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Original article

Interaction between rs6446482 polymorphisms in the *WFS1* gene in type 2 diabetes patients

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ABSTRACT

Background: Type 2 diabetes mellitus (T2DM) is a chronic metabolic condition characterized by high blood glucose levels. Mutations in the *WFS1* gene cause β-cell death, leading to Wolfram syndrome and diabetes. There have been few studies that have been linked to T2DM and the rs6446282 polymorphism in the WFS1 gene, and there are no studies from Saudi Arabia. As a result, the purpose of this study was to investigate the association between the rs6446482 polymorphism in the WFS1 gene and T2DM in the Saudi population.

Methods: One hundred T2DM patients and 100 healthy controls were recruited based on inclusion and exclusion criteria. Genomic DNA was obtained from collected blood, and using oligonucleotides, PCR was performed followed by RFLP analysis. Statistical analysis was performed with SPSS software to compare the numerical and categorical data.

Results: This case-control study compared T2DM patients to healthy controls. Age, weight, BMI, FBG, HDL-c, TC, TGs, and family history ($p < 0.05$). The effect of the rs6446482 polymorphism was performed with PCR-RFLP analysis, and the study results confirmed the positive association with HWE ($p = 0.01$), genotypes (GG vs. CC: OR-3.29 [95% CI: 1.33–8.13]; $p = 0.007$), dominance (CC vs. CG + GG: OR-2.12 [95% CI: 1.24–3.92]; $p = 0.006$), and allele frequencies (G vs. C: OR-2.15 [95% CI: 1.37–3.38]; $p = 0.0007$), revealing a positive association. ANOVA results showed a negative association between the rs6446482 polymorphism and T2DM risk parameters such as age, weight, height, BMI and FBG.

Conclusion: The results of this study confirmed that the rs6446482 polymorphism is associated with T2DM in the Saudi population. This study recommends updating the meta-analysis studies in T2DM with the rs6446482 polymorphism and should also be performed in different modes of diabetes.

1. Introduction

Diabetes is defined by aberrant glucose levels. Insulin secretion, insulin action, or both are excessive in people with diabetes mellitus, a group of metabolic diseases caused by a variety of factors (Khan, 2021). According to the World Health Organization, approximately 422 million people worldwide have diabetes, and diabetes is directly responsible for 1.6 million fatalities per year (Wang et al., 2021). Type 2 diabetes (T2DM) is a prevalent type of diabetes, and it is often accompanied by age-related complications, including chronic diseases. T2DM is an increasingly prevalent worldwide condition that is connected to major complications, which results in a shorter lifespan and impaired quality of life (Alharbi et al., 2021a). There is a strong association between age and T2DM, and presently, more than half of adults have the disease (Bellary et al., 2021). According to the International Diabetes Federation, over 425 million people worldwide will have diabetes in 2017, with an additional 352.1 million suffering from the disease. Estimates place the population at 629 million by 2045 and 587 million by 2085. Approximately 79 percent of all diabetic patients live in countries with low to medium GDP (Maratni et al., 2021). The influence of insulin on target tissues such as muscle and liver causes an increase in weight and can lead to obesity, which is a key risk factor for diabetes (Khan et al., 2014). Because of its uncertain aetiology, there are various genetic alterations associated with insulin resistance and β-cell dysfunction that contribute to the development of T2DM (Liu et al., 2021). Diabetes prevention in high-risk patients requires both lifestyle and pharmacological

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intervention. Family history and genetic information can help to identify high-risk individuals. A number of studies have shown that insulin resistance is a significant factor contributing to the development of T2DM, as well as a deficiency in insulin secretion. Numerous single nucleotide polymorphisms (SNPs) related to susceptibility to T2DM have been found through genome-wide association studies or GWAS (Khan et al., 2015d; Khan et al., 2015a). It is expected that the majority of T2DM patients will develop micro- and macrovascular complications (Ayelign et al., 2021).

