CHAPTER 2
TO STUDY THE ROLE OF RESISTIN IN TYPE 2 DIABETES

To study the role of Resistin in Type 2 Diabetes.

2.1 Introduction

T2D is marked by peripheral insulin resistance in the liver, muscle and adipose tissue (AT) [Samuel and Shulman, 2012]. ER stress, mitochondrial dysfunction and obesity are some of the factors associated with T2D. Adipose tissue is an energy depository that secretes bioactive molecules called adipokines. They modulate appetite, satiety, insulin sensitivity, lipid metabolism and glucose metabolism. They also stimulate other biological processes like cell adhesion, angiogenesis, hypertension, adipogenesis and bone morphogenesis [Di Raimo *et al.,* 2015; Pramanik *et al.,* 2018]. The pro-inflammatory and anti-inflammatory adipokines are in a state of equilibrium and are involved in glucose homeostasis in the healthy state. Resistin, a pro-inflammatory adipokine, is secreted by the infiltrating macrophages into the adipose tissue [Pramanik *et al.,* 2018]. Resistin gene is located on chromosome 19p13.2 and its levels are reported to be significantly elevated in both genetic and diet-induced models of obesity [Jamaluddin *et al.,* 2012]. Elevated resistin protein levels in T2D pathogenesis inhibit the insulin signalling pathway. Resistin activates SOCS-3 leading to IRS1/2 degradation and induction of insulin resistance. Moreover, resistin binds to ACAP1 to activate the cAMP/PKA signalling pathway, which further induces NF-kB activation and transcription of downstream inflammatory cytokines [Steppan *et al.,* 2005].

The putative role of resistin in the pathogenesis of human obesity and diabetes led to genetic association studies in different populations [Engert *et al.,* 2002; Ma *et al.,* 2002; Pizzuti *et al.,* 2002; Osawa *et al.,* 2004]. GWAS studies have identified prominent SNPs in the *resistin* promoter region that contribute to the circulating resistin protein levels. However, a few reports suggested contradictory results. Promoter polymorphisms of *resistin* have indicated the increased risk to T2D susceptibility by elevating circulating resistin levels [Engert *et al.,* 2004; Osawa *et al.,* 22004]. High circulating resistin levels have increased the risk of obesity, insulin resistance and T2D [Tuttolomondo *et al.,* 2010; Chen et al., 2009; Gharibeh *et al.,* 2010]. Besides T2D, promoter polymorphisms of *resistin* are linked with NAFLD [Zhang *et al.,* 2015], chronic kidney disease [Axelsson *et al.,* 2006], CAD [Tang *et al.,* 2008], PCOS [Urbanek *et al.,* 2003] and hypertrophic cardiomyopathy [Hussain *et al.,* 2010].

Hence, in the current study, *resistin* genetic variants in polymorphic sites (rs34861192 G/A, rs1862513 C/G and rs3219175 G/A) were studied and correlated with alterations in its protein levels. Alongside, a genotype-phenotype correlation with various metabolic parameters was also performed to understand their association.

2.2 Materials and Methods

2.2.1 Study subjects

The study was abided by the principles of the Helsinki Declaration and approved by Institutional Ethical Committee for Human Research (IECHR), Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India (FS/IECHR/2016-9). The importance of the study was explained to all the participants and a written consent was taken from each individual. We recruited age, sex and ethnically matched 502 controls (252 males and 250 females) and 469 T2D patients (253 males and 216 females) for the study. T2D patients with FBG>125mg/dL and not suffering from any other diseases were recruited for the study. The diabetes awareness camps were conducted for Gujarati community. Age-matched controls were randomly selected from the Gujarati community by community screening program over the same period. Controls showed FBG <110 mg/dL with no prior history of T2D.

2.2.2 Anthropometric parameters, Lipid profiling and DNA extraction

BMI was calculated by quantifying height and weight of all the study participants. Participants were subjected to overnight (12 h) fasting and FBG was measured by TRUEresult glucometer (Nipro Diagnostics, India). The venous blood samples (3ml) for biochemical analysis were obtained in K_3EDTA coated tubes (J. K. Diagnostics, Rajkot, India). Plasma was separated and lipid profile [TC (Total Cholesterol), TG (Triglycerides) and HDL (High-Density Lipoprotein)] was assayed by commercially available kits (Reckon Diagnostics P. Ltd, Vadodara, India). LDL (Low-Density Lipoprotein) was calculated using Friedewald's (1972) formula. Genomic DNA was extracted from the whole blood using a QIAamp DNA Blood Mini Kit (Qiagen, Germany). DNA purity was measured by calculating the ratio of absorbance at 260/280 nm by Cary 60 UV-Vis spectrophotometer (Agilent, California, USA). The integrity of DNA was checked by 0.8 % agarose gel electrophoresis. The DNA was stored at -20°C until further analysis.

2.2.3 Genotyping of *resistin* gene polymorphisms

Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) was used for genotyping of *resistin* gene three polymorphisms. The primer details of *resistin* gene SNPs are shown in Table 2.1. The reaction mixture (20µl) consisted of 3µl (50ng) of genomic DNA, 11µl of NFW, 2.0µl of 10X PCR buffer, 2.0µl of 2.5mM dNTPs (Sigma Chemical Co, St.Louis, Missouri, USA), 1.0µl each of 10µM forward and reverse primers (MWG Biotech, India) and 0.2µl of 5U/µlTaq Polymerase (Bangalore Genei, India). Amplification was executed using Applied Biosystems 96 well Thermal cycler (California, USA) as per the procedure of initial denaturation at 95°C for 5 minutes followed by 39 cycles each at 95°C for 30 seconds, 59-67°C (product specific; given in Table 2.1) for 30 seconds and 72°C for 30seconds, followed by final extension at 72°C for 10 minutes. The information of the restriction enzymes (REs) (Thermo Fisher Scientific, Wilmington, DE, USA) and digested products are mentioned in Table 2.1. The amplified products (15µl) were digested with 1U of the corresponding RE in a total reaction volume of 20µl as per the manufacturer's instruction. The amplified PCR products (5µl) and digested products (20µl) were assessed by electrophoresis on a 3.5% agarose gel stained with ethidium bromide (EtBr) along with a 50bp/100bp DNA ladder (MBI Fermentas, St.Leon-Rot, Germany). All the gels were visualized under UV transilluminator using Gel Doc EZ System (Bio Rad Laboratories, California, USA).

