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Circulatory Omentin-1 levels but not genetic variants influence the pathophysiology of Type 2 diabetes

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ABSTRACT

Objective: Omentin-1, an anti-inflammatory protein, is secreted by the visceral adipose tissue. Altered levels of Omentin-1 are associated with obesity and Type 2 Diabetes (T2D). Although Omentin-1 is implicated in the insulin signaling pathway, the relationship between the genetic variants of *Omentin-1* and T2D is not yet explored. The current study evaluates the association of *Omentin-1* polymorphisms (rs2274907 A/T and rs1333062 G/T), its transcript and protein levels, and genotype-phenotype correlation with metabolic parameters and T2D susceptibility.

Methods: Plasma and Peripheral Blood Mononuclear Cells (PBMCs) were separated from venous blood taken from 250 controls and 250 T2D patients recruited from Gujarat, India. Genomic DNA was isolated from PBMCs and genotyping of *Omentin-1* variants was performed by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). RNA was isolated from Visceral Adipose Tissue (VAT) samples of 12 controls and 10 patients, and transcript levels of *Omentin-1* were assessed by qPCR. Plasma Omentin-1 levels were estimated by ELISA. Fasting Blood Glucose, Body Mass Index (BMI) and plasma lipid profile were considered for the genotype-phenotype correlation analysis.

Results: Our study revealed no association of *Omentin-1* genetic variants with T2D risk ($p > 0.05$). However, the AT genotype of *Omentin-1* rs2274907 A/T polymorphism was associated with increased BMI ($p = 0.0247$). Plasma Omentin-1 levels were significantly decreased ($p < 0.0001$) however, increased VAT *Omentin-1* transcript levels ($p = 0.0127$) were observed in T2D patients.

Conclusion: Our findings suggest that decreased circulatory Omentin-1 levels could pose a risk towards T2D susceptibility.

1. Introduction

Insulin resistance at the level of the liver, muscle, and adipose tissue along with impaired insulin secretion are the hallmarks of Type 2 Diabetes (T2D) [1]. In the past few decades, obesity has been identified as one of the prime factors that lead to T2D. Adipose tissue (AT) serves not only as an energy depository but also as an organ that secretes bioactive molecules called adipokines (pro- and anti-inflammatory). The pro-inflammatory and anti-inflammatory adipokines are in a state of equilibrium and they play an important role in regulating lipid metabolism, insulin sensitivity, glucose metabolism, appetite and satiety [2]. *Omentin-1*, the anti-inflammatory adipokine gene, is located on chromosome 1q22-q23 and is secreted by visceral adipose tissue (VAT) [3]. Circulating Omentin-1 levels were reported to be reduced in obese subjects and have been negatively correlated with markers of obesity, such as Body Mass Index (BMI), waist circumference, and circulating leptin [4]. Omentin-1 has been implicated in insulin signaling pathway by Akt activation and consequently increased insulin sensitivity [5]. Reports suggest that reduced *Omentin-1* gene expression and circulating plasma Omentin-1 concentrations are associated with impaired glucose tolerance in T2D patients [6,7]. Moreover, fasting serum Omentin-1 levels have been negatively correlated with fasting insulin and

Abbreviations: T2D, Type 2 Diabetes; FBG, Fasting Blood Glucose; TC, Total Cholesterol; HDL, High Density Lipoprotein; TG, Triglycerides; LDL, Low Density Lipoprotein; BMI, Body Mass Index; PCR-RFLP, Polymerase Chain Reaction-Restriction Fragment Length Polymorphism

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Homeostatic Model Assessment-Insulin Resistance (HOMA-IR) [7].

There are a few studies on the genetic variants of *Omentin-1* where Val109Asp rs2274907 has been exclusively studied Non-alcoholic Fatty Liver Disease (NAFLD) [8], Coronary Artery Disease (CAD) [9,10], psoriasis [11], high calorie-diet intake [12], breast cancer [13] and rheumatoid arthritis [14]. There is only one report on *Omentin-1* 3′ UTR rs1333062 in Indian population showing an association with diabetes [15]. Hence, we aimed to investigate *Omentin-1* genetic variants (Exon 4 Val109Asp rs2274907 and 3′ UTR rs1333062), *Omentin-1* transcript levels in VAT along with its plasma levels, and genotype-phenotype correlation with various metabolic parameters.