It is crucial to identify genes to better understand disease pathophysiology and to improve diagnosis, prevention, and treatment (Khan et al., 2015c). Gene polymorphisms, the most common genetic variation in humans, are not always associated with a specific disease, while gene mutations are known to cause hereditary diseases. SNPs, which represent the substitution of one nucleotide for another, are one of the most common types of nucleotide polymorphisms in humans (Chiarella et al., 2019). Glucose metabolism and insulin secretion are influenced by genetic polymorphisms, and the bulk of these genes are involved in β-cell function. There are many genes that contribute to T2DM susceptibility that have been found by GWAS, candidate gene approaches, and linkage analysis. By employing a combined study of loci to produce genetic risk scores, it is now possible to predict the incidence of T2DM and, as a result, promote early diagnosis (Witka et al., 2019). Multiple members of families with several affected individuals were mapped to the Wolfram Gene (WFS1), which encodes wolframin, located on chromosome 4p16.1. When β-cells die as a result of a mutation in the WFS1 gene, it is known as Wolfram syndrome. Mutations in the WFS1 gene result in a lack of wolframin function due to cellular depletion. Homozygotes or compound heterozygotes have a greater risk of psychiatric hospitalization and DM, whereas heterozygotes are at an increased risk of hearing loss (Kytövuori et al., 2013; Minton et al., 2002; Cheng et al., 2013). WFS1 gene polymorphism studies were carried out in different forms of diabetes in the global population but there have been no genetic studies carried out in the Saudi population (Cheng et al., 2013; Domenech et al., 2002; Lauenborg et al., 2009; Nyaga et al., 2018). Based on previous documented studies with the rs6446482 polymorphism in the WFS1 gene (Singh et al., 2012; Franks et al., 2008; Mita et al., 2008), the present study was designed. The aim of this study was to investigate the genetic polymorphism (rs6446482) in the *WFS1* gene in Saudi patients confirmed with T2DM in the adult population.

2. Materials and methods

2.1. IRB statement

This study was designed with the approval of an ethical grant from the Institutional Review Board within the premises of the university hospital. The basic criteria were designed as the participants who signed the consent form and met the inclusion criteria for both the cases and controls. In this study, only participants who signed informed consent forms were recruited, and participants who provided oral consent due to the pandemic crisis were excluded from this study. Helsinki criteria were followed for the collection of human participants' samples after obtaining written informed consent (Roggli et al., 2008).

2.2. Sample size for case-control study

The sample size for both the T2DM cases and the control individuals was estimated using the Survey System Creative Research Systems online tool. Using the aforementioned formula, the sample size was calculated to be 25 units with a 95% confidence level and a 6% margin of error. The sample size for each group was calculated to be 97 people. This study included 200 participants, 100 of whom were type 2 diabetes patients and 100 of whom were healthy controls (Hameed et al., 2021).

2.3. Enrolment of the participants

Based on American Diabetes Association criteria, 100 T2DM cases and 100 healthy controls were selected for this study. T2DM patients fulfilled the inclusion criteria if their fasting plasma glucose levels did not exceed 7.0 mmol/L. The exclusion criteria for T2DM cases were validated as if normal glucose levels had been acquired and the patients were over the age of 20. The inclusion criteria for the control subjects were that they had normal glucose levels and no endocrine problems and that they were not using any medications. Exclusion criteria for this study included participants who had been diagnosed with any metabolic diseases, were taking medication, or had abnormal glucose levels. All cases and controls were drawn from the Diabetic Clinic Unit of King Khalid University and its hospital.

2.4. Sample collection and anthropometric evaluations

Body mass index (BMI) was calculated using height in centimetres and weight in kilograms. In both T2DM cases and controls, anthropometric measures such as age, sex, weight, and height were recorded (Alharbi et al., 2021b). For biochemical and molecular analysis, a 5 ml blood sample was aliquoted. Three millilitres of biochemical sample were utilized with the serum sample for glucose and lipid profiles. The remaining 2 ml of EDTA sample was used to isolate DNA and perform molecular analysis.

2.5. Serum analysis

FBG and lipid profile markers such as triglycerides (TGs), total cholesterol (TC), high-density lipoprotein cholesterol (HDLC), and low DL-C (LDLC) were measured in all 200 subjects. The serum was separated by centrifuging the coagulant tube, and the FBG and lipid profile values were calculated with the relevant kits (Alharbi et al., 2015).

2.6. DNA and oligonucleotide analysis

EDTA blood (0.6 ml) was used to extract genomic DNA in triplicate using Promega DNA isolation kits according to the manufacturer's instructions. The quality and amount of 200 DNA samples were evaluated using a NanoDrop spectrophotometer. Each DNA sample was treated so that 10 ng of genomic DNA was obtained. Polymerase chain reaction (PCR) of the WFS1 gene for the rs6446482 polymorphism was performed using 50 μl of reaction and 25 μl of the GoTaq master mix kit (PROMEGA, USA), which was comprised of buffer, $MgCl_2$, dNTPs, and Taq DNA polymerase. The remaining products, comprising 19 μl of filtered water and 2 μl of both forward and reverse primers, were added to the reaction mixture. Four microlitres of 10 ng genomic DNA was added separately to finish the reaction. Over 40 cycles of denaturation (94 °C-5 m), initial denaturation (94 °C-45 s), annealing (60 °C-45 s), extension (72 °C-1 m), and final extension (72 °C-10 m), genotyping was completed. The primer sequences were F: CGTAGGGACTGTGCTCTGGT and R: AAGATGGCAGCCGTGATAGT. The band size of the PCR result was planned to be 343 bp, which was verified on a 2.0% agarose gel stained with 10 μl ethidium bromide. The restriction site on the C-T rs6446482 polymorphism was cut using NlaIV, an NEB restriction enzyme. A restriction fragment length polymorphism (RFLP) study was performed for 18 h at 37 °C to cleave the restriction site, and the following band widths were found after digestion: AA-343 bp, AG-343/198/144, and GG-198/144 bp.

