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Original Article

Association of *CD36* gene variants rs1761667 (G > A) and rs1527483 (C > T) with Type 2 diabetes in North Indian population

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ABSTRACT

Introduction: Type 2 diabetes mellitus (T2DM) affects huge populations in India and abroad. Genetic polymorphisms (SNPs) in scavenger receptors such as CD36 have been implicated in the pathogenesis of diabetic atherosclerosis and cardiovascular diseases. Since *CD36* gene expression contributes to T2DM, we proposed to study the association of two of its polymorphisms.

Methods: A population of 400 subjects from North India was analyzed according to clinical parameters. Out of them 150 controls and 250 T2DM patients were genotyped for two SNPs namely rs1761667 (G > A) and rs1527483 (C > T) in the *CD36* gene using polymerase chain reaction and restriction fragment length polymorphism (PCR–RFLP) followed by statistical analysis.

Results and discussion: No association of rs1527483 (C > T) SNP was observed in T2DM patients. The GA genotype was prevalent in 76.0% diabetic population and a highly significant genotypic association of rs1761667 (G > A) SNP in *CD36* gene was observed in them (P = <0.001; OR 3.173; CI 1.098–9.174). Sample characteristics showed a highly significant association with lipid profile (P = <0.001). The 'GA' genotype in combination with CC genotype showed a significant association with TC (P = 0.020), LDL (P = 0.005) and VLDL (P = 0.029). In addition, the haplotype analysis CC/GA (−/+) and CT/GA (−/+) showed a strong association with TC and LDL (P < 0.05). The presence of −31118 A^{*} allele in haplotypes showed strong association with T2DM (P = <0.005).

Conclusion: Out of the two *CD36* gene polymorphisms tested, rs1761667 SNP is significantly associated with T2DM with the GA heterozygous genotype showing highest frequency among the T2DM patients.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a part of metabolic syndrome (MetS) which is affected both by environmental, as well as genetic factors. Many studies suggest that net lipid accumulation, caused by an imbalance between fatty acid delivery/synthesis and fatty acid oxidation, results in the activation of a serine kinase cascade [1,2]. This in turn, inhibits insulin signaling, resulting in insulin resistance in liver and skeletal muscle, the organs responsible for majority of glucose disposal. CD36 (FAT, SCARB3, GP88, glycoprotein IV (gpIV) and glycoprotein IIIb (gpIIIb)) is a broadly expressed 88 kDa membrane transporter glycoprotein that acts as a facilitator of fatty acid (FA) uptake, a signaling molecule and a receptor for a wide range of ligands [3]. In addition to FAs, CD36 binds to native lipoproteins and functions in the uptake of cholesteryl esters, facilitates the uptake of oxidized low/high-density lipoproteins and cholesterol [4–8]. As a result of its many ligands and functions, CD36 could impact a variety of conditions linked with the metabolic syndrome, including diabetes, insulin resistance, inflammation and atherosclerosis [9,10].

The *CD36* gene spans 36 Kb (7q11.2–7q21.11) and is comprised of 15 alternatively spliced exons that are differentially regulated by several upstream promoters [11,12]; CD36 plays an important role in lipid metabolism and its gene polymorphisms are related to hypertension [13], MetS and high-density lipoprotein cholesterol (HDL-C) [14]. Since there have been very few studies on the association of genetic variants in the *CD36* gene with T2DM [15–17], we proposed to investigate two probable *CD36* gene

Abbreviations: CD36, cluster of differentiation 36; oxLDL, oxidized low density lipoproteins; SNPs, single nucleotide polymorphisms; FAT, fatty acid transporter; GP88, glycoprotein 88; EDTA, ethyl diamine tetra acetic acid; NEB, New England Biolabs.

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Table 1
Primer sequences, PCR conditions, amplicon sizes, restriction enzymes with product sizes of SNPs in *CD36* gene.