Table 2.1 Primers and restriction enzymes (REs) used for genotyping of *resistin* gene polymorphisms.

SNP/ Primer	Sequence (5' to 3')	Annealing Temp	Amplicon Size (bp)	RE	Digested products (bp)
rs34861192 *resistin* -638 A/G	**FP:** TGAGCCACCG GCCATAAAC **RP:** CCTCTTGCTA GGCATGGTGC	59°C	267	*TaqI*	178+89
rs1862513 *resistin* -420 C/G	**FP:** GGGCTCAGCT AACCAAATCC **RP:** GCCTCCTTA GTAGCTGGGACT	64°C	391	*BbsI*	199+192
rs3219175 *resistin* -358 A/G	**FP:** CTTGTCCGCA CCATGCCTAG **RP:** CCTCTTGGGG TGGGGCTTC	67°C	304	*AluI*	221+84

FP: Forward Primer; RP: Reverse Primer; bp: base pair

2.2.4 Determination of plasma resistin protein levels

The plasma levels of resistin were estimated by the enzyme-linked immunosorbent assay (ELISA) kit for human resistin (RayBio, Norcross, GA, USA) with the sensitivity of 2ng/ml. All the plasma estimations were carried out in duplicate to ensure % Coefficient of Variation (CV) below 10%. The readings were measured to estimate the protein levels against a standard curve using MultiSkan reader (Thermo Fischer, USA).

2.2.6 Statistical analyses

The clinical characteristics (metabolic and lipid profile) and resistin protein levels were compared using the t-test or Mann Whitney test between controls and T2D patients followed by one-way ANOVA for multi-group comparison. Evaluation of the Hardy-Weinberg equilibrium (HWE) was performed for all the *resistin* polymorphisms in patients and controls by comparing the observed and expected frequencies of the genotypes using chi-square analysis. The distribution of the genotypes and alleles frequencies of the studied polymorphisms for patients and control subjects were compared using the chi-square test with 2x2 contingency table. *p*-values less than 0.025 for genotype and allele distribution were considered as statistically significant as per Bonferroni's corrections. Odds ratio (OR) with respective confidence interval (95% CI) for disease susceptibility was also calculated. Haplotypes, Linkage Disequilibrium (LD) coefficients (D'= D/Dmax) and r^2 values for the pair of the most common alleles at each site were obtained using http://shesisplus.bio-x.cn/SHEsis.html [Yong *et al.,* 2005]. For the genotype-phenotype association analysis, primarily all the parameters were checked for the normality test and accordingly further analyses were carried out. All the genotype-phenotype correlation analyses were carried out in T2D patients after adjusting for the disease susceptibility. Correlation analysis was performed by Spearman's correlation test. All the analyses were carried out in Prism 6 software (GraphPad, USA).

2.3 Results: Baseline characteristics

Clinical parameters were assessed, which showed a significant difference between controls and patients (Table 2.2). Patients had significantly elevated FBG levels ($p<0.0001$) compared to healthy controls. Moreover, obesity related parameters like BMI ($p<0.0001$), TC ($p=0.0025$) and TG ($p<0.0001$) were significantly increased, while HDL was significantly reduced in T2D patients (males: $p=0.0097$, females: $p<0.0001$) as compared to controls. LDL/HDL ratio, a marker for cardiovascular disease (CVD), was significantly high in T2D female patients as compared ($p=0.0007$) to control females. However, a similar trend

was not observed in T2D male patients. On the other hand, the TC/HDL ratio was significantly high in both T2D male and female patients than controls (males: p=0.0260, females: p<0.0001).

Table 2.2 Clinical characteristics of the controls and T2D patients.

	Controls (n=502)	**Patients** (n=469)	***p* value**
Age (years)	39.64±16.35	55.92±10.53	-
Sex: Male	252 (50.2%)	253 (53.94%)	
Female	250 (49.8%)	216 (46.06%)	-
FBG (mg/dl)	100.8 ±8.993	155.4±62.14	**<0.0001**
BMI (Kg/m^2)	24.29±5.094	27.24±5.810	**<0.0001**
TC (mg/dl)	160.9 ±42.22	166.7 ± 37.88	**0.0025**
TG (mg/dl)	124.4 ±63.06	187.7±94.46	**<0.0001**
HDL (mg/dl):Male	36.50±9.519	34.00±8.465	**0.0097**
Female	42.94±9.036	38.46±9.587	**<0.0001**
LDL (mg/dl)	95.32±41.79	95.10±37.52	0.9322
LDL/HDL: Male	3.089±1.307	3.223±1.376	0.2074
Female	2.515±1.014	2.939±1.398	**0.0007**
TC/HDL: Male	4.733±1.0823	5.045±1.878	**0.0260**
Female	3.881±1.354	4.631±1.804	**<0.0001**
Onset age (years)	NA	50.65 ± 10.10	-
Duration of disease (years)	NA	8.06 ± 7.3	-
Family history	NA	64 (14%)	-

Data are presented as Mean±SD; Statistical significance was considered at p<0.05.

