



Association of resistin (rs3745367) and urotensin II (rs228648 and rs2890565) gene polymorphisms with risk of type 2 diabetes mellitus in Indian population

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Received: 21 August 2020 / Accepted: 6 November 2020 / Published online: 2 December 2020
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Abstract

Insulin resistance may become the most powerful predictor of future development of type 2 diabetes mellitus (T2DM) and a therapeutic target for the treatment of the same. Both Resistin, an adipose derived peptide hormone and Urotensin II a potent vasoconstrictor, are reported to be involved in the development of insulin resistance and T2DM but the results remain contradictory. Therefore, investigations were carried out to study the association of T2DM and single nucleotide polymorphism (SNP) in Resistin (RETN) gene at rs3745367 (+299 G > A) and Urotensin II (UTS2) gene at rs228648 (+143 G > A) and rs2890565 (+3836 C > T) in a North Indian population. Method: The present case–control study, conducted from August 2017 to July 2020, involved 168 T2DM patients and 102 healthy controls. SNPs rs3745367, rs228648 and rs2890565 were amplified from genomic DNA in the studied samples by polymerase chain reaction (PCR) using specific primers. The amplified products were genotyped by restriction fragment length polymorphism (RFLP) using particular restriction endonucleases. Clinical parameters viz. glycosylated haemoglobin (HbA1c), fasting blood glucose (FBG), high density lipoprotein cholesterol (HDL-C), triglycerides (TG), total cholesterol (CHL) and fasting insulin were determined by enzymatic methods. Result and conclusion: A statistically significant association between T2DM and RETN gene at SNP rs3745367 ($p = 0.001$) and UTS2 gene at SNP rs2890565 ($p = 0.001$) was observed. In RETN gene SNP rs3745367, insulin and homeostasis model assessment of insulin resistance (HOMA-IR) were found to be higher in GA + AA combined genotype than in GG genotype for T2DM subjects. Regression analysis revealed that SNP rs2890565 and HOMA-IR were independently associated with the risk of development of T2DM when three SNPs were taken as independent variable adjusted for clinical variables. Among four haplotypes, A/T was found associated with increased risk of T2DM as determined for rs228648 and rs2890565 of UTS2 gene. It can be concluded from these results that polymorphism at rs3745367 of RETN gene and at rs2890565 of UTS2 gene are associated with risk of T2DM in North Indian population.

Keywords Genetic model · Gene polymorphism · Resistin · Single nucleotide polymorphism · Type 2 diabetes mellitus · Urotensin

Introduction

Type 2 diabetes mellitus (T2DM) is a chronic disease that develops due to multiple factors and it is characterized by insulin resistance, impaired insulin secretion or both [1]. Globally 415 million people diagnosed with this disease and number is expected to increase more than 640 million by 2040 [2]. T2DM is considered to be a polygenic disease and many genetic markers including cytokines have been reported to be involved in development of T2DM [3–6]. Resistin is a cysteine rich adipose derived peptide hormone encoded by resistin (RETN) gene [7]. It is secreted in humans by monocytes, macrophages and adipocytes and

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reported to regulate the glucose, energy and lipid homeostasis [8–10]. Resistin treated macrophages and adipocytes showed accumulation of lipids whereas a decreased fatty acid uptake in skeletal muscle cells by resistin treatment leads to lipid toxicity and insulin resistance [11–13]. Some studies reported an increased RETN gene expression when there is insulin resistance, T2DM and obesity [14, 15] but others failed to show any change in RETN expression in same conditions [16–18]. A genome wide association study showed that polymorphisms of rs3745367 and rs1423096 in RETN gene and haplotypes (A/G and G/G) determined by these SNPs were significantly associated with higher resistin levels and increased T2DM risk [19]. Although, worldwide several association studies were conducted on various genetic variations of RETN gene viz. -420 C > G in promoter region, + 156 C > T and + 299 G > A in intron 2 (SNP rs3745367), + 1084 G > A and + 62 G > A in 3' untranslated region, however most of them showed negative association with T2DM except SNP + 299 G > A which showed association with T2DM in some ethnicities whereas in other, contradictory results have been observed [20–24]. As association between different SNPs of RETN gene and T2DM except rs3745367 could not be observed in different population studies and information regarding the association of SNP rs3745367 with T2DM in Indian population is lacking, so the current study was planned.