SNP	Primer sequence	Annealing temp. (°C)	Product size (bp)	Restriction enzyme/allele sizes
SNP (C > T) rs1527483	F: 5′-CGCTACAACAATTTTATAGATTTTGAC-3′	55	252	*Taq* I CC 160,70,22
25,444 intron 11	R: 5′-TGAAATAAAAATAATCTTGTCGATGA-3′			CT 230,160,70,22 TT 230,22
SNP (G > A) rs1761667	F: 5′-CAAAATCACAATCTATTCAAGACCA-3′	56	190	*Hha* I GG 138, 52
−31118 Promoter 5′ region of exon 1A	R: 5′-TTTTGGGAGAAATTCTGAAGAG-3′			GA 190, 138, 52 AA 190

polymorphisms that might have an important role to play in T2DM and related complications. This is perhaps the first report of *CD36* gene polymorphism study in T2DM patients from Northern India.

2. Material and methods

2.1. Patients and clinical evaluation

Type 2 diabetic patients, 22–77 years of age (n = 250) were enrolled from the outpatient Diabetes Clinic of Chhatrapati Shahuji Maharaj Medical University (CSMMU), Lucknow under the supervision of expert clinicians. Age/sex-matched normal healthy controls (n = 150) were screened from healthy staff members of both Universities. The study was approved by the Medical Ethical Committee of CSMMU and a written informed consent was taken from all subjects enrolled for the study. Controls showing a normal oral glucose tolerance test were included in the study, whereas those having a history of coronary artery disease or other metabolic disorders were excluded. Subjects with fasting glucose concentrations $\geqslant$126 mg/dl or 2-h glucose concentrations $\geqslant$200 mg/dl after a 75-g oral glucose tolerance test were categorized in the diabetes group. Medical records of these patients were reviewed to ascertain diabetes-associated complications. A self-administered questionnaire was used to record the clinical history of diabetes, associated complications such as hypertension as well as family history. All patients were on oral hypoglycemic agents to maintain a normal glucose level in their blood.

Estimations of plasma glucose (mg/dl), serum insulin (mg/dl) and lipid profile (total serum cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and serum triglycerides) were done using commercially available *Ecoline* kits from Merck by double beam spectrophotometer at 550 nm (TGL-C), 510 nm (serum creatinine), 500 nm (total cholesterol) and 560 nm (HDL-C) [18]. Height, weight and waist circumference were measured to calculate body mass index (BMI) and waist hip ratio (WHR). Systolic and diastolic blood pressures were measured in the sitting position with an appropriately sized cuff after a 5 min rest. Clinical details of patients and controls were recorded.

2.2. DNA extraction and CD36 gene polymorphisms

Five milliliter of blood sample was taken in EDTA vials from both the groups. Genomic DNA was extracted from peripheral blood leucocytes using the standard salting out method [19]. Two *CD36* single nucleotide polymorphisms (SNPs) viz. G > A (rs1761667) in the −31118 promoter region of exon 1A and C > T (rs1527483) in intron 11 were genotyped in 150 controls and 250 T2DM patients using polymerase chain reaction and restriction fragment length polymorphism (PCR–RFLP) method. Primers for the SNPs were designed and restriction enzymes (REs) were identified using the Primer 3 and NEB cutter softwares respectively. Details including the location of SNPs in the *CD36* gene, primer sequences and REs with product sizes are presented in Table 1. PCR was performed in a 25 μl reaction mixture containing genomic DNA (100–150 ng), 10 pmol of each primer, 200 μM dNTPs, and 0·5 U of Taq DNA polymerase (MBI-Fermentas, USA) in a gradient Master Cycler (Eppendorf, USA). The PCR products were digested with the respective restriction enzymes, resolved on 2% agarose and 12% polyacrylamide gels.