2.3.1 Genetic analysis of the *resistin* gene polymorphisms

The three *resistin* SNPs were genotyped using PCR-RFLP and the representative gel images are shown in Figure 2.1. The genotype and allele frequencies of the explored *resistin* polymorphisms are summarized in Table 2.3. The distribution of genotype frequencies for all the polymorphisms investigated was consistent with Hardy-Weinberg equilibrium in both patient and control groups (p>0.05). *Resistin* rs1862513 C/G was significantly associated with T2D individuals (genotype and allele frequencies, p<0.0001). The CG genotype of *resistin* rs1862513 was associated with increased risk for T2D with an Odds Ratio (OR) of 0.3975, while the mutant homozygous TT genotype increased the risk by 0.26-fold. There was no difference in genotype and allele frequencies of *resistin* (rs3219175 A/T) among diabetic patients and controls. None of the polymorphisms of rs3219175 A/T were found to be associated with T2D (p>0.05) and hence discontinued after an initial assessment of 250 samples. *Resistin* rs34861192 G/A was found to be monomorphic in controls as well as T2D patients and was therefore discontinued for further analysis.

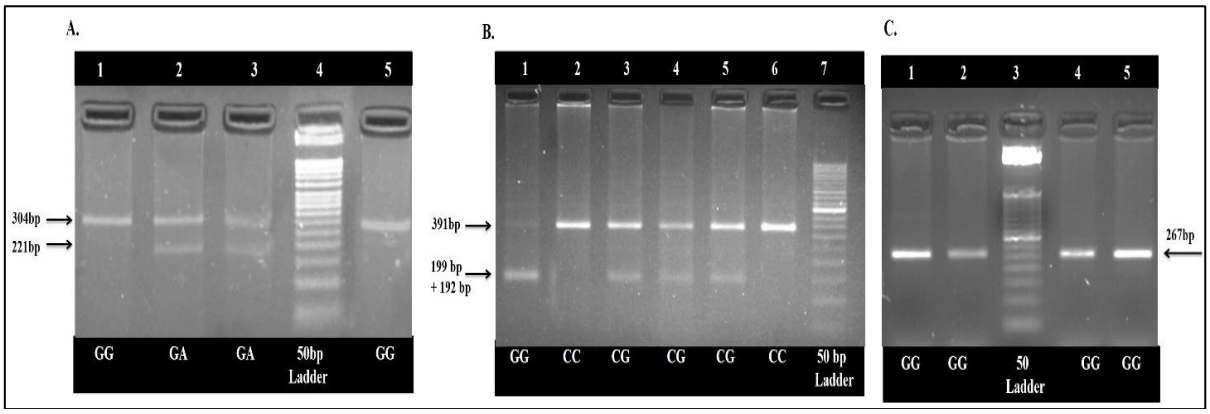

Figure 2.1 Representative gel images of *resistin* promoter SNPs. A) PCR-RFLP analysis of *resistin* rs3219175 A/G polymorphism on 3.5% agarose gel: lanes 1 and 5 show homozygous (GG) genotypes; lanes 2 and 3 show heterozygous (GA) genotypes; AA genotype was not observed. Lane 4 shows a 50bp DNA ladder. B) PCR-RFLP analysis of *resistin* rs1862513 C/G polymorphism on 3.5% agarose gel: lane 1 shows homozygous (GG) genotype; lanes 2 and 6 show homozygous (CC) genotypes; lanes 3, 4 and 5 show heterozygous (GC) genotypes. Lane 7 shows a 50 bp DNA ladder. C) PCR-RFLP analysis of *resistin* rs34861192 G/A polymorphism on 3.5% agarose gel: lanes 1, 2, 4 and 5 show homozygous (GG) genotypes. Lane 3 shows a 50 bp DNA ladder.

Table 2.3 Genotype and allele frequencies distribution of *resistin* polymorphisms in controls and T2D patients.

SNP	Genotype /allele	Controls	Patients	*p* for association	OR	95% CI
***Resistin* (rs34861192) G/A**		100	100	NA	NA	NA
	GG	100 (100.00)	100 (100.00)			
	GA	0 (0.00)	0 (0.00)			
	AA	0 (0.00)	0 (0.00)			
	G	200 (1.00)	200 (1.00)	NA	NA	NA
	A	0 (0.00)	0 (0.00)			
***Resistin* (rs1862513) C/G**		n=38 2	n=469			
	CC	63 (16.89)	172 (34.13)	R	-	
	CG	188 (48.82)	204 (48.58)	**0.0001[a]**	0.3975	0.2800 to 0.5641
	GG	131 (34.69)	93 (17.29)	**0.0001[a]**	0.2600	0.1756 - 0.3850
	C	314 (0.41)	573 (0.58)			
	G	450	425	**0.0001[b]**	0.5175	0.4275

		(0.59)	(0.42)			- 0.6266
***Resistin* (rs3219175) G/A**		n=250	n=250			
	GG	220 (93.72)	209 (91.08)	R	-	-
	GA	15 (6.18)	21 (8.71)	0.3448[a]	1.377	0.7073 - 2.681
	AA	0 (0.10)	0 (0.21)	-	-	-
	G	455 (0.97)	439 (0.95)			
	A	15 (0.03)	21 (0.05)	0.3456[b]	1.343	0.7067 - 2.599

n: number of patients/controls, R: reference group, CI: Confidence Interval, Odds Ratio: the allele frequency distribution, P: patients, C: controls, [a]Patients vs Controls (genotype) by the chi-square test with 2×2 contingency table. [b]Patients vs Controls (allele) by the chi-square test with 2×2 contingency table. Statistical significance was considered at $p<0.025$ as per Bonferroni's correction.

2.3.2 Haplotype analysis of the *resistin* polymorphisms

The estimated frequencies of the haplotypes for *resistin* SNPs (global $p=0.049$) did not vary significantly between T2D patients and controls (Table 2.4).

Table 2.4 Distribution of haplotype frequencies of *resistin* in controls and T2D patients.

