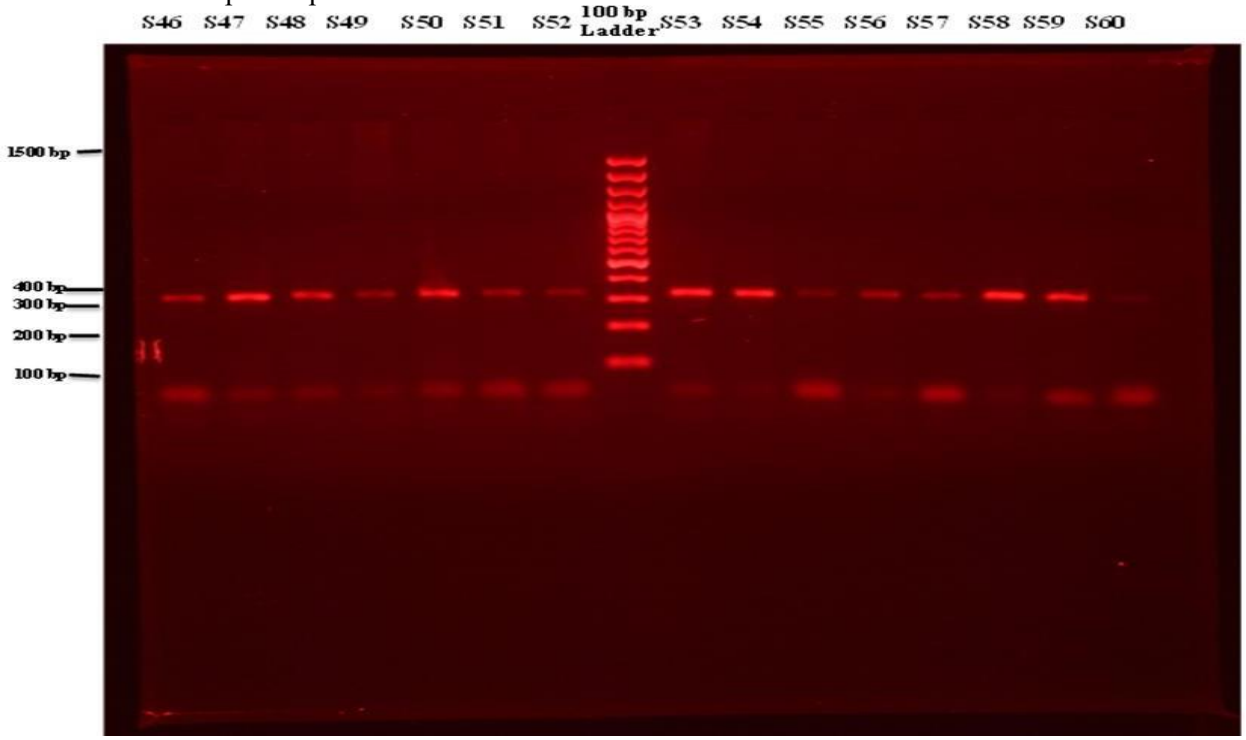

Figure 11: representative 2% (w/v) agarose gel of SNX19 amplified product. L1:100bp ladder,

Lane S46-S60: amplified product

There were 2 CC genotypes, 41 homozygous GG genotypes, and 17 were heterozygous CG genotypes, indicating variations in the SNX19 gene sequence among the samples (Figure 12).