Urotensin 2 (UTS2) gene specify an 11 amino acid cyclic peptide called Urotensin II (U-II), which is most potent mammalian vasoconstrictor identified to date that exerts its biological effects via interaction with a member of a G-protein coupled receptor superfamily, originally termed GPR14 [25–27]. Recent evidence suggests that vascular factor dysfunction contributes to insulin resistance [28]. In a study conducted on bovines, it was observed that both UTS2 and UTS2 receptor (UTS2R) genes were significantly associated with regulation of skeletal muscle fat accumulation and fatty acid metabolism [29]. U-II reportedly reduces glucose-induced insulin secretion in the perfused rat pancreas [30] and also the genetic markers present in UTS2 gene were reported to be involved in the prevalence of T2DM [31–33]. GGT (605G, 143G and 3836T) haplotype in UTS2 gene are associated with higher plasma level of U-II and insulin, and higher homeostasis model assessment of insulin resistance index (HOMA-IR) and β -cell function (HOMA- β) [33]. The present study was performed to assess possible association between RETN gene polymorphism at SNP rs3745367 and UTS2 gene polymorphisms at SNPs rs228648 and rs2890565 with T2DM in North Indian population as information is lacking in this population.

Materials and methods

Study population

The present study comprised of 270 subjects divided in two groups. First group consisting of 168 T2DM subjects aged 45–85 years and second group had 102 healthy control subjects aged 45–83 years from different parts of Haryana (India). Subjects suspected to have liver, kidney, lungs, coronary artery disease (CAD) and other major chronic or inflammatory diseases were excluded from the study. Diabetes was diagnosed according to the Indian council of Medical Research (ICMR), New Delhi criteria for diabetes which include fasting blood glucose > 126 mg/dl and glycosylated haemoglobin > 6.5% and information about the duration of diabetes was obtained from medical records. Subjects who were 45 or more years of age, no past history of diabetes, normal fasting blood glucose (FBG) and < 5.6% glycosylated haemoglobin (HbA1c) were included in the study as control subjects. The study was approved by the Ethical committee of Kurukshetra University, Kurukshetra and all the subjects who participated in the study had signed the informed consent form.

Body measurements and blood collection

Age, sex, height, weight and personal history were revealed in standardized questionnaire for all the enrolled subjects. Body mass index (BMI) was calculated by dividing the weight in kilogram (Kg)/height in meter square (m^2). After 10–12 h fasting, 5 ml venous blood was collected from all the subjects in the morning. Blood was collected in two types of vials, plain vial for serum collection and in ethylene diammine tetra acetate (EDTA) coated vial for FBG, HbA1c evaluation. Fasting insulin concentration was determined by enzyme linked immunosorbent assay (ELISA). HbA1c, high density lipoprotein cholesterol (HDL-C), triacylglycerides (TG), total cholesterol (CHL) were measured using enzymatic methods. Homeostasis model assessment (HOMA-IR) score were calculated by using the formula $\text{fasting insulin (IU/ml)} \times \text{fasting blood glucose (mg/dl)} / 405$.

DNA extraction and genotyping

DNA was extracted from whole blood stored at -20°C in EDTA coated vials using Qiamp DNA extraction mini kit (QIAGEN, New Delhi, India) according to manufacturer instructions and stored at -20°C for further use. Quality of DNA was checked on UV-spectrophotometer.