Haplotype (rs1862513 C/G, rs3219175 G/A)	Patients (Freq. %; n=382)	Controls (Freq. %;n=502)	*p* for Association	$p_{(global)}$	Odds Ratio [95%CI]
CA	12.64 (0.022)	6.73 (0.021)	-	0.049	-
CG	267.36 (0.485)	132.27 (0.408)	0.030		1.359 [1.029-1.796]
GA	6.36 (0.011)	6.27 (0.019)	-		-
GG	282.64 (0.482)	178.73(0.552)	0.030		0.736 [0.557-0.972]

CI represents the Confidence Interval. (Frequency<0.03 in both control & patients has been dropped and was ignored in the analysis).

2.3.3 Linkage Disequilibrium analysis of the *resistin* polymorphisms

The LD analysis shown that *resistin* SNPs (rs1862513 C/G; rs3219175 G/A) were in moderate linkage (D'=0.64, r^2= 0.01) (Figure 2.2).

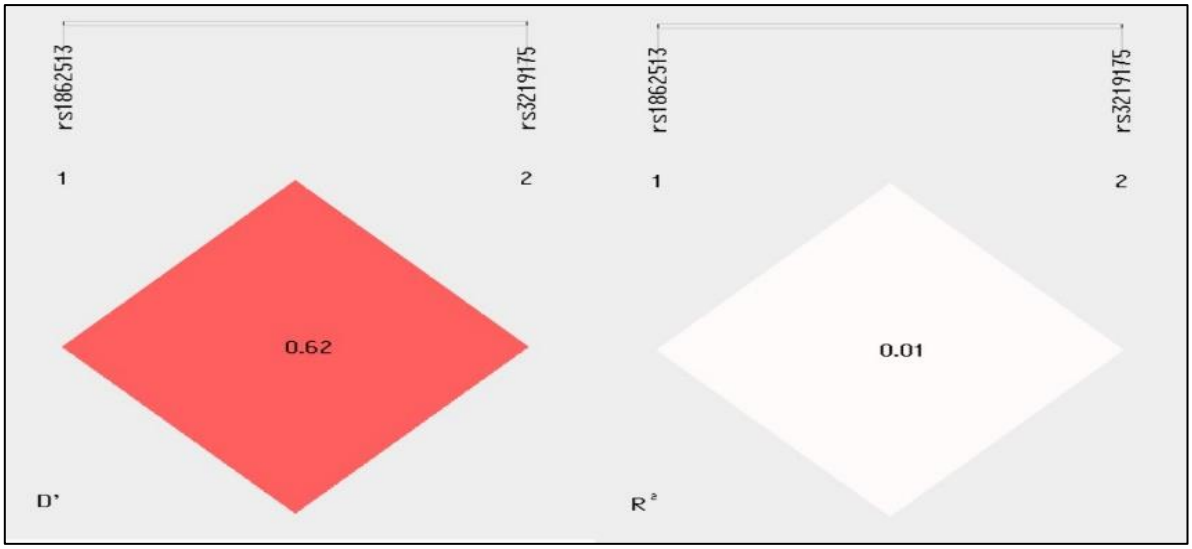

Figure 2.2 Linkage Disequilibrium (LD) block: LD block with respect to *resistin* rs1862513 and rs3219175 polymorphisms in Gujarat population.

2.3.4 The association of the *resistin* polymorphisms with the metabolic profile

Resistin rs1862513 CC genotype was associated with increased FBG (p=0.0035), BMI (p=0.0004) and TC levels (p=0.0190) but not associated with TG, LDL and HDL levels (p>0.05). While *resistin* rs3219175 G/A did not show association with the metabolic profile (p>0.05) (Table 2.5).

Table 2.5 Genotype-phenotype association analyses of *resistin* polymorphisms with metabolic profile.

Genotype	FBG mg/dl	BMI kg/m^2	TG mg/dl	TC mg/dl	LDL mg/dl	HDL mg/dl Male	HDL mg/dl Female
***Resistin* rs1862513 C/G**							
CC	140.8 (58.1)	27.2 (6.3)	169.8 (35.6)	174.9 (97.3)	105.7 (28.7)	34.9 (9.1)	39.7 (10.4)
CG	97.7 (8.6)	25.0 (5.2)	168.6 (38.7)	164.2 (22.1)	108.2 (32.9)	36.2 (9.8)	40.1 (10.0)
GG	100.1 (8.1)	25.3 (4.9)	171.4 (44.5)	152.2 (77.4)	111.1 (34.0)	34.1 (9.7)	38.1 (10.0)
p value	**0.003**	**0.0004**	0.9430	**0.0190**	0.4219	0.088	0.216
***Resistin* rs3219175 G/A**							
GG	119.9 (46.2)	25.4 (5.2)	155.5 (89.1)	156.6 (35.6)	105.6 (30.9)	36.6 (9.2)	36.5 (11.3)
GA	131.2 (65.7)	25.9 (6.1)	151.2 (87.9)	155.1 (37.8)	106.9 (34.9)	34.1 (8.2)	39.9 (11.1)
AA	-	-	-	-	-	-	-
p value	0.967	0.4321	0.5678	0.6567	0.8977	0.361	0.6888

Data are presented as Mean ±SE. Statistical significance was considered at p<0.05.

2.3.5 Plasma resistin protein levels and their association with *resistin* polymorphisms and metabolic profile

Plasma resistin protein levels were monitored in 40 controls, and 40 patients and a significant increase (p=0.0129) was observed in T2D patients (Figure 2.3A). Our results showed no association of *resistin* polymorphisms with plasma resistin protein levels. (p>0.05) (Figure 2.3B). Spearman's correlation analysis revealed no correlation between resistin protein levels and the metabolic profile parameters (p>0.05) (Table 2.6).