2. Materials and methods

2.1. Study subjects

The study was carried out in agreement with the principles of 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 written consent was taken from each individual. We recruited age, sex and ethnically matched 250 controls (142 males and 108 females) and 250 T2D patients (123 males and 127 females) for the study (Table S1). Samples of visceral (omental) adipose tissue were taken from the individuals undergoing bariatric surgery and fasting clinical parameters of all the study subjects are as described previously [16]. The patients showing Fasting Blood Glucose (FBG) > 125 mg/dL and suffering from no other diseases were recruited from diabetes awareness camps. Ethnically and geographically matched controls were randomly chosen 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. Anthropometric measurements, DNA isolation, and lipid profiling

BMI was estimated by measuring the height and weight of all the subjects. Venous blood samples (3 ml) for biochemical assessments were acquired from the subjects after 12 h of overnight fasting in K_3EDTA coated tubes (J. K. Diagnostics, Rajkot, India). Plasma was separated and stored at −20 °C for estimating lipid profile parameters. FBG, Total Cholesterol (TC), Triglycerides (TG) and High-Density Lipoprotein (HDL) were assayed by commercially available kits (Reckon Diagnostics P. Ltd, Vadodara, India). Low Density Lipoprotein (LDL) was calculated using Friedewald's (1972) formula. Genomic DNA was extracted from the whole blood using QIAamp DNA Blood Mini Kit (Qiagen, Germany). DNA purity was assessed by calculating the ratio of absorbance at 260/280 nm by Cary 60 UV–Vis spectrophotometer (Agilent, California, USA). The integrity of genomic DNA was assessed by 0.8% agarose gel electrophoresis. The DNA was stored at −20 °C until further analysis.

2.3. Genotyping of Omentin-1 polymorphisms

Omentin-1 polymorphisms (rs2274907 and rs1333062) were genotyped by performed by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). The primers used for genotyping of these polymorphisms are as shown in Table S2. 20 µl of the reaction mixture included 3 µl (50 ng) of genomic DNA, 11 µl of nuclease-free water, 2.0 µl of 10X PCR buffer, 2.0 µl of 2.5 mM dNTPs (Sigma Chemical Co, St.Louis, Missouri, USA), 1.0 µl each of 10 µM forward and reverse primers (MWG Biotech, India) and 0.3 µl of 3U/µl Taq Polymerase (Bangalore Genei, India). Amplification was performed using Applied Biosystems 96 well Thermal cycler (California, USA) as per the protocol of initial denaturation at 95 °C for 5 min followed by 39 cycles each at 95 °C for 30 s, 59–67 °C for 30 s and 72 °C for 30 s, followed by final extension at 72 °C for 10 min. 5 µl of the amplified products were analyzed by electrophoresis on a 2.0% agarose gel stained with ethidium bromide along with a 50 bp DNA ladder (MBI Fermentas, St.Leon-Rot, Germany) and photographed. Details of the restriction enzymes (Thermo Fisher Scientific, Wilmington, DE, USA) and digested products are mentioned in Table S2. 15 µl of the amplified products were digested with 1U of the corresponding restriction enzyme in a total reaction volume of 20 µl as per the manufacturer's instruction. A 50 bp DNA ladder (MBI Fermentas, St.Leon-Rot, Germany) was used as a marker. All the gels were visualized under UV transilluminator using Gel Doc EZ System (Bio Rad Laboratories, California, USA) (Fig. S1).

2.4. Determination of Omentin-1 transcript levels

RNA isolation and cDNA synthesis: Total RNA was isolated from VAT by Trizol method. RNA integrity and purity were verified by 1.5% agarose gel electrophoresis/ethidium bromide staining and O.D. 260/280 absorbance ratio 1.9 respectively. Further, RNA was treated with DNase I (Puregene, Genetix Biotech) before cDNA synthesis to avoid DNA contamination. One microgram of total RNA was used to prepare cDNA using the Transcriptor High Fidelity cDNA Synthesis Kit (Roche Diagnostic GmbH, Mannheim, Germany) according to the manufacturer's instructions in the Eppendorf Mastercycler gradient (USA Scientific, Inc., Florida, USA).The expression of *Omentin-1* and *GAPDH* transcripts was monitored by LightCycler®480 Real-time PCR (Roche Diagnostics GmbH, Manneheim, Germany) using gene-specific primers (Eurofins, Bangalore, India) as shown in Table S2. Expression of *GAPDH* gene was used as a reference. Real-time PCR was performed as described previously [16].

2.5. Determination of plasma Omentin-1 levels

The plasma levels of Omentin-1 were estimated by the enzyme-linked immunosorbent assay (ELISA) kit for human Omentin-1 (RayBio, Norcross, GA, USA) with the sensitivity of 2 ng/ml. All the plasma estimations were carried out in duplicates to ensure % Coefficient of Variation (CV) below 10%.

