DOI: https://doi.org/10.54393/pjhs.v6i1.2448

PAKISTAN JOURNAL OF HEALTH SCIENCES
(LAHORE)
https://thejas.com.pk/index.php/pjhs
ISSN (E): 2790-9352, (P): 2790-9344
Volume 6, Issue 01 (January 2025)

Original Article

OPEN ACCESS

Association of Klotho Gene Polymorphisms with Type 2 Diabetes Mellitus

Sahar Mudassar[1], Abubakar Sarfraz[2], Amina Shahid[2], Ejaz Ahmad[3], Imran Joher[4] and Anam[5]

[1]Department of Pathology, Rashid Latif Medical College, Lahore, Pakistan
[2]Department of Biochemistry, Pak Red Crescent Medical College, Lahore, Pakistan
[3]Department of Biochemistry, Niazi Medical and Dental College, Sargodha, Pakistan
[4]Department of Medicine, Sharif Medical and Dental College, Lahore, Pakistan
[5]Department of Physiology, Niazi Medical and Dental College, Sargodha, Pakistan

ARTICLE INFO

Keywords:
Diabetes, Genotype, Lipid, Glucose Level

How to Cite:
Mudassar, S., Sarfraz, A., Shahid, A., Ahmad, E., Joher, I., & Anam, . (2025). Association of Klotho Gene Polymorphisms with Type 2 Diabetes Mellitus: KL Gene Polymorphisms and T2DM. Pakistan Journal of Health Sciences, 6(1), 295–301. https://doi.org/10.54393/pjhs.v6i1.2448

***Corresponding Author:**
Sahar Mudassar
Department of Pathology, Rashid Latif Medical College, Lahore, Pakistan
drsaharmphil@gmail.com

Received date: 12th November, 2024
Acceptance date: 23rd January, 2025
Published date: 31st January, 2025

ABSTRACT

Genetic variants in the Klotho gene could influence the way β-cells function and effectively glucose functions, and this might influence the development of Type 2 Diabetes Mellitus. **Objectives:** To investigate the association between Klotho gene polymorphisms rs677332 and to determine the risk of developing type II diabetes in a case-control study. **Methods:** This case-control study was conducted from Feb 2024 to July 2024 at the Department of Pathology, Rashid Latif Medical College, Lahore. The total number of participants was n=586, sample n=293 case diabetics and 293 controls. DNA was extracted from blood samples and genotyped using Polymerase Chain Reaction followed by restriction digestion and validated through Sanger sequencing. To evaluate the genetic and clinical data, statistical tests were performed with SPSS version 25.0 and PLINK (v1.07). Logistic regression analysis, adjusted for age, sex, and region, was used to determine associations between Klotho polymorphisms and Type 2 Diabetes Mellitus. Fasting blood glucose levels were used as a reference variable in multiple nominal regression. **Results:** The SNPs rs677332 polymorphism and type 2 diabetes were significantly correlated, underscoring the importance of age, BMI, and heredity in diabetes risk. Logistic regression confirmed that individuals in the AA genotype were linked to a 73% rise in the likelihood of diabetes (OR=1.73, p=0.004). **Conclusions:** The rs677332 polymorphism of the Klotho gene may serve as a potential protective factor against Type 2 Diabetes Mellitus. The outcomes report the significance of Klotho gene variants for metabolic health and indicate the possible advantages of genetic screening for early treatment.

INTRODUCTION

Two-thirds of Asians and Westerners suffer from Type 2 Diabetes Mellitus (T2DM), a common illness. The development of type 2 diabetes in later life is known to be influenced by lifestyle factors like obesity and inactivity. However, there have been numerous scientific studies done to date to identify the hereditary factors that contribute to the risk of T2DM. It has been determined that some genetic mutations cause type 2 diabetes (T2DM), and some of these mutations regulate the body's glucose levels [1]. A protein that is highly involved in metabolism, insulin control, and aging is encoded by the Klotho (KL) gene. KL genetic polymorphisms may impact glucose metabolism, insulin resistance, and beta-cell function, which could be linked to type 2 diabetic mellitus [2]. Beta-glucuronidase, which can be found in the membrane of the blood vessel as a single-transmembrane glycoprotein, is made up by KL. Its anti-aging properties have been demonstrated in the klotho loss-of-function (kl-/kl-) mice, whose ageing symptoms, such as osteoporosis and arteriosclerosis, were comparable to those observed in elderly humans. Furthermore, the lifespans of the co-isogenic wild type mice were shorter than that of the genetically altered mice that overexpressed Klotho [3]. Recent advancements in genome-wide association studies (GWASs) have identified hundreds of genetic variants associated with diabetes, most of which could be in critical genes vital in modulating