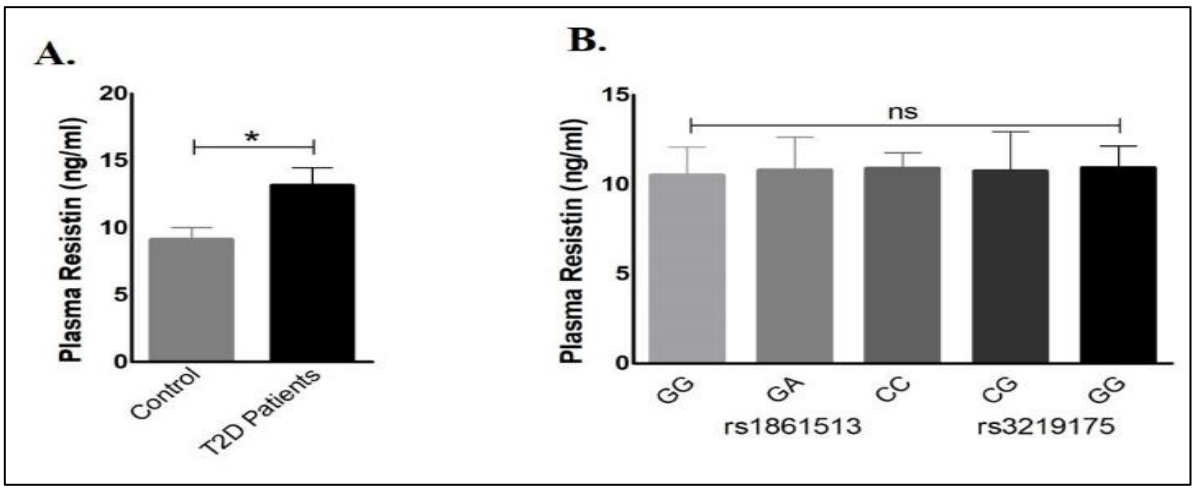

Figure 2.3 Plasma resistin protein levels in A) controls vs patients. A significant increase in plasma resistin protein levels in T2D patients (p=0.0129) as compared to controls was observed (controls n=40; T2D patients n=40). **B) Association of *resistin* polymorphisms with plasma resistin protein levels.** *Resistin* polymorphisms showed no association with resistin protein levels (p>0.05)

Table 2.6 Correlation analysis of plasma resistin protein levels with the metabolic profile.

Parameters	r^2	*p value*
BMI (Kg/m^2)	0.0107	0.9314
FBG (mg/dl)	0.0200	0.8986
Triglycerides (mg/dl)	-0.1306	0.3656
Total Cholesterol (mg/dl)	0.0000	0.9998
HDL (mg/dl): Male Female	0.2527 0.0914	0.2335 0.6372
LDL (mg/dl)	0.0624	0.6908

p>0.05, non-significant; n=40

2.4 Discussion

Investigating genetic variants for T2D in the Indian population is essential due to its high-risk for T2D and related metabolic traits. However, research carried out was restricted, often limited to a replication of GWAS. Though previous GWAS did not identify *resistin* rs1862513 as a T2D susceptible locus, it has been shown that the gene contributes towards T2D risk in multiple population studies. This risk may be attributed to factors such as epigenetic effects and ethnic differences.

The genetic variants of *resistin* and resistin levels in T2D patients suggest *resistin* rs1862513 CC genotype and higher plasma resistin levels to be associated with T2D. *Resistin* rs34861192 GG genotype was the only genotype present in all the samples in the studied cohort. Neither we found any association of *resistin* 3219175 G/A with T2D nor with any other parameters. A similar trend was reported in the Malaysian population [Apalasamy *et al.,* 2015]. An earlier study in Japanese subjects reported increased resistin levels to be associated with *resistin* rs1862513 C/G polymorphism [Osawa *et al.,* 2004; Osawa *et al.,* 2005; Osawa *et al.,* 2007]. These were further supported by studies in Korean individuals [Cho *et al.,* 2004] and Iranian women [Takhshid *et al.,* 2015] but not in Caucasians [Hivert *et al.,* 2009; Menzaghi *et al.,* 2006].

Association of *resistin* genetic variants and correlation of resistin protein levels with BMI, FBG, and plasma lipid profile were also assessed. We have found CC of *resistin* rs1862513 C/G SNP to be significantly associated with metabolic risk factors noticeable by elevated FBG, BMI and TC levels in T2D patients. The CC genotype is associated with other factors, as observed in the various populations (Table 2.7).

Table 2.7 Association of *resistin* rs1862513 CC genotype with various disorders.

Genotype	Associated with	Population	References
CC	Insulin resistance, hyperglycemia and hypertriglyceridemia	Finnish	Ukkola *et al.,* 2008
CC	Increased FBG and HbA1c levels, T2D	Iranian	Solaleh *et al.,* 2009
CC	CAD, T2D	Iranian	Emamgholipour *et al.,* 2010

The elevated resistin protein levels due to *resistin* rs1862513 C/G polymorphism is through specific binding of transcription factors Sp1 and Sp3 to the promoter element. This binding leads to enhanced promoter activity, as explained by Osawa *et al.* [Osawa *et al.,* 2005]. Apparently, rs1862513 C/G seems to be a vital decisive polymorphic site for *resistin* gene transcription. Furthermore, DNA methylation (DNAm), an epigenetic modification, plays a crucial role in regulating gene expression. *Resistin* rs1862513 C/G is CpG SNP that modulates the CpG sequence, thereby regulating resistin protein levels [Osawa *et al.,* 2005]. Thus DNAm, along with the binding of the transcription factors, explain increased resistin protein levels. Accordingly, increased resistin protein levels were observed in obesity induced T2D individuals [Steppan *et al.,* 2002; Gokhale *et al.,* 2014].

