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Study of CI962235 (Ins1361A), rs3212018 (16 bp del) and rs1049673 (G>C) *CD36* Gene Polymorphisms in T2DM Patients of North India

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Single Nucleotide Polymorphisms (SNPs) in *CD36*, a macrophage scavenger receptor have been implicated in the pathogenesis of diabetic atherosclerosis and cardiovascular diseases. *CD36* is responsible for the uptake of free fatty acids specially Oxidized Low Density Lipoprotein (Ox-LDL) during diseased conditions. Aim of the present research was to study the association of genetic polymorphisms in *CD36* gene and risk of developing type 2 diabetes mellitus (T2DM) in a North Indian population. Three SNPs in *CD36* gene *viz.* CI962235 (Ins1361A) at 317 codon in exon 10, rs3212018 (16 bp del) in exon 14 and rs1049673 (G>C) in exon 15 were screened in 200 each of healthy controls and T2DM patients. Clinical evaluation was done using commercial kits while the methods used for genotyping were Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) and Single Strand Conformation Polymorphism (SSCP). Statistical analysis was done by Fisher's exact test and chi-square (χ^2) statistics using SPSS (version-15.0). A significant increase in the clinical parameters such as Systolic Blood Pressure (SBP), Fasting Plasma Glucose (FPG), Post Prandial Glucose (PPG), Triglyceride (TG), Low Density Lipoprotein (LDL) and Very Low Density Lipoprotein (VLDL) was observed in T2DM cases when compared to controls by multivariate logistic regression analysis ($p<0.05$). Genotyping studies showed that out of three SNPs, rs3212018 was polymorphic in our population. Homozygous deletion (-/-) was found in 76% of T2DM cases in contrast to 72% of healthy controls. The present and past association studies of *CD36* gene variants showed that this is an important candidate gene in T2DM.

Key words: *CD36*, T2DM, single nucleotide polymorphisms, PCR, RFLP, single strand conformation polymorphism

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INTRODUCTION

T2DM is considered to be a life-threatening disease that is associated with long-term and debilitating complications, attributed deaths and economic burden to nations. A number of factors including genetics, ethnicity, dyslipidaemia, migration, diet and lifestyle have been associated with the prevalence of T2DM in Asians. India is a developing Asian country with fast industrialization and modern lifestyle. This has led to an enormous increase in the number of people with diabetes in both rural as well as urban areas which is causing a high burden to the country's economy (Samatha *et al*., 2012). The current reports revealed that India has 63 million diabetic people compared with 61.3 million of the previous year, which is expected to rise to 116.2 million by the end of 2030 (IDF, 2012). This equates to approximately four new cases every ten seconds or almost ten million per year (IDF, 2012; Parikh *et al*., 2011). T2DM is associated with a greater risk of mortality from Cardiovascular Disease (CVD) and dyslipidemia, which is characterized by raised triglycerides, low high density lipoprotein and high low density lipoprotein particles. The molecular mechanisms involved in the development of diabetes remain poorly understood. Besides globally, studies in Indians have also revealed that genetic factors along with environment play an important role in the pathogenesis of T2DM (Amini and Janghorbani, 2007).

CD36 is a multispecific, integral 88-kDa membrane glycoprotein expressed on the surface of a wide variety of cell types including adipocytes, skeletal muscle cells, platelets, endothelial cells and monocytes/macrophages. It is also known as Fatty Acid Translocase (FAT) and platelet glycoprotein IV or IIIb (Tuomisto *et al*., 2005; Fernandez-Real *et al*., 2010). *CD36* has emerged as an important molecule not only in atherogenesis but also in T2DM. Since *CD36* is a fatty acid transporter in heart muscles and adipocytes, the discrepancy of *CD36* being protective depends on whether or not a pro-inflammatory environment generates pathologic *CD36* ligands. Under abnormal conditions such as obesity and hyperlipidaemia, ligands affect inflammatory and insulin signaling pathways *via CD36* (Nicholls *et al*., 2011).