DOI: https://doi.org/10.54393/pjhs.v6i1.2448

insulin secretion and sensitivity. Furthermore, a recent analysis has successfully grouped diabetes-related variants into five clusters, i.e., beta cell, proinsulin, obesity, lipodystrophy, and liver/lipid, representing five diabetes-causing pathways which are corresponding to insulin production, insulin processing, adiposity, fat redistribution, and lipid metabolism, respectively. Using the genetic risk score (GRS) generated by diabetes-related and pathway-specific variants, we could comprehensively explore the potential modifying effect of genetic predisposition to T2D or diabetes-causing pathway on the association between serum pyrethroids and T2D risk [4]. Chronic metabolic disease known as type 2 diabetes mellitus (T2DM) mainly affects the body's glucose metabolism. Insulin is a hormone that is necessary for controlling blood sugar levels, and in type 2 diabetes, the pancreas may not generate enough of it. Insulin helps cells absorb glucose so they can use it as fuel [5, 6]. The pancreatic beta cells' ability to secrete insulin decreases as the illness worsens. Insulin resistance occurs in the body, which means that cells do not react to the hormone as well. This leads to insulin resistance, and elevated blood glucose levels as the cells get less able to uptake glucose [7]. Fall in insulin secretion and action leads to the retention of glucose in the blood stream (hyper-glycaemia). Long-term managing of hyper-glycaemia with may lead to several complications in the long run including failure of the kidney, nerve damage, heart disease and problems with the eyes [8]. Type 2 diabetes mellitus (T2DM) is a pandemic metabolic disease characterized by increased blood sugar and caused by resistance to insulin in peripheral tissues and damage to pancreatic beta cells. Kruppel-like Factor 14 (KLF-14) is proposed to be a regulator of metabolic diseases, such as diabetes mellitus (DM) and obesity. Adiponectin (ADIPOQ) is an adipo-cytokine produced by the adipocytes and other tissues and was reported to be involved in T2DM [9]. Klotho (KL) gene appears to have important function in regulation of glucose homeostasis through modulation of pancreatic beta-cell function and glucose utilization. The KL gene variants may affect the KL levels or activity and, in turn, contribute to elevated oxidative stress and inflammation as well as dysregulation of signaling through the insulin/IGF-1 and Wnt pathways. These pathways are important for the growth, survival and function of the beta cells and insulin secretion. When KL function is impaired through polymorphisms, then beta cells are rendered vulnerable to dysfunctions which make the insulin producing rate to decline and the body cells' sensitivity to insulin reduce. This impairment of insulin secretion and insulin sensitivity leads to dysglycaemia and further to the development of T2D. Hence, genetic variation within the KL gene is strongly associated with insulin secretion dysfunction, disturbed glucose homeostasis, and T2D development [10]. Polymorphism in KL gene can alter pancreatic β-cell structure and function, which can lead to reduced insulin release capability when facing high blood glucose levels. The well-known hallmark of type 2 diabetes is reduced βcell function, which leads to chronic hyperglycemia. KL gene can modulate the glucose-stimulated manufacture of insulin. Mutations in this gene could affect β-cell response to glucose, leading to compromised insulin secretion, which accompanies the ability to maintain normal plasma glucose levels. Because KL participates in multiple metabolic pathways, including those associated with glucose homeostasis and insulin signaling, KL is a strong candidate gene for T2DM susceptibility. The KL gene contains SNPs (e.g. rs677332) that have been associated with T2DM and insulin resistance.

The study aimed to investigate the association between KL gene polymorphisms rs677332 and the risk of developing type II diabetes in a case control study.