2.3. Statistical analysis

Allele frequencies, genotype frequencies and carriage rates of the alleles in all the groups were compared using 2 × 2 contingency table by Fisher's exact test. The Hardy–Weinberg equilibrium at individual loci was assessed by chi-square (χ^2) statistics using SPSS (v 15.0). Allele frequency was calculated as the number of occurrences of the test allele in the population divided by the total number of alleles. Carriage rate was calculated as the number of individuals carrying at least one copy of test allele divided by the total number of individuals. Association of various clinical parameters with different *CD36* (SNPs C/T and G/A) genotypes in T2DM patients and controls was calculated by 2 × 2 paired t-test. Differences were considered statistically significant for $P < 0.05$. The strength of association of SNP-SNP combinations was determined by Odds ratio (OR) at 95% confidence interval (CI) using Logistic Regression Analysis (SPSS). Multivariate Logistic regression analysis was used to examine the association of biochemical parameters with different haplotypes of the two SNPs. Data were presented as mean ± SD.

3. Results

3.1. Characteristics of the sample population

Out of the total number of subjects (n = 400) included in the study, 150 were healthy controls with an average age of 47.07 ± 6.01 years, their fasting and post prandial glucose levels were 109.69 ± 51.96 and 143.69 ± 21.46 mg/dl, respectively. The average age of the patients (n = 250) was 48.39 ± 9.91 years, their fasting and postprandial glucose levels were 166.14 ± 65.76 and 266.40 ± 88.45 mg/dl, respectively. The patients average systolic BP was slightly elevated (135.22 ± 19.35 mm Hg) and the mean diastolic pressure was almost normal (84.27 ± 10.77 mm Hg). Total cholesterol (225.83 ± 34.83 mm/dl) and LDL-C (158.00 ± 37.04 mm Hg) levels were slightly raised and HDL-C was low (43.47 ± 5.26 mm Hg) in patients. Therefore, the lipid profile in patients showed a significant difference when compared to control subjects ($P < 0.001$). However, no significant difference was observed in BMI, WHR and serum creatinine levels between the T2DM and control groups ($P > 0.05$) (Table 2).

3.2. Genetic analysis

PCR–RFLP results showing the pattern of genotypes for the two SNPs (rs1527483 and rs1761667) in the *CD36* gene have been represented in Fig. 1a and b.

The allele and genotype frequency distribution and carriage rate of *CD36* gene polymorphisms among 150 controls and 250 patients

Table 2
Sample characteristics for 400 subjects from North Indian population.

Sample characteristics	Controls (n = 150)	Patients (n = 250)	P-value
Age (years)	47.07 ± 6.01	48.39 ± 9.91	0.064
Basal metabolic index, BMI (kg/m^2)	23.43 ± 3.83	24.49 ± 4.60	0.701
Waist hip ratio, WHR	0.97 ± 0.06	0.92 ± 0.08	1.000
Fasting plasma glucose, F (mg/dl)	109.69 ± 51.96	166.14 ± 65.76	0.216
Post prandial plasma glucose, PP (mg/dl)	143.69 ± 21.46	266.04 ± 88.45	0.313
Blood pressure systolic, SBP (mmHg)	117.40 ± 5.48	135.22 ± 19.35	0.454
Blood pressure diastolic, DBP (mmHg)	76.80 ± 6.48	84.27 ± 10.77	0.846
Total-cholesterol, TC (mg/dl)	171.65 ± 41.02	225.83 ± 34.83	**<0.001**
Triglyceride, TGL (mg/dl)	151.87 ± 64.23	112.04 ± 16.88	**<0.001**
HDL-cholesterol, HDL (mg/dl)	59.07 ± 13.93	43.47 ± 5.26	**<0.001**
LDL-cholesterol, LDL (mg/dl)	82.21 ± 48.94	158.00 ± 37.04	**<0.001**
VLDL-cholesterol, VLDL (mg/dl)	30.37 ± 12.85	22.44 ± 3.56	**<0.001**
Serum creatinine, S. Cret. (mg/dl)	1.08 ± 0.16	1.04 ± 0.09	0.912