The gene encoding *CD36* is located at chromosome 7, at locus q11.2. It is 36 Kb long and is comprised of 15 alternatively spliced exons that are differentially regulated by several upstream promoters (Armesilla and Vega, 1994; Rac *et al*., 2007; Gautam and Banerjee, 2011). It was reported that hepatic insulin resistance with high plasma FFA and triglycerides occurred due to a homozygous disruption on the *CD36* locus (Ma *et al*., 2004; Nagarajan *et al*., 2012). In the present work an attempt was made to study the association of three single nucleotide polymorphisms (SNPs) in *CD36* gene with T2DM in a North Indian population.

MATERIALS AND METHODS

Study design: The blood samples were collected during January 2011 to March 2012 at the King George's Medical University (KGMU), Lucknow, India under the supervision of expert clinicians according to World Health Organization (WHO) inclusion-exclusion criteria. The number of samples genotyped was 200 each of normal healthy controls and T2DM patients with an age ranging from 22-76 years. Age/sex-matched normal controls were screened from healthy staff members of both universities. Informed and written consent was obtained from all the participants. The study protocol was approved by the Institutional Ethics Committee of KGMU. Medical records of T2DM patients were reviewed to ascertain diabetes-associated complications. Clinical details, family history of diabetes and associated complications were recorded. The details of inclusion and exclusion criteria for patients and controls were as follows:

Inclusion criteria for patients: Elevated glucose concentrations i.e. fasting plasma glucose/ post prandial glucose/ Random blood sugar (FPG/PPG/RBS) on more than one occasion i.e.:

- **FPG:** ≥126 mg dL^{-1} (7.0 mmol L^{-1}) of fasting (8 h or O/N) glucose in venous whole blood
- **Two hour post prandial (PPG):** ≥200 mg dL^{-1} (11.1 mmol L^{-1}) after consumption of 1.7g kg^{-1} b.wt. of anhydrous glucose in 200 mL water within 1-2 min (OGTT)/75 g glucose
- **RBS:** ≥200 mg dL^{-1} (11.1mmol L^{-1}) with classical symptoms

Exclusion criteria for patients:

- Pregnant or nursing mother
- Patients diagnosed of psychotic disorder or hospitalized for depression
- Patients with other types of diabetes such as Mature Onset Diabetes in Young (MODY), Latent Autoimmune Diabetes in Adults (LADA), Gestational Diabetes (GD) and diabetes due to any type of pancreatic injury
- Patients having fluctuations in glucose readings in multiple examinations

Criteria for selection of normal subjects: Normal healthy age/sex matched controls with no family history of any specific disease/allergy/infection/inflammatory responses have been included in the study.

Clinical analysis: Peripheral blood samples (2 mL each) were collected with and without EDTA for DNA extraction and biochemical estimations, respectively.

Anthropometric assessment: Body Mass Index (BMI) and Waist Hip Ratio (WHR) were calculated by measuring height, weight and waist circumference, systolic and diastolic blood pressures were measured for anthropometric assessment. Standing body height (to the nearest 0.5 cm) was measured with a commercial stadiometer. A digital scale, with an accuracy of ±100 g, was used to measure Body Weight (BW). The Waist Circumference (WC) was measured in a horizontal plane, midway between the inferior margin of the ribs and the superior border of the iliac crest. Hip Circumference (HC) was measured at the fullest point around the buttocks with a metallic tape. The measurements were taken thrice and the mean was taken in all cases. WC (cm) was divided by HC (cm) to calculate Waist to Hip Ratio (WHR). Body Mass Index (BMI) (kg m^{-2}) was calculated by dividing weight (in kilograms) by the square of height (in meters), as a measure of total adiposity. Systolic and diastolic blood pressures were measured in the sitting position with an appropriately sized cuff after a 5 min rest.

