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Case-Control Study on *KCNJ11* Gene Polymorphism and Type 2 Diabetes Mellitus

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ABSTRACT

Background: *Type 2 diabetes mellitus (T2DM) it is a complex metabolic condition marked by insulin resistance and dysfuction of pancreatic β-cells. Genome-wide association studies (GWAS) indicate that multiple genes, notably potassium inwardly-rectifying channel, subfamily J, member 11 (KCNJ11), have a major role in susceptibility to type 2 diabetes mellitus (T2DM). Aim of study: To estimate the risk of KCNJ11 gene polymorphism (rs5215) in the development of type 2 diabetes.* ***Methods:*** *A case-control research was done involving 300 participants, including 150 individuals with Type 2 Diabetes Mellitus and 150 healthy controls. Genotypic data was obtained by allele-specific Polymerase Chain Reaction (AS-PCR), while biochemical measurements involved fasting blood glucose, serum insulin levels, and lipid profiles.* ***Results:*** *genotyping of the SNP rs5215 under codominant model show highly significant variation between diabetic and control group with T/C genotype (OR = 0.48, 95% CI = 0.29 – 0.78, P = 0.006),dominant model (T/C-C/C) show statistically significant result (OR = 0.56, 95% CI = 0.35 – 0.89, P = 0.013), The minor allele frequency (C) was decreased in T2DM patient compared to controls(OR = 0.79, 95% CI = 0.89 – 1.77, P = 0.19), the association did not reach statistical significance.* ***Conclusion:*** *Single nucleotide polymorphism of the KCNJ11 gene (rs5215) is insignificantly linked to susceptibility of type 2 diabetes mellitus in the AL-Najaf population.*

Keywords: KCNJ11, rs5215 SNP, T2DM.

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INTRODUCTION

Diabetes mellitus (DM) is comprises a group of metabolic disorders marked by persistent hyperglycemia, either from defective insulin secretion, insulin action, or a combination of them [(1)]. leading to disturbances in glucose, protein, and lipid metabolism[(2)], The main cause of premature death, primarily from cardiac disease, is type 2 DM [(3)] The latest edition of the International Diabetes Federation (IDF) Diabetes Atlas indicates that over 500 million individuals globally have diabetes, with predictions suggesting a 20% increase by 2030 and a 46% increase by 2045[(4)]. Chronic hyperglycemia in diabetes mellitus may result in damage, dysfunction, and failure of the end organs, including the retina, kidneys, neurological system, heart, and blood vessels [(5)] . Numerous environmental factors have been linked to obesity that cause type 2 diabetes, including being overweight, eating a high-fat diet, getting older, and not exercising [(6)], Furthermore , a group of candidate genes known as their correlated with type 2 diabetes due to its impact in the development of metabolic disorder [(7)(8)], Several Genome wide association studies (GWAS) identified a number of single nucleotide polymorphism (SNPs) located in intragenic or intergenic regions have been associated with higher risk of developing type 2 diabetes [(9)], All of these SNPs correlated with T2DM have been shown

to influence insulin production , insulin resistance, and glucose or lipid metabolism [10]. ABCC8, KCNJ11, and PPARG are represent the most widely studied genes associated with diabetes [11]. they strongly affect insulin action , glucose metabolism, pancreatic beta cell function, and other metabolic processes like energy intake and expenditure, as well as lipid metabolism [12]. KCNJ11 is a significant candidate gene in the risk of T2DM because of its role in regulating glucose-induced insulin production [13]. The KCNJ11 gene, which encodes the Kir6.2 protein subunit, belongs to the inward rectifying potassium channel J subfamily. The Kir6.2 protein encodes the pore-forming subunit of the ATP-sensitive potassium channel (KATP) in pancreatic beta cells. An increase in the KATP activity in pancreatic beta cells is associated with decreased insulin production, rising fasting plasma glucose levels, impaired pancreatic cell function, and the development of diabetes mellitus [14]. Studies indicate that KCNJ11 is significantly expressed in pancreatic β-cells [15]. Polymorphic loci, including rs5215 within the gene area have been associated with diabetes [16]. KCNJ11 gene is placed onto the short arm of chromosome 11, exactly at 11p15.1 It is a single exon gene of 4.08 kilobases, starting from 17385246 bp to 17389331bp [17].