2.7. Statistical analysis

For statistical analysis, the 25th version of SPSS software was applied. Hardy Weinberg equilibrium (HWE) was utilized to compare genotype frequencies in controls. Student's *t*-test was performed to compare the two groups of clinical data. Numerical and categorical factors were evaluated while comparing T2DM patients to controls. All genetic models of genotypes and allele frequencies were assessed in T2DM cases and controls. One-way ANOVA was performed between the rs6446482 polymorphism and T2DM details (Khan et al., 2019).

3. Results

3.1. Clinical investigations

In this case-control study, 200 subjects were divided into 100 T2DM cases and 100 controls. In T2DM cases, 30% of females and 70% of male participants were present, whereas in controls, 42% of females and 58% of male individuals were present. T2DM patients and controls had mean ages of 55.73 ± 10.58 and 41.45 ± 8.25, respectively. T2DM patients had a BMI of 27.33 ± 1.78, and controls had a BMI of 25.95 ± 3.54. T2DM patients' and controls' weights were determined to be 74.34 ± 10.88 and 72.34 ± 8.54, respectively. T2DM patients and controls had approximately identical heights (162.71 ± 8.70 vs. 162.62 ± 8.61). The diabetes levels for FBG were determined to be 13.16 ± 5.32 and 5.22 ± 0.61 for controls. T2DM patients had HDL-c values of 0.87 ± 0.38 and LDL-c levels of 3.82 ± 1.06, whereas controls had 0.67 ± 0.24 and 3.80 ± 0.82. T2DM patients had high levels of TC and TG (5.65 ± 1.26 and 2.27 ± 1.31), whereas controls had low levels (5.19 ± 0.98 and 1.57 ± 0.71). There was a statistically significant connection between T2DM family history and cases and controls (p = 0.01). The levels of age, weight, BMI, FBG, HDL-c, TC, and TG were found to be significantly related to anthropometric and biochemical data (p < 0.05). Other factors, such as sex, height, and LDL-c levels, were found to be linked with T2DM patients and controls (P > 0.05). The clinical features of T2DM patients and healthy controls are shown in Table 1.

Table 1
Baseline features between T2DM patients and controls involved in this study.

Characteristics	Cases (n = 100)	Controls (n = 100)	P Values
Gender (female/male)	30/70	42/58	0.58
Age	55.73 ± 10.58	41.45 ± 8.25	0.001
BMI	27.33 ± 1.78	25.95 ± 3.54	0.002
Weight	74.34 ± 10.88	72.34 ± 8.54	0.01
Height	162.71 ± 8.70	162.62 ± 8.61	0.91
FBG [mmol/L]	13.16 ± 5.32	5.22 ± 0.61	<0.0001
HDL-c [mmol/L]	0.87 ± 0.38	0.67 ± 0.24	0.001
LDL-c [mmol/L]	3.82 ± 1.06	3.80 ± 0.82	0.87
TC [mmol/L]	5.65 ± 1.26	5.19 ± 0.98	0.01
TG [mmol/L]	2.27 ± 1.31	1.57 ± 0.71	<0.0001
Family History	36 (0.36)	21 (0.21)	0.01

3.2. Genotype investigation

In this study, only the rs6446482 polymorphism was genotyped between 100 T2DM patients and 100 controls. The call rate of rs6446482 loci was found to be greater than 95%, which contributed to the dependability of the results. The HWE analysis was carried out on control subjects and revealed a significant association (p = 0.01). In T2DM cases, the CC, CG, and GG genotypes were found to be 49%, 32%, and 19%, whereas in controls, the CC, CG, and GG genotypes were found to be 68%, 24%, and 08%, respectively. The C and G allele frequencies were found to be 0.65 and 0.35 in T2DM patients and 0.80 and 0.20 in control subjects, respectively. When T2DM cases were compared to control subjects, genotypes (GG vs. CC: OR-3.29 [95% CI: 1.33–8.13]; p = 0.007), dominant (CC vs. CG + GG: OR-2.12 [95% CI: 1.24–3.92]; p = 0.006), and allele frequencies (G vs. C: OR-2.15 [95% CI: 1.37–3.38]; p = 0.0007) revealed a positive association. Both recessive (CC + CG vs. AA: OR-0.67; 95% CI: [0.36–1.25] & p = 0.20) and codominant models (CC + GG vs. CG: OR-0.37 [95% CI: 0.15–0.89]; p = 0.02) revealed a negative association (see Table 2).

