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Original article

Eleven genetic variants of seven important candidate genes involved in manifestation of type 2 diabetes mellitus

Madhukar Saxena [a,1], Daoud Ali [b,1,*], Dinesh Raj Modi [a], Hussain Al Ali [c], SA Hussain [b], S Manohrdas [b]

[a] Department of Biotechnology, Babasaheb Bhimrao Ambedkar University, Vidya Vihar, Rai Bareilly Road, Lucknow, India
[b] Department of Zoology, College of Science, King Saud University, Saudi Arabia
[c] Centre of Excellence for Genomics, King Abdulaziz City for Science and Technology, Riyadh, Saudi Arabia

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ABSTRACT

Background: Diabetes especially type 2 diabetes mellitus (T2DM) is securing the rank under five around the world and leading cause of death cardiac disease and cancer. T2DM is a severe inflammatory state mainly associated to obesity and enhanced oxidative stress.
Objectives: We have investigated eleven genetic polymorphisms of seven important genes viz. IL-10, TNF-α, IL-6, adiponectin, IL-1, IL-18, and Vitamin D-receptor. We have focused to investigate the haplotypes, gene-gene interactions and their role in determining individual susceptibility to T2DM and their manifestation.
Methods: Eleven genetic variants of seven important candidate genes in 440 T2DM individuals and 440 controls were genotyped using conventional polymerase chain reaction (PCR) and PCR restriction fragment length polymorphism (PCR-RFLP). All calculations were done by SPSS software and LD were analysed by haploview (SHEsis software).
Results: Genotypic frequency of rs1800872, rs1800795, rs2241766, rs17846866 and rs1946518 showed highly significant association in our population. While allele frequencies of rs1800795, rs2241766 and rs1946518 showed highly significant association. The allele set of "CGGAGGTAFTB", "CGGAGTTAFTB" and "CGGATGTCFTB" increase the chance of diabetes up to 1.6 times. This study reflects that these individuals are more susceptible of having T2DM.
Conclusions: The present study will provide a new insight in the development and the manifestation of T2DM. These genetic studies showing gene interaction for the susceptibility of the disease may be used as prognostic markers and alter treatment strategies for T2DM.

1. Introduction

Diabetes especially type 2 diabetes mellitus (T2DM) is securing the rank under five around the world and leading cause of death cardiac disease and cancer. From our previous reports which revealed that South east Asia including India has 78.3 million diabetics which are expected to increase up to 140.2 million till 2040 (Bid et al., 2008; Banerjee and Saxena, 2012a,2012b; Saxena et al., 2012a,2012b; IDF, 2015). The complex interactions and associations among lymphoid cells are usually mediated by a group of proteins which are collectively designated as cytokines. They include immune modulating agents like interleukins and inteferons (Banerjee and Saxena, 2012a,2012b). Apart from this the major sub group of cytokines, the chemokines which are of low molecular weight cytokines that alter the behaviour of leukocyte. Traditionally the cytokines have been classified into pro- and anti-inflammatory. They are antagonistic in behaviour for each other i.e. proinflammatory effector functions are inhibited by anti-inflammatory cytokines. These inflammatory mediators have been proposed to be involved directly or indirectly in causing T2DM and their manifestation. As Asian Indians have more prone and have a greater susceptibility to T2DM, they are a good site of population for carrying out such genetic studies (Bid et al., 2009a,2009b,2010). Polymorphisms (SNPs), mainly those within