Genotypes were determined by PCR–RFLP method. The PCR amplification was performed in a 25 μl reaction mixture containing master mix 2X (GoTaq® Green Master Mix, Promega, India), 7.5 pmol of each forward and reverse primer

(sequence shown in Table 1), 40–60 ng of genomic DNA and best quality water. Assay conditions included an initial denaturation at 95 °C for 5 min, followed by 35–40 cycles of denaturation at 95 °C for 30 s, reannealing at 50–60 °C for 1 min and extension at 72 °C for 1 min, and a final extension at 72 °C for 10 min.

Amplified products of RETN and UTS2 gene containing SNPs of interest were run on 1.5% agarose gels which were stained using ethidium bromide and visualised on UV-transilluminator. A 10 µl aliquot of the PCR products was completely digested with 2–5 units of restriction enzymes and restriction digested PCR products were resolved on 3% agarose gel. G to A transition mutation in resistin gene at SNP rs3745367 generates an *AluI* restriction enzyme site. Wild type DNA in resistin gene SNP rs3745367 yielded four bands with 243, 55, 52 and 23 bp, while the mutated DNA showed five bands with 158, 85, 55, 52 and 23 bp. Transition mutation from C to T in UTS2 gene polymorphism at SNP rs228648 creates a *NlaIII* site. Wild type DNA yielded two bands of 109 and 32 bp and mutated DNA showed 3 bands with 57, 52 and 32 bp. Transition mutation from G to A in UTS2 gene at SNP rs2890565 eliminates a *CviQI* site. The wild type DNA expected to show three bands with 161, 84 and 18 bp whereas mutated DNA yielded two bands with 245 and 18 bp.

Statistical analysis

The Hardy–Weinberg equilibrium for genotypic distributions was examined using the χ^2 goodness-of-fit test. Data for the biochemical measurements was analysed by Student's *t*-test and data are presented as means \pm SD. Differences were considered to be significant at $p < 0.05$. To evaluate the association between type 2 diabetes as dependent variable and potential clinical parameters, logistic regression was applied. Haplotype analysis was done using SHEsis online software.

Results

Analysis of clinical and demographic profile

Current study is comprised of 168 T2DM patients and 102 healthy individuals as control. Biochemical parameters

and demographic profile of both T2DM and control subjects are shown in Table 2. Results showed a significant increase in FBG, HbA1c%, fasting insulin, HOMA-IR, total CHL and low density lipoprotein cholesterol (LDL-C) in T2DM group (Table 2).

Genotyping analysis of genetic markers

Out of three SNPs, two were detected to be highly polymorphic (rs3745367 and rs2890565), whereas SNP (rs228648) showed no significant difference in case and control population. Genotypic differences of SNPs in T2DM and control group are shown in Tables 3 and 4 as accessed by chi square test.

Table 2 Comparison of demographic data, clinical and biochemical characteristics of studied subjects

Parameters	Case	Controls	p-value
SEX (M/F)	100/68	57/45	
AGE (Yrs)	57.58 \pm 8.8	57.16 \pm 9.49	0.745
BMI(Kg/m ²)	25.45 \pm 4.02	24.50 \pm 4.29	0.072
SBP (mmHg)	133.22 \pm 16.52	134 \pm 12.6	0.869
DBP (mmHg)	88.02 \pm 13.79	85.84 \pm 9.74	0.130
FBG (mg/dl)	183.08 \pm 63.15	103.1 \pm 10.24	<0.000*
HbA1c %	7.50 \pm 1.18	5.47 \pm 0.45	<0.000*
TG (mg/dl)	162.71 \pm 37.23	154.0 \pm 45.2	0.102
HDL-C (mg/dl)	49.46 \pm 11.98	55.39 \pm 9.05	<0.000*
LDL-C (mg/dl)	106.96 \pm 41.26	93.81 \pm 25.30	0.001*
Total CHL (mg/dl)	188.80 \pm 42.74	179.95 \pm 26.88	0.037*
Insulin (IU/ml)	6.72 \pm 5.44	4.96 \pm 3.49	<0.000*
HOMA- IR index	3.15 \pm 3.56	1.28 \pm 0.96	<0.000*