Many studies have indicated an association between resistin protein levels and lipid profile [Owecki *et al.*, 2009; Jové *et al.*, 2003; Asano *et al.*, 2010; Chen *et al.*, 2005]. Evidently. elevated resistin protein levels contribute to the progression/development of T2D. We have observed increased plasma FFA and TNF-α levels in the same T2D patients. Interestingly, resistin is shown to elevate TNF-α levels [Silswal *et al.*, 2005; Aquilante *et al.*, 2008]. Previous studies suggested FFA mediated elevated TG and Very Low-Density Lipoprotein (VLDL) production related to diabetic dyslipidemia It is shown that TNF-α creates a surge of hepatocellular TG levels [Julius *et al.*, 2003; Bays *et al.*, 2013]

Further, it is also shown that the influence of FFA on hepatic TG production leads to an elevated hepatic release of VLDL, which encompasses apolipoprotein B (apo B) [Julius *et al.*, 2003; Bays *et al.*, 2013]. ApoB is a crucial protein factor of VLDL, and any irregularities related to the metabolism of ApoB contribute towards the development of dyslipidemia and the associated increased risk of CVD [Packard *et al.*, 2000]. Direct involvement of resistin in the synthesis of apoB has been shown to influence hepatic lipid and lipoprotein levels [Costandi *et al.*, 2011]. Besides, resistin is also known to reduce the expression and secretion of other adipokines such as adiponectin and leptin, affecting hepatic lipoprotein regulation [Kim *et al.*, 2004]. Our lab studies on TNF-α and adiponectin conform to these results [Patel *et al.*, 2019; Palit *et al.*, 2020]. Thus, the present study shows an association between *resistin* rs1862513 C/G SNP, plasma resistin protein and lipid levels in the Indian population and contributes to understand resistin's role in dyslipidemia, obesity and T2D.

Hence, the current data suggest that lifestyle, diet and ethnic differences play a significant role in the pathogenesis of T2D. An overall mechanism involving *resistin* genetic polymorphisms and its protein levels in obesity and dyslipidemia associated with T2D is shown schematically (Figure 3.4).

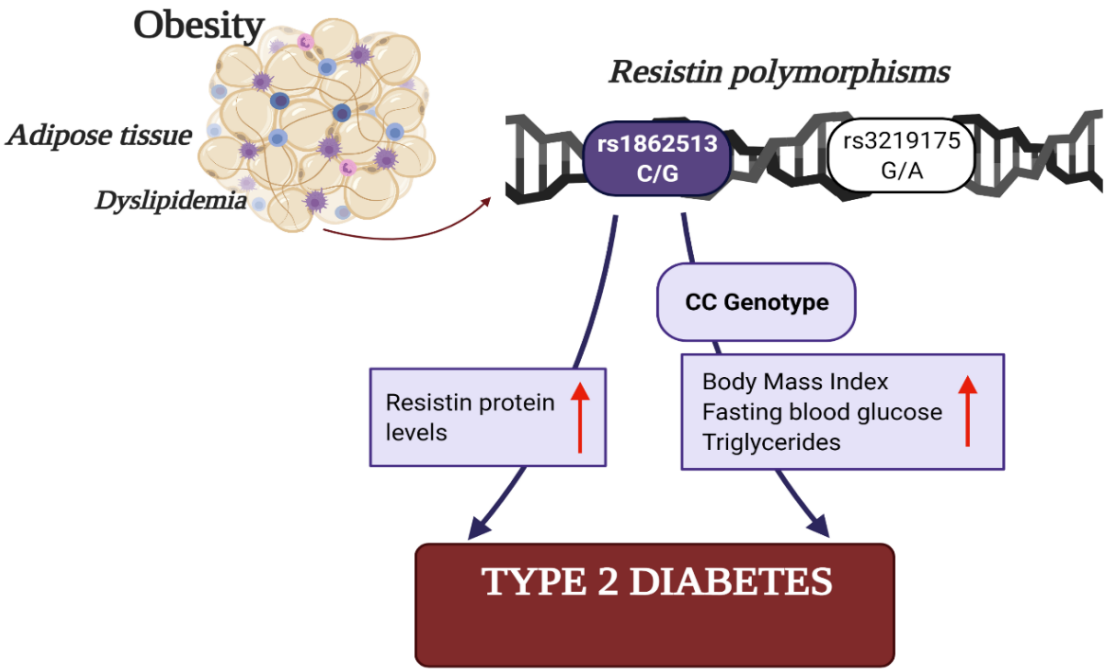

Figure 2.4 Role of resistin in T2D: *Resistin* rs1862513 C/G polymorphism is associated with the risk of T2D while CC genotype is associated with increased BMI, FBG and TC levels. Besides, in T2D individuals, resistin protein levels are increased. Altogether, rs1862513 CC contributes to the increased risk of T2D in Gujarat population. However, rs3219175 is not associated with risk of T2D.

References:

1. Aquilante CL, Kosmiski LA, Knutsen SD, Zineh I. Relationship between plasma resistin concentrations, inflammatory chemokines, and components of the metabolic syndrome in adults. Metabolism. 2008;57(4):494-501.
2. Apalasamy YD, Rampal S, Salim A, Moy FM, Su TT, Majid HA, Bulgiba A, Mohamed Z. Polymorphisms of the resistin gene and their association with obesity and resistin levels in Malaysian Malays. Biochemical genetics. 2015;53(4-6):120-31.
3. Asano H, Izawa H, Nagata K, Nakatochi M, Kobayashi M, Hirashiki A, Shintani S, Nishizawa T, Tanimura D, Naruse K, Matsubara T. Plasma resistin concentration determined by common variants in the resistin gene and associated with metabolic traits in an aged Japanese population. Diabetologia. 2010;53(2):234-46.