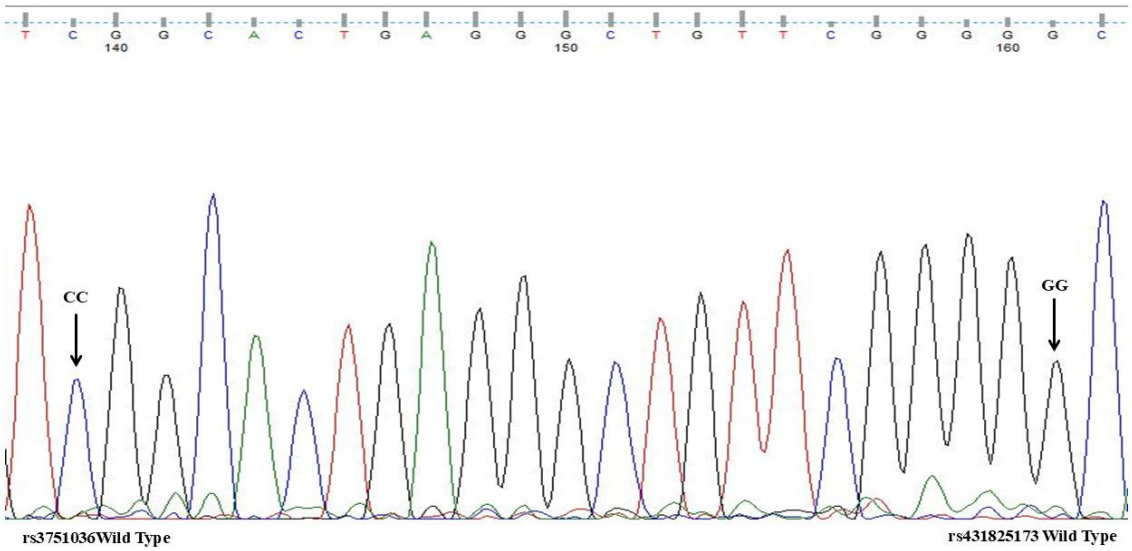

Figure 12: Finch TV generated chromatogram of SNX19, wild type rs3751036 (CC) and wild type rs431825173 (GG)

The results show that the observed counts are close to the expected counts, with a high p-value (0.989) indicating a strong likelihood of Hardy-Weinberg Equilibrium (HWE). This suggests that the population is likely in equilibrium, and the observed values are consistent with the expected values under HWE (Table 3).

Table 3: Distribution of genotypes of the SNX19 (rs3751036) gene. Hardy-Weinberg equilibrium was used to get the expected and Chi-square values.

Genotypes	Observed values	Expected values	X^2 test value	P value
Homozygote reference	2	1.8	0.21	0.989
Heterozygote	17	17.3		
Homozygote variant	41	40.8		

The Association of SNX19 Genotypes with HBA1C Level and Clinical Outcome

It has been found that there was no significant association between HbA1c levels and the genotypes of rs3751036 (CC wild-type, GC heterozygous, and GG homozygous mutant) (Table 4). The p-values for baseline HbA1c and rs3751036 genotypes (0.442) and for second-visit HbA1c and genotypes (0.588) were both greater than the significance level of 0.05. The results for SNX19 (rs3751036) genotypes and HbA1c levels are presented in Table 4. Individuals with a decrease of ≥0.8% in HbA1c were classified as "Responders," while those with a reduction of <0.8% were classified as "Non-Responders."(Rashid, Shahzad, Mahmood, & Khan, 2019) . The responder percentages were 100% for the wild-type allele CC, 80.5% for the GG allele, and 41.2% for the GC allele (Figure 13). It has been observed that SNX19 gene polymorphism has no impact on clinical outcomes in the context of the percentage HbA1c reduction after three months of therapy.