Biochemical assessment: Serum was collected from the blood in plain vials (without EDTA) after centrifugation for 10 min at 3000 rpm at 4°C. Estimations of plasma glucose (mg dL^{-1}), serum insulin (mg dL^{-1}) and lipid profile (total serum cholesterol, TC; High density lipoprotein-cholesterol, HDL-C and serum triglycerides, TG) were made using commercially available Ecoline kits (Merck) by double beam spectrophotometer at 550 (TGL-C), 510 (S. Creatinine), 500 (TC) and 560 nm (HDL-C). LDL-Cholesterol (LDL-C) was estimated by using Friedewald's formula (Friedewald *et al*., 1972) as has been shown below:

$$LDL\text{-}C = TC\text{-}HDL\text{-}C\text{-}(TG/5) \text{ and } VLDL\text{-}C = TG/5$$

Genetic analysis

DNA isolation and SNP genotyping: Genomic DNA was extracted from EDTA-blood samples using salting out method with slight modifications and checked on 0.8-1% agarose gel (Miller *et al*., 1988; Gautam *et al*., 2011). The quality and quantity of DNA was estimated using a biophotometer (Eppendorf, USA).

Three known single nucleotide polymorphisms (SNPs) designated as CI962235 (Ins1361A) at 317 codon in exon 10, rs3212018 (16 bp del) 'GCACAAATAAAGCACT' at 2085-2070 position of transcript in exon 14 and rs1049673 (G>C) at 2484 position of transcript in exon 15 of *CD36* gene were genotyped in 400 subjects (200 each of controls and T2DM patients). SNPs were selected based on their minor allele frequency (MAF) and functional relevance. Genotyping was done by using polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) and single strand conformation polymorphism (SSCP). Primers for each SNP were designed and restriction enzymes identified using Primer 3 and NEB cutter softwares, respectively (Table 1). Sample size for each of the SNPs was calculated using Quanto programme (v.1.2.1) using 2 tailed analysis (Power: 90; Disease prevelance: 3%; error: 5%).

PCR was performed in a 25 μL reaction mixture containing genomic DNA (150 ng), 10 pmol of each primer, 400 μM dNTPs and 0.25 Units of Taq DNA polymerase (Bioscience, India) in a gradient Master cycler (Eppendorf, USA). We performed each SNP genotyping in duplicate and genotyping success rate was 99.5%. The primers, PCR conditions and results of restriction digestion are shown in Table 1. The digested products were resolved on 10-12% polyacrylamide gels, stained with ethidium bromide or silver staining and documented in a gel documentation system (Vilber-Lourmat, France).

Preparation of SSCP gel: Polyacrylamide gels (10-12%) were prepared from 30% bis-acrylamide stock (29% acrylamide + 1% bis-acrylamide), 1X TBE, 10% APS and TEMED in cold. Ten μLof each PCR product was diluted with equal volume of denaturation buffer (95% formamide, 0.5 M EDTA, 0.05% xylene cyanol) in 0.5 mL tubes. The samples were denatured at 95°C for 6-7 min and immediately snapped on ice. 30-45 ng (6-8 μL) of denatured samples were loaded on the gel. The gel was pre-run at 200 V for 30 min. Samples were applied and gel was electrophoresed at 50 V for 4-6 h in 1X TBE buffer at 20-25°C. After completion of the run, gels were silver stained and documented.

Table 1: Polymorphisms in *CD36* gene, their location, primer sequences, PCR conditions and restriction enzymes with product sizes

rs ID, Nucleotide changes and position of the polymorphisms	Primer sequences	Product size (bp)	Annealing temp (°C)	Restriction enzymes	Allele sizes
CI962235, Ins 1361A at 317 codon in exon 10	F: 5' TCCGACGTTAATCTGAAAGGA 3' R: 5' TAAAATTTTCTGCCACCATTCT 3'	387	56	AseI	Homo ins 278, 110 Hetero ins 387, 278,110 Homo no 387
rs3212018, 16 bp del at 2085-2070 at exon 14	F: 5' CATGTCTAGCCACTGATCATTTTT 3' R: 5' TCAGGACTTTTCTGGATTTGG 3'	313	56	No RE	No del (+/+) 313 Hetero del (+/-) 313, 297 Homo del (-/-) 297
rs1049673, G>C at exon 15	F: 5' CTGTCATAATCGCCTCATAAAGAC 3' R: 5' CAAATGTCTTTTTGTTCTTCATCC 3'	250	55	No RE	.....