3.3. ANOVA analysis

Table 3 demonstrates the ANOVA results, which was performed between rs6446482 polymorphisms in T2DM risk parameters such as age, weight, height, BMI and FBG. In GG genotypes, age (57.94 ± 12.65), weight (73.13 ± 8.27), and BMI (27.75 ± 1.92) were found to be high. Height (163.15 ± 9.50) was shown to be increased in CG genotypes. FBG (13.63 ± 5.12) levels were observed to be elevated in CC genotypes. None of the genotypes had a positive correlation. ANOVA demonstrated the significance of risk factors associated with the rs6446482 polymorphism.

Table 2
Genotype and allele frequencies between T2DM and control subjects in the rs6446482 polymorphism in the *WFS1* gene.

Genotypes/Allele	Cases	Controls	OR (95%CI)	P Value
CC	49 (49%)	68 (68%)	Locus	Locus
CG	32 (32%)	24 (24%)	1.85 (0.97–3.52)	0.05
GG	19 (19%)	08 (08%)	3.29 (1.33–8.13)	0.007
CC vs. CG + GG	49 (49%)	68 (68%)	2.12 (1.24–3.92)	0.006
CC + CG vs. GG	51 (51%)	32 (32%)	0.37 (0.15–0.89)	0.02
CC + GG vs. CG	68 (68%)	76 (76%)	0.67 (0.36–1.25)	0.20
C allele	130 (0.65)	160 (0.80)	Locus	Locus
G allele	70 (0.35)	40 (0.20)	2.15 (1.37–3.38)	0.0007

Table 3
Association of ANOVA with the rs7923837 polymorphism in T2DM risk.

	CC = 49	CG = 32	GG = 19	P Value
Age	56.75 ± 11.01	52.75 ± 7.93	57.94 ± 12.65	0.05
BMI	27.27 ± 1.79	27.18 ± 1.69	27.75 ± 1.92	0.82
Height	163.05 ± 8.12	163.15 ± 9.50	161.21 ± 9.04	0.61
Weight	72.67 ± 8.23	71.35 ± 9.33	73.13 ± 8.27	0.71
FBG	13.63 ± 5.12	12.51 ± 5.83	13.06 ± 5.07	0.68

4. Discussion

Based on the available data, the WFS1 gene is connected with Wolfram syndrome (WS), also known as diabetes insipidus, insulin-deficient diabetes mellitus, optic atrophy, and deafness. The inheritance pattern of WS was documented as autosomal recessive, and mutations in the *WFS1* gene were documented. Despite the fact that early-onset diabetes is a feature of this multisystem disease, individuals with recessive loss of function did not have a genetic explanation for WS. If insulin production is impaired, the *WFS1* gene's hereditary contribution to diabetes risk is limited. Endoplasmic stress is produced by β-cell degeneration as a result of tungsten protein misfolding. Despite the possibility of autosomal recessive mutations, several dominant mutations have been reported. Numerous studies have revealed a connection between wolframin and diabetes. A high degree of WFS1 expression was found in pancreatic islets and insulinoma β-cell lines despite its abundance. Homeostasis of the ER and diabetes are well-known relationships between them. This is because T2DM patients have increased insulin production as a result of insulin resistance. As a result of enhanced WFS1 expression in wild-type mice, ER stress and β-cell dysfunction can be observed in WFS1 knockout mice. One of the functional roles of the *WFS1* gene appears to be a membrane glycoprotein that is inherently endoglycosidase H-sensitive. As part of the ubiquitin-protease pathway, WFS1 negatively controls transcription Factor 6, which is implicated in ER stress signalling. Wolframin is abundantly expressed in the pancreas, and it may contribute to the formation of a protein precursor of insulin by folding a preproinsulin precursor. Previous data show that the amount of pancreatic islet expression is much greater than that found in pancreatic exocrine cells. Progressive loss of β-cells, poor glucose tolerance, and cell cycle progression occurs as a result of deficiency in the Wolframin component in mice. This is associated with the activation of ER stress and the unfolded protein response. This supports the hypothesis that WFS1 is involved in the regular operation of β-cells, but its involvement during embryogenesis is not yet known. There is substantial evidence that the WFS1 protein interacts with the mesenchymal and/or epithelial contacts that occur during pancreatic development (Rigoli et al., 2011; Khan, 2021; Elek et al., 2015).