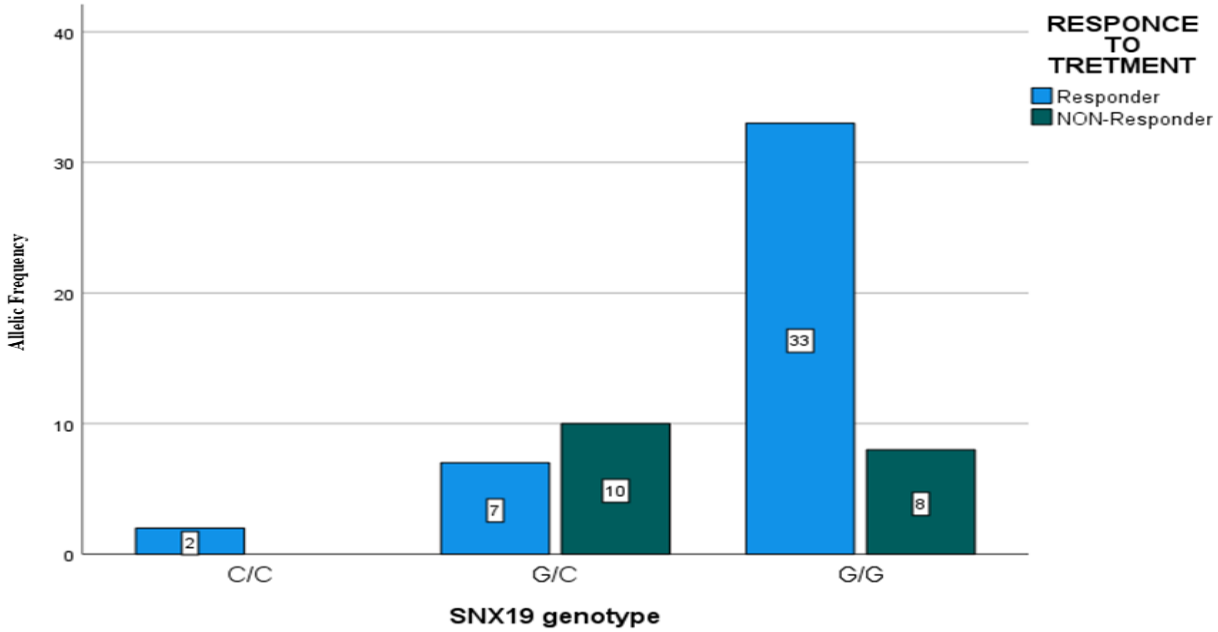

Figure 13: Association of SNX19 rs3751036 genotypes with Clinical outcome: A retrospective analysis

Table 4: Association analysis of SNX19 rs3751036 genotypes with HbA1c levels in patients with T2D. One-way ANOVA and Post-Hoc Tukey test results

Dependent Variable	(I) SNX19 genotype	(J) SNX19 genotype	Mean Difference (I-J)	Sig.	P-Value
HbA1c 1st Visit (6.60-12.50)	C/C	G/C	1.40529%	.503	0.442
		G/G	0.96829%	.704	
	G/C	C/C	-1.40529%	.503	
		G/G	-0.43700%	.638	
	G/G	C/C	-0.96829%	.704	
		G/C	0.43700%	.638	
HbA1c 2nd Visit (4.78-11.40)	C/C	G/C	0.01971%	1.000	0.588
		G/G	0.56134%	.912	
	G/C	C/C	-0.01971%	1.000	
		G/G	0.54164%	.584	
	G/G	C/C		-0.56134%	.912
		G/C		-0.54164%	.584

Discussion

This study included 60 patients with Type 2 Diabetes, who had an average age of 54.37 ± 11.61 years. The mean weight of the patients was 68.52 ± 8.198 kg, and their height mean value of 5.44 ± 0.39 feet. The Body Mass Index (BMI) of patients with a mean BMI of 25.29 ± 4.54. The patients received medication at varying doses, ranging from 500 to 1000 mg/day, with a mean dose of 840 ± 210.76 mg/day. The mean Glycated Hemoglobin levels at the initial visit were 9.3 ± 1.7%, while at the follow-up visit, the HbA1c levels were 8.4 ± 1.9%. T2DM was highly prevalent among the mean age of 54.37 years of patients in the current study. Our results are consistent with those of another study that showed the middle age group to have the highest prevalence of T2DM (Nanditha et al., 2016). In the same way, a different study carried out in Karachi, Pakistan, found that people in the middle age range (36–56) had a greater prevalence of diabetes mellitus and identified risk factors like obesity, sedentary behavior, and elevated HbA1c values (Muzaffar, 2008). Furthermore, the incidence of diabetes rose with age. In comparison, a survey done between 1990 and 2019 found that 56% of respondents between the ages of 15 and 39 had diabetes. This could be because of the various changes that occurred during their formative years, such as adjustments to their daily routine, health care, and relationships with friends, family, and coworkers, as well as changes to their lifestyle (occupation, education, living arrangement, and daily routine). Additionally, genetic diversity has a beneficial impact; those with a favorable family history of diabetes are more likely to get the disease. Like this study, a sizable portion of the patients had a favorable family history of diabetes. A higher incidence of type 2 diabetes related to family history. The highest risk of developing type 2 diabetes was found in those with a biparental history of the disease (Smith et al., 2024).