Statistical analysis: Comparison of clinical parameters between controls and T2DM patients were carried out by multivariate logistic regression analysis. Allelic and genotypic frequencies along with carriage rates of polymorphic alleles in patient and control groups were evaluated by Fisher's exact test/Pearsons chi-square (χ^2) test. Hardy-Weinberg equilibrium at individual locus was assessed by chi-square (χ^2) statistics using SPSS (v. 15.0). All p-values were statistically significant for $p<0.05$. Odds Ratio (OR) at 95% Confidence Interval (CI) was determined to describe the strength of association by logistic regression model. The OR was calculated for a combination of both homozygous and heterozygous genotypes.

RESULTS

Clinical analysis: In univariate analysis, the age, sex, BMI, systolic blood pressure, FPG, PPG, TC, TG, HDL, LDL and VLDL were found to be significant risk factors for diabetes ($p<0.05$). However, the multivariate logistic regression analysis revealed that systolic blood pressure, FPG, PPG, TG, LDL and VLDL were significant risk factors for diabetes ($p<0.05$) (Table 2).

Genetic analysis: The band pattern of SNP Ins1361A is shown in Fig. 1. All digested products showed monomorphic condition (no insertion of A nucleotide). After confirmation on 1.5% agarose gel the PCR products for SNP rs3212018 was directly checked on a 8-10% polyacrylamide gel (Fig. 2a). In controls the frequency of rs3212018 (16 bp del) *i.e.*, '-/-'genotypes in *CD36* gene was observed in 72% control individuals while the same was found in 76% patients (Table 3). The genotypic status was confirmed by sequencing of samples representing all three genotypic conditions and analysis by multiple sequence alignment using CLUSTAL-W software (Fig. 2b). The carriage rate of no del (-) allele of rs3212018 in next generation was found to be 2.02 times higher in T2DM patients [CI (95%) = 2.02{0.180-22.645}]. However, no significant association was observed in genotype frequency, allele frequency and carriage rate ($p>0.05$). Moreover, in rs1049673 (G>C) at exon 15 of *CD36* gene only one condition *i.e.*, four band patterns were observed with no shift (Fig. 3).

DISCUSSION

The association between *CD36* over-expression and presence of atherosclerotic risk factors, particularly diabetes, shown in this study is in agreement with a common etiology of the disease (Saxena *et al*., 2012). The role of *CD36* in lipid metabolism and metabolic syndrome prompted us to investigate the association of *CD36* gene variants with T2DM. Earlier studies from our laboratory have shown that out of several genetic variants *viz*. 478C>T in exon 4, dinucleotide deletion (delAC) in exon 5, rs1761667 (G>A) in -31118 promoter region of exon 1A and rs1527483 (C>T) in intron 11, one SNP rs1761667 (G>A) showed a highly significant association with T2DM (Love-Gregory *et al*., 2008; Banerjee *et al*., 2010). Moreover, there was no evidence of deletion mutation in *CD36* gene in our North Indian population (Gautam *et al*., 2011). A single nucleotide insertion of A at nt 1361 of transcript (codon 317) in exon 10 causes a frameshift and leads to the appearance of a premature stop codon resulting in a marked reduction in the level of macrophage *CD36* mRNA. This mutation was found to be responsible

Table 2: Multivariate logistic regression analysis of clinical parameters of 200 each of controls and T2DM patients by adjusting other variables for associated risk factors