4. Axelsson J, Bergsten A, Qureshi AR, Heimbürger O, Barany P, Lönnqvist F, Lindholm B, Nordfors L, Alvestrand A, Stenvinkel P. Elevated resistin levels in chronic kidney disease are associated with decreased glomerular filtration rate and inflammation, but not with insulin resistance. Kidney international. 2006;69(3):596-604.
5. Bays HE, Toth PP, Kris-Etherton PM, Abate N, Aronne LJ, Brown WV, Gonzalez-Campoy JM, Jones SR, Kumar R, La Forge R, Samuel VT. Obesity, adiposity, and dyslipidemia: a consensus statement from the National Lipid Association. Journal of clinical lipidology. 2013;7(4):304-83.
6. Chen BH, Song Y, Ding EL, Roberts CK, Manson JE, Rifai N, Buring JE, Gaziano JM, Liu S. Circulating levels of resistin and risk of type 2 diabetes in men and women: results from two prospective cohorts. Diabetes care. 2009;32(2):329-34.
7. Chen CC, Li TC, Li CI, Liu CS, Wang HJ, Lin CC. Serum resistin level among healthy subjects: relationship to anthropometric and metabolic parameters. Metabolism. 2005;54(4):471-5.
8. Cho YM, Youn BS, Chung SS, Kim KW, Lee HK, Yu KY, Park HJ, Shin HD, Park KS. Common genetic polymorphisms in the promoter of resistin gene are major determinants of plasma resistin concentrations in humans. Diabetologia. 2004;47(3):559-65.
9. Costandi J, Melone M, Zhao A, Rashid S. Human resistin stimulates hepatic overproduction of atherogenic ApoB-containing lipoprotein particles by enhancing ApoB stability and impairing intracellular insulin signaling. Circulation research. 2011;108(6):727-42.
10. Di Raimo T, Azzara G, Corsi M, Cipollone D, Vasco VR, Businaro R. Adipokines and their involvement as a target of new drugs. Journal of Pharmacovigilance. 2015.
11. Emamgholipour S, Hossein-Nezhad A, Kh M, Mohajerani SA, Shirzad M. Genetic variants at the resistin gene promoter might have a role in atherogenesis in patients with coronary artery disease. Journal of Diabetes and Metabolic Disorders. 2010;9:13.
12. Engert JC, Vohl MC, Williams SM, Lepage P, Loredo-Osti JC, Faith J, Doré C, Renaud Y, Burtt NP, Villeneuve A, Hirschhorn JN. 5′ flanking variants of resistin are associated with obesity. Diabetes. 2002;51(5):1629-34.

13. Gharibeh MY, Al Tawallbeh GM, Abboud MM, Radaideh A, Alhader AA, Khabour OF. Correlation of plasma resistin with obesity and insulin resistance in type 2 diabetic patients. Diabetes & metabolism. 2010;36(6):443-9.
14. Gokhale NH, Acharya AB, Patil VS, Trivedi DJ, Setty S, Thakur SL. Resistin levels in gingival crevicular fluid of patients with chronic periodontitis and type 2 diabetes mellitus. Journal of Periodontology. 2014;85(4):610-7.
15. Hivert MF, Manning AK, McAteer JB, Dupuis J, Fox CS, Cupples LA, Meigs JB, Florez JC. Association of variants in RETN with plasma resistin levels and diabetes-related traits in the Framingham Offspring Study. Diabetes. 2009;58(3):750-6.
16. Hussain S, Asghar M, Javed Q. Resistin gene promoter region polymorphism and the risk of hypertrophic cardiomyopathy in patients. Translational Research. 2010;155(3):142-7.
17. Jamaluddin MS, Weakley SM, Yao Q, Chen C. Resistin: functional roles and therapeutic. British journal of pharmacology. 2012;165(3):622-32.
18. Kim KH, Zhao L, Moon Y, Kang C, Sul HS. Dominant inhibitory adipocyte-specific secretory factor (ADSF)/resistin enhances adipogenesis and improves insulin sensitivity. Proceedings of the National Academy of Sciences. 2004 Apr 27;101(17):6780-5. considerations for cardiovascular disease. British journal of pharmacology. 2012;165(3):622-32.
19. Julius U. Influence of plasma free fatty acids on lipoprotein synthesis and diabetic dyslipidemia. Experimental and clinical endocrinology & diabetes. 2003;111(05):246-50.
20. Joshi SR, Saboo B, Vadivale M, Dani SI, Mithal A, Kaul U, Badgandi M, Iyengar SS, Viswanathan V, Sivakadaksham N, Chattopadhyaya PS. Prevalence of diagnosed and undiagnosed diabetes and hypertension in India—results from the Screening India's Twin Epidemic (SITE) study. Diabetes technology & therapeutics. 2012;14(1):8-15.
21. Jové M, Planavila A, Cabrero À, Novell F, Ros E, Zambón D, Laguna JC, Carrera MV. Reductions in plasma cholesterol levels after fenofibrate treatment are negatively correlated with resistin expression in human adipose tissue. Metabolism. 2003;52(3):351-5.
22. Menzaghi C, Coco A, Salvemini L, Thompson R, De Cosmo S, Doria A, Trischitta V. Heritability of serum resistin and its genetic correlation with insulin

resistance-related features in nondiabetic Caucasians. The Journal of Clinical Endocrinology & Metabolism. 2006;91(7):2792-5.

