Journal of Diabetes & Metabolic Disorders
https://doi.org/10.1007/s40200-022-01131-y

RESEARCH ARTICLE

Genetic variation in *DPP-IV* gene linked to predisposition of T2DM: A case control study

Archna Bhargave[1] · Kiran Devi[1] · Imteyaz Ahmad[1] · Anita Yadav[2] · Ranjan Gupta[1]

Received: 18 July 2022 / Accepted: 15 September 2022

Abstract

Purpose *DPP-IV* is a ubiquitously expressed cell surface protein that can be presented in soluble forms. It has recently gained medical importance as its inhibitors are widely being used as treatment of T2DM. The present research aims to resolve whether genetic variants of *DPP-IV* have association with susceptibility to T2DM.

Method Two variants of *DPP-IV* were detected in 100 controls and 100 T2DM by PCR–RFLP technique. Demographic characteristics were recorded. Clinical characteristics were analyzed by enzymatic method. Statistical analysis was performed using SPSS-21.

Results Demographic and clinical characteristics differ significantly between two groups. The genetic variation in SNP rs3788979 and SNP rs7608798, both in case and control, were in accordance with Hardy–Weinberg Equilibrium (p value > 0.05). Both SNPs rs3788979 and rs7608798 were significantly related to T2DM (p- < 0.05). Minor G allele of rs3788979 was linked with the susceptibility of T2DM (*p*-value-0.000; OR- 4.235). T allele of SNP rs7608798 conferred the risk of diabetes with OR-2.235.

Conclusion This is the first attempt to investigate the association of *DPP-IV* gene with T2DM in Indian population. The finding of study concludes that genetic variation in *DPP-IV* gene may considerably increase the risk of developing T2DM.

Keywords Type 2 diabetes mellitus · Dipeptidyl Peptidase- IV · Single nucleotide Polymorphism · Gene polymorphism · And Genetic model

Introduction

Type 2 Diabetes Mellitus (T2DM) is a heterogeneous metabolic disorder, comprising 90% of the global cases of diabetes mellitus (DM). It results from both/either peripheral resistance of insulin and reduced secretion of insulin [1, 2]. Around 463 million people were affected by diabetes mellitus in 2019 worldwide and the count is estimated to rise to 700 million people by 2045. In India, in 2019,77 million people have diabetes mellitus and by 2045, 134 million people are expected to be affected by it [3]. Uncontrolled diabetes not only has dire consequences on health and well-being of an individual but also imposes an economic burden on the individual as well as on the nation [4, 5]. The total expenditure on diabetes was USD 760 billion in 2019 which is assumed to rise to USD 845 billion in 2045 [6].

The susceptibility to T2DM is determined by an interplay of genetic and metabolic factors [5]. Recently several genes ascertaining the existence of T2DM have garnered attention such as *ABCA1*, *SOD2, SLC30A8*, *KCNJ11, ADIPOQ, TCF7L2*, *IL-10*, *PON2*, and *DPP- IV* [7].

DPP-IV gene present on chromosome 2(2q24.3) encodes for Dipeptidyl Peptidase -IV (DPP-IV). DPP-IV also known as CD26, is a ubiquitously expressed type II transmembrane glycoprotein [8]. Usually, it is cleaved from the membrane in a

✉ Ranjan Gupta
r.gupta@kuk.ac.in

Archna Bhargave
archna.bhargave90@gmail.com

Kiran Devi
kiranduhan302@gmail.com

Imteyaz Ahmad
ahmadimteyaz99@gmail.com

Anita Yadav
ayadav@kuk.ac.in

[1] Department of Biochemistry, Kurukshetra University, Kurukshetra 136119, Haryana, India

