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Original Research Article

Association of Dipeptidyl Peptidase 4 Gene Single Nucleotide Polymorphism with Type 2 Diabetes Mellitus

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Abstract:

Background: Dipeptidyl Peptidase 4 (DPP-4), a ubiquitous serine peptidase, cleaves N-terminal dipeptides from various substrates, such as GLP-1 and GIP, which are involved in controlling energy homeostasis. DPP-4 is identified as an important target for glycemic control due to its direct and indirect effects on glucose and lipid metabolism. There are various reports of DPP-4 release from adipose tissue, the liver and the pancreas. It is also known as T-cell antigen CD 26. DPP-4 polymorphisms are important targets for understanding the pathogenesis and genetics of diabetes mellitus.

Aim: To study the association between SNP in the DPP4 gene in patients with type 2 diabetes mellitus.

Methods: 50 patients with type 2 diabetes mellitus and 50 healthy controls were recruited, and blood parameters were assessed (RBS, AST, ALT, and ALP). The blood sample was subjected to DNA extraction, amplification was done by ARMS PCR, and polymorphism identified using agarose gel electrophoresis. The results were statistically analyzed and tabulated.

Results: The mean random blood sugar level among the participants with T2DM was 222.4 mg/dL, and among healthy participants, it was 116.37 mg/dL. The mean AST among T2DM and healthy controls was 26.74 IU/L and 25.04 IU/L, respectively, with no significant difference ($p = 0.278$). The mean ALT among T2DM and healthy controls was 28.83 IU/L and 20.96 IU/L, respectively. There was a highly significant ALT among the participants with T2DM ($p = 0.001$). Genotype distribution for DPP-4 at rs12617656 among T2DM and controls does not obey Hardy-Weinberg law. The dominant model and recessive model showed a significant association of rs12617656 with the south Indian population, with odds ratio of 6.0 (1.242–28.99) and 1.601 (0.613–4.176), respectively.

Conclusions: We found an increase in the frequency of homozygous patients (C/C) for DPP-4 (rs 12617656) single nucleotide polymorphism in patients with T2DM. Identification of single nucleotide polymorphisms of DPP-4 among families contributes to the early identification of susceptible individuals to start a specific DPP4 inhibitor drug that will be more effective than other anti-diabetic drugs.

Keywords: DPP4, Incretin, Type II Diabetes Mellitus, SNP rs12617656.

Introduction

Diabetes mellitus is a complex metabolic disorder characterized by hyperglycemia due to dysfunction of islet cells in the endocrine pancreas, which increases insulin resistance and decreases insulin secretion. [1] Insulin maintains glucose homeostasis by regulating the uptake of glucose into skeletal muscle and adipose tissue by the GLUT-4 receptor. [1]

Incretin hormone is like glucagon-like peptide 1 (GLP-1) and GIP (Glucose-Dependent Insulinotropic Polypeptide) and contributes to the control of insulin release by acting on the islet cells of the pancreas. Incretin hormones are synthesized by the uptake of glucose in the diet, which has insulinotropic activity.[2]

They are responsible for 60% of insulin secretion postprandial.[3] The DPP-4 enzyme, which regulates the incretin hormone by terminating its action, causes chronic hyperglycemia due to diabetes, which leads to dysfunction, damage, and failure of

function in various organs like the kidney, eyes, nerves, blood vessels, and heart.

DPP-4, a ubiquitous serine peptidase, cleaves N-terminal dipeptides from various substrates, such as GLP-1 and GIP, which are involved in controlling energy homeostasis. It is a type II transmembrane glycoprotein with a molecular weight of 110 kDa that cleaves a penultimate X-proline and X-alanine from the N-terminal side of the polypeptide. They are shed from the membranes of cells and found in circulating plasma. [4] One of the important factors in the pathophysiology of T2DM [5] is the proteolytic cleavage of incretin hormones, which is regulated by the DPP-4 enzyme.

Other than its enzymatic role, it also has a multifunctional role as a signaling protein as well as a ligand for the extracellular matrix.

Hence, in this case-control study, the frequency of the DPP-4 gene polymorphism rs12617656 (T<C) located in chromosome 2q24.3 was studied among cases of diabetes mellitus in the South Indian population.

Aim and Objective: To find out if there is any association of SNP (Single Nucleotide Polymorphism) in the DPP-4 gene of patients with T2DM and to compare them with healthy individuals.

Materials and Methods

The study was conducted at Rajiv Gandhi Government General Hospital, Chennai, after obtaining Institutional Ethics Committee on 100 subjects of age group of more than 30 years, of whom 50 were known cases of T2DM and 50 were apparently healthy subjects who served as controls.