23. Ma X, Warram JH, Trischitta V, Doria A. Genetic variants at the resistin locus and risk of type 2 diabetes in Caucasians. The Journal of Clinical Endocrinology & Metabolism. 2002;87(9):4407-10.
24. Onuma H, Tabara Y, Kawamura R, Ohashi J, Nishida W, Takata Y, Ochi M, Nishimiya T, Ohyagi Y, Kawamoto R, Kohara K. Dual effects of a RETN single nucleotide polymorphism (SNP) at–420 on plasma resistin: genotype and DNA methylation. The Journal of Clinical Endocrinology & Metabolism. 2017;102(3):884-92.
25. Osawa H, Yamada K, Onuma H, Murakami A, Ochi M, Kawata H, Nishimiya T, Niiya T, Shimizu I, Nishida W, Hashiramoto M. The G/G genotype of a resistin single-nucleotide polymorphism at− 420 increases type 2 diabetes mellitus susceptibility by inducing promoter activity through specific binding of Sp1/3. The American Journal of Human Genetics. 2004;75(4):678-86.
26. Osawa H, Onuma H, Ochi M, Murakami A, Yamauchi J, Takasuka T, Tanabe F, Shimizu I, Kato K, Nishida W, Yamada K. Resistin SNP-420 determines its monocyte mRNA and serum levels inducing type 2 diabetes. Biochemical and biophysical research communications. 2005;335(2):596-602.
27. Osawa H, Tabara Y, Kawamoto R, Ohashi J, Ochi M, Onuma H, Nishida W, Yamada K, Nakura J, Kohara K, Miki T. Plasma resistin, associated with single nucleotide polymorphism− 420, is correlated with insulin resistance, lower HDL cholesterol, and high-sensitivity C-reactive protein in the Japanese general population. Diabetes care. 2007;30(6):1501-6.
28. Owecki M, Nikisch E, Miczke A, Pupek-Musialik D, Sowinski J. Serum resistin is related to plasma HDL cholesterol and inversely correlated with LDL cholesterol in diabetic and obese humans. Neuroendocrinology Letters. 2010;31(5):673.
29. Patel R, Palit SP, Rathwa N, Ramachandran AV, Begum R. Genetic variants of tumor necrosis factor-α and its levels: A correlation with dyslipidemia and type 2 diabetes susceptibility. Clinical nutrition. 2019;38(3):1414-22.
30. Palit SP, Patel R, Jadeja SD, Rathwa N, Mahajan A, Ramachandran AV, Dhar MK, Sharma S, Begum R. A genetic analysis identifies a haplotype at

adiponectin locus: association with obesity and type 2 diabetes. Scientific reports. 2020;10(1):1-0.

31. Pramanik S, Rathwa N, Patel R, Ramachandran AV, Begum R. Treatment avenues for type 2 diabetes and current perspectives on adipokines. Current diabetes reviews. 2018;14(3):201-21.
32. Pizzuti A, Argiolas A, Di Paola R, Baratta R, Rauseo A, Bozzali M, Vigneri R, Dallapiccola B, Trischitta V, Frittitta L. An ATG repeat in the 3′-untranslated region of the human resistin gene is associated with a decreased risk of insulin resistance. The Journal of Clinical Endocrinology & Metabolism. 2002;87(9):4403-6.
33. Packard CJ, Demant T, Stewart JP, Bedford D, Caslake MJ, Schwertfeger G, Bedynek A, Shepherd J, Seidel D. Apolipoprotein B metabolism and the distribution of VLDL and LDL subfractions. Journal of lipid research. 2000;41(2):305-17.
34. Samuel VT, Shulman GI. Mechanisms for insulin resistance: common threads and missing links. Cell. 2012;148(5):852-71.
35. Rathwa N, Patel R, Palit SP, Ramachandran AV, Begum R. Genetic variants of resistin and its plasma levels: Association with obesity and dyslipidemia related to type 2 diabetes susceptibility. Genomics. 2019;111(4):980-5.
36. Santilli F, Liani R, Di Fulvio P, Formoso G, Simeone P, Tripaldi R, Ueland T, Aukrust P, Davi G. Increased circulating resistin is associated with insulin resistance, oxidative stress and platelet activation in type 2 diabetes mellitus. Thrombosis and Haemostasis. 2016;116(12):1089-99.
37. Silswal N, Singh AK, Aruna B, Mukhopadhyay S, Ghosh S, Ehtesham NZ. Human resistin stimulates the pro-inflammatory cytokines TNF-α and IL-12 in macrophages by NF-κB-dependent pathway. Biochemical and biophysical research communications. 2005;334(4):1092-101.
38. Solaleh E, Arash HN, Azam N, Mazaher R, Bagher L. Promoter resistin gene polymorphism in patients with type 2 diabetes and its influence on concerned metabolic phenotypes. Journal of Diabetes and Metabolic Disorders. 2009;8:17.
39. Steppan CM, Wang J, Whiteman EL, Birnbaum MJ, Lazar MA. Activation of SOCS-3 by resistin. Molecular and cellular biology. 2005;25(4):1569.
40. Steppan CM, Lazar MA. Resistin and obesity-associated insulin resistance. Trends in endocrinology & Metabolism. 2002;13(1):18-23.

41. Ukkola O, Kunnari A, Kesäniemi YA. Genetic variants at the resistin locus are associated with the plasma resistin concentration and cardiovascular risk factors. Regulatory peptides. 2008;149(1-3):56-9.
42. Urbanek M, Du Y, Silander K, Collins FS, Steppan CM, Strauss JF, Dunaif A, Spielman RS, Legro RS. Variation in resistin gene promoter not associated with polycystic ovary syndrome. Diabetes. 2003;52(1):214-7.
43. Takhshid MA, Zare Z. Resistin–420 C/G polymorphism and serum resistin level in Iranian patients with gestational diabetes mellitus. Journal of Diabetes & Metabolic Disorders. 2015;14(1):1-7.
44. Tang NP, Wang LS, Yang L, Zhou B, Gu HJ, Sun QM, Cong RH, Zhu HJ, Wang B. A polymorphism in the resistin gene promoter and the risk of coronary artery disease in a Chinese population. Clinical endocrinology. 2008;68(1):82-7.
45. Tuttolomondo A, La Placa S, Di Raimondo D, Bellia C, Caruso A, Sasso BL, Guercio G, Diana G, Ciaccio M, Licata G, Pinto A. Adiponectin, resistin and IL-6 plasma levels in subjects with diabetic foot and possible correlations with clinical variables and cardiovascular co-morbidity. Cardiovascular diabetology. 2010;9(1):1-7.
46. Yong YO, He L. SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. Cell research. 2005;15(2):97.
47. Zhang CX, Guo LK, Qin YM, Li GY. Interaction of polymorphisms of resistin gene promoter-420C/G, glutathione peroxidase-1 gene Pro198Leu and cigarette smoking in nonalcoholic fatty liver disease. Chinese medical journal. 2015;128(18):2467.