[2] Department of Biotechnology, Kurukshetra Univerity, Kurukshetra, India

Published online: 12 October 2022

cell-type-specific manner by the process known as shredding, which allows it to get released into blood circulation in soluble form (sDPP-IV). Functionally sDPP-IV is a circulating soluble multifunctional enzyme that exhibits serine exopeptidase activity. As a result, it cleaves dipeptides from the number of substrates and gets involved in several processes like signalling, immune cell activation, and cardiovascular regulation [2, 9–12]. Incretins such as GLP-1 (glucagon-like peptide-1) and GIP (glucose-dependent insulinotropic peptide) are one of the physiological substrates of DPP-IV. Both GLP-1 and GIP are released after meal intake under neural control and stimulate the release of insulin from beta cells of the pancreas in a glucose-dependent fashion. Thus, oral inhibitors of DPP- IV which inhibit DPP- IV enzyme by more than 80% for up to 24 h and enhance meal-related concentration of GLP and GIP are widely being fostered for treatment of T2DM [13]. Researchers have found that genetic polymorphism in *DPP-IV* alters its expression and predisposes individuals to several diseases. One of the recent studies shows that *DPP-IV* restriction sites rs12617656, rs466443and rs7633162 are associated with T2DM and rs3788979 is associated with a high risk of Covid-19 disease [2, 14]. Similarly, Bouchard [15] found that homozygotes for minor allele T of rs7608798 *DPP-IV* are at lower risk of hyperglycemia/diabetes (*p*-value-0.002). Another study by Snarska [16] elucidated that A allele of rs7608798 is strongly associated with acute pancreatitis.

Review of scientific literature reveals that majority of the prior studies for genetic variants of *DPP-IV* association with disease and plasma lipid levels have been conducted on Caucasians of European ancestry [14, 15]. However, close to the negligible attempts have been made for understanding the same or with T2DM, in the Indian population. Keeping in mind this gap in knowledge and acknowledging that Indian population is highly predisposed to T2DM incidence, the present study was carried out. Also, the current study is aimed to understand the association of *DPP-IV* with clinical parameters that may reveal new insights in understanding T2DM occurrence and progression at genetic level. This may open new avenues in early diagnosis of the disease.

Materials and method

Subjects

The present study was conducted with 200 subjects including 100 controls (healthy) and 100 T2DM patients (who were on follow-up treatment). The subjects geographically belonged to different regions of Haryana (India). The inclusion criteria considered while selecting T2DM patients were strictly according to diagnostic criteria of the Indian Council of Medical Research (ICMR) which involves fasting plasma glucose $\geq$ 126 mg/dl 'or' HbA1c $\geq$ 6.5%. Subjects diagnosed with T1DM and other severe diseases of kidney, liver, and coronary artery disease were excluded from the study. Subjects with > 40 years old, normal fasting plasma glucose concentration (100 mg/dl) and HBA1c < 5.6% were considered as controls in the study and were recruited from outpatient department (OPD) of hospitals who were on regular body check-ups.

Pregnant and lactating mothers were excluded from both groups. Subjects without complete information were also excluded from the study. The study protocol was approved by Institutional Human Ethical Committee Kurukshetra University, Kurukshetra Haryana and all subjects had signed written informed consent.

Sample collection

A questionnaire was designed for recording demographic characteristics such as age, gender, alcohol consumption, and smoking habits. Body mass index (BMI) was evaluated by dividing the weight in kilogram (Kg)/height in meter square (m^2). Blood samples (5 ml) were drawn early in the morning from subjects after 10–12 h of overnight fasting in K_2EDTA coated vials.

The clinical parameters such as Plasma sugar fasting (PSF), glycated haemoglobin (HbA1c), total cholesterol (TC), triacylglycerol (TG), high-density lipoprotein (HDL) were measured using enzymatic methods.

Genomic DNA extraction and genotyping

Salting out method of Millller et al. [17] with minor modifications was used for extracting genomic DNA from whole blood samples. Integrity of extracted DNA samples was evaluated on 1% agarose gel and purity was ascertained using A260/A280 ratio. The samples with ratio in the range of 1.6 to 2.0 were considered for PCR-based analysis.