Data shown as mean \pm standard deviation

BMI body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *FBG* fasting blood glucose, *HbA1c*% glycated haemoglobin, *TG* triacylglycerides, *HDL-C* high density lipoprotein cholesterol, *LDL-C* low density lipoprotein cholesterol, *Total CHL* total cholesterol, *HOMA-IR* homeostasis model assessment for insulin resistance

* $p < 0.05$ statistically significant

Table 1 The primer sequence, product length and restriction enzyme used for restriction digestion

Gene	SNP	Primer sequence	PCR Product	Restriction enzyme
RETN	rs3745367	F-GAGAGGATCCAGGAGGTCG R-GTGAGACCAAACGGTCCCT	373 bp	<i>AluI</i>
UTS2	rs228648	F-GGAAACCAACGTATTTTCATC R-GCAAAAAGAGGCAACTTACAGC	141 bp	<i>NlaIII</i>
UTS2	rs2890565	F-GTGCCTGTCTGTCTGCATTCA R-GAGTCCTGTAAAACCGTACAG	263 bp	<i>CviQI</i>

Table 3 Allelic frequency and genotypic distribution of RETN gene polymorphism in T2DM and control subjects

SNP(rs3745367)	Case	Control	χ^2	OR (95% C.I)	p-value
GG	59 (35.1%)	52 (50.9%)			
GA	84 (50%)	46 (45%)			
AA	25 (14.8%)	04 (3.9%)	11.29		0.003*
Allelic(G vs A)			10.06	1.84 (1.26–2.69)	0.001*
Additive (GG vs GA)			3.26	0.62 (0.37–1.04)	0.087
(GG vs AA)			10.47	0.18 (0.05–0.55)	0.001*
Dominant (GG vs GA+AA)			6.59	1.92 (1.16–3.17)	0.014*
Recessive (AA vs GA+ GG)			7.95	0.23 (0.07–0.69)	0.001*
Heterozygous (GA vs GG+AA)			0.61	0.82 (0.50–1.43)	0.434

SNP single nucleotide polymorphism, OR odd ratio, C.I confidence interval

*p<0.05 statistically significant

Table 4 Allelic frequency and genetic distribution of UTS2 gene polymorphism in T2DM and control subjects

	Case	Control	χ^2	OR (95% C.I)	p-value
SNP(rs228648)					
GG	30 (17.8%)	19 (18.6%)			
GA	74 (44%)	48 (47%)			
AA	64 (38.1%)	35 (34.3%)	0.396		0.82
Allelic(G vs A)			0.271	1.09 (0.77–1.56)	0.601
Additive(GG vs GA)			0.004	1.02 (0.51–2.02)	0.945
(AA vs GG)			0.17	0.86 (0.42–1.75)	0.647
Dominant (GG vs GA+ AA)			0.02	2.98 (1.32–6.72)	0.872
Recessive (AA vs GA+ GG)			0.39	0.84 (0.50–1.41)	0.532
Heterozygous (GA vs GG + AA)			0.23	1.12 (0.68–1.85)	0.631
SNP(rs2890565)					
CC	134 (79.7%)	94 (92.1%)			
CT	27 (16%)	08 (7.8%)			
TT	07 (4.1%)	00 (0.00%)	8.72		0.01*
Allelic (C vs T)			10.54	3.40 (1.56–7.41)	0.001*
Additive (CC vs CT)			4.31	2.36 (1.03–5.43)	0.037*
(TT vs CC)			4.80	∞	0.028*
Dominant (CC vs CT+TT)			7.42	2.98 (1.32–6.72)	0.006*
Recessive (TT vs CT+CC)			4.36	∞	0.036*
Heterozygous (CT vs CC+TT)			3.81	0.44 (0.19–1.02)	0.05*