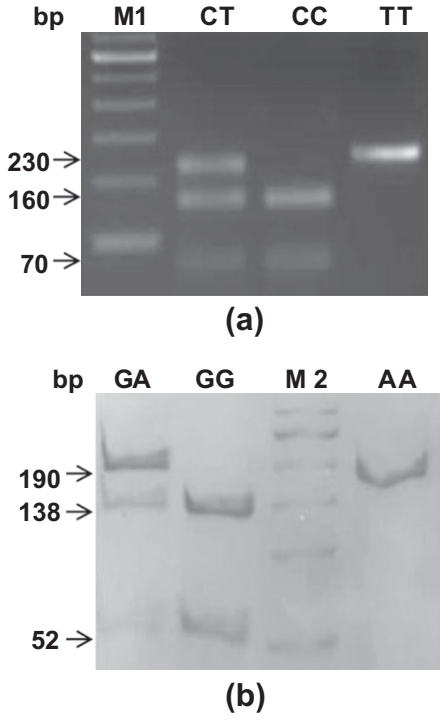

Fig. 1. (a) Ethidium bromide stained agarose gel of SNP (C > T) rs1527483 showing different genotypes; CT: 230, 160, 70 bp; CC: 160, 70; TT: 230; M1: 100 bp ladder (b) silver stain polyacrylamide gel of SNP (G > A) rs1761667; GA: 190, 138, 52; GG: 138, 52; AA: 190; M2: 50 bp ladder.

are shown in Table 3. In case of both SNPs, the allele and genotype frequencies in control and patient groups were in Hardy–Weinberg Equilibrium (HWE). The minor allele frequencies (MAF) for both SNPs in our analysis were $\geqslant$0.1 (Table 3). For C/T polymorphism, no allelic and genotypic associations were observed in the present study (P > 0.05). Most subjects were homozygous wild type (CC), while the TT genotype was rare in the study population (TT genotype frequency $\leqslant$ 0.01). Allele frequencies of C and T were almost same in controls and T2DM patients (Table 3).

In case of the G/A promoter polymorphism, although no allelic association was observed (P = 0.244), the frequency of the GA genotype was significantly higher in T2DM (76.0%) when compared to controls (49.0%, P = <0.001). SNP (G > A) genotypes showed a highly significant association (P < 0.001; OR 3.173; CI 1.098–9.174) with T2DM when compared to controls (Table 3).

Haplotype analysis using logistic regression analysis showed the possible effect of two genotypes, i.e. double combinations of SNPs (C > T) and (G > A) on the risk of developing T2DM. Out of the nine possible haplotypes, we obtained only six with frequencies ranging from 6% to 37% in this population (Table 4). The frequency of recessive genotypes (AA and TT) was very low in the population, so their combination with other genotypes was not considered.

Results showed significant association in case of CC/GG and CC/GA haplotypes (P = <0.05). The risk of CC/AA (+/+, +/−) also showed significant association (P = <0.05) while the risk of CT genotype with GG/GA was highly significant (P = <0.05) (Table 4). The risk of GA and GG without CT genotype was 0.3–2.6 times higher (P = <0.001), (OR; 0.290 and 2.597; CI 95%; 0.178–0.474 and 1.567–4.303). The risk of diabetes in the combination CT/GA (−/−) also showed significant association (P = 0.025).

Analysis of the association of haplotypes (double combinations of genotypes) with biochemical parameters using multivariate logistic regression brought forth some interesting results. We found that the subjects without CC/GG genotypes (−/−) showed significant association with TGL and VLDL (P = 0.006). In case of CC/GG (−/+) subjects significant association was observed with HDL, LDL and S. Cret. (P = <0.05). Subjects with CC/GA genotypes (+/+) showed significant association with TC (P = 0.020), LDL (P = 0.005) and VLDL (P = 0.029) and subjects without GA showed significant association with HDL (P = 0.003) and LDL (P = 0.005). Subjects with haplotype CC/GA (−/+) showed significant association with TC (P = 0.005) and LDL (P = <0.001) while CC/GA (−/−) with TC (P = 0.007), LDL (P = 0.001) and S. Cret. (P = 0.009). Subjects with CC genotype in combination with AA (+/+) also showed significant association with TC and LDL (P = 0.003). Subjects having CC without AA genotype (+/−) and CT with GG (+/+) showed significant association with HDL (P = 0.034, 0.036). In case of CT/GG (+/+) subjects significant association was observed with LDL (P = 0.001), but CT/GG (+/−) and CT/GA (+/−) showed significant association with TGL and VLDL (P = 0.006) and TC, LDL, S. Cret. (P = 0.007, 0.001, 0.009) respectively. In case of CT/GA (−/+) and (−/−) genotypes, TC, LDL, VLDL (P = <0.05) and S. Cret. (P = <0.001) showed significant association, respectively (Table 5). Other haplotypes such as CT/AA, TT/GG etc. were not found in our population.