Genotyping of selected SNPs were determined using the PCR–RFLP technique. Both SNPs rs3788979 and rs7608798 are located in the intron region. The polymerase chain reaction (PCR) technique was carried out in 25 µl reaction mixture containing 0.5 µl each forward and reverse primer, 2.5 µl $MgCl_2$, 0.5 µl dNTPs, 16.5 µl nuclease-free water, 2.5 µl Taq buffer, 1 µl Taq DNA polymerase and 1 µl DNA (approx 50 ng) as template for amplification. The PCR conditions included denaturation initially for 5 min at 94ºC, followed by 35 cycles of denaturation at 94ºC for 45 s, annealing at appropriate temp for 45 s, extension at 72ºC for 45 s; final extension for 5 min at 72ºC. Amplified PCR products were resolved by electrophoresis (5 V/60 min) using 1.5% agarose gel in Tris Borate-EDTA (TBE) buffer containing 0.5 µg/ml of ethidium bromide. 100 bp (Genei) molecular size ladder was used to determine the size of the bands. The gel was viewed and photographed on a UV Transilluminator (Figs. 1 and 2). The details of primers sequence, and annealing temperature is shown in Table 1

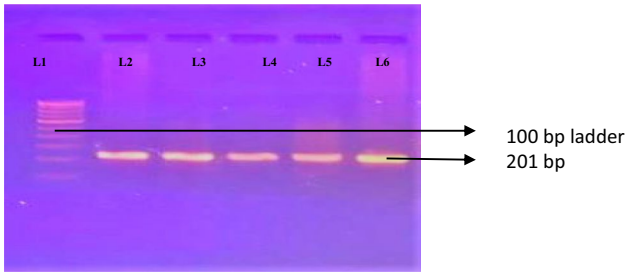

Fig. 1 PCR of rs3788979 of *DPP-IV*. Lane 1 100 bp ladder, lanes 2, 3, 4, & 5 shows 201 bp product

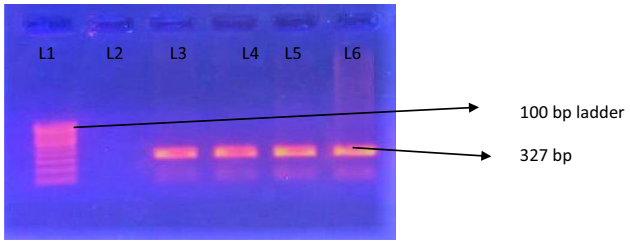

Fig. 2 PCR of rs7608798 of *DPP-IV*. Lane 1 shows100bp Ladder, lane 2 shows blank, lane 3, 4, 5, 6 shows 327 bp product

For SNP rs3788979, 201 bp amplified PCR product was digested using 2 units of HaeIII (New England Biolabs) at 37ºC in 10 µl reaction mixture for 1 h and resolved on 3% agarose gel. Transition mutation at SNP rs3788979 of *DPP-IV* results in loss of restriction site for the enzyme resulting in 201 bp product, with wild type yielding 173 bp & 28 bp product while homozygous mutated resulted in 201 bp product (Fig. 3).

For SNP rs7608798, 327 bp PCR product was digested at 65ºC for 1 h with BsrD1 (New England Biolab) and was resolved on 2% agarose gel. Transition mutation creates BsrD1 site. Homozygous wild type resulted in one band of 327 bp and homozygous mutated results in two bands of 184 and 143 bp (Fig. 4). Restriction enzymes were identified using online molecular tool NEB cutter, GenScript and confirmed using Restriction Mapper.

Statistical analysis

Gene counting method was used to estimate allele and genotype frequencies in all subjects. Chi-square goodness of fit test was employed to ascertain that genotype and allele distribution for each polymorphism follows Hardy -Weinberg Equilibrium (HWE) ($p > 0.05$). Quantitative data is presented as mean ± Standard deviation, whereas qualitative data is summarized as frequency (percentage). Chi-square test was applied to assess the statistical difference between control and T2DM subjects in the categorical data like gender, allele and genotype distribution. Odds ratio (OR) and 95% confidence Interval (CI) was calculated by using logistic regression. Student's t-test was used to compare continuous and normally distributed quantitative demographic and clinical characteristics of T2DM and control subjects. All statistical analyses were performed using