2.6. Statistical analyses

The clinical characteristics of the study subjects were compared using the *t*-test. Hardy-Weinberg equilibrium (HWE) was performed for *Omentin-1* polymorphisms in patients and controls by comparing the observed and expected frequencies of the genotypes using the chi-square analysis. The distribution of genotype and allele frequencies of *Omentin-1* polymorphisms for patients and control subjects were compared using the chi-square test with 2x2 contingency tables. *p*-values < 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 calculated. Haplotype and linkage disequilibrium (LD) analysis were carried out using http://shesisplus.bio-x.cn/SHEsis.html [17]. For analyses of the transcript and protein levels, unpaired *t*-test and one-way ANOVA were applied. Post hoc Tukey test was applied for multiple group analysis. All the genotype-phenotype correlation analyses were carried out in T2D patients. All the analyses were carried out in GraphPad Prism 5 software. The statistical power of detection of the association with the disease at the 0.025 level of significance was determined by using the G* Power software

2.7. Bioinformatics analysis

In silico prediction tools PANTHER [18], POLYPHEN [19], I-MUTANT [20], were employed to predict the sequence based impact on the protein due to single amino acid variation and the details are provided

Table 1
Genotype and allele frequencies distribution of *Omentin-1* polymorphisms in T2D patients and controls.

SNP	Genotype	Controls (Frequency)	Patients (Frequency)	*p* for HWE	*p* for Association	Odds ratio	(95% CI)
		(n = 250)	(n = 235)				
(rs2274907)	TT	206	189	(C)	R	-	-
Omentin-1	TA	44	46	0.2285	0.1992[a]	1.378	0.8436 to 2.250
Exon 4 Val109Asp A/T	AA	0	0	(P) 0.1087	-	-	-
	T	430 (0.93)	416 (0.90)		0.2212[c]		
	A	34 (0.07)	44 (0.10)			1.338	0.8381 to 2.135
		(n = 250)	(n = 235)				
	TT	45	35	(C)	R	-	-
(rs1333062)	TG	109	105	0.1541	0.4167[a]	1.239	0.7387 to 2.077
Omentin-1	GG	96	95	(P)	0.3681[a]	1.272	0.7526 to 2.151
3'UTR				0.4993			
G/T	T	199 (0.40)	175 (0.37)		0.4119[b]		
	G	301 (0.60)	291 (0.63)			1.114	0.8602 to 1.444

n:Number of Patients/ Controls, R:Reference group, HWE: Hardy-Weinberg Equilibrium, CI: Confidence Interval, Odds ratio is based on allele frequency distribution. (P) refers to Patients and (C) refers to Controls.

[a] Patients vs. Controls (genotype) using chi-square test with 2 × 2 contingency table.

[b] Patients vs. Controls (allele) using chi-square test with 2 × 2 contingency table. Statistical significance was measured at $p < 0.025$ as per Bonferroni's correction.

in supporting data.

3. Results

3.1. Clinical parameters

The clinical parameters of 250 controls and 250 patients used for genetic association study are as shown in Table S1.

3.2. Association of Omentin-1 polymorphisms

The genotype and allele frequencies of the explored *Omentin-1* polymorphisms (rs2274907 A/T and rs1333062 G/T) are summarized in Table 1. The distribution of genotype frequencies for all the polymorphisms were in agreement with Hardy-Weinberg expectations in both patient and control groups ($p > 0.025$). Our results suggest no difference in genotype as well as allele frequencies of *Omentin-1* SNPs among diabetic patients and controls. None of the polymorphisms of *Omentin-1* were found to be associated with T2D ($p > 0.05$), and were hence discontinued after an initial assessment of 250 samples. This study has 85% statistical power for the effect size 0.1 to detect association of *Omentin-1* polymorphisms at $p < 0.025$ in T2D patients and controls

3.3. Haplotype and linkage disequilibrium (LD) analysis

The estimated frequencies of the haplotypes obtained for rs2274907 A/T and rs1333062 G/T did not differ significantly between patients and controls (global $p = 0.853$) (Table 2). None of the haplotypes were found to be associated with T2D. The LD analysis revealed that the two polymorphisms of *Omentin-1* were in moderate association (D'=0.56, $r^2 = 0.05$) (Fig S2).

3.4. Association of Omentin-1 polymorphisms with FBG, BMI and plasma lipids:

Omentin-1 rs2274907 AT genotype was found to be associated with increased BMI ($p = 0.0247$) (Table 3). However, it was not associated with FBG and plasma lipids ($p > 0.05$). Further, rs1333062 G/T did not show any association with FBG, BMI and plasma lipids ($p > 0.05$).