The study found no significant variation in demographic and clinical variables between genders, except for height. Males had a significantly higher mean height (5.63 ± SD) compared to females (5.28 ± SD) ($p=0.0001$). Independent Sample T-Test revealed no significant differences between males and females in age, weight, BMI, dose, and HbA1c levels at the first and second visits. People may ask why diabetes and gender are related. This is due to the biological differences between males and females. These variations influence the expression of specific genes, which can affect different organs and systems and are influenced by sex chromosomes and hormones. Moreover, metabolic alterations generally impact the physiology and mechanism of both sexes, but women are more likely to be impacted by these changes (Harreiter & Kautzky-Willer, 2018).

The sequence results showed polymorphism in 58 out of a total of 60 samples for SNX19 rs3751036. The NCBI-generated nucleotide BLAST result revealed that the sequenced region corresponded to a non-coding transcript variant present in a gene regulatory region. According to the dbSNP database, rs3751036 was in the flanking region of rs431825173. Gene expression and function can be profoundly impacted by single nucleotide polymorphisms (SNPs) in flanking regions of genes, such as introns and untranslated regions (UTRs), as they can modify regulatory elements, splicing sites, and miRNA binding sites (Treacy & Borisenko, 2012). According to the available literature, there are no existing studies on rs431825173 and rs3751036 SNPs. Therefore, we conducted this research to investigate the effect of polymorphisms in the SNX19 gene on Type 2 Diabetes (T2D) individuals. This is because the role of SNX19 in Type 1 Diabetes (T1D) has been established, and it is known to play a crucial role in insulin secretion. Our study aims to explore the potential association between SNX19 gene variants and T2D susceptibility. (Harashima et al., 2012). The dbSNP indicates that the SNX19 gene has many reported SNPs. For our investigation, we chose rs431825173, a Level 2 SNP (missense SNP). A particular kind of SNP known as Level 2 SNPs is grouped according to how functionally they affect gene expression. Interestingly, compared to Level 1, Level 2 SNPs are thought to be more severe since they may cause shortened proteins, loss of protein function, and possible links to diseases (Abrahams et al., 2021). The reason we selected a single SNP (rs431825173) for our study instead of analyzing the entire exon was due to financial constraints. Whole-exome sequencing or genotyping would have provided more comprehensive data, but it was not feasible within our budget. Therefore, we prioritized the analysis of a single, potentially high-impact SNP to maximize the value of our resources.

Conclusion

SNX19 gene is not deleterious and has no association with changes in HbA1c level and clinical outcome in type 2 diabetic patients.

Recommendations

● Conducting replication studies with larger and more diverse sample sizes can help validate the findings of this study

● Larger sample sizes increase the statistical power and reliability of the results, providing more robust evidence for the association between SNX19 gene polymorphism and HbA1c level

● The whole exon sequencing of SNX19 gene will provide important information about this gene, because the distribution of this gene is reported in Kidney, CNS, Bone marrow, Heart and Pancreas.

Abbreviations

SNX19 (sorting nexin 19), SNP (Single nucleotide polymorphism), HBA1c (glycated hemoglobin), Type 2 Diabetes (T2D), DNA (Deoxy Ribo Nucleic Acid)

Acknowledgement

I am thankful to all those who helped in providing the resources, chemicals and reagents. I am also thankful to the Advanced Center for Genomic Technology (ACGT) Khyber Medical University, Peshawar.

Declaration of Conflict of Interest

All authors have no conflict of Interest

References

Abrahams, L., Savisaar, R., Mordstein, C., Young, B., Kudla, G., & Hurst, L. D. (2021). Evidence in disease and non-disease contexts that nonsense mutations cause altered splicing via motif disruption. Nucleic Acids Research, 49(17), 9665-9685.

Al-Rifai, R. H., Majeed, M., Qambar, M. A., Ibrahim, A., AlYammahi, K. M., & Aziz, F. (2019). Type 2 diabetes and pre-diabetes mellitus: a systematic review and meta-analysis of prevalence studies in women of childbearing age in the Middle East and North Africa, 2000–2018. Systematic Reviews, 8(1), 268. doi: 10.1186/s13643-019-1187-1

Amatya, B., Lee, H., Asico, L. D., Konkalmatt, P., Armando, I., Felder, R. A., & Jose, P. A. (2021). SNX-PXA-RGS-PXC subfamily of SNXs in the regulation of receptor-mediated signaling and membrane trafficking. International journal of molecular sciences, 22(5), 2319.