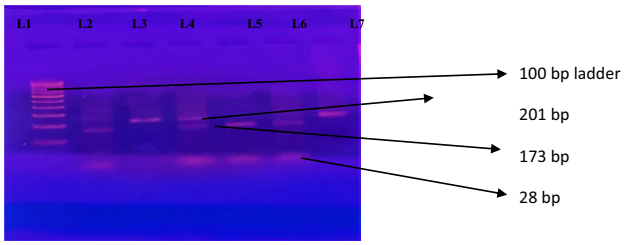

Fig. 3 RFLP of rs3788979 SNP. Lane 1 100 bp ladder, Lanes2,5 & 6 shows 173 and 28 bp product, lanes 3 & 7 shows 201 bp product, lane 4 shows 201, 173 & 28 bp product

IBM SPSS Statistics version 21.0 software. Data analyzed were defined statistically significant at $p < 0.05$. The power of the study assessed was 89.2% and 75.4% at 5% level of significance for SNPs rs3788979 and rs7608798 respectively as calculated by OSSE—an online sample size estimator.

Results

Comparative analysis of demographic characteristics

The case–control study comprised 100 subjects in T2DM group and 100 age-matched subjects in control group. The demographic characteristics have been highlighted in Table 2. Results revealed that smokers were significantly higher in case group than control group (p- value -0.000). A significant difference was observed between two groups when traditional risk

Table1 Primer sequence, Annealing temperature and product

Gene	Variant	Primer sequence	Annealing temperature	Product size
DPP-IV	rs3788979	F-CCACCCCTGATCTTCCTTTT R-GCAGTAGGGAATGGTTTGCT	58ºC	201
	rs7608798	F-GCCTGACCATGAAAAGGTTGC R-CATTCAGGACAAGAGTCTTAC CCTA	53.2 ºC	327

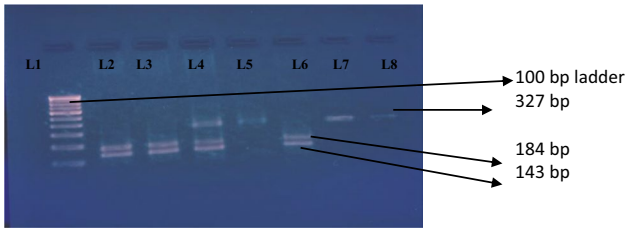

Fig. 4 RFLP of rs7608798. Lane1 shows 100 bp ladder, lanes 2, 3, 6 shows 143&184 bp product, lane 4 shows 143,184&327 bp product, lane 5, 7 &8 shows 327 bp product

factor i.e. exercise was considered (*p*-value- 0.000). Significant difference exists between the T2DM patients and control subjects when diet was considered.

Comparative analysis of anthropometric and clinical characteristics

Comparative analysis of anthropometric and clinical characteristics of both T2DM and control group is shown in Table 3. The study revealed that levels of plasma sugar fasting (PSF), HBA1c were significantly higher in T2DM patients than control subjects (*p*-value- 0.000). Lipid profile measuring total cholesterol (TC), triacylglycerides (TG), high-density lipoprotein (HDL-C), low-density lipoprotein (LDL-C), very low-density lipoprotein (VLDL-C) and apolipoprotein also differ significantly between two groups (*p*-value-0.000). Body Mass Index (BMI), systolic blood pressure (SBP) anthropometric characteristics were significantly lower in control subjects than T2DM subjects. Though HDL-Cholesterol of control group was higher than the case group, no significant difference was observed (*p*-value-0.656).

Genotype distribution of *DPP- IV* genetic variants (rs3788979 and rs7608798) inT2DM and control groups

The genotype distribution of rs3788979 for both control and case were in agreement with Hardy- Weinberg Equilibrium

Table 2 Demographic characteristics of T2DM and control subjects

Demographic characteristics	T2DM	Control	*p* value
Age(Yrs)	49.33 ± 12.02	48.84 ± 13.1	0.391
Gender(F/M)	46/54	47/53	0.443
Smoking (Y/N)	56/44	21/79	0.000*
Alcohol(Y/N)	52/48	46/54	0.198
Exercise(Y/N)	23/72	52/48	0.000*
Diet (Veg / NonVeg)	60/40	78/22	0.003*
			0.442