	Unadjusted			Adjusted		
Factors	OR	95%CI	p-value	OR	95%CI	p-value
Age (years)	1.02	1.01-1.03,	<0.01	0.99	0.96-1.03	0.85
Sex						
Male	0.65	0.52-0.81	<0.01*	0.56	0.24-1.33	0.19
Female	1.00	(ref)	1.00 (ref)			
BMI kg m^{-2}	1.16	1.11-1.20	<0.01*	1.07	0.96-1.20	0.30
WHR	1.10	0.72-1.67	0.67	0.10	0.03-1.28	0.10
SBP (mmHg)	1.08	1.07-1.09	<0.01*	1.09	1.06-113	<0.01*
DBP (mmHg)	1.01	0.99-1.02	0.25	0.99	0.94-1.05	091
FPG (mg dL^{-1})	1.04	1.03-1.05	<0.01*	1.04	1.03-1.05	<0.01*
PP (mg dL^{-1})	1.08	1.06- 1.09	<0.01*	1.07	1.04-1.09	<0.01*
TC (mg dL^{-1})	1.03	1.02-1.03	<0.01*	0.99	0.98-101	0.36
TGA (mg dL^{-1})	0.99	0.98-0.99	0.01*	0.97	0.96-0.99	0.002*
HDL (mg dL^{-1})	1.02	1.01-1.02	0.001*	0.97	0.93- 1.01	0.09
LDL (mg dL^{-1})	1.04	1.03-1.04	<0.01*	1.07	1.05-1.08	<0.01*
VLDL (mg dL^{-1})	1.16	1.11-1.20	<0.01*	1.21	1.04-1.41,	0.01*
S. CRET (mg dL^{-1})	0.80	0.34-1.85	0.60	2.01	0.10-41.25	0.65

*Significant

Table 3: Allele, genotypic frequencies and carriage rates of SNP rs3212018 (16 bp del)

	(rs3212018, 16 bp del at exon 14) allele frequencies					
	Controls		Patients			
	Count (n = 400)	Frequency	Count (n = 400)	Frequency	p-values	CI (95%)
No del (+)	342	(0.855)	358	(0.870)	0.605111	1.136885
Homo del (-)	58	(0.145)	52	(0.130)		[0.698924~1.849282]
	Genotype frequencies					
	Controls		Patients			
	Count (n = 200)	Frequency	Count (n = 200)	Frequency	p-values	CI (95%)
No Del (+/+)	144	(0.720)	152	(0.760)	0.555	0.549 [0.60-4.010]
Hetero Del (+/-)	54	(0.270)	44	(0.221)		
Homo Del (-/-)	02	(0.010)	4	(0.019)		
	Carriage rate					
	Controls		Patients			
	Count (n = 400)	Frequency	Count (n = 400)	Frequency	p-values	CI (95%)
No del (+)	198	(0.495)	204	(0.650)	0.568	2.02 [0.180-22.645]
No del (-)	02	(0.005)	4	(0.129)		
Homo del (+)	56	(0.140)	50	(0.158)	0.334	1.367 [0.725-2.581]
Homo del (-)	144	(0.360)	158	(0.50)		

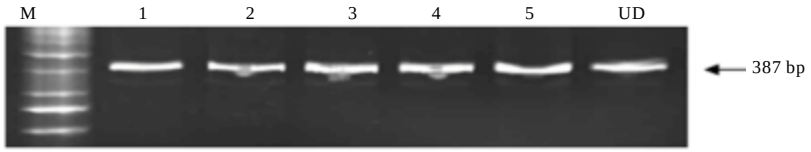

Fig. 1: Polyacrylamide gel showing genotype of SNP CI962235 (Ins 1361A) lanes 1-5 No ins, UD, Undigested, M 50 bp ladder