One of the highest rates of diabetes in the Arab world is found in Saudi Arabia, specifically T2DM. There are other epigenetic mechanisms, such as DNA methylation, histone modification and noncoding RNA, that have been implicated in the pathogenesis of this complicated disease, caused by fast environmental and behavioural changes in the Gulf population (Al Safar et al., 2018). According to the International Diabetes Federation, Saudi Arabia is one of the top 10 countries in the world, with the highest diabetes rates among persons aged 20 to 79. It is also worth noting that during the past three decades, the prevalence of T2DM has risen from 7% in 1989 to 32% in 2009. There are still many unknowns in regard to T2DM patients' glycaemic management (Alzaheb and Altemani, 2018). However, the level of impairment differed between studies conducted in Saudi Arabia, other Middle Eastern countries, and other countries throughout the world. A previous study suggested that Saudi Arabia spent approximately 14% of its overall health budget on diabetes care. The study suggests that diabetes patients' health and quality of life should be improved to lower the social and personal expenditures associated with diabetic health care in Saudi Arabia (Alshayban and Joseph, 2020). The frequency of T2DM was high in the Gulf region. Bahrain, Oman, and Kuwait reported high prevalence rates of 25.7%, 16.1% and 21%, respectively. As a result of the high prevalence of diabetes in Saudi Arabia, the number of diabetic patients has continuously expanded in recent decades (Al Mansour, 2020). Most of the studies focusing on detecting the susceptibility loci for T2DM have been done in European populations. To accurately understand the gene-environment interactions that increase the risk of developing diabetes, association studies performed in the Middle East populations must be replicated in populations of people who have a higher risk of developing diabetes, including populations in the Gulf States, such as those found in the Arabian Peninsula. In addition, the Saudi Arabian population is not genetically uniform and has unique genetic origins as well as a high prevalence of some diseases caused by complicated mutations. Even though obesity is strongly connected with one of the greatest prevalence frequencies, there are many other risk factors to consider.

In this study, the rs6446482 polymorphism was studied in the Saudi population with 100 T2DM cases and 100 controls, and the current study results showed a significant association with the GG genotype (GG vs. CC: OR-3.29 [95% CI: 1.33–8.13]; p = 0.007), dominant genotype (CC vs. CG + GG: OR-2.12 [95% CI: 1.24–3.92]; p = 0.006), and allele frequencies (G vs. C: OR-2.15 [95% CI: 1.37–3.38]; p = 0.0007). In this study, all T2DM patients were confirmed to be overweight subjects, and previous studies recorded obesity as one of the risk factors for T2DM as well as GDM (Khan et al., 2015b). Age, weight, BMI, FBG, family history and HDL-C, TC and TGs showed a positive association between T2DM and control subjects ($p < 0.05$).

The role of the WFS1 gene in diabetes is connected through insulin-producing pancreatic β-cells ceasing to function. Four SNPs (rs10010131, rs6446482, rs752854, and rs734312 (H611R)) in the *WFS1* gene have been demonstrated to be associated with T2DM in Caucasians (Sandhu et al., 2007). In this study, only the rs6446482 polymorphism was studied, and this study showed a positive association. WFS1 and T2DM risk SNPs were also replicated in a previous study (Franks et al., 2008). Global studies performed with the rs6446482 polymorphism showed all forms of association with T2DM (Singh et al., 2012; Han et al., 2010; Lee et al., 2008; Domínguez-Cruz et al., 2020; Mita et al., 2008; Domenech et al., 2002) and gestational diabetes (El Noury et al., 2018; Lauenborg et al., 2009) and type 1 diabetes (Nyaga et al., 2018). WFS1 gene polymorphisms rs734312 and rs10010131 were found to have a negative relationship with T2DM in meta-analysis studies (Cheng et al., 2013).

In terms of strengths and limitations, this study has certain drawbacks: (I) in this study, only one polymorphism was studied, (ii) a limited sample size was enlisted, and (iii) no protein investigations were performed. This study strength was completed with 100 equal T2DM cases and controls. This study was well designed due to the use of a specific restriction enzyme. There were no obese or morbidly obese subjects in the controls. This was a sex-based study in both the T2DM cases and controls ($p = 0.58$).

5. Conclusion

This study confirms that the rs6446482 polymorphism has a positive association with T2DM patients. This study suggests that meta-analysis studies involving the rs6446482 polymorphism in T2DM be upgraded.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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