* *p*-value < 0.05 considered as statistically significant

Table 3 Anthropometric and clinical characteristics of T2DM and control subjects

Characteristics	T2DM	Control	*p* value
BMI (Kg/m2)	27.52 ± 3.32	24.22 ± 3.35	**0.000***
DBP(mmHg)	82.99 ± 9.62	81.13 ± 9.552	0.081
SBP(mmHg)	142.05 ± 16.09	123.59 ± 11.64	**0.000***
PSF (mg/dl)	187.47 ± 58.23	86.94 ± 7.99	**0.000***
HbA1c(%)	7.52 ± 1.71	5.47 ± 0.5	**0.000***
TC(mg/dl)	186.03 ± 46.18	150.61 ± 32.93	**0.000***
TG(mg/dl)	193.31 ± 45.00	110.41 ± 21.39	**0.000***
HDL-C(mg/dl)	46.52 ± 16.19	47.37 ± 9.89	0.328
LDL-C(mg/dl)	105.63 ± 40.54	58.62 ± 20.17	**0.000***
VLDL-C(mg/dl)	36.32 ± 19.87	23.19 ± 9.54	**0.000***
Apo B	100.77 ± 28.20	62.43 ± 14.07	**0.000***

Values are Mean ± SD. Student's t-test was used., **p*-value < 0.05 considered as statistically significant. *BMI* body mass index, *DBP* diastolic blood pressure, *SBP* systolic blood pressure, *PSF* plasma sugar fasting, *HbA1c* glycated haemoglobin, *TC* total cholesterol, *TG* triglycerides, *HDL-C* high density lipoprotein cholesterol, *LDL-C* low density lipoprotein cholesterol, *VLDL-C* very low density lipoprotein cholesterol, *Apo B* apolipoprotein B

(χ2- 2.349 p- value – 0.309 for control, χ2-3.738, and *p*-value -0.154 for T2DM). The genotype distribution frequencies of AA, AG, and GG genotype of rs3788979 of *DPP- IV* in T2DM subject was 49%, 36%, 15%, and in the control subject was 75%, 21%, and 4% respectively. The substantial dissimilarity in the distribution of genotype between the two groups (*p*-value-0.000) was observed. The GG genotype frequency in T2DM subjects was significantly higher as compared to control subjects. The frequency of A and G allele was 85.5% and 14.5% in control subjects and 67% and 33% in T2DM group. It was observed that G allele is more frequent in case group as compared to control subjects with p value of 0.000 (Table 4).

The genotype distribution of *DPP-IV* rs7608798 was also found in accordance with Hardy- Weinberg equilibrium (χ2-1.148, *p*-value-0.563 for control, χ2-0.425, and *p*-value-0.808 for T2DM). Genotype distribution of both case and control groups differ significantly (*p*-value- 0.000**).** TT genotype was found to be more prevalent in T2DM subjects when compared with control subjects. CT genotype was found to be significantly higher in T2DM group than in the control group. It was observed that T allele is the predominant allele inT2DM subjects with OR-2.235 and p value of 0.000 (Table 4).

Association of *DPP-IV* variant (rs3788979 and rs7608798) with T2DM

To explore association of *DPP-IV* rs3788979 with T2DM risk, genotype distribution under dominant and recessive genetic

Table 4 *DPP-IV* rs3788979 and rs7608798 genotype and allele distribution frequencies inT2DM and Control Subjects

Variant			T2DM 100(%)	Control 100(%)	Fischer exact / $\chi 2$	OR	95% CI	*p*-value
rs3788979	Genotype	AA	49	75	15.767			**0.000***
		AG	36	21				
		GG	15	4				
	Alleles	G	66 (33)	29(14)	18.899	2.904	1.776–4.749	**0.000***
		A	134 (67)	171 (85)				
	Dominant Model	AG + GG	51	25	14.346	3.122	1.716–5.683	**0.000***
		AA	49	75				
	Recessive Model	GG	15	4	7.037	4.235	1.353–3.255	**0.004***
		AA + AG	85	96				
rs7608798	Genotype	CC	27	54	15.581			**0.000***
		CT	53	36				
		TT	20	10				
	Alleles	T	93 (46.5)	56(28)	14.642	2.235	1.475–3.386	**0.004***
		C	107 (53.5)	144(72)				
	Dominant Model	CT + TT	73	46	15.126	3.174	1.757–5.733	**0.000***
		CC	27	54				
	Recessive Model	TT	20	10	3.922	2.250	0.994–5.092	**0.024***
		CC + CT	80	90				