Persons with an age >30 years, both male and female, and T2DM patients willing to give written informed consent were included in the study.

Persons with an age <30 years, type 2 diabetic patients, those who are on DPP4 inhibitors, pregnancy, and autoimmune diseases were excluded from the study.

Study Procedure: The blood was collected in a K2-EDTA tube used for DNA extraction. DNA was extracted from whole blood with the help of the Puri Fast Human Blood Genomic DNA Mini Spin Prep Kit. The kit utilizes silica-based membrane technology in the form of a convenient spin column, which yields purified DNA.

Cells are incubated with proteinase K in the presence of chaotropic salt, resulting in lysis. This immediately inactivates all nucleases. The nucleic acids that are released from the cell bind selectively to special glass fibers pre-packed in the purification filter tube.

Bound nucleic acids are purified in steps of repeated rapid "wash and spin," which removes the contaminating cellular components. Finally, the low-salt elution will release the nucleic acids from the glass fiber. This method eliminates the use of organic solvent extractions and nucleic acid precipitation, thereby leading to rapid purification of many samples simultaneously.

All the DNA extraction steps were carried out at room temperature except the incubation step at 56°C.

The eluted nucleic acid (i.e., DNA) was analyzed for the measurement of DNA by spectrophotometer. DNA concentration and purity were measured.

The maximum absorbance of pure DNA samples occurs over a broad peak at around 260 nm. At 280 nm, it absorbs only about half as much UV light compared to 260 nm. DNA absorbs UV light due to the heterocyclic rings of the nucleotides. The ratio of absorbance at 260 nm and at 280 nm (A260/A280) is used to assess the purity of the DNA sample, and it should be around 1.8.

ARMS PCR, apart from amplifying the target gene like conventional PCR, will also produce different sized products depending on the allelic variation present. This is the major advantage of ARMS PCR over conventional PCR. Therefore, ARMS PCR is a one-step process in which there is no need for further restriction digestion of the amplified products.

Arms PCR Reagent Composition

Table 1

Reagent	Volume	Mutant
Red Dye PCR Master Mix	10 µL	10 µL
T allele Primer Mix	2.5 µL	-
C allele Primer Mix		2.5 µL
Purified DNA sample	7.5 µL	7.5 µL
Total	20 µL	20 µL

The above contents are added to a 0.2-ml fresh PCR tube, then transferred to a thermocycler and made into a setting to amplify my products.

Amplification Protocol for PCR:

Table 2

	Steps	Time	Temperature
35 Cycles	Initial denaturation	5 min	95°C
	Denaturation	30 sec	95°C
	Annealing	30 sec	60°C
	Extension	30 sec	72°C
	Final extension	5 min	72°C

After the PCR process is over, the product of PCR is interpretation, which is done after electrophoresis.

The DNA extracted was identified by agarose gel electrophoresis. Low EEO (electro endosmosis) agarose was preferred for performing DNA gel electrophoresis. The extracted DNA was subjected to gel electrophoresis and visualized under a GELDOC gel documentation instrument using UV light.

Statistical Analysis: Statistical analyses were performed using Statistical Package for Social Sciences software (SPSS version 23, USA). A normality test was done, and it was found that all variables were normally distributed. Depending upon the nature of the data, appropriate parametric statistical tests were chosen, and a p-value of < 0.05 was considered to be significant.

The frequency of genotype distribution among controls and cases was compared using the chi-square test (X2). All the biochemical parameters among cases and controls are tested by an unpaired t-test. The level of significance of the p value was set at <0.05. If the p-value<0.001 was considered to be strongly significant, by using logistic regression analysis, the odds ratio of two-tailed p-values and the 95% CI (confidence interval) were analyzed. The frequency of genotype distribution was tested using Hardy-Weinberg equilibrium.

Results

Table 3: Mean Distribution of Random Blood Sugar among T2DM and Healthy Controls

Random Blood Sugar (mg/dL)	**Mean ± SD**		**P-Value**
	T2DM	**Controls**	
	222.4 ± 94.64	116.37 ± 13.89	0.001**
Serum Urea in mg/dL	**Mean ± SD**		**p-Value**
	T2DM	**Controls**	
	26.59 ± 7.640	20.04 ± 5.571	0.001**
Serum Creatinine in mg/dL	Mean ± SD	**p-Value**	
	T2DM	Controls	
	0.924 ± 0.242	0.657 ± 0.155	0.001**
** H- Highly Significant * S-Significant			

Table 3 shows the mean distribution of random blood sugar among T2DM and healthy controls. The mean random blood sugar level among the participants with T2DM was 222.4 mg/dL, and among healthy participants, it was 116.37 mg/dL. There was a highly significant random blood sugar level among participants with T2DM ($p = 0.001^{**}$).