The first predominant variant of the KCNJ11 gene was denoted rs5215. The National Center for Biotechnology Information (NCBI) provides the nucleotide sequences for single nucleotide polymorphism (SNP).

*rs5215 This SNP is T/C within exon 1: Major allele = T, Minor allele = C. The T nucleotide is replaced by C nucleotide.

Several studies have investigated the association between KCNJ11 single nucleotide polymorphism (SNP) and type 2 diabetes [18].which focusing on the rs5215 SNP.

Current study intends to examine the correlation of KCNJ11 rs5215 polymorphism with T2DM in Alnajaf population. Also, it aims to investigate the effect of the polymorphism on major metabolic parameters such as fasting blood glucose, insulin, lipids, and HOMA-IR. Finding such associations will aid in identifying genetic predispositions and allow clinicians to more effectively intervene with tailored strategies guided by precision medicine principles .

METHODS

Study population

The current research is a case-control approach, consisting of 150 diabetic patients (the pathological case group) and 150 apparently healthy (the control group). The study's duration spanned from September 2024 to March 2025. Patients had been selected from the Al-Najaf Centre for Diabetes and Endocrinology, situated in Al-Sadr Medical City in the Al-Najaf Governorate. the diagnosis was established according to the criteria recommended by the American Diabetes Association (ADA), which involves fasting plasma glucose (FPG) ≥126 mg/dL and/or HbA1c ≥6.5%, along with specific clinical signs and symptoms [19]. The participants in the control group were of comparable age and had no familial or personal medical history of diabetes or cardiovascular disease. Standard procedures had been followed for performing anthropometric measurements, including height and weight, for calculating Body Mass Index (BMI) utilising the formula: weight (kg)/height (m^2). Ethical approval was obtained from the Faculty of Medicine at the University of Kufa.

Biochemical Measurements

Five milliliters of blood were drawn from each participant via peripheral vein puncture following an overnight fast. Subsequently, the blood samples were divided into two aliquots.first part: Three milliliters of blood were transferred into a plain tube and allowed to coagulate at 37°C for approximately 15 minutes, followed by centrifugation at 2000 xg

for 10-15 minutes. The resulting serum was separated and stored at -20°C for the determination of phenotype parameters, second part: two milliliters of blood were combined with EDTA in a tube for genetic analysis purposes.

The enzymatic assessment of fasting blood glucose has been done by using the glucose oxidase-peroxidase assay [20] , the lipid profile include triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C). All estimates were calculated using enzymatic colorimetric techniques. Low-density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C) have been measured using the Friedewald equation [21], Serum insulin levels were evaluated using a sandwich ELISA kit, and the diagnosis of insulin resistance was determined by the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) approach, involving the corresponding formula: HOMA-IR = [Glucose (mg/dL) x Insulin (μU/mL)] 405 [22].

DNA Extraction

Geneaid DNA mini kit (Korea) was used to extract genomic DNA from whole blood samples. DNA concentration and purity were determined via absorbance measurements using BIODROP. Absorbance at 260 nm corresponds to DNA concentration, with nucleic acids exhibiting maximum absorbance at this wavelength, while proteins absorb at 280 nm [23]. The A260/A280 ratio was utilized to gauge DNA purity, with a ratio falling in the range of (1.8- 2.0) indicative of pure DNA. Additionally, absorbance at 230 nm, reflecting other contaminants, was considered by calculating the A260/A230 ratio.