Chi-square test was used. OR-Odd Ratio, * $p < 0.05$ is statistically significant

models was analyzed using logistic regression. Under dominant genetic model it was assumed that allele G increases the risk of disease. The AG and GG genotypes under dominant genetic model were found significantly correlated with T2DM ($\chi 2$-14.346; OR-3.122; 95% CI-1.716–5.683; *p*-value-0.0.000). The AA genotype of rs3788979 was ascertained as a protective factor against T2DM risk. *DPP-IV* rs3788979 genetic variant also had significant association under recessive model with T2DM ($\chi 2$- 7.037, OR- 4.235, 95% CI-1.353–13.255, *p*-value-0.008) (Table 4).

Further, association of *DPP-IV* rs7608798 SNP with T2DM risk was investigated using logistic regression. The CT and TT genotype of SNP rs7608798 genotypes under the dominant genetic model were significantly correlated with T2DM (OR-3.174, CI: 1.757–5.733, *p*-value- 0.000) (Table 4). The CC genotype of rs7608798 SNP was found as a protective factor against the risk of disease.

Comparative analysis of *DPP-IV* genotype (rs3788979 and rs7608798) with clinical parameters under dominant model in control and T2DM subjects

Comparative analysis of rs3788979 SNP genotypes with clinical parameters in T2DM and control was investigated using the dominant genetic model (AG + GG vs. AA). No significant variation was seen between rs3788979 SNP and clinical parameters in both T2DM and the control group ($p > 0.05$) (Table 5). Similarly, no significant association was observed between clinical parameters and genotypes of rs7608798 (p-value > 0.05) in both case and control groups. But the levels of clinical parameters were found higher in CT + TT genotype as compared to the CC genotype in the T2DM subjects (Table 6).

Discussion

The prevalence of diabetes is escalating at an alarming rate increasing the overall burden not only on the family but also on the economy of the nation [18]. Researchers around the world are carrying extensive research for understanding and unravelling the pathophysiological mechanism of T2DM and its related complications.

DPP-IV gene has gained considerable interest in recent years due to the gluco-regulatory activity of DPP-IV on GIP and GLP-1(incretin hormones), the main regulators of postprandial insulin secretion. Studies carried out on subjects with T2DM in Malaysian population by Ahmed [19] showed that these subjects have lower levels of active GLP-1 and high levels of sDPP-IV. This speculates that there is more degradation of GLP-1 & GIP resulting in deterioration of glycemic control. Ryskjaer [20] also showed a positive association of DPP- IV activity with plasma glucose and HbA1c.

Reviewing the literature verifies that *DPP-IV* gene is highly polymorphic with its different loci associated withT2DM [2] and number of other diseases [14, 21, 22]. rs12617656, rs4664443and rs12617656 of *DPP-IV* gene were shown to be significantly associated with T2DM in

Table 5 Analysis of *DPP-IV* rs3788979 genotype with clinical parameters under dominant model