4. Discussion

The role of CD36 in lipid metabolism, metabolic syndrome and T2DM prompted us to investigate its SNP association with T2DM in our North Indian population. In the present study, we demonstrated that the GA heterozygous genotype of a promoter polymorphism (rs1761667) in the *CD36* gene was more prevalent in the T2DM patients and showed association with diabetes in the North Indian population. A similar association of a promoter polymorphism (rs1527479) in the *CD36* gene with insulin resistance and

Table 3
Allele, genotype and carriage rate frequencies (F) of SNPs and their association status with T2DM.

SNPs	Allele frequency		Genotype frequency		Carriage rate		Association		
	Controls F (*n)	T2DM patients F (*n)	Controls F (**n)	T2DM patients F (**n)	Controls F(***n)	Patients F(***n)	Allele (df = 1)	Genotype (df = 2)	Carrier rate (df = 1)
SNP (C > T) rs1527483	C = 0.89 (268)	C = 0.88 (439)	CC = 0.80 (120) CT = 0.19 (28)	CC = 0.76 (190) CT = 0.24 (59)	C = 0.99 (148)	C = 0.99 (249)	$\chi^2 = 0.429$	$\chi^2 = 2.331$	$\chi^2 = 0.493$
Intron 11	T = 0.11 (32)	T = 0.12 (61)	TT = 0.01 (02)	TT = 0.00 (01)	T = 0.20 (30)	T = 0.24 (60)	$P = 0.512$	$P = 0.312$	$P = 0.483$
SNP (G > A) rs1761667	G = 0.64 (193)	G = 0.60 (301)	GG = 0.40 (60) GA = 0.49 (73)	GG = 0.22 (56) GA = 0.76 (189)	G = 0.89 (133)	G = 0.98 (245)	$\chi^2 = 1.356$	**$\chi^2 = 35.24$**	$\chi^2 = 0.887$
Promoter	A = 0.36 (107)	A = 0.40 (199)	AA = 0.11 (17)	AA = 0.02 (05)	A = 0.60 (90)	A = 0.78 (194)	$P = 0.244$	**$P = < 0.001$**	$P = 0.346$

* Number of respective alleles.
** Genotypes.
*** Carriers of alleles in the study population.

Table 4
Distribution of double combinations of the SNPs (C > T) and (G > A) in *CD36* gene in T2DM patients and controls.

