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The Association of Single Nucleotide Polymorphism (+1893CC/AA) of INPPL1 Gene with Type 2 Diabetes Mellitus in AL-Najaf Population

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Abstract

Background: Insulin resistance in the insulin target tissue and insufficient insulin production by pancreatic β-cells are the hallmarks of chronic type 2 diabetes mellitus (T2DM). Certain INPPL1 gene variants have been associated with type 2 diabetes in Indian, Japanese, British, and French populations, according to researches.

Objectives: Evaluate the relationship between the risk of type 2 diabetes mellitus (T2DM) in the AL-Najaf community and single nucleotide polymorphisms (SNPs) in the INPPL1 gene.

Methods: A case-control research was conducted with a sample size of 200 individuals. The INPPL1 gene's single nucleotide polymorphism (+1893CC/AA) was genotyped using the PCR-RFLP technique.

Results: The frequency of the A allele ($p < 0.001$) was shown to be considerably greater in T2DM patients, and SNP +1893CC/AA was found to be strongly linked with T2DM.

Conclusion: The vulnerability of the AL-Najaf community to type 2 diabetes mellitus has been strongly connected to the single nucleotide polymorphism (+1893CC/AA) in the INPPL1 gene, according to the results.
Key words: Type 2 diabetes mellitus, INPPL1, Gene polymorphism.

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Introduction: Diabetes mellitus is a progressive and chronic endocrine disorder characterized by hyperglycemia in which high levels of glucose in the blood. Diabetes mellitus disrupts the control of glucose, lipid, and protein metabolism, leading to significant macro- and micro-vascular problems. Furthermore, individuals with DM lack or have insufficient levels of insulin, the crucial hormone that regulates the body's glucose balance (1). Iraq is a diverse nation with a population of 40,222,493 individuals as of 2021. The total number of adults in the population is 19,914,400, with 1,505,000 of them being affected with diabetes (2)

The classification of diabetes mellitus includes type 1, which is caused by the death of pancreatic beta cells by autoimmune cells (3), and type 2. The predominant form of diabetes is T2DM, which represents over 90% of all cases (4). Gestational diabetes refers to glucose intolerance that occurs during pregnancy (5). Other types of diabetes mellitus are associated with monogenic deficiencies in β- cells function, also referred to as monogenic defects of the β-Cells (6).

The etiology of T2DM can be attributed to genetic factors, environmental variables, or potential medicinal consequences (7). Numerous environmental variables, including a high-cholesterol diet, a lack of exercise, obesity, stress, and lifestyle changes, considerably raise the risk of T2DM (8)(9). Insulin secretory dysfunction, the main cause of β-cell impairment and what gives rise to type 2 diabetes, can be brought on by failures in the synthesis of insulin itself or any of the insulin precursors (10) , as well as by disruption of the secretion process(11).

The complex polygenic nature of the disease has been shown by several genome-wide association studies conducted in recent years (12)(13). Most of these genetic locations increase the risk of type 2 diabetes by primarily affecting the synthesis of insulin (14). More than 30 SNPS linked to diabetes have so far been found and these genes are thought responsible for It characterization of about 10% of the of DM occurrence chance (15).

The INPPL1 gene, also known as inositol polyphosphate phosphatase-like 1, is located on human Chromosome 11 (16). It consists of 28 exons and spans a length of 3777 nucleotides (17). INPPL1 promoter is regulated by Specificity protein 1 (Sp1) transcription Factor. Which is a zinc finger family transcription factor which can regulate a number of genes that influence cell survival and proliferation (18).

This gene produces an SH2-containing 5'-inositol phosphatase protein, which is widely expressed at high levels of mRNA in the brain, adipose tissue, placenta, liver, and skeletal muscle (19). Research has shown that INPPL1 plays a crucial role in inhibiting insulin signaling. This process is accomplished by removing phosphate groups from phosphatidylinositol-3,4,5-trisphosphate, which is the active byproduct of the enzyme. This byproduct stops phosphoinositide 3-kinase (20)

and thus prevent the translocation of glucose transporter 4 from the cytosol to the plasma membrane, which results in the uptake of glucose (21).

The decrease of INPPL1 in the number of animals has enhanced insulin sensitivity by augmenting insulin signaling. Moreover, another study performed suggests that it has a significant impact in the development of insulin resistance and type 2 diabetes (22).

The (+1893CC/AA) variant identified in the INPPL1 gene is a regularly reported single nucleotide polymorphism (SNP) that is associated with an increased risk of type 2 diabetes mellitus (T2DM)(17).