Clinical Parameter	Control		*p*-value	T2DM		*p*-value
	AA	AG + GG		AA	AG + GG	
BMI(Kg/m2)	24.75 ± 3.35	24.48 ± 3.61	0.36	27.32 ± 3.07	27.70 ± 3.56	0.28
DBP(mmHg)	80.03 ± 8.92	81.45 ± 9.76	0.22	83.06 ± 8.91	82.92 ± 10.66	0.47
SBP(mmHg)	125.26 ± 13.27	126.48 ± 15.08	0.34	140.27 ± 15.03	143.04 ± 16.68	0.205
PSF(mg/dl)	86.86 ± 7.45	87.16 ± 9.61	0.43	184.71 ± 46.50	190.12 ± 68.02	0.32
HbA1c(%)	5.47 ± 0.50	5.48 ± 0.51	0.39	7.64 ± 1.81	7.45 ± 1.61	0.26
TC(mg/dl)	152.18 ± 30.87	145.88 ± 38.78	0.20	185.90 ± 46.15	186.17 ± 46.61	0.48
TG(mg/dl)	111.64 ± 21.65	107.32 ± 20.73	0.20	198.64 ± 44.07	188.18 ± 45.66	0.12
HDL-C(mg/dl)	47.36 ± 9.61	47.41 ± 10.89	0.49	45.35 ± 11.88	47.65 ± 19.52	0.24
LDL-C(mg/dl)	57.54 ± 19.64	61.86 ± 10.09	0.17	106.83 ± 41.95	104.30 ± 39.88	0.38
VLDL-C(mg/dl)	23.80 ± 10.09	21.53 ± 7.68	0.15	37.30 ± 18.48	35.37 ± 21.27	0.31
Apo B	61.54 ± 13.81	63.56 ± 14.47	0.24	100.73 ± 28.82	100.83 ± 27.73	0.49

Data shown as mean ± standard deviation, **p*-value < 0.05 considered as statistically significant

Table 6 Analysis of *DPP-IV* rs7608798 genotype with clinical parameters under dominant model

Clinical Parameter	Control		*p*-value	T2DM		*p*-value
	CC	CT + TT		CC	CT + TT	
BMI(Kg/m2)	24.50 ± 3.45	23.89 ± 3.23	0.18	27.19 ± 3.36	27.66 ± 3.31	0.23
DBP(mmHg)	82.37 ± 9.57	79.67 ± 9.42	0.08	81.92 ± 10.48	83.34 ± 9.32	0.25
SBP(mmHg)	122.92 ± 11.23	124.00 ± 12.65	0.20	141.09 ± 16.21	142.47 ± 16.14	0.35
PSF(mg/dl)	86.23 ± 8.64	86.75 ± 7.63	0.16	180.22 ± 49.62	190.15 ± 61.59	0.22
HbA1c(%)	5.45 ± 0.54	5.58 ± 0.45	0.10	7.90 ± 1.90	7.37 ± 1.62	0.08
TC(mg/dl)	147.22 ± 33.50	156.34 ± 30.64	0.08	181.11 ± 53.32	187.86 ± 43.52	0.25
TG(mg/dl)	112.55 ± 22.85	108.09 ± 19.66	0.23	185.10 ± 35.57	196.21 ± 47.91	0.14
HDL-C(mg/dl)	46.65 ± 10.62	48.22 ± 8.99	0.21	47.82 ± 11.74	46.04 ± 17.60	0.31
LDL-C(mg/dl)	58.20 ± 22.03	59.12 ± 17.63	0.41	103.27 ± 44.87	106.51 ± 39.03	0.36
VLDL-C(mg/dl)	22.73 ± 9.3	23.94 ± 9.94	0.28	34.41 ± 17.79	37.05 ± 20.66	0.28
ApoB	64.12 ± 16.79	61.40 ± 12.14	0.17	100.54 ± 27.33	101.28 ± 30.52	0.45

Data shown as mean ± standard deviation, **p*-value < 0.05 considered as statistically significant

Malaysian population [2]. The current study also observed substantial association of *DPP-IV* gene polymorphism with T2DM. *DPP-IV* gene loci rs3788979 was significantly associated with T2DM with frequency of GG genotype to be significantly higher in T2DM subjects than control indicating that individuals with this type of genotype are more prone to develop T2D. In coherence with a study conducted on the Chinese population by Xing et al. [23], proposing allele A of rs3788979 to be related with lower serum TG level and BMI, the present study also reported allele A of SNP rs3788979 of *DPP-IV* as a protective allele in diabetes incidence.