Genotyping

KCNJ11 polymorphisms (rs5215) were genotyped using polymerase chain reaction-allele specific (PCR-AS), the DNA sample was amplified using the following primer:

Reverse primer: CAAGAAAGGCAACTGCAACG,

Allele T:GTGGTGTGGGCACTTTGATAlleleC:GTGGTGTGGGCACTTTGAC and analyze PCR products on 2.5% agarose (60 min and 75V) then visualized under UV light , The presence or absence of a specific PCR product indicates the presence or absence of the corresponding allele. Amplification was performed utilizing 2 PCR tube in a total reaction volume of 25 µl in each tube as the following: tube1/ 12.5µl GoTaq Green Master Mix (Promega, U.S.A), 2µl of reverse primer, 2µl of allele T primer ,2.5µl of nuclease free water, and 6µl genomic DNA solution as template.

Tube2/ 12.5µl GoTaq Green Master Mix (Promega, U.S.A), 2µl of reverse primer, 2µl of allele C primer ,2.5µl of nuclease free water, and 6µl genomic DNA solution as template. The PCR reaction program of the protocol of rs5215 SNP were; (initial denaturation) 95 °C for 5 min followed by 35 cycles of (denaturation) 95 °C for 30s,(annealing) 60 °C for 30 s,(extension) 72 °C for 1 min. and (final extension) at 72 °C for 7min. The amplification product of rs5215 SNP was 917 bp for allele T and allele C , analyzed on 2.5% agarose.

Statistical Analysis

All statistical analyses were done with SPSS software version 25. Continuous variables were expressed as mean ± standard deviation (SD) and evaluated using Student's t-test. Biochemical variations among the genotypes were assessed using one-way ANOVA. Every statistical analysis were performed at a significance level below 0.05. The Hardy-Weinberg equilibrium (HWE) was determined using χ^2 testing to check if observed genotype frequencies deviated from estimated proportions (24) . Additionally, odds ratios (ORs) with 95% confidence intervals (CIs) have been calculated from logistic regression

analysis to investigate the effect of genetic factors on the probability of developing diabetes.

RESULTS

The results of biochemical and anthropometric values for ages and BMI in the patients group versus the control group revealed insignificant variations according to the P value (0.077) and (0.617) for age and BMI respectively. Other parameters were found to change in T2DM patients when compared with healthy individuals. Levels of TG, TC, VLDL-C, LDL-C, FBG, insulin and HOMA-IR exhibited significant increases ($p<0.001$) in the patient's group when compared with the group of control. Yet, HDL-C levels exhibited significant reduction ($P= <0.008$) in T2DM patients when compared with the control group as shown in Table (1).

Genotyping results of rs5215 SNP for patients as well as control group was analyzed under numerous inheritance models as shown in Table (2), The codominant and additive model shows highly significant variation, The dominant model show a significant results, Recessive model showed insignificant variations while Frequency of allele (C) show insignificant difference, Adjusting for age, sex, and BMI, revealed no statistically significant differences in the results.

The product of PCR was electrophoresed on 2.5% agarose (60min and 75V) and immediately visualized under the UV light. The amplification product of KCNJ11 gene SNP rs5215 T/C was found to be 1917bp (Allele T and Allele C).as shown in Figure (1). The genetic power to identify an influence at rs5215 was merely 82%, regarded adequate. Additionally, there was a notable deviation from Hardy-Weinberg equilibrium ($p < 0.02$) in the control group. The minor allele frequency (C allele) was not identified as a risk factor for diabetes in the current study (p-value=0.19).

Table 1: Anthropometric and biochemical factors values of T2DM and control.