3.5. Bioinformatics analysis

The positive genotype-phenotype association for *Omentin-1* rs2274907 AT genotype with increased BMI suggests their crucial role in Omentin-1 activity. Therefore, we further investigated the impact of polymorphism on Omentin-1 protein using bioinformatics tools. *Omentin-1* rs2274907 A/T polymorphism results in aspartate to valine substitution at position 109 of Omentin-1 protein [21]. PANTHER and POLYPHEN tools showed that *Omentin-1* rs2274907 is probably benign suggesting that the substitution does not affect the phenotype nor has damaging effects on the function of Omentin-1 protein. I-MUTANT predictions revealed decreased stability of *Omentin-1* rs2274907 variant as compared to its native structure (Table 4).

3.6. Relative gene expression of Omentin-1 and its association with Omentin-1 SNPs, and a correlation with metabolic profile

Significantly increased *Omentin-1* transcript levels were observed in T2D patients as compared to controls after normalization with *GAPDH* expression as suggested by the significant ($p < 0.0127$) mean ΔCt values (Fig. 1A). Moreover, a $2^{-\Delta\Delta Cp}$ analysis showed approximately 4.2 fold change in the expression of *Omentin-1* transcript levels in patients as compared to controls as shown in Fig. 1B. Further, there was no significant difference observed between *Omentin-1* transcript levels and its SNPs ($p > 0.05$) as shown in Fig. 1C. Spearman's correlation analysis

Table 2
Distribution of haplotype frequencies of *Omentin-1* polymorphisms in T2D patients and controls.

Haplotype (*Omentin-1* rs2274907 A/T and rs1333062 G/T)	Patients(Freq. %) (n = 230)	Controls(Freq. %) (n = 250)	*p* for association	$p_{(global)}$	Odds ratio [95%CI]
TT	142(0.307)	135(0.322)	0.064	0.853	1.296 [0.983 ~ 1.707]
TG	276(0.597)	249(0.595)	8.60×10^5		1.641 [1.283 ~ 2.099]
AT	28(0.06)	29(0.069)	0.684		1.116 [0.654 ~ 1.905]

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

Table 3
Genotype-phenotype association analysis of *Omentin-1* 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
***Omentin-1* rs2274907 A/T**							
TT (n = 189)	118.7(45.53)	25.6(5.42)	157.0(85.52)	161.3(36.86)	100.1(29.86)	36.4(9.92)	41.3(9.63)
AT (n = 46)	127.8(45.15)	27.0(5.55)	168.6(86.17)	166.3(32.72)	106.1(28.89)	33.8(7.62)	41.3(8.60)
AA (n = 0)	–	–	–	–	–	–	–
p value	0.1369	**0.0247**	0.1763	0.2010	0.0825	0.1248	0.8184
***Omentin-1* rs1333062 G/T**							
TT (n = 35)	119.7(54.22)	25.4(6.03)	144.6(74.93)	162.0(37.56)	101.8(25.16)	36.0(9.18)	41.8(11.10)
TG (n = 105)	120.1(43.27)	25.9(5.31)	155.8(88.89)	163.0(34.15)	103.6(32.24)	36.5(10.41)	41.9(9.49)
GG (n = 95)	120.3(41.22)	25.8(5.22)	163.9(88.55)	159.3(37.12)	98.7(29.83)	35.8(8.14)	40.8(8.59)
p value	0.9150	0.4323	0.1852	0.3773	03,678	0.8933	0.5850

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

Table 4
In-silico analysis of *Omentin-1* rs2274907 A/T polymorphism.

Amino acid change	PANTHER	POLYPHEN	I-MUTANT
Asp109Val	probably benign	benign	Decrease

revealed no correlation between *Omentin-1* transcript levels and BMI, FBG or plasma lipids ($r^2 = 0$, $p > 0.05$) (Table 5).