SNP single nucleotide polymorphism, OR odd ratio, C.I confidence interval

*p<0.05 statistically significant

Association of RETN gene polymorphism (rs3745367) with T2DM

AA genotype of RETN gene SNP at rs3745367 was significantly higher in T2DM group as compared to control group ($\chi^2 = 11.29$, $p = 0.003$). Allelic frequency of A allele was found to be increased in T2DM group as compared to control group (G VS A, OR = 1.84, 95% CI: 1.26–2.69, $p = 0.001$). Significant differences in genotype distribution of polymorphism rs3745367 were seen in three genetic models viz. additive, recessive model and dominant genetic model. (Table 3).

Association of UTS2 gene polymorphisms (rs228648 and rs2890565) with T2DM

For UTS2 gene polymorphism at SNP rs228648, differences in genotypic distribution in two study groups could not be observed ($\chi^2 = 0.396$, $p = 0.82$). Significant differences could not be observed between allele frequencies of T2DM and control subjects (G vs A, OR = 1.09, 95% CI: 0.77–1.56, $p = 0.60$) (Table 4).

Significant differences were observed in genotypic distribution of UTS2 gene polymorphism at SNP rs2890565 between T2DM and control groups ($\chi^2 = 8.72$, $p = 0.01$). Allele frequency of T allele was found to be significantly higher in T2DM group as compared to control group (12.2 vs 3.9%, C vs T, OR = 3.4, 95% CI: 1.56–7.41, $p = 0.001$). In addition, three genetic models viz. additive, recessive and dominant genetic model showed significant association (Table 4).

Comparisons of genotypes with clinical profiles of subjects

Clinical comparisons between T2DM and control subjects were performed using dominant model (GG vs GA + AA). There was a statistical significant association in both T2DM and control subjects having GA + AA genotypes with p-value of 0.01 and 0.04 respectively regarding insulin levels for RETN gene SNP rs3745367. There was lowered HOMA-IR in T2DM subjects having GG genotypes with p-value of 0.004 (Table 5). Significant association for clinical parameters with gene polymorphism at SNP rs228648 and rs2890565 of UTS2 gene could not be observed except SBP which was found to be higher in CT + TT genotype as compared to wild type (CC) for SNP rs2890565 in both T2DM and control groups (p -value = 0.04 and 0.01) (Table 6) and HDL-C was found to be lowered in GA + AA genotypes compared to wild type (GG) for SNP rs228648 (p -value = 0.008) (Table 7).

Haplotype analysis

SHEsis online software was used for haplotype analysis of UTS2 gene SNPs (Shi and He 2005). All possible haplotypes were examined from the observed genotypes of studied group for UTS2 gene SNPs rs228648 (G > A) and rs2890565 (C > T) and haplotype (A/T) was found to be significantly associated with the increase risk of developing T2DM (Table 8).

Table 5 Statistical analysis of clinical parameters in association with genotype distribution of SNP rs3745367