In the current study, *DPP-IV* gene variant rs7608798 was also reported to be associated with T2DM. The SNP rs7608798 is reported to have protective effect against coronary artery disease in women of Taiwanese population [24]. However, no association of this SNP with coronary artery stenosis was observed in CAD and T2D patients of Chinese Han population [25]. The frequency of minor allele T was more in T2DM patients than in control subjects, indicating it as a risk allele in the present study. These findings are contrary to the study of Bouchard [15] reporting T allele carriers to be at lower risk of diabetes. The observed difference may be due to diversity in genetic makeup and environmental setup.

Genetic variants of *DPP-IV* gene are also associated with clinical parameters in number of studies carried out in South Asian cohort [26] and Chinese Han population [25], which are inconsistent with the results of present study. No significant correlation was found between *DPP-IV* rs3788979 and rs7608798 genotypes and the circulating clinical parameters. To the best of my knowledge, only few studies have demonstrated the association of *DPP-IV* gene with T2DM in general, and rarely any study has been carried out on Indian population. As considerable efforts are being expanded for identifying individuals at risk of T2DM, the findings of present study appears to be of great relevance.

Apart from genotyping for the SNPs rs3788979 and rs7608798 *DPP-IV* gene polymorphism, the current study

has also examined the relationship of several non-genetic risk factors with the risk of T2DM. The study revealed a significant association of smoking with T2DM which is in accordance with a study carried out by Wannamethee [27] who associated cigarette smoking with an increased risk of diabetes (RR- 1.52; 95%CI-1.10–2.10). Nicotine, an alkaloid present in cigarettes alters glucose homeostasis and also increases blood serum levels of heavy metals such as arsenic, lead & cadmium leading to development of diabetes [28]. Not performing any exercise has been related with an increased risk of diabetes in our study which is in accordance with study of McAuley [29] who revealed that insulin sensitivity can be increased by an intensive exercise program. Another lifestyle factor is diet which also showed an association with risk of diabetes incidence. Vegetarian diet has been associated with improvement in lipid profile and glucose levels in serum thus reducing the risk of T2DM [30]. HbA1c values were found to be reduced by high wheat fibre diet and low-GI legume diet [31]. The result of our study corroborates well with the previous studies that being on a vegetarian diet reduces the risk of diabetes.

On statistically analyzing the values of PSF, HbA1c, Cholesterol, LDL and VLDL, significant difference exists among control and T2DM patients in present study. Similar results were obtained in the studies carried out by Ha [32] in which significant differences in circulating levels of glucose, LDL, high-sensitive C-reactive protein, triglyceride, interleukin-6, and tumour necrosis factor-alpha between diabetic and non-diabetics were identified. Another study carried out on the Iraqi population reported raised levels of serum glucose, total cholesterol, triglycerides, very-low-density lipoprotein, low-density lipoprotein, and malondialdehyde among type 2 diabetes mellitus patients as compared to control [33]. The present study did not reveal any significant difference in High-Density Lipoproteins (HDL) levels in control and T2DM patients, which according to Sorrentino [34] may be due to extended-release niacin therapy given to T2DM patients.

Conclusion

The current study concluded that *DPP-IV* gene polymorphism is associated with type 2 diabetes mellitus suggesting its role in pathogenesis of T2DM. In Indian population GG genotype at rs3788979 increases the susceptibility to T2DM, as G allele is more frequent in T2DM subjects. Allele T of SNP rs7608798 was more common in T2DM subject and may be predisposing risk factor for type 2diabetes mellitus in the studied population. However further studies with large sample size is required to ascertain the association between *DPP- IV* gene polymorphism and T2DM.

Acknowledgements We would like to acknowledge the contribution of blood donors in this study.

Author contributions Archna Bhargave - Conceptualization, Methodology, data collection and analysis, writing of original draft for publication

Kiran Devi - Methodology

Imteyaz Ahmed - Methodology, Data analysis

Anita Yada - Conceptualization

Ranjan Gupta - Conceptualization, Methodology, resources, data analysis and editing of original draft

All authors have read and approved the final draft.

Declarations

Ethics approval The study protocol was approved by Institutional Human Ethical Committee Kurukshetra University, Kurukshetra Haryana.

Consent to participate Informed consent was obtained from all the participating individuals.

Consent to publish The participants provided the written consent to publish their data.

Competing interest No potential conflict of interest reported by author.

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