METHODS

This case-control study was conducted for the duration of six months from Feb 2024 to July 2024 at the Department of Pathology, Rashid Latif Medical College, Lahore. The age of the patient was 50 to 65 years. Inclusion criteria were patients having confirmed diagnosis with diabetes II, FBG> 126 mg/DI, HbA1c > 6.5%, written authorization was acquired for genetic analysis along with data usage from patients. Exclusion criteria were excessive blood glucose level, metabolic disorder, and metformin medication use. Purposive sampling was employed to select participants meeting specific inclusion criteria related to ensuring relevant data for genetic analysis. The formula sample size comparing two proportions (control and case group) with binary outcomes (presence or absence of T2DM) yields the following useful formula: n= Zα/2·(p1(1–p1) + p2(1–p2)) / (p1-p2)2. Proportion of allele frequency in case group p1=0.60, proportion of allele frequency in control group p2=0.40, (p1–p2=0.20), and α=0.05 [11]. The total sample size of participants was n= 586 and divided into n= 293 case and 293 controls group. In this experiment, coagulant tubes and an anticoagulant vacutainer containing EDTA were used to collect 5ml of venous blood for biochemical and molecular analysis. PCR was used for DNA extraction and genotyping to identify specific KL polymorphisms. DNA was extracted from 244 EDTA blood samples using the Qiagen DNA extraction kit (Qiagen, USA) following the manufacturer's protocol. The extracted DNA was diluted in TE buffer and stored at -20°C until further use. For genotyping, the DNA samples were prepared by amplifying the KL rs677332 polymorphism using polymerase chain reaction (PCR). The reaction mix included PCR master mix, specific primers, purified water, and genomic DNA. The

DOI: https://doi.org/10.54393/pjhs.v6i1.2448

amplified PCR products were then digested using the HPY1888III restriction enzyme and analyzed on a 2% agarose gel to confirm genotyping results. Primers were designed using Primer3 or NCBI Primer-BLAST tools. Here is a suggested primer pair for amplifying the KL rs677332 region. Forward Primer: 5'- TGC TGG AAG AGA AGT GGA AGC -3'. Reverse Primer: 5'- AGA GTC CTC CGA GGC TCA TT -3'. To evaluate metabolic status, fasting blood glucose (FBG), insulin, and HbA1c were tested in addition to genetic data. For evaluating metabolic health in a study on KL gene polymorphisms, biochemical assays are essential. After an 8-hour fasting, fasting blood glucose (FBG) was measured; were indicative of diabetes. An average blood glucose level over the previous two to three months was provided by hemoglobin A1c (HbA1c), with values above 6.5% indicating diabetes. Clinical and genetic data imported from Excel were statistically analyzed by using SPSS version 25.0. For comparison of diabetic patients and controls, t-test, Hardy-Weinberg Equilibrium (HWE), and multiple logistic regression were used. Using fasting blood glucose (FBG) as a reference, multiple nominal regression analysis was also carried out and significant level of $p<0.05$. The study was approved by the Institutional Review Board of Rashid Latif Medical College, Lahore under the reference number IRB00010673. Informed consent was obtained from all study participants prior to enrollment in the study.

RESULTS

A comparison of the clinical and demographic traits of the control group with the diabetes cases is shown in table 1. With a mean age of 56.4 years compared to 52.5 years ($p<0.001$), the results show that diabetics were much older than controls. This implies a risk of diabetes that varies with age. Furthermore, the diabetes group's body mass index (BMI) is significantly higher (28.5 kg/m2) than that of the control group (25.1 kg/m2), and the p-value (<0.001) further supports this finding. Additionally, the diabetes cohort's fasting blood glucose (FBG) levels are much higher (151.5 mg/dL) than the controls' (90.0 mg/dL), and this difference is statistically significant ($p<0.001$). Furthermore, supporting the presence of diabetes is the fact that the glycated hemoglobin (HbA1c) levels in cases of the disease (7.9%) are significantly higher than in controls (5.7%). The gender ratio is approximately equal between controls and diabetic patients, as revealed by the Chi-Square Test's finding of no significant difference in sex distribution ($p=0.18$) between the two groups in table 1.