The mean serum urea level among the participants with T2DM was 26.59 mg/dL, and among healthy participants, it was 20.04 mg/dL. There was a highly significant serum urea level among the participants with T2DM ($p = 0.001^{**}$).

The mean serum creatinine level among the participants with T2DM was 0.924 mg/dL, and among healthy participants, it was 0.657 mg/dL. There was a significant increase in serum creatinine levels among the participants with T2DM ($p = 0.001^{**}$).

Table 4: Comparison of Mean AST, ALT and ALP among the Participants of T2DM and Healthy Controls

Variable	Mean ± SD		P-Value
	T2DM	**Controls**	
AST in IU/L	26.74 ± 8.263	25.04 ± 7.454	0.278
ALT in IU/L	28.83 ± 12.38	20.96 ± 8.720	**0.001****
ALP in IU/L	91.65 ± 17.27	86.57 ± 19.63	0.168
** H- Highly Significant, * S- Significant			

Table 4 shows the comparison of mean AST, ALT, and ALP among the participants with T2DM and healthy controls. The mean AST among T2DM and healthy controls was 26.74 IU/L and 25.04 IU/L, respectively, with no significant difference (p = 0.278). The mean ALT among T2DM and healthy controls was 28.83 IU/L and 20.96 IU/L, respectively. There was a highly significant ALT among the participants with T2DM (p = 0.001**). The mean ALP was found to be 91.65 IU/L and 86.57 IU/L among participants with T2DM and healthy controls. There was no significant difference in the ALP between T2DM and controls (p = 0.168).

Table 5: Hardy-Weinberg Equilibrium for DPP-4 Gene: rs12617656 (T2DM and Controls)

Group	Count	Gene Type			Chi-Square Value (Σ^2)	p-Value
		Heterozygous (T/C)	Non-risk Homozygous(T/T)	Risk Homozygous(C/C)		
T2DM	Observed	31	9	10	6.303	**0.043***
	Expected	33	11	6		
Controls	Observed	35	13	2		
	Expected	33	11	6		
Total	Observed	66	22	12		
	Expected	66	22	12		
Chi-square test, ** H- Highly Significant, * S- Significant						

Table 5 shows the Hardy-Weinberg equilibrium for the DPP-4 gene rs12617656 allele. The observed and expected count for heterozygous (T/C), non-risk homozygous (T/T), and risk homozygous (C/C) alleles among cases (T2DM) and controls shows a significant difference (p = 0.043**), showing disequilibrium. Thus, the genotype distribution for DPP-4 at rs12617656 among T2DM and controls does not obey Hardy-Weinberg law.

$p^2 + 2pq + q^2 = 1$

p = frequency of homozygous dominant genotype (T/T) q = frequency of homozygous recessive genotype (C/C) pq = frequency of heterozygous genotype (T/C).

Table 6: Genotype Distribution of DPP-4 Gene at rs12617656 among T2DM and Controls

Genotype	N (%)		Chi-Square(χ^2)	p-Value
	T2DM	Controls		
Heterozygous (T/C)	31 (62)	35 (70)	49.520	0.001**
Non-risk Homozygous (T/T)	9 (18)	13 (20)		
Risk Homozygous (C/C)	10 (20)	2 (4)		
Chi-square test, ** H- Highly significant, * S-Significant				

Table 6 shows the genotype distribution of the DPP-4 gene at rs12617656 among T2DM and controls. The percentages of heterozygous alleles (T/C) among T2DM and controls were 62% and 70%, respectively. The percentage of non-risk homozygous alleles (T/T) among T2DM and controls was 18% and 20%, respectively. The percentage of risk homozygous alleles (C/C) among T2DM and controls was 20% and 4%, respectively. There was a significant increased frequency of risk homozygous allele (C/C) among T2DM (p = 0.001**).