* Corresponding author at: Department of Zoology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia.
E-mail address: aalidaoud@ksu.edu.sa (D. Ali).
[1] Both the authors have equally contributed in the manuscript.
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the regulatory regions of cytokines genes often have to be involved at expression levels and can be disease modifiers. Individuality in the inheritance of this polymorphic genes lead to change in their immune responses. This might change the severity as well as the duration of inflammation, thereby leading to the progression of T2DM (Dinarello, 1996; Wilson et al., 1997; Bidwell et al., 1999). All genes of IL-1 family are polymorphic and most of these polymorphisms have been shown to be involved in either the susceptibility or the severity of inflammatory conditions and diseases (Haukim et al., 2002). The pro-inflammatory cytokine IL-18, a member of IL-1 family is playing a crucial role in the cascade of inflammation. It is highly expressed in atherosclerotic plaques and plays an important role in the destabilization of plaques (Mallat et al., 2002). A polymorphic variation in IL-6 gene might increases risk for T2DM development and was already reported (Vozarova et al., 2003; Illig et al., 2004; Saxena et al., 2014). While, the predominance nature of IL-10 genotype in T2DM is mainly due to its protective nature against the inflammation and encouraging humoral responses which delay the activation of inflammatory cytotoxic reactions involved in destruction of pancreatic β-cell. IL-10 is involved to down regulate the secretion of mainly pro-inflammatory cytokines secreted from monocytes/macrophages (Sankaran et al., 1999). Moreover, VDR gene is also a novel candidate gene and contributes to the T2DM susceptibility (Saxena et al., 2018). VDR is a part of the steroid/thyroid hormone receptor family and is involved in regulating the normal functions of the pancreas especially the secretion of insulin. They play the major role in regulating the β-cell insulin secretion (Saxena et al., 2018). It would be quite interesting to know whether the polymorphisms in aforesaid genes taken together would predispose and unwinding the hidden related facts in the T2DM manifestation. In our earlier reports, we have thoroughly went from anthropometric and biochemical parameters in T2DM in association with individual as well as in combination of polymorphisms with cytokine gene (Saxena et al., 2009,2012a,2012b; Saxena et al., 2015). We have investigated eleven genetic polymorphisms of seven important genes viz. IL-10, TNF-α, IL-6, adiponectin, IL-1, IL-18, and Vitamin D-receptor. We have focused to investigate the gene-gene interactions and their role in determining individual susceptibility to T2DM and their manifestation.

2. Material and methods

2.1. Molecular and biochemical studies

2.1.1. Patient selection and clinical evaluation

Enrolled Type 2 diabetes patients (n = 440) from the OPD of King George's Medical University (KGMU), Lucknow, India parallelly with age/Sex-matched normal healthy controls (n = 440) with proper written consent after due approval of IEC including all anthropometric and biochemical data.

2.1.2. DNA extraction and genotyping

Genomic DNA was extracted from peripheral blood mononuclear cells (PBMCs) using salting out method (Saxena et al., 2009,2012a,2012b). Eleven genetic variants of seven important candidate genes viz. IL-10-592A/C (rs1800872), TNF-a-308G/A (rs1800629), IL-6-174G/C (rs1800795), IL-6-597G/A (rs1800797), Adiponectin + 45G/T (rs2241766;Q1), Adiponectin + 10211 T/G (rs17846866;Q2), IL-1b-511C/T (rs16944), IL-18-607 A/C (rs1946518), Vitamin D Receptor (VDR) FokIT/C (rs2228570), VDR TaqIC/T (rs731236) and VDR BsmIA/G (rs1544410) were genotyped using conventional polymerase chain reaction (PCR) in a 15 μl reaction mixture containing 100 ng of template DNA, buffer (100 mM Tris, pH 9.0; 500 mM KCl; 15 mM MgCl2; 0.1% gelatin), 200 lM dNTP, 10 pmol of each primer and 1.0 unit Taq DNA polymerase followed by PCR restriction fragment length polymorphism (PCR-RFLP). The minor allele frequency (MAF) $\geq$ 0.01 for genotyped SNPs were included in the present study. The primers designed by Primer 3.0 online software. Other methodological regarding sequences of primers, condition of PCR reactions, size of amplicon (bp), type of restriction endonucleases (RE) required were as per Table 1 (Bid et al., 2009a,2009b). To maintain the efficacy and quality of genotyping random duplicate samples in ~ 20% of the samples were performed. The PCR products digested with RE as per Table 1 (Fermentas, USA) respectively were electrophoresed on 12.5% polyacrylamide gels. The genotyping results were cross checked in 20% random duplicates and success rate in more than 98% concordance was obtained.