Table 1: Association Between Controls and Diabetic Cases for rs677332 Polymorphism

Variables	Groups	Mean ± SD	Test Statistic	p-Value
Age (Years)	Controls (n=293)	52.5 ± 8.9	t=3.20	<0.001
	Diabetic (n=293)	56.4 ± 9.5		
BMI (kg/m^2)	Controls (n=293)	25.1 ± 3.6	t=5.90	<0.001
	Diabetic (n=293)	28.5 ± 4.0		
FBG (mg/dL)	Controls (n=293)	90.0 ± 11.5	t=11.30	<0.001
	Diabetic (n=293)	151.5 ± 31.8		
HbA1c (%)	Controls (n=293)	5.7 ± 0.5	t=14.20	<0.001
	Diabetic (n=293)	7.9 ± 1.4		
Sex (M/F)	Controls (n=293)	165 / 125	χ^2=1.75	0.18
	Diabetic (n=293)	161 / 135		

Table 2 shows the genotype frequencies of the rs677332 polymorphism in both diabetic patients and control subjects. There is a clear correlation between the AA genotype and a higher risk of developing diabetes, as seen by the considerably higher AA genotype frequencies in the diabetic group (44.9%) compared to 30.0% in the control group ($p = 0.003$). In contrast, the prevalence of the AG genotype is considerably lower in cases of diabetes (37.2%) compared to controls (50.0%), suggesting that this genotype may offer some protection against the development of diabetes. However, the p-value for this comparison is not stated directly. There was no statistically significant difference in the frequencies of the GG genotype between the two groups (20.0% in controls and 17.9% in diabetics), indicating that higher risk of diabetes was not linked to this genotype in table 2.

Table 2: Genotype Frequencies of rs677332 Polymorphisms in Diabetic Cases and Control Subjects

Polymorphism	Genotype	Control Subjects (n=293)	Diabetic Cases (n=293)	p-Value	Interpretation
rs677332	AA	87 (30.0%)	133 (44.9%)	<0.003	Significant Association; Higher in Diabetic Cases.
	AG	145 (50.0%)	110 (37.2%)	<0.005	Significant Association; Lower in Diabetic Cases.
	GG	58 (20.0%)	53 (17.9%)	0.088	No Significant Difference.

To reveal the relationship between genotypes of rs677332 and Type 2 Diabetes Mellitus (T2DM). With an odds ratio (OR) of 2.10 (95% CI: 1.45 - 3.06) and a p-value of 0.003, the data demonstrate that those with the AA genotype had a markedly elevated risk of T2DM. This implies that the risk of developing diabetes is more than twice as high for those who carry the AA genotype as for people who do not. With an OR of 0.63 (95% CI: 0.45 - 0.88) and a p-value of 0.012, on the other hand, the AG genotype appears to lessen the risk of T2DM, suggesting that those who carry it may be at a lesser risk for illness. On the other hand, the GG genotype does not significantly correlate with T2DM (OR = 0.88, p = 0.78), meaning that it has no effect on the risk of getting diabetes in table 3.

DOI: https://doi.org/10.54393/pjhs.v6i1.2448

Table 3: Association of rs677332 Genotypes with Diabetes Type 2

Geno-type	Control Subjects (n=293)	Diabetic Cases (n=293)	Odds Ratio (OR)	95% Confidence Interval (CI)	p-Value	Interpretation
AA	87 (30.0%)	133 (44.9%)	2.10	1.45-3.06	0.003	AA Genotype Associated with Increased Risk of T2DM.
AG	145 (50.0%)	110 (37.2%)	0.63	0.45-0.88	0.012	AG Genotype may Confer Resistance to T2DM.
GG	58 (20.0%)	53 (17.9%)	0.88	0.58-1.33	0.78	No Significant Association: GG Genotype is Not Protective.

Results presents the findings of a multivariate logistic regression study aimed at determining risk variables for diabetes. The results highlight the significance of age as a risk factor, showing that every year of age beyond 50 raises the likelihood of diabetes by 5% (OR=1.05, p=0.016). Moreover, there may be gender variations in the risk of diabetes, as being female is linked to a 52% increased chance of getting the disease (OR=1.52, p=0.004). BMI also demonstrates a strong correlation, emphasizing the significance of obesity in the incidence of diabetes. For every unit increase, there is an 8% increase in the likelihood of diabetes (OR=1.08, p=0.006). The AA genotype rs677332 is linked to a 73% rise in the likelihood of diabetes (OR=1.73, p=0.004), confirming its important role in the risk of developing diabetes. Lastly, with an odds ratio of 2.12 (p<0.001), a family history of diabetes is a powerful predictor, meaning that those who have one are more than twice as likely to get the illness as in table 4.