Genotypes	Controls ($n = 150$)	Patients ($n = 216$)	*P*-value	OR (95% CI)
CC&GG				
(+&+)	45 (30.0%)	38 (17.59%)	0.011	1.0 (Ref.)
(+&−)	75 (50.0%)	142 (65.74%)	0.950	1.032 (0.387–2.753)
(−&+)	11 (7.38%)	9 (4.16%)	0.307	1.737 (0.603–5.006)
(−&−)	19 (12.67%)	27 (1.25%)	0.075	2.314 (0.918–5.831)
CC&GA				
(+&+)	56 (37.34%)	136 (62.96%)	<0.001	1.0 (Ref.)
(+&−)	64 (42.67%)	44 (20.37%)	0.114	1.709 (0.880–3.321)
(−&+)	19 (12.66%)	27 (12.5%)	0.307	0.576 (0.200–1.659)
(−&−)	11 (7.33%)	9 (4.17%)	0.042	0.484 (0.240–0.975)
CC&AA				
(+&+)	15 (10%)	5 (2.32%)	0.022	1.0 (Ref.)
(+&−)	105 (70.0%)	175 (81.02%)	0.047	164.198 (0.000–4.9×10^{11})
(−&+)	4 (2.66%)	0 (0.0%)	0.557	682.054 (0.000–2.0×10^{12})
(−&−)	26 (17.34%)	36 (16.67%)	0.546	820.991 (0.000–2.4×10^{12})
CT&GG				
(+&+)	9 (6.0%)	9 (4.17%)	0.002	1.0 (Ref.)
(+&−)	19 (12.67%)	27 (12.50%)	0.604	1.308 (0.475–3.604)
(−&+)	51 (34.0%)	39 (18.05%)	<0.001	2.597 (1.567–4.303)
(−&−)	71 (47.34%)	141 (65.28%)	0.092	1.858 (0.905–3.817)
CT&GA				
(+&+)	17 (11.34%)	26 (12.03%)	<0.001	1.0 (Ref.)
(+&−)	11 (7.34%)	9 (4.17%)	0.203	0.641 (0.323–1.272)
(−&+)	57 (38.0%)	136 (62.96%)	<0.001	0.290 (0.178–0.474)
(−&−)	65 (43.34%)	45 (20.83%)	0.025	0.343 (0.135–0.872)

T2DM was reported in the Caucasian population at risk for MetS [17]. However, the allele and genotype frequencies of C/T (rs1527483) polymorphism in intron 11 were similar in controls and T2DM patients and did not show any association with T2DM. This suggested that the regulatory region of the gene is responsible for the varied expression of the *CD36* gene in normal and diseased conditions since a variant located in the *CD36* upstream promoter determines the binding site for transcription factors [13]. A study in Italian men has shown that a haplotype of five SNPs spanning the *CD36* gene was associated with higher free fatty acid and triglyceride levels thereby modulating lipid metabolism and cardiovascular risk [13]. It was also suggested that since CD36 modulates the uptake of modified lipoproteins and is related to insulin resistance through its contribution to fatty acid metabolism, the variants of the *CD36* gene may contribute to the pathogenesis of diabetes in African Americans [14]. A recent study on the variants of the *CD36* gene in Boston and Puerto Rican adults also revealed its association with metabolic syndrome which can increase the risk of cardiovascular disease and T2DM [20]. However, the minor allele frequency of the SNP G > A (rs1761667) was slightly lower in our population (0.36) compared to 0.46 in Puerto Ricans [20]. A genetic study in the French diabetic population revealed a rare non-sense mutation in the *CD36* gene while a promoter variant −178A/C was significantly associated with adiponectin levels and represented a putative marker for insulin resistance [15,16]. Another common polymorphism (478 C/T) associated with CD36 deficiency was reported in Japanese patients with heart disease [21].

Evidence from the literature has shown that *CD36* expression in monocytes is up regulated by oxidized low density lipoprotein and its level increases in T2DM, hyperglycemia and related atherosclerosis probably through its contribution to disturbed fatty acid metabolism, [22–24] suggesting a possible connection between atherosclerosis and insulin resistant states through CD36 [25]. The increased hepatic CD36 protein expression in response to diets rich in fatty acids and/or to obesity contributes to aberrant liver fatty acid uptake and subsequent dyslipidemia [26] and the increased foam cell formation in T2DM is caused by decreased macrophage cholesterol efflux to HDL. Reports have suggested that it was associated with abnormal glucose metabolism, and altered serum lipids and CD36 deficiency is a risk factor for MetS [27].