Parameters	Control Mean±SD	T2DM Mean±SD	*P-Value*
No (M/F)	150(71/79)	150(67/83)	
Age (y)	50.65 ±6.48	52.16 ±8.18	0.077
BMI (Kg/m2)	29 ±5.17	29.31 ±5.34	0.617
TG (mg/dl)	114.99 ±12.72	241.95 ±35.39	0.001
TC (mg/dl)	144.85 ±22.70	249.15 ±23. 45	0.001
VLDL-C (mg/dl)	24.61 ±4.68	47.29 ±10.90	0.001
LDL-C (mg/dl)	97.10 ±12.51	152.58 ±10.29	0.001
HDL-C (mg/dl)	43.86 ±8.62	41.39±7.31	0.008
FBG (mg/dl)	93.83 ±8.63	168.67 ±26.24	0.001
Insulin (µU/L)	6.38 ±5.23	11.61 ±6.92	0.001
HOMA-IR	1.49 ±1.38	4.80 ±2.89	0.001

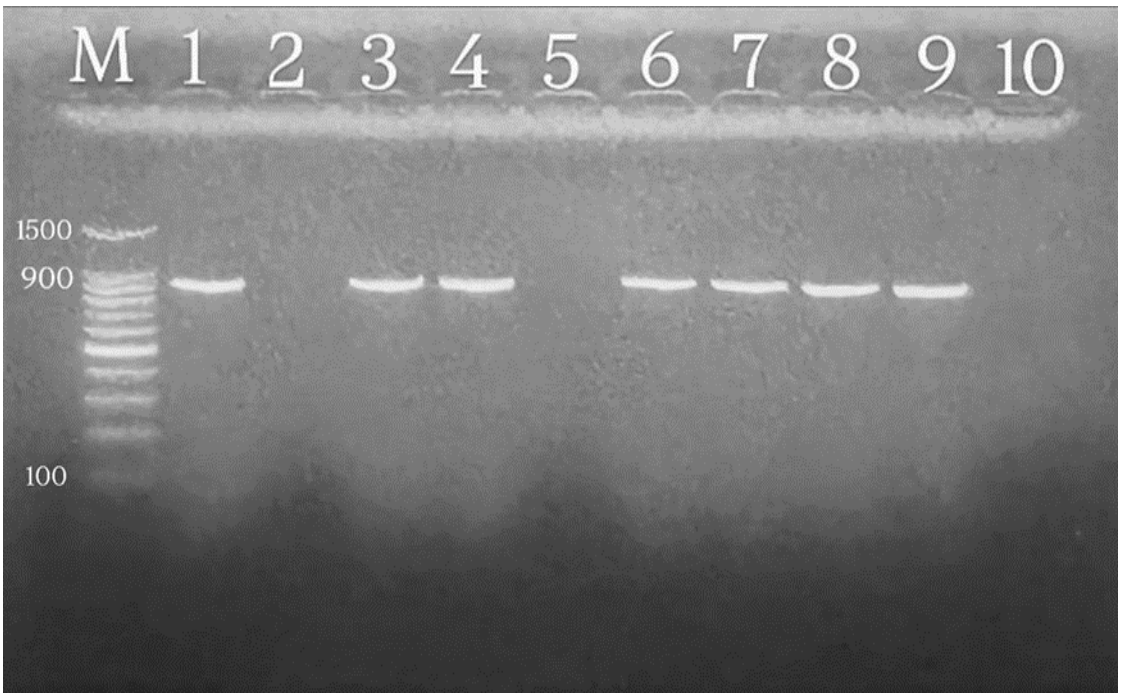

Figure 1: AS-PCR product of KCNJ11 rs5215 on 2.5% agarose gel electrophoresis. M:Ladder.Lane 1&9 indicates genotype (TT) 917bp.Lane 3,4,7&8 indicates genotype (TC) 917bp.Lane 6 indicates genotype (CC) 917bp.

Table 2: Genotype and allele frequency results of SNP rs5215 T/C KCNJ11 gene in T2DM and control subjects.