3.7. Plasma Omentin-1 levels and its association with Omentin-1 SNPs, and a correlation with metabolic profile

Plasma Omentin-1 levels showed a significant decrease

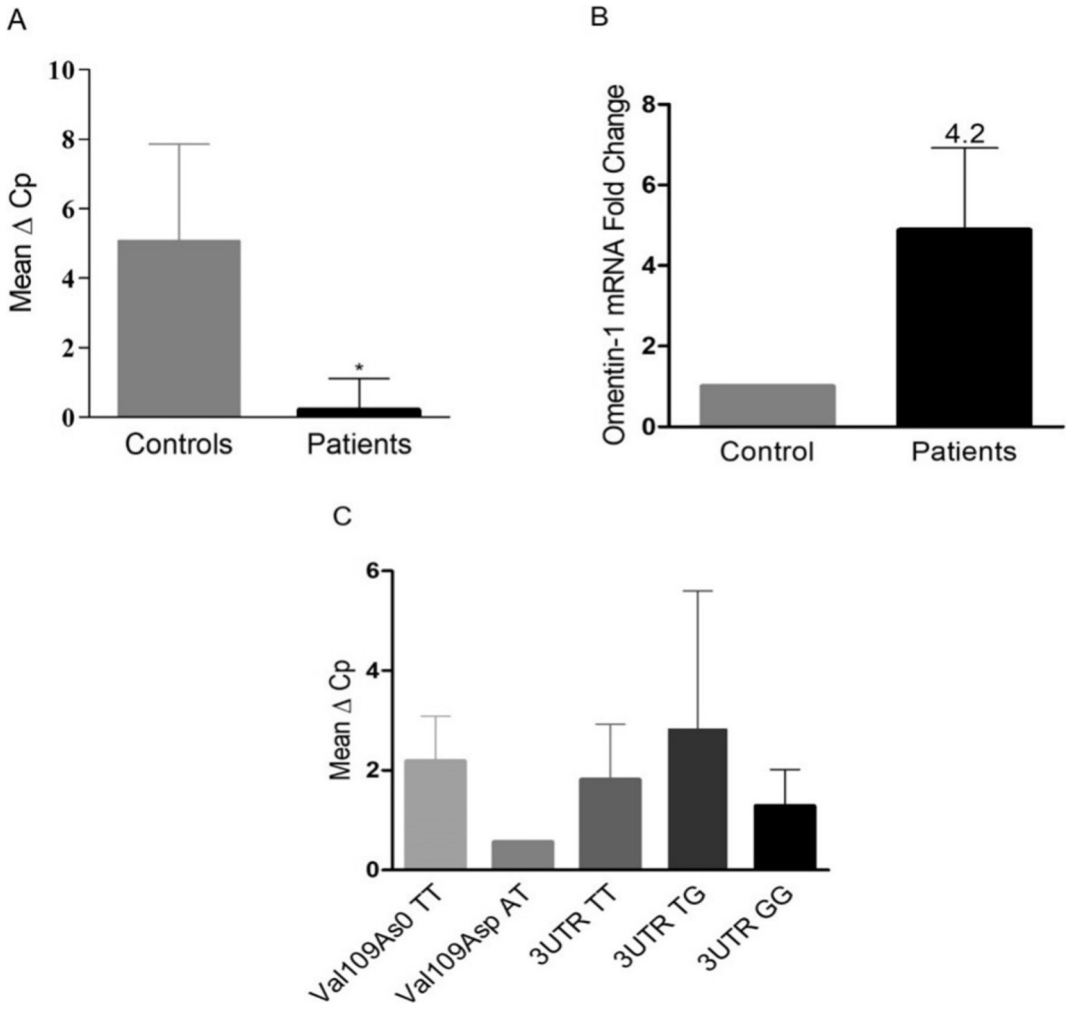

Fig. 1. (A) Relative gene expression of VAT *Omentin-1* in controls and patients: Significant increase in *Omentin-1* transcript levels was observed in patients (Mean ΔCt ± SEM: 5.06 ± 2.79 vs 0.20 ± 0.90; $p = 0.0127$). **(B) Relative fold change of *Omentin-1* expression in controls and patients.** T2D patients showed 4.2 fold increase in *Omentin-1* mRNA expression as determined by the $2^{-\Delta\Delta Cp}$ method (Controls n = 12; T2D patients n = 10). **(C) Association of *Omentin-1* polymorphisms with *Omentin-1* transcript levels.** *Omentin-1* polymorphisms with *Omentin-1* transcript levels showed no association with Omentin-1 transcript levels ($p > 0.05$).

Table 5
Correlation analysis of *Omentin-1* transcripts with metabolic profile.

Parameters	r^2	p
BMI (Kg/m^2)	0.2571	0.6583
FBG (mg/dL)	−0.4000	0.7500
TG (mg/dL)	0.4000	0.7500
TC (mg/dL)	0.3491	0.7568
HDL (mg/dL): Male	0.5678	0.6789
Female	0.9876	0.5678
LDL (mg/dL)	0.4000	0.7500

$p > 0.05$, non-significant. n = 10.

Table 6
Correlation analysis of plasma Omentin-1 with metabolic profile.

Parameters	r^2	p
BMI (Kg/m^2)	−0.0127	0.9020
FBG (mg/dL)	0.2427	0.1538
TG (mg/dL)	0.1728	0.2401
TC (mg/dL)	0.0940	0.4865
HDL (mg/dL): Male	0.1420	0.4541
Female	0.3000	0.1642
LDL (mg/dL)	0.1192	0.4520

$p > 0.05$, non-significant. n = 40.