Some studies have reported alterations in FFAs and TGL with a common haplotype in *CD36*, especially the SNPs rs1761667 and rs1049673 have been associated with elevated plasma FFAs in white men without diabetes [13]. In our study, rs1527483 (C > T) showed highly significant association with lipid levels ($P = < 0.001$) when considered alone. However, on haplotype analyses significant associations between *CD36* SNPs and biochemical parameters were evident (Table 5). A significant difference was observed in total cholesterol (TC) with CC genotype in combination with GA and AA, but CT without GA was also associated with it. Subjects without CC/GA genotype (−/−) were also associated with TC. TC also showed significant association with GA genotype minus CC and CT. Significant differences in TGL levels were also observed in subjects without CC and GG genotypes but subjects with CT/GG (+/−) were also associated with it. HDL is only associated with CC genotype except CT/GG (+/+) in combination without GA and AA. LDL showed its association with CC genotype in combination with GA, without GA and with AA, but CT genotype showed its associa-

Table 5
Association comparison of haplotypes of rs1527483 and rs1761667 with biochemical parameters.

Genotypes		Biochemical parameters *P*-value					
		TC	TGL	HDL	LDL	VLDL	S. Cret.
CC&GG	+/+	0.745	0.572	0.536	0.564	0.636	0.348
	+/−	0.636	0.720	0.577	0.851	0.955	0.804
	−/+	0.402	0.772	**0.029**	**<0.001**	0.772	**0.023**
	−/−	0.447	**0.006**	0.889	0.549	**0.006**	0.462
CC&GA	+/+	**0.020**	0.258	0.990	**0.005**	**0.029**	0.620
	+/−	0.240	0.392	**0.003**	**0.045**	0.151	0.275
	−/+	**0.005**	0.070	0.611	**<0.001**	0.070	0.807
	−/−	**0.007**	0.382	0.091	**0.001**	0.382	**0.009**
CC&AA	+/+	**0.003**	0.065	0.276	**0.003**	0.065	0.287
	+/−	0.449	0.380	**0.034**	0.224	0.394	0.953
	−/−	0.993	0.446	0.943	**0.027**	0.568	0.867
CT&GG	+/+	0.058	0.701	**0.036**	**0.001**	0.701	0.232
	+/−	0.447	**0.006**	0.889	0.549	**0.006**	0.890
	−/+	0.745	0.572	0.536	0.564	0.636	0.348
	−/−	0.636	0.720	0.577	0.851	0.955	0.804
CT&GA	+/+	0.690	0.700	0.669	0.306	0.715	0.413
	+/−	**0.007**	0.382	0.091	**0.001**	0.382	**0.009**
	−/+	**0.020**	0.258	0.111	**0.005**	**0.029**	0.620
	−/−	0.487	0.226	0.704	0.092	0.199	**<0.001**

tion with GG and without GA genotypes. Subjects showed its significant association with VLDL only in case of CC/GG (−/−), CC/GA (+/+), CT/GG (+/−) and CT/GA (−/+). In case of S. Cret. subjects without CC and GA, subjects with CT and without GA, subjects without CT and GA, subjects with GG and GG without CC showed a highly significant association. The association of these haplotypes with biochemical parameters indicates that somehow they are responsible for the manifestation of the disease, for example, although CT genotype was not prevalent among the North Indian diabetic patients but in combination with the high-risk genotype GA showed significant associations with TC, LDL, VLDL etc. suggesting that the selected SNPs have a synergistic effect on T2DM. In addition, the presence of the minor allele A^* of rs1761667 in the -31118 promoter region of the *CD36* gene seemed to contribute greatly in elevating the risk of developing this disorder.

5. Conclusions

In conclusion, this is perhaps the first study to examine the association of *CD36* polymorphisms in North Indian adults, a high-risk urban population. Considering that CD36 plays a crucial role in T2DM, we showed that the 5' promoter SNP rs1761667 has a significant association with T2DM patients in this population. Our results suggest that individuals having a 'GA' genotype might be susceptible to T2DM and may be at risk of developing related complications. Moreover, it has provided a lead for future studies to examine the role of other *CD36* variants in the development of T2DM in India and other ethnic populations.

6. Competing interests

The authors declare that they have no competing interests.

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