Parameter	Case			Control		
	GG	GA+AA	p-value	GG	GA+AA	p-value
BMI(Kg/m ²)	25.52 ± 3.95	25.41 ± 4.08	0.864	25.24 ± 4.77	23.79 ± 3.67	0.087
SBP (mmHg)	131.61 ± 13.17	134.33 ± 16.59	0.246	134.42 ± 11.86	132.58 ± 13.34	0.463
DBP (mmHg)	87.72 ± 13.17	88.19 ± 14.18	0.830	86.5 ± 9.08	85.16 ± 10.43	0.491
FBG (mg/dl)	177.49 ± 54.70	186.11 ± 67.33	0.370	104.0 ± 13.29	105.36 ± 12.83	0.600
Hb1Ac %	7.45 ± 1.19	7.52 ± 1.18	0.715	5.44 ± 0.43	5.50 ± 0.47	0.503
TG (mg/dl)	164.09 ± 42.56	161.96 ± 34.18	0.741	155.17 ± 49.20	152.24 ± 41.13	0.744
HDL-C (mg/dl)	49.89 ± 11.10	49.23 ± 12.47	0.725	56.40 ± 10.08	54.35 ± 7.82	0.252
LDL-C (mg/dl)	104.72 ± 42.88	108.09 ± 44.63	0.632	93.26 ± 25.99	94.38 ± 24.81	0.824
Total CHL(mg/dl)	186.94 ± 42.07	190.76 ± 45.49	0.585	180.69 ± 27.93	179.19 ± 26.01	0.779
Insulin (IU/ml)	5.71 ± 3.75	7.85 ± 6.92	0.01*	3.95 ± 2.94	5.28 ± 3.64	0.045
HOMA- IR index	2.44 ± 1.61	3.82 ± 4.55	0.004*	1.03 ± 0.83	1.40 ± 1.02	0.057

Data shown as mean ± standard deviation

BMI body mass index, SBP systolic blood pressure, DBP diastolic blood pressure, FBG fasting blood glucose, HbA1c% glycated haemoglobin, TG triacylglycerides, HDL-C high density lipoprotein cholesterol, LDL-C low density lipoprotein cholesterol, Total CHL total cholesterol, HOMA-IR homeostasis model assessment for insulin resistance

* $p < 0.05$ statistically significant

Table 6 Statistical analysis of clinical parameters in association with genotype distribution of SNP rs2890565

Parameter	Case			Control		
	CC	CT+TT	p-value	CC	CT+TT	p-value
BMI(Kg/m ²)	25.62 ± 4.09	24.78 ± 3.70	0.249	24.58 ± 4.3	23.95 ± 4.62	0.337
SBP (mmHg)	134.69 ± 15.5	127.44 ± 19.22	0.04*	132.77 ± 12.52	142.25 ± 10.33	0.016*
DBP (mmHg)	88.76 ± 12.76	85.14 ± 17.21	0.252	85.37 ± 9.8	91.37 ± 7.42	0.05*
FBG (mg/dl)	178.39 ± 55.12	201.5 ± 86.57	0.140	104.3 ± 13.28	108.87 ± 9.07	0.193
Hb1Ac %	7.45 ± 1.17	7.70 ± 1.21	0.281	5.47 ± 0.45	5.46 ± 0.42	0.948
TG (mg/dl)	162.28 ± 35.76	164.39 ± 43.08	0.795	154.36 ± 45.32	146.36 ± 46.39	0.640
HDL-C (mg/dl)	49.93 ± 12.18	47.62 ± 11.13	0.290	55.3 ± 8.68	56.46 ± 13.41	0.810
LDL-C (mg/dl)	104.49 ± 37.84	116.28 ± 56.84	0.253	94.54 ± 25.52	85.3 ± 22.24	0.267
Total CHL(mg/dl)	186.66 ± 39.66	196.79 ± 58.38	0.339	181 ± 26.9	171.04 ± 27.01	0.316
Insulin (IU/ml)	7.14 ± 5.84	7.46 ± 6.99	0.246	4.55 ± 3.33	2.64 ± 3.85	0.176
HOMA- IR index	3.24 ± 3.29	3.6 ± 5.47	0.714	1.2 ± 0.95	1.05 ± 0.57	0.504

Data shown as mean ± standard deviation

BMI body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *FBG* fasting blood glucose, *HbA1c*% glycated haemoglobin, *TG* triacylglycerides, *HDL-C*, high density lipoprotein cholesterol, *LDL-C* low density lipoprotein cholesterol, *Total CHL* total cholesterol, *HOMA-IR* homeostasis model assessment for insulin resistance

*p < 0.05 statistically significant

Table 7 Statistical analysis of clinical parameters in association with genotype distribution of SNP rs228648