2.2. Statistical analysis

Allele frequencies and genotypic frequencies were compared by 2×2 and 2×3 contingency table by Chi-square test ($\chi 2$) and Fisher's exact test respectively followed by HWE assiation at individual locus usingChi-squire statistic.

Moreover, SHEsis software were used for multiple regression analysis (MRA) to analyze the gene-gene interaction association along with Pairwise Linkage disequilibrium (LD) based on D' statistics and correlation coefficient (r^2) (Shi and He, 2005). Studied Gene-Gene Interactions, Logistic regression was used to study the combination of gene-gene interactions between eleven variants of eight genes.

3. Results

The biochemical parameters showed significant association with FBS, PPBS, TC, TGL, HDL, LDL and VLDL ($P < 0.001$) when compared with healthy controls (Table 2). All the genotyping data for individual SNPs in T2DM patients (n = 440) and age-sex matched healthy controls (n = 440) have been summarized and the genotypic frequency of rs1800872, rs1800795, rs2241766, rs17846866 and rs1946518 showed highly significant association in our population. While rs1800629 and rs731236 showed significant association in our population. The rs1800797, rs16944, rs2228570 and rs1544410 showed no significant association (Table 3). When we assessed the allele frequencies of these variants we found that rs1800795, rs2241766 and rs1946518 showed highly significant association and rs2241766 increases the chance of having diabetes by twice [OR; 1.955(95% CI;1.595–2.395)]. Only allele of rs1800629 showed significant association in our population ($p = 0.010$) (Table 4). Further we have gone to analyse the carriage rate allele frequencies of individual alleles and found that 'C' allele of rs1800795 and rs1800872, both 'T' and 'G' allele of rs2241766 and 'A' allele of rs1946518 showed highly significant association. While recessive allele of rs1544410 i.e. allele 'b' also showed highly significant association (Table 5). Later on we go for gene gene interaction analysis of all studied variants of IL-10-592A/C (rs1800872), TNF-a-308G/A (rs1800629), IL-6-174G/C (rs1800795), IL-6-597G/A (rs1800797), Adiponectin + 45G/T (rs2241766;Q1), Adiponectin + 10211 T/G (rs17846866;Q2), IL-1b-511C/T (rs16944), IL-18-607 A/C (rs1946518), Vitamin D Receptor (VDR) FokIT/C (rs2228570), VDR TaqIC/T (rs731236) and VDR BsmIA/G (rs1544410) and found very interesting results. We found that the allele set

"AGGATTTCFTB", "CAGATTTCFTB", "CGCATTTCFTB", "CGGAGGTAFTB", "CGGAGTTAFTB", "CGGATGTCFTB", "CGGATTTCFTB", "AGGATTTAFTB", "CGCATTTAFTB", "CGGATGTAFTB", "CGGATTCCFTB" and "CGGATTTAFTB" showed significant association in the manifestation of the disease (Table 6).

Table 1
List of SNPs with position, primer sequences, PCR conditions, amplicon sizes and restriction endonucleases (RE).

Genes	SNP [Position (rs no.)]	Primer Sequences (5′–3′)	PCR Conditions (Tm/Cy)	Amplicon Size (bp)	RE
IL-10	−592A/C (rs1800872)	F-CCTAGGTCACAGTGACGTGG R-GCTAGTCAGGTAGTGCTCACC	63/35	411	RsaI
TNF-α	−308G/A (rs1800629)	F-AGGCAATAGGTTTTGAGGGCCAT R-TTGGGGACACAAGCATCAAGG	64/35	147	NCoI
IL-6	−174G/C (rs1800795)	F-TTGTCAAGACATGCCAAGTGGT R-GGGAAAATCCCACATTTGATAA	57/44	231	NlaIII
	−597G/A (rs1800797)	F-GGAGTCACACACTCCACCT R-CTGATTGGAAACCTTATTAAG	61.5/35	527	FokI
Adiponectin	+45G/T (rs2241766)	F-GGCTCAGGATGCTGTTGCTGG R-GCTTTGCTTTCTCCCTGTGTCT	60/35	327	BspHI
	+10211 T/G (rs1784686)	F-GCTAAGTATTACAGATTTCAGGGCAG R-CAGCAACAGCATCCTGAGC	60/35	222	PleI
IL-1β	−511C/T (rs16944)	F-TGGCATTGATCTGGTTCATC R-GTTTAGGAATCTTCCCACTT	60/35	304	SacI
IL-18	−607 A/C (rs1946518)	F-CTTTGCTATCATTCCAGGAA R-TAACCTCATTCAGGACTTCC	60/35	300	MseI
VDR	FokI T/C (rs2228570)	F-AGCTGGCCCTGGCACTGACTCTGCTCT R-ATGGAAACACCTTGCTTCTTCTCCCTC	58/35	265	Fok I
	TaqI C/T (rs731236)	F-CAACCAAGACTACAAGTACCGCGTCAGTGA R-AACCAGCGGGAAGAGGTCAAGGG	63/35	740	Taq I
	BsmI A/G (rs1544410)	F-CAGAGCATGGACAGGGAGCAA R-GCAACTCCTCATGGCTGAGGTCTC	63/35	825	Bsm I