($p < 0.0001$) in T2D patients (Fig. 2A). Further, the levels of Omentin-1 were significantly low ($p = 0.017$) in obese patients compared to obese controls (Fig. 2B). Further, no association was found between Omentin-1 plasma levels and its SNPs ($p > 0.05$) as shown in Fig. 2C. Spearman's correlation analysis revealed no correlation between Omentin-1 protein levels and BMI, FBG and plasma lipids ($r^2 = 0$, $p > 0.05$) (Table 6).

4. Discussion

There are numerous studies on the association of adipokine genetic variants in T2D but with few being explored in the Indian population. The present study was designed to determine genetic risk factors from one of the strongly linked chromosomal regions 1q21-23 in Gujarat population for T2D.

Our results revealed that the genetic variants of *Omentin-1* (rs2274907 A/T and rs1333062 G/T) are not associated with T2D. Similar observations were reported in the Caucasian population [21,22] though not in Polish and North Indian population [23,14]. Further, our association analysis revealed rs2274907 AT genotype to be significantly associated with increased BMI in T2D patients. In context to this, it is also reported to be associated with the increased risk towards NAFLD [8]. *Omentin-1* rs2274907 polymorphic (A/T) site is present in exon-4 and is reported to result in a change of amino acid from Asp (GAC) to Val (GTC) at position 109 [21]. Our *in silico* analysis revealed the site as benign, having no major structural effect on the protein activity.

The transcript as well as protein levels of Omentin-1 reveal quite an intriguing picture of increased mRNA levels and decreased protein levels in T2D patients. Though studies carried out by other research