Parameter	Case			Control		
	GG	GA+AA	p-Value	GG	GA+AA	p-Value
BMI(Kg/m ²)	24.96 ± 3.39	25.57 ± 4.15	0.367	25.29 ± 3.76	24.36 ± 4.43	0.349
SBP (mmHg)	133.86 ± 14.9	133.21 ± 16.88	0.832	134.57 ± 10.55	133.27 ± 13.04	0.915
DBP (mmHg)	88.37 ± 9.67	87.92 ± 13.77	0.830	87.1 ± 6.14	85.55 ± 10.4	0.388
FBG (mg/dl)	182.89 ± 63.05	183.52 ± 63.45	0.960	107.47 ± 19.2	104.02 ± 11.21	0.448
Hb1Ac %	7.41 ± 1.18	7.53 ± 1.18	0.617	5.49 ± 0.52	5.46 ± 0.43	0.815
TG (mg/dl)	149.01 ± 46.11	165.96 ± 34.42	0.058	146.14 ± 28.04	155.47 ± 48.27	0.264
HDL-C (mg/dl)	54.71 ± 11.66	48.42 ± 11.81	0.008*	55.92 ± 9.04	55.27 ± 9.11	0.777
LDL-C (mg/dl)	116.66 ± 50.99	105.82 ± 42.17	0.278	87.61 ± 22.42	95.23 ± 25.83	0.198
Total CHL(mg/dl)	201.18 ± 54.53	187.23 ± 43.4	0.190	172.76 ± 20.99	181.6 ± 27.9	0.123
Insulin (IU/ml)	5.65 ± 5.23	7.22 ± 5.98	0.246	6.03 ± 4.35	4.35 ± 3.05	0.119
HOMA- IR index	2.4 ± 2.09	3.43 ± 3.95	0.148	1.65 ± 1.28	1.15 ± 0.83	0.107

Data shown as mean ± standard deviation

BMI body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *FBG* fasting blood glucose, *HbA1c*% glycated haemoglobin, *TG* triacylglycerides, *HDL-C* high density lipoprotein cholesterol, *LDL-C* low density lipoprotein cholesterol, *Total CHL* total cholesterol, *HOMA-IR* homeostasis model assessment for insulin resistance

*p < 0.05 statistically significant

Regression analysis

Multiple logistic regression analysis was done using SPSS software in which T2DM was taken as dependent variable and three polymorphisms were taken as independent variable. It was observed that SNP rs3745367 and rs2890565 were independently associated with T2DM ($p = 0.01$ and $p = 0.009$ respectively) and HOMA-IR and SNP rs2890565 were associated with T2DM after adjusted for age, sex and HOMA-IR ($p = 0.00$ and $p = 0.01$ respectively) (Table 9).

Discussion

Studies elucidated that resistin protein might impair insulin action, glucose tolerance, decreased glucose uptake in skeletal muscle cells and play a role in obesity [34, 35]. As SNP rs3745367 is present in 2nd intron of RETN gene that has no known regulatory function, however studies have shown that SNPs in the non-coding region, such as the 3-untranslated region can affect gene expression [36]. RETN gene variant at SNP rs3745367 and obesity were reported as

Table 8 Analysis of association between haplotypes in UTS2 gene with T2DM

Haplotype	Case	Control	χ^2	p-value	OR (95%CI)
A C	182.7 (0.544)	113.7 (0.557)	0.097	0.755	0.94 (0.66–1.34)
A T	23.3 (0.069)	4.2 (0.021)	6.136	0.013*	3.48 (1.22–9.91)
G C	114.3 (0.340)	82.2 (0.403)	2.185	0.139	0.76 (0.53–1.09)
G T	15.7 (0.047)	3.7 (0.018)	2.964	0.085	2.63 (0.84–8.26)