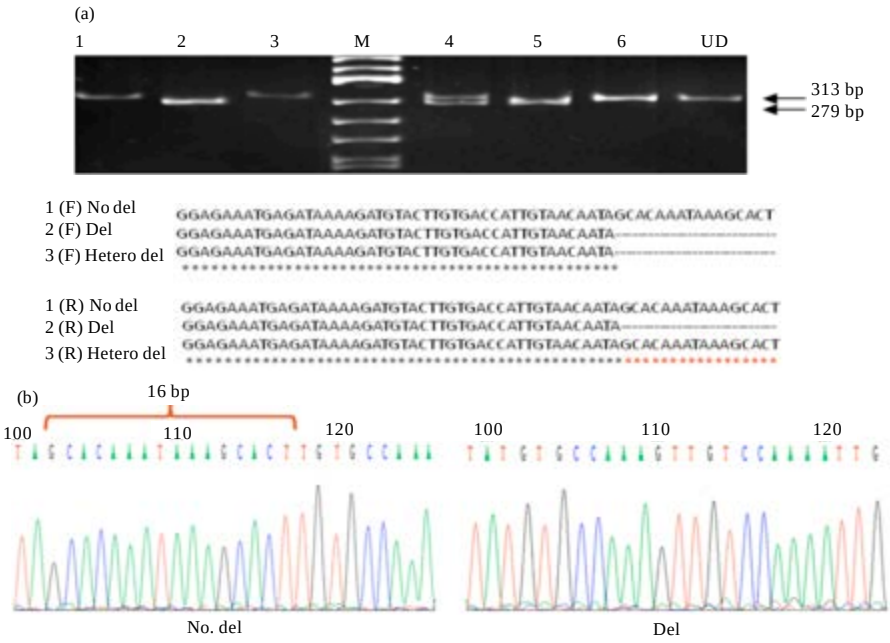

Fig. 2(a-b): (a) Polyacrylamide gel showing genotype of rs 3212018 (16 bp del), Lanes 1, 3, 6, (+/+), Lanes 2, 5 (-/-), UD, Undigested, M ϕ x174 Hin f 1 digest, (b) Multiple alignment using CLUSTAL-W and sequening showing rs3212018 (16 bp del)

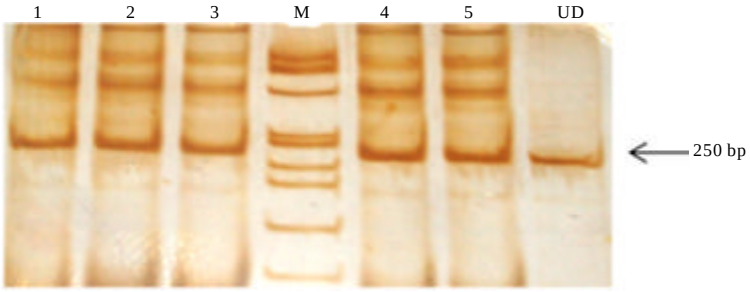

Fig. 3: Silver staiming of polyacrylamide gel showing monomorphic condition of SNP rs 1049673 (G>C) in patients samples, Lane UD, Undigested, Lane, M, ϕ x174 Hae III digest

for type 1 *CD36* deficiency in Japanese (Kashiwagi *et al*., 1996). Although SNPs rs3212018 (16 bp del) and rs1049073 (G>C) lie in the 3'UTR region which attribute their role in post translation modifications, we did not observe any significant association of these polymorphisms in North Indian population.

The limited power for analyzing binary traits of *CD36* gene associated with T2DM in the case control design was the major limitation of our study. Although, correction for multiple testing is not required for variants that already have strong priors of association with T2DM, the possibility exists that some of our weaker associations may represent false positive findings. The limitation of our study may be the small sample size, therefore, individual SNPs did not show significant association (Table 3) However, the *CD36* gene with its variants appears to be an important candidate gene and may have a predictive potential on disease susceptibility in the North Indian population. However, further investigations are required to elucidate the underlying pathophysiological consequences.

CONCLUSION

CD36 , being an important receptor molecule for modified lipoproteins, plays an important role in the regulation of lipid metabolism. Since lipid metabolism is closely associated with diabetes and in turn atherosclerosis, it may be worthwhile to study the gene variants at the *CD36* locus. Our previous studies have shown significant association of certain SNPs in *CD36* gene with T2DM in North Indian population. This is probably the first report on these *CD36* gene variants in a North Indian population. Further studies are required in larger cohorts to validate the results in different ethnic populations.

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REFERENCES

Amini, M. and M. Janghorbani, 2007. Diabetes and impaired glucose regulation in first-degree relatives of patients with type 2 diabetes in Isfahan, Iran: Prevalence and risk factors. Rev. Diabetic Stud., 4: 169-176.

Armesilla, A.L. and M.A. Vega, 1994. Structural organization of the gene for human *CD36* glycoprotein. J. Biol. Chem., 269: 18985-18991.