Table 4: Multiple Logistic Regression Analysis for Factors Associated with Diabetes

Variables	Odds Ratio (OR)	95% Confidence Interval (CI)	p-Value
Age	1.05	1.01-1.09	0.016
Sex (Female)	1.52	1.13-2.04	0.004
BMI	1.08	1.02-1.14	0.006
rs677332 Genotype AA	1.73	1.14-2.62	0.004
Family History of Diabetes	2.12	1.55-2.90	<0.001

SNP rs659117 in the KL gene has a Regulome DB score of 2, indicating moderate regulatory potential. It acts as an eQTL in tissues like the liver and pancreas, influencing gene expression. The SNP affects transcription factor binding (e.g., FOXO1, STAT3) and is in a DNase-hypersensitive region, suggesting its role in gene activation and insulin regulation, and further impact the pathogenesis of T2DM, see table 5.

Table 5: SNP rs659117 in the KL Gene has a Regulome DB Score

Feature	Value/Details	Interpretation
Regulome DB Score	2	A score of 2 suggests that the SNP has moderate regulatory potential, with potential impacts on gene regulation.
eQTL	Yes (Liver, Pancreas)	SNP rs659117 influences gene expression in liver and pancreas tissues, which are relevant to glucose metabolism.
TFBS (Transcription Factor Binding Sites)	Yes, Binding to FOXO1 and STAT3	The SNP affects regions bound by FOXO1 and STAT3, which are transcription factors involved in meta-bolic regulation and insulin signaling.
DNase Hypersensitivity	Yes, Located in Active Enhancer Region	The SNP is in an open chromatin region, likely to be involved in transcriptional regulation.
Protein Binding	Yes, Histone H3K4me3 Binding	The SNP influences a region bound by histone modification proteins, which is critical for gene activation.
Motif Impact	Yes, Alters Motif for STAT3 Binding	The SNP changes the STAT3 binding motif, suggesting it could modulate the transcription factor's role in regulating insulin sensitivity.

First band indicate AA genotype, second band indicate AG and third band indicate GG genotype. The band size 150kb, see figure 1.

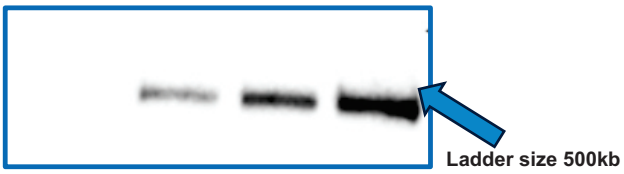

Figure 1: Gel Image of Genotype

DISCUSSION

Diabetes has become a common disorder in our century. An unregulated diet, inactivity, and a sedentary lifestyle have all contributed to the elevated incidence of type 2 diabetes, a chronic metabolic disease, in the 20th century. Moreover, every inherited family is influenced by genetics [12, 13]. Comparing the demographic, clinical, and genetic traits of diabetic patients and control participants allowed researchers to examine the potential link between the rs677332 polymorphism and diabetes. The findings revealed major variations in age, BMI, fasting blood glucose (FBG), and glycated hemoglobin (HbA1c) levels among the two groups as well as notable relationships with specific genotypes of the rs677332 polymorphism. In the present study, the mean age of the diabetes group was 56.4 years, which was substantially older than the mean age of the control group (52.5 years). This result is in line with multiple studies that have shown aging to be a significant risk factor diabetic patient, emphasizing the significance of age-