Table 2
Clinical characteristics of controls and cases. Values are mean ± SD; P^{**}<0.01 ***<0.001 compared to control.

Clinical characteristics	Controls	Cases
Age (yr)	45.68 ± 9.11	48.10 ± 8.32
Fasting blood glucose (FBS) mg/dl	82.18 ± 7.68	169.32 ± 74.38***
Post-prandial blood glucose (PPBS) mg/dl	141.25 ± 14.10	278.15 ± 106.27***
Total cholesterol (TC) mg/dl	178.40 ± 27.35	226.51 ± 38.35***
Triglycerides (TGL) mg/dl	124.90 ± 44.24	114.16 ± 19.12***
High density lipoproteins (HDL) mg/dl	47.10 ± 13.15	44.98 ± 9.12**
Low density lipoproteins (LDL) mg/dl	66.13 ± 25.10	157.10 ± 51.47***
Very low density lipoproteins (VLDL) mg/dl	24.90 ± 7.40	23.01 ± 3.26***
Serum creatinine (SCRT) mg/dl	1.03 ± 0.11	1.05 ± 0.07

Allele set of "CGGAGGTAFTB", "CGGAGTTAFTB" and "CGGATGTCFTB" increase the chance of diabetes upto 1.6 times; the OR are 1.436 [95% CI; 0.796 ~ 2.587], 1.610 [95% CI; 0.890 ~ 2.912] and 1.466 [95% CI;0.791 ~ 2.717] respectively. This shows that these set of allele from IL to 10-592A/C (rs1800872), TNF-a-308G/A (rs1800629), IL-6-174G/C (rs1800795), IL-6-597G/A (rs1800797), Adiponectin + 45G/T (rs2241766;Q1), Adiponectin + 10211 T/G (rs17846866;Q2), IL-1b-511C/T (rs16944), IL-18-607 A/C (rs1946518), Vitamin D Receptor (VDR) FokIT/C (rs2228570), VDR TaqIC/T (rs731236) and VDR BsmIA/G (rs1544410) increased the risk of the disease upto 1.6 times. Linkage disequilibrium (D') and Correlation coefficient (r2) of IL-10-592A/C (rs1800872), TNF-a-308G/A (rs1800629), IL-6-174G/C (rs1800795), IL-6-597G/A (rs1800797), Adiponectin + 45G/T (rs2241766;Q1), Adiponectin + 10211 T/G (rs17846866;Q2), IL-1b-511C/T (rs16944), IL-18-607 A/C (rs1946518), Vitamin D Receptor (VDR) FokIT/C (rs2228570), VDR TaqIC/T (rs731236) and VDR BsmIA/G (rs1544410) eleven variants with T2DM in North Indian population is shown in (Fig. 1).