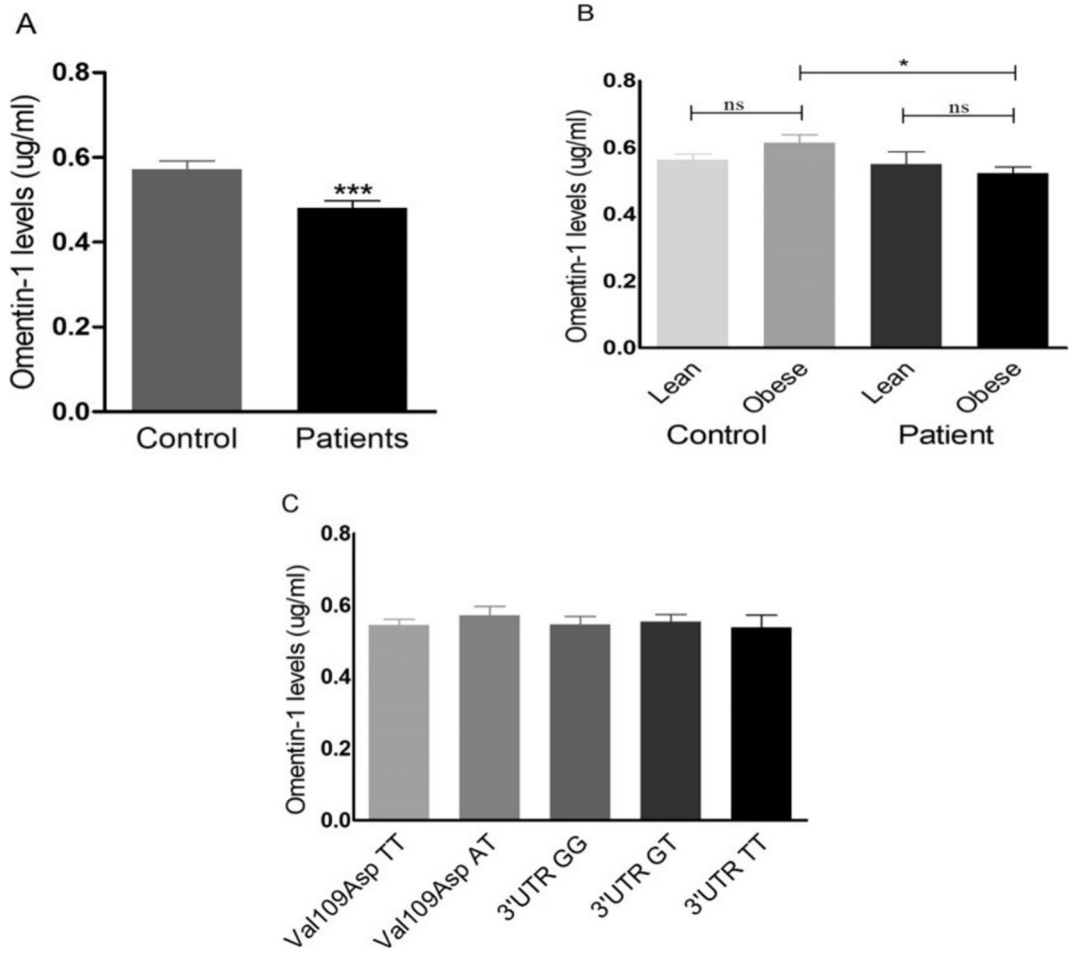

Fig. 2. Plasma Omentin-1 levels in (A) controls vs. patients (B) control (lean vs. obese) and Patients (lean vs. obese). Our results showed a significant decrease in plasma Omentin-1 levels in T2D patients ($p < 0.0001$) compared to controls; obese T2D patients showed a significant decrease compared to obese controls ($p = 0.017$) (Controls n = 40; T2D patients n = 40). **(C) Association of *Omentin-1* polymorphisms with plasma Omentin-1 levels.** *Omentin-1* polymorphisms showed no association ($p > 0.05$) with plasma Omentin-1 levels.

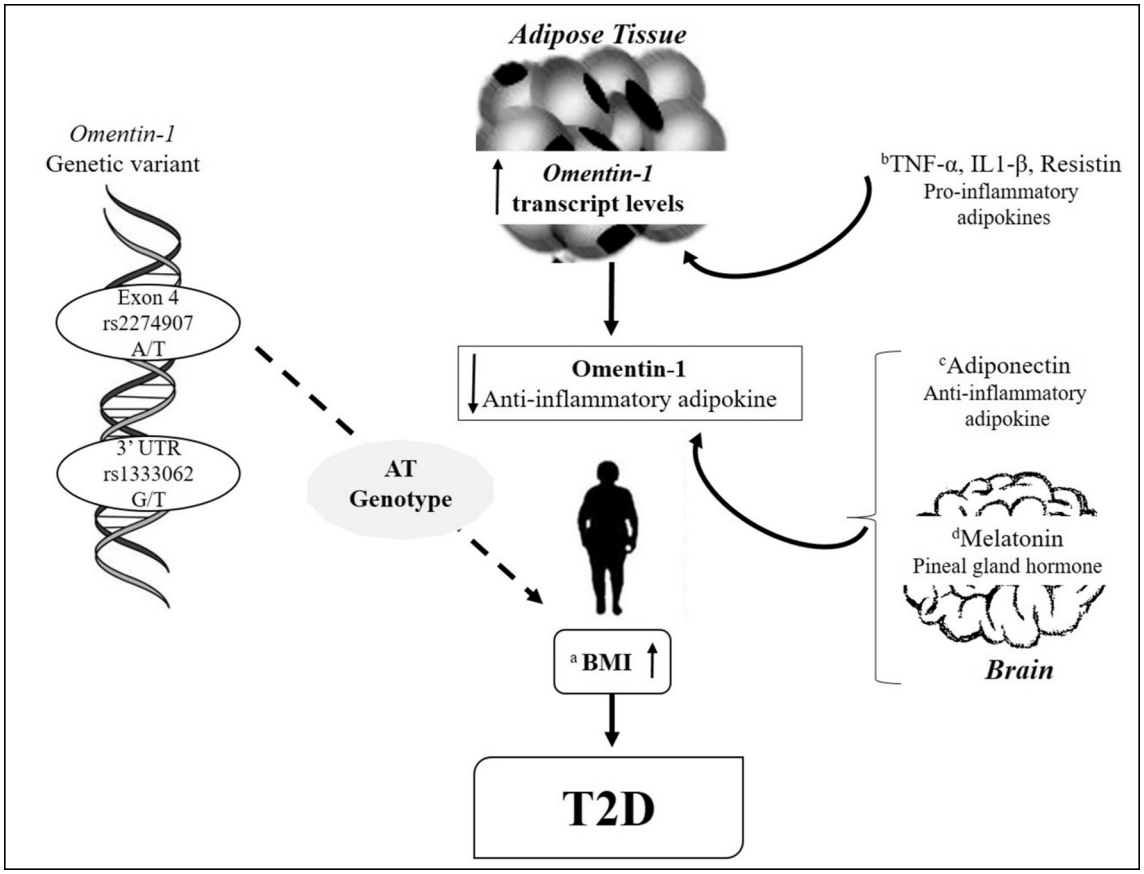

Fig. 3. Role of Omentin-1 in T2D: [a]The genetic variants of *Omentin-1* are not associated with T2D susceptibility, however the AT genotype (rs2274907) is associated with an increased BMI. In obese individuals, Omentin-1 might be regulated by multiple factors at transcriptional as well as translational levels. Our previous studies demonstrate increased [b]pro-inflammatory adipokines, decreased [c]anti-inflammatory adipokines and [d]melatonin levels. Thus, these factors might contribute to *Omentin-1* VAT transcript levels and plasma protein levels, which might play a role in the development of obesity-induced T2D condition.