Genotyping rs5215 T/C	Control N=150(%)	Patient N=150(%)	OR 95%CI	P value	Adjusted OR 95%CI	P value
Codominant						
T/T	57(38%)	78(52%)	1.00(Reference)	0.006	1.00(Reference)	0.006
T/C	81(54%)	54(36%)	0.48(0.29-0.78)		0.48(0.30-0.79)	
C/C	12(8%)	18(12%)	1.11(0.49-2.53)		1.13(0.50-2.57)	
Dominant						
T/T	57(38%)	78(52%)	1.00(Reference)	0.013	1.00(Reference)	0.015
T/C-C/C	93(62%)	72(48%)	0.56(0.35-0.89)		0.57(0.36-0.90)	
Recessive						
T/T-T/C	138(92%)	132(88%)	1.00(Reference)		1.00(Reference)	

Genotyping rs5215 T/C	Control N=150(%)	Patient N=150(%)	OR 95%CI	P value	Adjusted OR 95%CI	P value
C/C	12(8%)	18(12%)	1.58(0.72-3.48)	0.25	1.60(0.73-3.51)	0.24
Additive						
T/T-C/C	69(46%)	96(64%)	1.00(Reference)	0.0014	1.00(Reference)	0.0015
T/C	81(54%)	54(36%)	0.47(0.29-0.75)		0.47(0.30-0.76)	
Allele						
T	195(65%)	210(70%)	(Reference)	0.19		
C	105(35%)	90(30%)	0.79(0.89-1.77)			

DUSSCUTION

The current study explored the association of the KCNJ11 rs5215 (T>C) polymorphism with Type 2 Diabetes Mellitus (T2DM) susceptibility in the Alnajaf population . In contrast to results from several studies in different populations, we observed no significant association between the rs5215 variant and T2DM risk under any genetic model (codominant, dominant, recessive, and additive), even after adjusting for potential confounders such as age, gender, and BMI. These results are consistent with previous studies conducted in other Middle Eastern populations. For instance, similar null associations were reported in Iraqi populations [24], as well as among Saudi Arabian population [17], and Iranian population [25]. On the other hand, substantial associations between rs5215 and T2DM risk have been established in korean [26] and Indians populations [27].

The gap between our results and those of other populations indicates a possible ethnic-specific influence. Variations in linkage disequilibrium patterns, gene-environment interactions (e.g., diets, physical activity), and genetic heterogeneity likely account for the differences in the effect of rs5215 among population. Alnajaf population may have unique environmental exposures or genetic backgrounds that influence the impact of KCNJ11 on glucose metabolism. While genotyping was performed by using approved AS-PCR protocols, additional validation by alternative genotyping techniques would enhance the reliability of our findings.

The genetic power for rs5215 was 82%, adequate for detecting moderate effects, therefore supporting the reported relationships. The Hardy–Weinberg equilibrium (HWE) test for the control group indicated a deviation (p=0.02), suggesting potential concerns such as population stratification, genotyping errors, or an actual correlation with illness susceptibility.

It is essential to acknowledge the limitations of this study. Firstly, the sample size of diabetic patients and control individuals is relatively small. Secondly, factors related to socioeconomic behavior, such as lifestyle, smoking, and dietary habits, may introduce confounding variables. Consequently, further research is required to address and control for these potential confounders.

CONCLUSIONS

Polymorphisms in KCNJ11 gene SNPs rs5215 are not associated with T2DM in Alnajaf population.

Recommendations

A larger sample size is necessary to examine the link between the rs5215 SNPs of the KCNJ11 gene and conditions incidence, Future research should investigate various SNPs of the KCNJ11 gene in the Alnajaf population. These investigations can identify the SNPs that are more prevalent in this governorate and are implicated in the pathophysiology of T2DM, Assess the effects of the KCNJ11 gene on type 1 diabetes mellitus.

Ethical approval

The present study Which is conducted by (RAM, HA,ZDZ) was approved by the local Department of Biochemistry and Kufa ethical committee.

Statement of permission and conflict of interests

The authors declare that they have no competing interests.

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