Harashima, S. i., Horiuchi, T., Wang, Y., Notkins, A. L., Seino, Y., & Inagaki, N. (2012). Sorting nexin 19 regulates the number of dense core vesicles in pancreatic β-cells. Journal of diabetes investigation, 3(1), 52-61.

Harreiter, J., & Kautzky-Willer, A. (2018). Sex and gender differences in prevention of type 2 diabetes. Frontiers in endocrinology, 9, 220.

Hu, Y. F., Zhang, H. L., Cai, T., Harashima, S., & Notkins, A. L. (2005). The IA-2 interactome. Diabetologia, 48(12), 2576-2581. doi: 10.1007/s00125-005-0037-y

Jensen, E. T., Bertoni, A. G., Crago, O. L., Hoffman, K. L., Wood, A. C., Arzumanyan, Z., . . . Wu, M. (2020). Rationale, design and baseline characteristics of the Microbiome and Insulin Longitudinal Evaluation Study (MILES). Diabetes, Obesity and Metabolism, 22(11), 1976-1984.

Kawasaki, E. (2023). Anti-islet autoantibodies in type 1 diabetes. International journal of molecular sciences, 24(12), 10012.

Kharroubi, A. T., & Darwish, H. M. (2015). Diabetes mellitus: The epidemic of the century. World journal of diabetes, 6(6), 850.

Lyssenko, V., Jonsson, A., Almgren, P., Pulizzi, N., Isomaa, B., Tuomi, T., . . . Groop, L. (2008). Clinical risk factors, DNA variants, and the development of type 2 diabetes. New England Journal of Medicine, 359(21), 2220-2232.

Meo, S. A., Zia, I., Bukhari, I. A., & Arain, S. A. (2016). Type 2 diabetes mellitus in Pakistan: Current prevalence and future forecast. J Pak Med Assoc, 66(12), 1637-1642.

Muzaffar, F. (2008). To Observe Implementation of American Diabetes Association (ADA) Guidelines for Care of Type 2 Diabetics at Peripheral Diabetes Clinics (PDCs) in Pakistan.

Nanditha, A., Ma, R. C., Ramachandran, A., Snehalatha, C., Chan, J. C., Chia, K. S., . . . Zimmet, P. Z. (2016). Diabetes in Asia and the Pacific: implications for the global epidemic. Diabetes care, 39(3), 472-485.

Norton, L., Shannon, C., Gastaldelli, A., & DeFronzo, R. A. (2022). Insulin: The master regulator of glucose metabolism. Metabolism, 129, 155142. doi: 10.1016/j.metabol.2022.155142

Petersen, M. C., & Shulman, G. I. (2018). Mechanisms of insulin action and insulin resistance. Physiological reviews.

Raciti, G. A., Longo, M., Parrillo, L., Ciccarelli, M., Mirra, P., Ungaro, P., . . . Béguinot, F. (2015). Understanding type 2 diabetes: from genetics to epigenetics. Acta diabetológica, 52, 821-827.

Rashid, M., Shahzad, M., Mahmood, S., & Khan, K. (2019). Variability in the therapeutic response of Metformin treatment in patients with type 2 diabetes mellitus. Pakistan journal of medical sciences, 35(1), 71.

Saraiva, M., Vieira, P., & O'garra, A. (2019). Biology and therapeutic potential of interleukin-10. Journal of Experimental Medicine, 217(1), e20190418.

Smith, K. R., Meeks, H., Curtis, D., Brown, B. B., Kole, K., & Kowaleski-Jones, L. (2024). Family history of type 2 diabetes and the risk of type 2 diabetes among young and middle-aged adults. Chronic Diseases and Translational Medicine.

Treacy, M., & Borisenko, K. (2012). The local structure of amorphous silicon. Science, 335(6071), 950-953.

Meo, S. A., Zia, I., Bukhari, I. A., & Arain, S. A. (2016). Type 2 diabetes mellitus in Pakistan: Current prevalence and future forecast. J Pak Med Assoc, 66(12), 1637-1642.