OR odd ratio; C.I confidence interval

*p < 0.05 statistically significant

Table 9 Multiple regression analysis when T2DM was used as dependent variable and three SNPs were used as independent variable

	p-value ^a	OR (95%CI)	p-value ^b	OR (95%CI)
Age			0.560	0.80 (0.39–1.66)
Sex			0.840	0.94 (0.52–1.67)
HOMA-IR			0.000*	2.72 (1.92–3.87)
rs3745367	0.12*	0.52 (0.31–0.86)	0.238	0.70 (0.39–1.26)
rs2890565	0.009*	0.33 (0.14–0.76)	0.018*	0.34 (0.14–0.83)
rs228648	0.981	0.99 (0.51–1.91)	0.737	1.13 (0.53–2.40)

OR odd ratio; C.I confidence interval

*p < 0.05 statistically significant

p-value^a, unadjusted; p-value^b, adjusted for age, sex and HOMA-IR

potential risk factors for T2DM in a study conducted on Caucasians [37]. This SNP also affects serum resistin concentration which was found to be higher in patients with T2DM [38–40]. It might be a risk factor for stroke in patients with T2DM [41] and associated with unfavourable outcomes in T2DM patients [42]. Polymorphism of RETN gene rs3745367 is involved in the development of insulin resistance and T2DM as reported in Iraqi population [22], meta-analysis study [43] and observed in the present study. Contrarily, significant association between RETN gene polymorphism at SNP rs3745367 and risk of T2DM could not be observed in Japanese population [44]. Minor allele A was more frequent in T2DM patients than in the control group (p = 0.001) [23] and GG may be a protective factor against T2DM [43] and as reported in the present study. Serum resistin concentration as well as HOMA-IR was remarkably more in mutant genotype (GA + AA) as compared with wild type (GG) in German [45], Thailand [21] and Egyptian [46] T2DM subjects. In contrast, there was no difference in GG and GA + AA genotypes in Han Chinese patients thus neglecting the risk of T2DM incidence [47].

U-II protein may be involved in the pathogenesis of T2DM [48] as it may increase blood glucose level through the mobilization of glucose. Circulating U-II concentrations were significantly higher in T2DM patients with metabolic syndrome compared to those without metabolic syndrome (p < 0.001) but significant correlation could not be observed

between U-II concentrations and insulinemia in Belgium population [49]. Levels of U-II were found higher in women with gestational diabetes mellitus (GDM) and its level showed a positive correlation with insulin resistance marker (HOMA-IR) [50] and increased cardiovascular risk as observed in patients with polycystic ovary syndrome [51]. UTS2 gene SNPs at rs228648 and at rs2890565 were found essentially associated with T2DM in Han Chinese and Japanese population respectively [31, 52, 53] and these SNPs were suggested as risk factors in developing diabetic retinopathy (DR) in Caucasians [54]. SNP rs2890565 was found to be significantly associated with T2DM in our study. T Allele frequency was significantly higher in T2DM subjects as compared to control subjects (12.2 vs 3.9%, p = 0.001) so carrier of T allele may be prone to pathogenesis of T2DM but significant association could not be observed between clinical parameters, genotypes and T2DM in our study.

Conclusion

It can be concluded from the present study that individuals with AA genotype of RETN gene SNP rs3745367 are more prone to develop insulin resistance and T2DM as compared to those carrying GG genotype and G allele at this locus may be protective factor against T2DM. T allele

in UTS2 gene SNP rs2890565 is more frequent in T2DM subjects than in controls thus may be a predisposing factor in development of T2DM in Indian population.

Acknowledgement The authors are thankful to Dr. Ashok Chaudhary and Dr. Sapna Grewal, Bio and Nano Technology Department, Guru Jambheshwar University of Science and Technology (G.J.U), Hisar, Haryana, for providing access to instruments, which have been used in present research work, in their laboratory.

Author contributions All authors contributed to this work significantly and all are in agreement with the content of the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The research work was approved by the Ethical committee of Kurukshetra University, Kurukshetra and all the subjects who participated in the study had signed the informed consent form.

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