groups are in discord with our transcript results [4,24,25], it is important to note that these groups have not monitored the protein levels. Our results on the transcript levels are in agreement with the report of Schäffler et al. [5] who showed an increase in *Omentin-1* transcript levels as a response to elevated levels of pro-inflammatory adipokines. It could at best be explained as a defence mechanism elicited under obesity-induced changes in the micro-environment of adipose tissue [5,26]. As an explanation of elevated anti-inflammatory levels, Li et al. have suggested it to be a stimulation induced by various pro-inflammatory cytokines besides differential binding frequencies of NF-κB, a major adipokine regulator [27]. In support of these findings, we have also observed an increased expression of pro-inflammatory adipokines such as TNF-α [16], IL1β [28] and resistin [29]. Furthermore, epigenetic modifications like miRNA regulation, DNA methylation, and post-translational modifications have also been suggested to regulate mRNA expression of adipokines [27,30,31]. In this context, the observed increased mRNA expression could be due to any of these reasons.

As against the transcript levels, plasma Omentin-1 levels were significantly lowered in T2D patients. Studies by other research groups substantiate our results on protein levels [4,32]. There are several explanations put forward for the reduced circulatory Omentin-1 levels in diabetic conditions. First of all, the incidence of decreased Omentin-1 in the circulation could be a consequence of either inhibited translation or decreased stability of mRNA or protein. Secondly, Yan et al. [7] have shown circulating Omentin-1 levels and adiponectin levels to have a direct correlation. Interestingly, we have observed reduced adiponectin levels in our population [33]. One of the studies has suggested that adiponectin may have a regulatory influence on Omentin-1 levels [34]. However, future studies are needed in this direction to unravel the intricate relations if any. Dysregulation of blood glucose levels with the increased propensity towards T2D and diabetic complications have been shown to be associated with sleep disturbances [35]. Moreover, it has also been reported that circadian rhythms can influence metabolic processes of adipose tissue and also expression and secretion of adipokines [36,37]. Such regulation is likely to be mediated by melatonin by way of its action on VAT either through its membrane receptors or via an action on the sympathetic nervous system [38]. The possible mechanisms of action of melatonin on Omentin-1 may be corresponding to its effect on the levels of adiponectin. From our previous study, we have observed reduced plasma melatonin levels in T2D patients [39]. The reduced Omentin-1 levels might contribute towards the progression/development of T2D. The underlying mechanism for the differential expression of mRNA and protein levels needs to be investigated in depth through *in-vivo* studies.

As discussed above, in obesity-induced diabetic individuals, there are altered levels of pro-inflammatory (TNF-α) and anti-inflammatory (adiponectin) adipokines. Omentin-1 is reported to manifest its anti-inflammatory activity by inhibiting TNF-α through JNK pathway in healthy individuals [40]. Circulatory Omentin-1 is used as a biomarker of diabetes, obesity, atherosclerosis, inflammatory disease, metabolic syndrome, and cancer [6,2] and in this context, the same could be considered in our Gujarat T2D population. However, its polymorphic sites are not associated with the disease. Further studies on *Omentin-1* expression in larger sample size are required to validate our results.

To our knowledge, this is the only study that ascribes an association between *Omentin-1* polymorphisms, its transcript and protein levels with biochemical parameters in Gujarat population. Thus, our results contribute to an understanding of the role of Omentin-1 in obesity-

induced T2D.

The current study suggests *Omentin-1* might be regulated by multiple factors at transcriptional as well as translational levels, while genetic polymorphisms are not associated with T2D. We observed an association of the AT genotype of rs2274907 with increased BMI levels. The reduced Omentin-1 protein levels might be influenced by increased pro-inflammatory adipokines and epigenetic modifications. These factors are known to be induced by a sedentary lifestyle and an unhealthy diet. The Omentin-1 levels might also be regulated by anti-inflammatory adipokine and melatonin. Thus, all these factors could be involved in the development of dyslipidemia and obesity-induced T2D (Fig. 3).

5. Conclusion

Our study suggests that although *Omentin-1* genetic variants are not associated with T2D, its reduced protein levels could play a role in T2D susceptibility.

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Competing Interests

The authors declare that no competing interests exist.

Author Contributions

RB conceived the idea and designed the experiments. MN provided adipose tissue samples. NR, RP, and SP performed the experiments. SJ performed the bioinformatics data analysis. NR did the data acquisition, performed the data analysis and wrote the original draft. RB and AVR contributed to the critical revision and approval of the manuscript.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cyto.2019.03.011.

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