4. Discussion

Diabetes is basically a condition with an involvement of pro-inflammatory state having an enhanced level of circulating cytokines and suggests a causal role for inflammation in its etiology. Previous reports documented that hyperglycemia is associated with T2DM and acutely enhanced level of peripheral cytokines viz. IL-1, TNF-α and IL-6 (Tsiavou et al., 2004). A significant association of IL-4 gene variants have also been reported with increased risk of T2DM along with other related complications in north Indians (Achyut et al., 2007). However, as per our previous reports in IL-1Ra gene variants we have reported four different alleles in north Indians with T2DM. Most of the T2DM individuals were homozygous genotype I as per our previous report[2] and its association with coronary artery disease (CAD) individuals with and without T2DM (Marculescu et al., 2002). It was documented that these genetic variants increases the risk of having T2DM upto 3.4 times. Other IL-1 gene variants viz. IL-1 α-889, IL-1 β + 3954 (rs 1143634) and IL-1 β-511) in T2DM as well as periodontitis were also reported (Struch et al., 2008; Lopez et al., 2009). One of the case-control study of IL-1β gene variant found significant association in genotypic frequencies (P < 0.0001) but no association was observed in allele frequencies of −511C and −511 T. While a new sub member of IL-1 family, the IL-18 gene variant have been vastly studied by other groups in T2DM associated complications. One of the renowned research group i.e. Opstad et al. (2011) studied three IL-18 gene variants +183 A/G, −137 G/C and −607C/A in T2DM individuals and reported that only IL-18 + 183 A/G was significantly associated with T2DM manifestation. However, in the population of china only −607C/A gene variant was found to be associated with T2DM (Huang et al., 2010). Controversial results were reported by European researchers on IL-18 gene variants with T2DM and other metabolic diseases and disorders (Thompson et al., 2007; Rafiq et al., 2008; He et al., 2010). In contrast to the previous reports we found that in north Indians, −607C/A gene variants showed significant genotypic association (P = 0.002) and most of the individuals were heterozygous. Moreover, lots of controversies on the results on IL6-174 G/C and -597 A/G gene variants with T2DM or insulin resistance. Apart from this, 'G' allele of -174 G/C SNP was found to be associated with increased T2DM risk in Americans and Spanish Caucasians but not in the Finnish Diabetes Prevention Study (DPS) (Kubaszek et al., 2003a,2003b).

Furthermore, nondiabetic individual was found to be associated with IL6-174C/C genotype in higher insulin sensitivity (Fernandez-Real et al., 2000; Kubaszek et al., 2003a,2003b). While -174 G/C was found to be associated with T2DM risk in North Indians and in the metagenomic Indian study (Mukhopadhyaya et al., 2010; Popko et al., 2010; Ferreira et al., 2011). Although TNFα-308 G/A was also a focused gene

Table 3
Genotypic frequencies of studied genetic variants.

Gene	Genotype			
		Controls (n = 440)	Patients (n = 440)	p-value
IL-10 rs1800872 (−592A/C)	**CC**	243	220	0.000
	CA	148	205	
	AA	49	15	
TNF-α rs1800629 (−308 G/A)	**GG**	248	288	0.022
	GA	167	132	
	AA	25	20	
IL-6 rs1800795 (−174 G/C)	**GG**	230	310	0.000
	GC	187	114	
	CC	23	16	
IL-6 rs1800797 (−597 G/A)	**AA**	259	267	0.764
	AG	161	151	
	GG	20	22	
AdipoQ rs2241766 (+45G/T;Q1)	**TT**	73	200	0.000
	TG	318	227	
	GG	49	13	
AdipoQ rs17846866 (+10211 T/G;Q2)	**TT**	270	221	0.000
	TG	121	176	
	GG	49	43	
IL-1β rs16944 (−511C/T)	**TT**	256	226	0.890
	TC	168	190	
	CC	16	24	
IL-18 rs1946518 (−607 A/C)	**CC**	245	126	0.000
	AC	167	277	
	AA	28	37	
VDR *FokI (T/C) (rs2228570)*	**FF**	86	74	0.567
	Ff	259	270	
	ff	95	96	
VDR *TaqI (C/T) (rs731236)*	**TT**	134	146	0.009
	Tt	237	196	
	tt	69	98	
VDR *BsmI (A/G) (rs1544410)*	**BB**	317	320	0.543
	Bb	89	94	
	bb	34	26	

Table 4
Allelic frequencies of studied genetic variants.