DOI: https://doi.org/10.54393/pjhs.v6i1.2448

related pathophysiological alterations that may predispose people to the disease [14]. Further supporting the literature that links obesity to a higher risk of diabetes type 2 by associating excess adipose tissue with insulin resistance is the considerably higher BMI (28.5 kg/m2) in the diabetic group [15]. The FBG and HbA1c level analysis provides additional evidence for the participant classification; diabetics had significantly higher levels (FBG: 151.5 mg/dL; HbA1c: 7.9%) than controls (FBG: 90.0 mg/dL; HbA1c: 5.7%). These findings support earlier studies, showing that diabetic patients typically have higher blood glucose levels, underscoring the importance of glycemic control in the management of diabetes [16]. In the current study finding that, the rs677332 polymorphism genotype frequencies showed a p-value of 0.003 and that the AA genotype was substantially more common in diabetic patients (44.9%) compared to controls (30.0%). This result is consistent with earlier research showing that some genetic variations can affect diabetes susceptibility and reveals a strong correlation between the AA genotype and T2DM [17]. In contrast, the AG genotype was found in diabetic cases in 37.2% of cases compared to controls in 50.0% of cases, suggesting a possible protective effect against diabetes and supporting earlier findings that certain genotypes may confer resistance to metabolic diseases. This reinforces the concept that different alleles/variants within the same gene may have different effects as well as the evidence that the GG genotype is not significantly associated with diabetes [18]. Current results were consistent with those from a multiple logical regression study that identified independently unfavorable prognostic factors for T2DM are age, sex, BMI, the rs677332 AA genotype and history of diabetes in the family. The risk of diabetes increased by 5% per year of age over the age of 40, and being female was associated with 52% higher risk of diabetes. These findings align with previous studies that highlight the differential impact of sex and age on diabetes outcomes and prevalence [19]. Furthermore, a unit increase in BMI raised the risks of diabetes by 8% which corroborates the finding with previous studies that cholesterol was obesity and insulin resistant [20]. There were 73% increased odds of diabetes associated with the rs677332 AA genotype, which argues for contributions of specific genes to type 2 diabetes risk through genetic predisposition. Consistent with this finding, family history emerged as a predictor of diabetes indicating the importance of interaction between genetic and environmental effects in the etiology of this condition. These observations correlate with some previous epidemiological findings that type 2 diabetes fall into the category of heritable diseases [21, 22]. The KL (Klotho) gene, while primarily associated with type 2 diabetes mellitus (T2DM), plays a broader role in various metabolic pathways. KL gene variants influence calcium-phosphorus regulation through fibroblast growth factor (FGF) signaling, which is crucial for mineral homeostasis and linked to conditions like chronic kidney disease (CKD) and osteoporosis. Additionally, KL polymorphisms are associated with aging and longevity by reducing oxidative stress and inhibiting cellular senescence, contributing to protection against cardiovascular diseases such as vascular calcification and atherosclerosis. These findings highlight the systemic relevance of KL variants, positioning them as potential biomarkers or therapeutic targets for metabolic and age-related diseases beyond T2DM [23]. We have expanded the discussion to emphasize the clinical and genetic implications of present findings, particularly the significant association of the AA genotype with an increased risk of Type 2 Diabetes Mellitus (T2DM). Additionally, we highlighted the potential protective role of the AG genotype, which appears to reduce the risk of T2DM. The AA genotype may increase the risk of Type 2 Diabetes Mellitus (T2DM) due to its potential effect on the expression or function of genes involved in glucose metabolism, insulin resistance, or β-cell function. Specific genetic variants in key genes, like those influencing insulin secretion or action, can lead to an impaired ability to regulate blood sugar levels. In the case of the AA genotype, it may be associated with higher expression of risk-related alleles or less favorable interactions with other metabolic pathways, making individuals more susceptible to insulin resistance, beta-cell dysfunction, and, ultimately, T2DM. [24]. We also discussed how these genetic insights could contribute to personalized medicine and risk prediction for T2DM, supporting the development of tailored preventive and therapeutic strategies.

CONCLUSIONS

Analysis of the rs677332 polymorphism, on the other hand, shows age, BMI and genetic factors. The latter is also a significant contributor to the risk of type 2 diabetes. This study complements previous research and lends support to the need for genetic screening and tailored prevention efforts in high-risk groups. KL gene polymorphisms play a significant role in various metabolic and age-related conditions. Future studies should explore their therapeutic potential and utility in early genetic screening, which could help identify individuals at risk and guide targeted interventions.

DOI: https://doi.org/10.54393/pjhs.v6i1.2448

Authors Contribution

Conceptualization: SM
Methodology: AS[1], AS[2]
Formal analysis: AS[1], A
Writing, review and editing: SM,EA, IJ, AS[2]

All authors have read and agreed to the published version of the manuscript

Conflicts of Interest

The authors declare no conflict of interest.