Banerjee, M., S. Gautam, M. Saxena, H.K. Bid and C.G. Agrawal, 2010. Association of *CD36* gene variants rs1761667 (G>A) and rs1527483 (C>T) with Type 2 diabetes in North Indian population. Int. J. Diabetes Mellitus, 2: 179-183.

Fernandez-Real, J.M., J.M. Mercader, F.J. Ortega, J.M. Moreno-Navarrete, P. Lopez-Romero and W. Ricart, 2010. Transferrin receptor-1 gene polymorphisms are associated with type 2 diabetes. Eur. J. Clin. Invest., 40: 600-607.

Friedewald, W.T., R.I. Levy and D.S. Fredrickson, 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem., 18: 499-502.

Gautam, S. and M. Banerjee, 2011. The macrophage Ox-LDL receptor, *CD36* and its association with type II diabetes mellitus. Mol. Genet. Metab., 102: 389-398.

Gautam, S., C.G. Agrawal, H.K. Bid and M. Banerjee, 2011. Preliminary studies on *CD36* gene in type 2 diabetic patients from north India. Indian J. Med. Res., 134: 107-112.

IDF, 2012. Clinical Guidelines Task force: Global guideline for type 2 diabetes. International Diabetes Federation, Brussels, Belgium.www.idf.org

Kashiwagi, H., Y. Tomiyama, S. Nozaki, S. Honda and S. Kosugi *et al*., 1996. A single nucleotide insertion in codon 317 of the *CD36* gene leads to *CD36* deficiency. Arterioscler. Thromb. Vasc. Biol., 16: 1026-1032.

Love-Gregory, L., R. Sherva, L. Sun, J. Wasson and T. Schappe *et al*., 2008. Variants in the *CD36* gene associate with the metabolic syndrome and high-density lipoprotein cholesterol. Hum. Mol. Genet., 17: 1695-1704.

Ma, X., S. Bacci, W. Mlynarski, L. Gottardo and T. Soccio *et al*., 2004. A common haplotype at the *CD36* locus is associated with high free fatty acid levels and increased cardiovascular risk in Caucasians. Hum. Mol. Genet., 13: 2197-2205.

Miller, S.A., D.D. Dykes and H.F. Polesky, 1988. A simple salting out procedure for extracting DNA from human nucleate cells. Nucleic Acids Res., 16: 1215-1215.

Nagarajan, P., M.J. Mahesh Kumar, R. Venkatesan, S.S. Majundar and R.C. Juyal, 2012. Genetically modified mouse models for the study of nonalcoholic fatty liver disease. World J. Gastroenterol., 18: 1141-1153.

Nicholls, H.T., G. Kowalski, D.J. Kennedy, S. Risis and L.A. Zaffino *et al*., 2011. Hematopoietic cell-restricted deletion of *CD36* reduces high-fat diet-induced macrophage infiltration and improves insulin signaling in adipose tissue. Diabetes, 60: 1100-1110.

Prikh, R., K. Pandia., M . Goyal, M. Sharma and M.S. Dolima, 2011. Trend of clinical drug trials in type 2 diabetes mellitus over last decade. Perspect. Clin. Res., 2: 64-66.

Rac, M.E., K. Safranow and W. Poncyljusz, 2007. Molecular basis of human *CD36* gene mutations. Mol. Med., 13: 288-296.

Samatha, P., M. Venkateswarlu and V. Siva Prabodh, 2012. Lipid profile levels in type 2 diabetes mellitus from the tribal population of Adilabad in Andhra Pradesh, India. J. Clin. Diag. Res., 6: 593-596.

Saxena, M., P. Vats, K. Agrawal and M. Banerjee, 2012. Expression of macrophage scavenger receptor *CD36* in humans: Its implication in type 2 diabetes mellitus. Ann. Biol. Res., 3: 3015-3021.

Tuomisto, T.T., M.S. Riekkinen, H. Viita, A.L. Levonen and S. Yla-Herttuala, 2005. Analysis of gene and protein expression during monocyte-macrophage differentiation and cholesterol loading: cDNA and protein array study. Atherosclerosis, 180: 283-291.