Gene	Allele				
		Controls (n = 880)	Patients (n = 880)	p-value	Odd's Ratio
IL-10 rs1800872 (−592A/C)	C	634	645	0.556	0.939 (0.761–1.158)
	A	246	235		
TNF-α rs1800629 (−308 G/A)	G	663	708	**0.010**	0.742(0.592–0.931)
	A	217	172		
IL-6 rs1800795 (−174 G/C)	G	647	734	**<0.000**	0.552(0.438–0.697)
	C	233	146		
IL-6 rs1800797 (−597 G/A)	A	679	685	0.732	0.962(0.769–1.203)
	G	201	195		
AdipoQ rs2241766 (+45G/T;Q1)	T	464	627	**<0.000**	0.450(0.370–0.548)
	G	416	253		
AdipoQ rs17846866 (+10211 T/G;Q2)	T	661	618	**0.022**	1.280(1.037–1.579)
	G	219	262		
IL-1β rs16944 (−511C/T)	T	680	642	0.076	1.218(0.979–1.515)
	C	200	238		
IL-18 rs1946518 (−607 A/C)	C	657	592	**<0.000**	1.955(1.595–2.395)
	A	223	351		
VDR *FokI (T/C) (rs2228570)*	F	431	418	0.535	1.061(0.880–1.279)
	f	449	462		
VDR *TaqI (C/T) (rs731236)*	T	505	488	0.414	1.082(0.896–1.306)
	t	375	392		
VDR *BsmI (A/G) (rs1544410)*	B	723	734	0.487	0.916(0.715–1.173)
	b	157	146		

variant in T2DM of several groups. In Arragonians, TNFα-308 G/A gene variant was found to be associated with T2DM (Vendrell et al., 2003). However, Greece and UK/Irish origin reports showed contended results with other reports from China, Japan and Mexico (, Boraska et al., 2010). Significant associations was reported from Croatian Caucasian, Finnish, Indian, Mexican and Taiwanese individuals on TNFα-308 G/A gene variants with T2DM. No association was observed in genotypic and allelic frequencies. The −592*A allele of IL-10-592 A/C gene variant was reported and found to be increased in T2DM individuals. The low frequency of T2DM in Taiwanese population may be attributed to the involvement of 'C' allele of IL-10-592 which is very rare in Caucasians. Many research groups have reviewed the Adiponectin gene variants in various ethnic groups.

Table 5
Carriage rate allele frequencies of studied genetic variants.

Gene	Carriage Rate								
		Controls (n = 440)	Patients (n = 440)	p-value	Odd's Ratio		Controls (n = 440)	Patients (n = 440)	p-value	Odd's Ratio
IL-10 rs1800872 (−592A/C)	C+	391	425	**<0.000**	0.282(0.155–0.510)	A+	197	220	0.121	0.811(0.622–1.057)
	C−	49	15			A−	243	220		
TNF-α rs1800629 (−308 G/A)	G+	415	420	0.445	0.790(0.423–1.445)	A+	192	152	**0.006**	1.467(1.117–1.926)
	G−	25	20			A−	248	288		
IL-6 rs1800795 (−174 G/C)	G+	417	424	0.254	0.684(0.356–1.313)	C+	210	130	**<0.000**	2.177(1.650–2.873)
	G−	23	16			C−	230	310		
IL-6 rs1800797 (−597 G/A)	A+	420	418	0.524	0.804(0.411–1.573)	G+	181	173	0.582	1.079(0.824–1.412)
	A−	20	22			G−	259	267		
AdipoQ rs2241766 (+45G/T ;Q1)	T+	391	427	**<0.000**	0.243(0.130–0.455)	G+	367	240	**<0.000**	4.189(3.062–5.732)
	T−	49	13			G−	73	200		
AdipoQ rs17846866 (+10211 T/G;Q2)	T+	391	397	0.509	0.864(0.561–1.332)	G+	170	219	**0.001**	0.635(0.486–0.831)
	T−	49	43			G−	270	221		
IL-1β rs16944 (−511C/T)	T+	424	416	0.198	1.529(0.801–2.919)	C+	184	214	**0.042**	0.759(0.582–0.991)
	T−	16	24			C−	256	226		
IL-18 rs1946518 (−607 A/C)	C+	412	403	0.247	1.351(0.811–2.249)	A+	195	314	**<0.000**	0.319(0.242–0.422)
	C−	28	37			A−	245	126		
VDR *FokI (T/C) (rs2228570)*	F+	345	344	0.935	1.013(0.736–1.396)	f+	354	366	0.295	0.832(0.590–1.173)
	F−	95	96			f−	86	74		
VDR *TaqI (C/T) (rs731236)*	T+	371	342	**0.013**	1.541(1.095–2.167)	t+	306	294	0.385	1.134(0.854–1.506)
	T−	69	98			t−	134	146		
VDR *BsmI (A/G) (rs1544410)*	B+	406	414	0.286	0.750(0.442–1.272)	b+	123	120	**<0.000**	0.472(0.343–0.649)
	B−	34	26			b-	317	320		