Source of Funding

The author received no financial support for the research, authorship and/or publication of this article.

REFERENCES

[1] Galicia-Garcia U, Benito-Vicente A, Jebari S, Larrea-Sebal A, Siddiqi H, Uribe KB, *et al.* Pathophysiology of Type 2 Diabetes Mellitus. International Journal of Molecular Sciences. 2020 Aug; 21(17): 6275. doi: 10.3390/Ijms21176275.

[2] Liu Y and Chen M. Emerging Role of A-Klotho in Energy Metabolism and Cardiometabolic Diseases. Diabetes & Metabolic Syndrome: Clinical Research & Reviews. 2023 Sep: 102854. doi: 10.1016/J.Dsx.2023.102854.

[3] Navarro-García JA, Salguero-Bodes R, González-Lafuente L, Martín-Nunes L, Rodríguez-Sánchez E, Bada-Bosch T, et al. The Anti-Aging Factor Klotho Protects against Acquired Long QT Syndrome Induced by Uremia and Promoted by Fibroblast Growth Factor 23. BMC Medicine. 2022 Jan; 20(1): 14. doi: 10.1186/s12916-021-02209-9.

[4] Jia C, Zhang S, An J, Cheng X, Li P, Zhang X, *et al.* Genetic Predisposition to Impaired Beta-Cell Function Modifies the Association Between Serum Pyrethroid Levels and the Risk of Type 2 Diabetes: A Gene-Environment Interaction Study. Ecotoxicology And Environmental Safety. 2024 Oct; 284. doi: 10.1016/J.Ecoenv.2024.116948.

[5] Jiang S, Young JL, Wang K, Qian Y, Cai L. Diabetic-Induced Alterations in Hepatic Glucose and Lipid Metabolism: The Role of Type 1 and Type 2 Diabetes Mellitus. Molecular Medicine Reports. 2020 Aug; 22(2): 603-611. doi: 10.3892/Mmr.2020.11175.

[6] Zhao X, An X, Yang C, Sun W, Ji H, Lian F. The Crucial Role and Mechanism of Insulin Resistance in Metabolic Disease. Frontiers in Endocrinology. 2023 Mar; 14: 1149239. doi: 10.3389/fendo.2023.1149239.

[7] Ruze R, Liu T, Zou X, Song J, Chen Y, Xu R, *et al.* Obesity and Type 2 Diabetes Mellitus: Connections in Epidemiology, Pathogenesis, and Treatments. Frontiers in Endocrinology. 2023 Apr; 14(1). doi: 10.3389/fpubh.2022.1047794.

[8] Su W, Chen M, Xiao L, Du S, Xue L, Feng R, *et al.* Association of Metabolic Dysfunction-Associated Fatty Liver Disease, Type 2 Diabetes Mellitus, and Metabolic Goal Achievement with Risk of Chronic Kidney Disease. Frontiers in Public Health. 2022 Nov; 10. doi: 10.3389/fpubh.2022.1047794.

[9] Elfaki I, Mir R, Tayeb F, Alalawy AI, Barnawi J, Dabla PK, *et al.* Potential Association of The Pathogenic Kruppel-like Factor 14 (KLF14) and Adiponectin (ADIPOQ) SNVs with Susceptibility to T2DM. Endocrine, Metabolic & Immune Disorders-Drug Targets. 2024 Jul; 24(9): 1090-1100. doi: 10.2174/0118715303258744231117064253.

[10] Alvarez MF, Fernandez G, Elfrida SS, Gomez ME. Investigation of KLF14 Promoter Methylation in Patients with Type 2 Diabetes. A Preliminary Analysis. Open Access Library Journal. 2024 Oct; 11(10): 1-5. doi: 10.4236/oalib.1111888.

[11] Politi C, Roumeliotis S, Tripepi G, Spoto B. Sample Size Calculation in Genetic Association Studies: A Practical Approach. Life. 2023 Jan; 13(1): 235. doi: 10.3390/life13010235.

[12] Reed J, Bain S, Kanamarlapudi V. A Review of Current Trends with Type 2 Diabetes Epidemiology, Aetiology, Pathogenesis, Treatments and Future Perspectives. Diabetes, Metabolic Syndrome and Obesity. 2021 Aug: 3567-3602. doi: 10.2147/DMSO.S319895.