The INPPL1 gene has been discovered to be associated with type 2 diabetes mellitus (T2DM) in populations from Britain, France, and Japan(20). A research done in the Chinese population to investigate the correlation between certain genetic variations (SNP) on the INPPL1 gene and the occurrence of type 2 diabetic mellitus. The findings demonstrated notable disparities in the genotype and allele frequency of the INPPL1 (+1893CC/AA) locus between individuals with T2DM and the healthy control group(23). A study was directed to consider the association between two specific genetic variants (SNPs) of the INPPL1 gene and the susceptibility to type 2 diabetes mellitus (T2DM) in the population of North India. A significant correlation found between the SNP +1893CC/AA and T2DM, with a significantly higher occurrence of this SNP in individuals with T2DM(24).

Materials and method:

Samples collection: A case-control research was conducted with a sample size of 200 individuals. The participants were categorized into: a group of 100 individuals with type 2 diabetes and a group of 100 healthy individuals. The study was conducted from December 2022 to December 2023.The study was conducted in the Postgraduate Laboratory Department of Biochemistry at the University of Kufa, specifically within the Faculty of Pharmacy. The protocol of the study received approval from the Ethical Committee of the Faculty of Pharmacy, Kufa University.

DNA extraction and genotyping: DNA is extracted from blood using Presto™ Mini gDNA Kit (Geneaid). INPPL1 polymorphisms (+1893CC/AA) were genotyped using (PCR-RFLP), then cheeked on an agarose gel using electrophoresis. SNP's amplification technique was carried out using appropriate primers (F-TAAGGGCCACATGGGCTATCACCCC and R-CCTGACACCCAGGGATACTGCAAGT) and master mixture.

According to instruction of the primer synthesiser company, the primers (originally lyophilized), were dissolved in the free ddH2O to obtain a final concentration of 100 µM/µl served as a stock solution that stored at -20 °C. A concentration of 10 µM/µl was prepared from the stock primers to be used as a work primer.

PCR reactions were performed in a total volume of 25 µl containing 12.5 µl commercially available PCR mastermix (GoTaq® Green Master Mix, Promega, India) (containing dNTP, Taq DNA polymerase, MgCl2, 10 × PCR buffer), 0.5 µl (15 pmol/ µl) each of forward and reverse primers, 50 ng of genomic DNA and 9.5 µl sterile nuclease free water. The PCR involves initial denaturation at 95 ◦C for 5 min followed by 35 cycle of denaturation at 95 ◦C for 30 s, annealing at 60 ◦C - 65 ◦C for 45 s, and extension at 72 ◦C for 45 s and final extension at 72 ◦C for 10 min for the complete amplification of all PCR fragments.

In the RFLP technique, restriction endonucleases are utilized to cut the amplicon produced by the amplification process at a particular spot, usually 4 or 6 bp of certain nucleotide sequences. Optimization of Digestion Conditions (RFLP) was achieved by using:

1) 10 units of Ddel restrictions enzyme (New England Biolabs).

2) 10µl of PCR product.

3) incubation time (overnight).

Tubes were centrifuged in a micro-centrifuge for some seconds at 2000xg, mixed through pipetting as well as incubated for a certain adequate time. Agarose gel-electrophoresis was used to assess the findings.

Statistical analysis: Using (SPSS.v.26.0 software) SPSS Inc, the mean levels of each characteristic via genotype were compared using the Student t-test and ANOVA. The chi-square test was also used to examine categorical data (alleles and genotypes). The results were expressed as a P-value, an odds ratio (OR), and a confidence interval (CI 95%).

Results: Table (1) shows that the evaluation of results of ages values in T2DM group versus the controls group revealed insignificant variations according to the P value (0.75). Other parameters were found to change in T2DM patients when compared with healthy individuals. Levels of BMI FBG, insulin and HOMA-IR revealed significant ($p<0.0001$) increases in T2DM group when compared with the control group.

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Table(1): Phenotype Parameters Values of T2DM and Control Groups.

Parameter	Mean ± SD		P Value
	T2DM Group (N=100)	Control Group (N=100)	
Gender (M\F)	(59\49)	(53\45)	
Age (Years)	49.89 ± 8.32	49.5 ± 9.17	0.75
BMI (Kg/M2)	27.16 ± 3.48	26.29 ± 2.46	0.039*
FSG (Mg/Dl) Median	183	88	<0.0001**
Insulin	6.32 ± 1.1	5.48 ± 0.94	<0.0001**
HOMA-IR	2.86 ± 0.81	1.23 ± 0.28	<0.0001**

Note: * = means that it's a significant result.

Table (2): INPPL1 Gene SNP (+1893CC/AA) Genotype and Allele Frequency Results in T2DM and Control Subjects.