Table 6
Summary of Significant Gene gene interactions. Global chi^2 is 257.401520 while df = 11 (frequency < 0.03 in both control & case has been dropped. Fisher's p value is 0.00e + 000; Pearson's p value is 0.00e + 000.

Loci chosen for hap-analysis: rs1800872 rs1800629 rs1800795 rs1800797 rs2241766 rs17846866 rs16944 rs1946518 rs2228570 rs731236 rs1544410	Case (freq)	Control (freq)	Chi2	Fisher's p	Pearson's p	Odds Ratio [95%CI]
A G G A T T T C F T B*	13.67(0.016)	29.39(0.033)	9.875	0.001686	0.001683	0.361 [0.187 ~ 0.698]
C A G A T T T C F T B*	22.76(0.026)	42.53(0.048)	11.574	0.000674	0.000673	0.407 [0.239 ~ 0.692]
C G C A T T T C F T B*	0.00(0.000)	51.30(0.058)	67.026	0.00e + 000	2.82e-016	–
C G G A G G T A F T B*	32.00(0.036)	18.78(0.021)	1.460	0.227055	0.226977	1.436 [0.796 ~ 2.587]
C G G A G T T A F T B*	33.84(0.038)	17.86(0.020)	2.519	0.112552	0.112483	1.610 [0.890 ~ 2.912]
C G G A T G T C F T B*	29.41(0.033)	16.89(0.019)	1.494	0.221614	0.221535	1.466 [0.791 ~ 2.717]
C G G A T T T C F T B*	99.48(0.113)	156.80(0.178)	40.820	1.82e-010	1.72e-010	0.366 [0.268 ~ 0.501]
A G G A T T T A F T B*	32.59(0.037)	0.00(0.000)	28.041	1.24e-007	1.21e-007	–
C G C A T T T A F T B*	37.32(0.042)	0.00(0.000)	32.326	1.38e-008	1.34e-008	–
C G G A T G T A F T B*	32.82(0.037)	0.00(0.000)	28.250	1.11e-007	1.09e-007	–
C G G A T T C C F T B*	38.12(0.043)	0.00(0.000)	33.060	9.47e-009	9.17e-009	–
C G G A T T T A F T B*	33.51(0.038)	0.00(0.000)	28.870	8.09e-008	7.92e-008	–

The study on urban Asian Sikhs, 5 tag SNPs of Adiponectin gene revealed that it has no relation in T2DM. However, in Whitehall II study, adiponectin was found independently a predictor of diabetes and GWAS showed significant association with the gene loci of Adiponectin. In +45 T/G gene variant, subjects with 'GG' and 'TG' genotypes were at greater risk of having T2DM in obese Iranians while, both these genotypes were related with gestational T2DM in the women of Malaysia. Our previous report reported for the first time that +10211 T/G polymorphism in intron 1 of Adiponectin gene is related with T2DM individuals in support with previous report in an Asian Indians. Result also in support with Pima Indians and French Caucasians. Linkage disequilibrium is the occurrence of some allele and genetic combinations in a population more or less often than would be expected from a random formation of haplotypes from other alleles depends on their frequencies.