[13] Galicia-Garcia U, Benito-Vicente A, Jebari S, Larrea-Sebal A, Siddiqi H, Uribe KB, Ostolaza H, Martín C. Pathophysiology of type 2 diabetes mellitus. International journal of molecular sciences. 2020 Aug 30;21(17):6275. doi: 10.3390/ijms21176275.

[14] Lima JE, Moreira NC, Sakamoto-Hojo ET. Mechanisms Underlying the Pathophysiology of Type 2 Diabetes: From Risk Factors to Oxidative Stress, Metabolic Dysfunction, and Hyperglycemia. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 2022 Feb; 874. doi: 10.1016/j.mrgentox.2021.503437.

[15] Barbieri M, Prattichizzo F, La Grotta R, Matacchione G, Scisciola L, Fontanella RA, *et al.* Is It Time to Revise the Fighting Strategy toward Type 2 Diabetes? Sex and Pollution as New Risk Factors. Ageing Research Reviews. 2024 Jul. doi: 10.3389/fcvm.2022.964743.

[16] Ortega MA, Fraile-Martínez O, Naya I, García-Honduvilla N, Álvarez-Mon M, Buján J, *et al.* Type 2 Diabetes Mellitus Associated with Obesity (Diabesity). The Central Role of Gut Microbiota and Its Translational Applications. Nutrients. 2020 Sep; 12(9): 2749. doi: 10.3390/nu12092749.

DOI: https://doi.org/10.54393/pjhs.v6i1.2448

[17] Zhang LY, Jin YJ, Jin QS, Lin LY, Zhang DD, Kong LL. Association between Resistin+ 299A/A Genotype and Nonalcoholic Fatty Liver Disease in Chinese Patients with Type 2 Diabetes Mellitus. Gene. 2013 Oct; 529(2): 340-344. doi: 10.1016/j.gene.2013.08.001.

[18] Ayelign B, Negash M, Andualem H, Wondemagegn T, Kassa E, Shibabaw T, *et al.* Association of IL-10 (– 1082 A/G) and IL-6 (– 174 G/C) Gene Polymorphism with Type 2 Diabetes Mellitus in Ethiopia Population. BMC Endocrine Disorders. 2021 Dec; 21: 1-8. doi: 10.1186/s12902-021-00738-1.

[19] Sarray S, Ezzidi I, Moussa S, Abdennebi HB, Mtiraoui N. Association Study between Adiponectin Gene Variants, Serum Levels and The Risk of Type 2 Diabetes in Tunisian Women: Insights from BMI stratification. Cytokine. 2024 Sep; 181. doi: 10.1016/j.cyto.2024.156695.

[20] Mamashli E, Goulding RP, Iranparvar M, Skishahr FS, Siahkouhian M, Ramezanzade R, *et al.* Association of Adiponectin Gene Single Nucleotide Polymorphisms with Environmental Risk Factors in Type 2 Diabetes Mellitus: An Updated Evidence of Haplotype-Based Analysis Study. Gene. 2025 Jan; 933. doi: 10.1016/j.gene.2024.148816.

[21] Cole JB and Florez JC. Genetics of Diabetes Mellitus and Diabetes Complications. Nature reviews nephrology. 2020 Jul; 16(7): 377-390. doi: 10.1038/s41581-020-0278-5.

[22] Goodarzi MO and Rotter JI. Genetics Insights in the Relationship between Type 2 Diabetes and Coronary Heart Disease. Circulation Research. 2020; 126(11): 1526-1548. doi: 10.1161/CIRCRESAHA.

[23] Aaldijk AS, Verzijl CR, Jonker JW, Struik D. Biological and Pharmacological Functions of the FGF19-and FGF21-Coreceptor Beta Klotho. Frontiers in Endocrinology. 2023 May; 14. doi: 10.3389/fendo.2023.1150222.

[24] Zenoaga-Barbăroșie C, Berca L, Vassu-Dimov T, Toma M, Nica MI, Alexiu-Toma OA, *et al.* The Predisposition for Type 2 Diabetes Mellitus and Metabolic Syndrome. Balkan Journal of Medical Genetics. 2023 Jul; 26(1): 21-6. doi: 10.2478/bjmg-2023-0003.