	Control Group (N= 100)		Patient Group (N= 100)		OR (CI %)	P Value
	NO.	%	NO.	%		
Genotyping						
Codominant						
CC	77	57	58	43	Reference Group	
CA	15	50	15	50	0.55 (0.33-0.91)	0.02*
AA	8	22.86	27	77.14	1.85 (1.01-3.38)	0.037*
Dominant						
CC	77	57	58	43	Reference Group	
AA + CA	23	35.38	42	64.62	1.83 (1.19-2.8)	0.004
Recessive						
CC + CA	92	55.76	73	44.24	Reference Group	
AA	8	22.86	27	77.14	3.38 (1.61-7.06)	0.0001
Frequency						
C	169	56.33	131	43.67	Reference Group	
A	31	31	69	69	2.23 (1.53-3.24)	
	200		200		400	

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The results of SNP +1893CC/AA of T2DM as well as control persons with numerous inheritance models are shown in (table 2). The codominant model showed that patients of homozygous (AA) (OR = 1.85, CI 95% = 1.01 – 3.38, P = 0.037) and heterozygous (CA) (OR= 0.55, CI 95% = 0.33-0.91, P = 0.02) genotypes significantly elevated; with respect to control group. The dominant model indicated that patients of (AA+CA) genotypes increased significantly (OR=0.82, CI 95%= 0.62 - 1.09, P=0.2) with respect to the controls. Recessive model showed that controls of AA genotypes significantly declined (OR =1.52, 95% CI = 0.78 – 2.95, P =0.14) with respect to the patients groups. Frequency of (A) in patient group significantly increased (OR= 2.23, CI 95%= 1.53 – 3.24, P= < 0.001) with respect to the controls group.

Discussion: Numerous genome-wide association studies of T2DM conducted in recent years have proven the polygenic nature of the disease (1). Most of these locations increase the risk of type 2 diabetes by primarily affecting production of insulin(13).

Single nucleotide polymorphisms in numerous locations responsible for regulation insulin secretion were found as a result of genome-wide research (15). Many SNPS linked to diabetes have been found and these genes are thought responsible for about 10% of the of DM occurrence chance (25). Comprehending the genetic contribution of several genes to type 2 diabetes mellitus in our current society is crucial. As a result, this problem might encourage the disease's management strategy to be improved. Examining how gene variation affects the risk of disease onset and resistance to it is a significant problem(7).

INPPL1 is a significant negative modulator of insulin signaling, according to researches and this is carried out by dephosphorylating Phosphatidyl Inositol-3, 4, 5-Trisphosphate, the enzyme's active byproduct, which inhibits phosphoinositide 3-. Additionally, In the T2DM populations of the British, French, and Japanese, certain INPPL1 polymorphisms have been linked to the disease.(20) Its contribution in insulin signaling has been directly demonstrated through in vivo investigations; INPPL1 reduction in animals has enhanced insulin sensitivity by increasing insulin signaling. also Its involved in insulin resistance, hypertension, and T2DM (22).

In this research FSG is measured, which is notably higher in the patient group compared to the control group due to inadequate release of insulin, which increases serum glucose levels. This study is supported by previous study that found that type 2 diabetes is caused by a lack of insulin, which disrupts the insulin secretory apparatus and reduces the volume of b cells. This inability to produce enough insulin results in an increase in free sugar glucose (26). The relationship between patients and healthy individuals insulin level in this research are significantly different as the insulin levels were higher in T2DM patient group.It may be confirmed by the findings of another study (27) that individuals with type 2 diabetes have high insulin levels since they are receiving therapies that help

regulate insulin levels such as glinides, sulfonylureas, and dipeptidyl-peptidase 4 inhibitors. Insulin resistance was assessed in this study, and it was shown to be considerably higher in the patients group than in the control group. IR has shown beneficial for both early disease diagnosis and therapy selection.

The results of SNP +1893CC/AA of T2DM as well as control individuals with different inheritance models are examined. The analysis of the data of SNP under the codominant demonstrated that patients of homozygous genotypes AA significantly elevated than controls with odds ratios of more than 1. This finding suggests that AA allele is associated with diabetes risk and or/development of T2DM in the studied sample, i.e., AL-Najaf population. while the patients with the heterozygous genotype CA under codominant demonstrated insignificant differences relative to the controls group with odds ratios of less than 1 and This indicates that there is no relationship between heterozygous genotype CA and development/ or risk of T2DM. This finding is in line with a recent study(24) that determined that the presence of the INPPL1 gene polymorphism at +1893CC/AA increases the vulnerability to T2DM in the population of North India. also This study aligns with a previous study (23) that found significant differences in the genotype and allele frequency of the (+1893CC/AA) locus between T2DM patients and the healthy control in Chinese population.

Conclusions: The +1893CC/AA SNP of INPPL1 gene is implicated in the pathogenesis of T2DM in AL-Najaf population. According to the allele frequency the presence of homogeneous AA genotype responsible to develop T2DM in AL-Najaf population.

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