Gene-gene interaction analysis of eight gene variants when taken together in our previous genetic report, CGGATTTC* and CGGAGTCA* set of allele combination was reported to increase the risk upto 7.5 and 4.248 times respectively. Similarly, in this present report we have observed that allele set of "AGGATTTCFTB", "CAGATTTCFTB", "CGCATTTCFTB", "CGGAGGTAFTB", "CGGAGTTAFTB", "CGGATGTCFTB", "CGGATTTCFTB", "AGGATTTAFTB", "CGCATTTAFTB", "CGGATGTAFTB", "CGGATTCCFTB" and "CGGATTTAFTB" in IL-10-592A/C (rs1800872), TNF-a-308G/A (rs1800629), IL-6-174G/C (rs1800795), IL-6-597G/A (rs1800797), Adiponectin + 45G/T

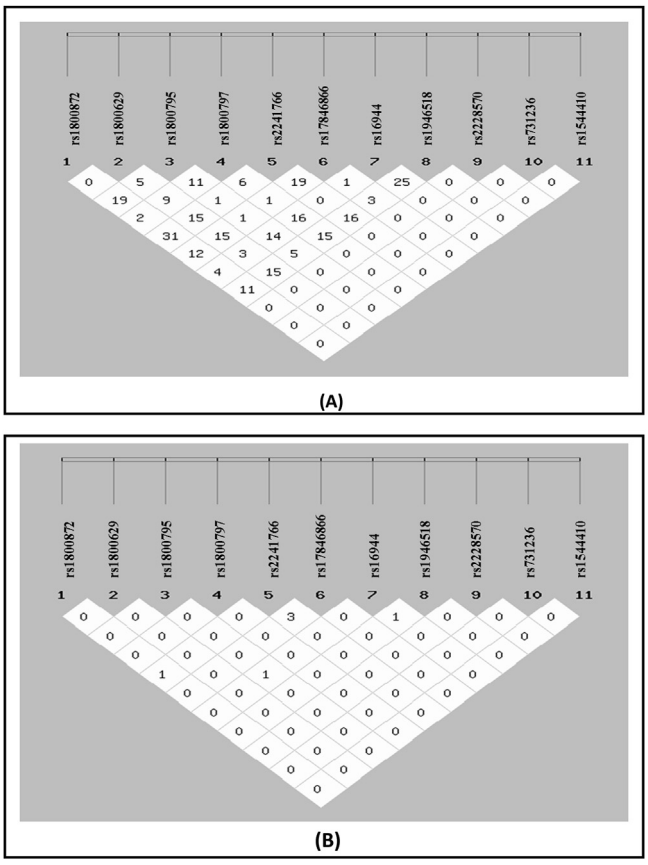

Fig. 1. **(A)** Linkage Disequilibrium (D') and **(B)** Correlation coefficient (r^2) of all eleven studied gene variants.

(rs2241766;Q1), Adiponectin + 10211 T/G (rs17846866;Q2), IL-1b-511C/T (rs16944), IL-18-607 A/C (rs1946518), Vitamin D Receptor (VDR) FokIT/C (rs2228570), VDR TaqIC/T (rs731236) and VDR BsmIA/G (rs1544410) were found to significantly associated and increases the risk of having T2DM.

Moreover, allele set of "CGGAGGTAFTB", "CGGAGTTAFTB" and "CGGATGTCFTB" increase the chance of diabetes upto 1.6 times in our population. This study reflects that these individuals are more susceptible of having T2DM. The present study will provide a new insight in the development and the manifestation of T2DM. Gene variant studies have shown a considerable level of variation amongst various ethnic groups around the globe. Therefore, it is mandatory to perform such genetic studies so that individuals can be benefitted. Individuals who are at high risk will be able to take prior precautionary measures so, they may avoid or delay the onset of disease. These genetic studies showing gene interaction for the susceptibility of the disease may be used as prognostic markers and alter treatment strategies for T2DM.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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