Table 7: Bivariate Logistic Regression Analysis for DPP4 Polymorphisms at rs12617656 with T2DM among South Indian

Model	Allele	Frequency		Chi-Square Association (Σ^2)	OR (Odds Ratio)	p-Value (95% CI)
		T2DM	Controls			
Additive	T/C	31	35	0.244	1.279	0.621 (0.481–3.401)
	T/T	9	13			
Dominant	T/C +T/T	40	48	6.061	6.000	**0.014** * (1.242-28.99)
	C/C	10	2			
Recessive	T/T	9	13	3.256	1.601	**0.041***(0.613-4.176)
	C/C +T/C	41	37			
** H- Highly Significant, * S- Significant						

Table 7 shows bivariate logistic regression analysis for DPP-4 polymorphism with T2DM among the south Indian population. The dominant model and recessive model showed a significant association of rs12617656 with the south Indian population, with odds ratios of 6.0 (1.242–28.99) and 1.601 (0.613–4.176), respectively. There was no significant association between the additive model and the south Indian population of T2DM and controls ($p > 0.05$).

Discussion

DPP-4 is identified as an important target for glycemic control due to its direct and indirect effects on glucose and lipid metabolism. There are various reports of DPP-4 release from adipose tissue, the liver, and the pancreas. It is also known as T-cell antigen CD 26. It is an integral cell membrane protein. DPP-4 is also observed to have protease-independent activity on GLP and glucagon. [6]

The DPP-4 enzymes are encoded by the DPP-4 gene. The DPP-4 gene is located on the chromosome 2q24.2 locus, with 26 exons and 25 introns located on the reverse strand. The DPP-4 gene is expressed elsewhere, but the organ-dependent percentage of expression varies from one cell to another type of cell in an organ. DPP-4 acts as a novel adipokine synthesized from adipose tissue that increases insulin resistance and stimulates inflammatory activity.

Polymorphism in DPP-4 was associated with type 2 diabetes mellitus, obesity, and metabolic syndrome. In a genomic-wide association study, various SNPs were identified in the DPP-4 locus.

Lee et al. conducted a study to evaluate the expression of DPP-4 in peripheral blood T cells and circulating sDPP-4 and to find the association with T2DM patients in the Korean population.[7] They found the expression of DPP-4 on CD4 and CD8 T cells to be significantly increased in patients with T2DM. [7] A. Bhargave et al. conducted a study in Haryana, India, to find the association of DPP-4 gene polymorphisms of various SNP sequences with T2DM. There was a significant association with SNPs rs3788979 and rs7608798 related to T2DM. In the Indian population, the GG genotype of SNP rs3788979 and the T allele of SNP rs7608798 increase the susceptibility to T2DM. [8]

Ahmed RH et al. conducted a study in the Malaysian population with different ethnic groups (Malay, Chinese, and Indian) to find the association of DPP-4 gene polymorphisms of various SNP sequences with T2DM. The association was found with SNPs rs12617656, rs7633162, and rs4664443. Such an association was more evident among the Indian ethnic group with respect to rs12617656. SNP rs4664443 G allele polymorphism in subjects with T2DM was associated with increased DPP-4 levels. No association was found between T2DM and the other SNPs rs1558957, rs1861978, rs2160927, rs17574, rs7608798, and rs1014444. [1]

The current study was conducted to find out whether the SNP rs12617656 C allele in the DPP-4 gene polymorphism in South Indian subjects is associated with T2DM and to evaluate the SNP rs 12617656 within the DPP-4 gene that could be associated with the sDPP-4 levels.

In our study, we found T2DM to have a strong association with the polymorphism in the SNP rs12617656 C allele of the DPP4 gene. The presence of associations with the south Indian population among the dominant and recessive models in the logistic regression is also in concurrence with the study by Ahmed et al. [1]

DPP-4 polymorphisms are also identified in various other conditions, like myocardial infarction, obesity, rheumatoid arthritis, etc.

In the DPP-4 locus, 44 informative HapMap SNPs with Hardy-Weinberg p-values and MAFs ≥ 0.05 were found, and all of them are intronic or located in the 3'-flanking region. [9]

The promoter region in the DPP-4 gene does not contain conventional TATAA or CCAAT sequences but is characterized by a cytosine- and guanine-rich promoter region. [10]

Conclusion

We found an increase in the frequency of homozygous patients (C/C) for DPP-4 (rs 12617656) single nucleotide polymorphism in patients with T2DM. DPP-4 gene polymorphism has a positive correlation with ALT. This indicates that an elevation of the liver-specific enzyme ALT has a positive correlation with fatty liver disease specific to the liver. The DPP-4 gene single nucleotide polymorphism association with T2DM implies a role in disease pathogenesis. Hence, the identification of single nucleotide polymorphism of DPP-4 among families contributes to the early identification of susceptible individuals to start a specific DPP4 inhibitor drug that will be more effective than other anti-diabetic drugs.

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