CHAPTER 4
TO STUDY THE ROLE OF VASPIN IN TYPE 2 DIABETES

To study the role of Vaspin in Type 2 Diabetes

4.1 Introduction

Central obesity is a chief player in developing metabolic syndrome and viewed as a risk factor for T2D. Adipocytes produce various biomolecules, together known as adipokines, which play a crucial role in metabolism, inflammation and immunity. Several other adipokines have come to light since leptin's discovery. The adipokines exhibit both pro- and anti-inflammatory properties and are crucial integrators of systemic metabolism with immune function. Homeostasis between pro-and anti-inflammatory macrophages marks normal body functions [Pramanik *et al.,* 2018]. Vaspin, a member of serpin A12, was initially discovered in visceral adipose tissue (VAT) of Otsuka Long-Evans Tokushima fatty rat [Hida *et al.,* 2005; Nakatsuka *et al.,* 2012]. It is an anti-inflammatory adipokine reported to inhibit kallikrein 7 protease activity that degrades insulin. It also stimulates cell proliferation, inhibits apoptosis and ameliorates ER stress in HepG2 cells [Nakatsuka *et al.,* 2012; Heiker *et al.,* 2013]. Limited studies establish the complementary effect of exogenous recombinant vaspin on insulin sensitivity and glucose tolerance [Hida *et al.,* 2005; Klöting *et al.,* 2011].

In humans, decreased vaspin protein levels are associated with increased BMI and reduced insulin sensitivity in adults [Choi *et al.,* 2011; Genc *et al.,* 2011] and obese women having polycystic ovary syndrome (PCOS) [Tan *et al.,* 2008; Polak *et al.,* 2017]. Thus, the emerging line of evidence supports the idea of vaspin being a significant factor involved in the progression of obesity-induced T2D. *Vaspin* comprising of 6 exons and 5 introns, is located on chromosome 14q32.13. SNPs of *vaspin* are well explored of which, intronic polymorphic sites (intron 2 rs77060950 G/T and intron 4 rs2236242 A/T) have been investigated in relation to various diseases like T2D [Kempf *et al.,* 2010; Teshigawara *et al.,* 2012], PCOS [Kohan *et al.,* 2014], metabolic syndrome [Hashemi *et al.,* 2012; Mehanna *et al.,* 2016], CAD [Li *et al.,* 2013], NAFLD [Hashemi *et al.,* 2013], and obesity [Ghany *et al.,* 2017]. The forecasts indicate that cases of T2D will escalate to 74.9 million by 2030 [Wild *et al.,* 2004] in India, with the Gujarat population being the second highest [Joshi *et al.,* 2014]. The present study investigates the distribution of genotypes and high-risk alleles of *vaspin* present in the intronic region (intron 2 rs77060950 G/T and intron 4 rs2236242 A/T) and their correlation with any alterations in their transcript and protein levels. Alongside, a genotype-phenotype correlation with various metabolic parameters has been made to understand their possible association.

4.2 Materials and Methods

4.2.1 Study subjects

Details regarding study subjects are described in **section 2.2.1** of chapter 2. Samples of visceral (omental) adipose tissue were taken from the individuals undergoing bariatric surgery and fasting clinical parameters of all the study subjects were considered. The individuals with FBG>125mg/dL and not suffering from any other diseases were recruited for the study as T2D patients.

4.2.2 Anthropometric parameters, Lipid profiling and DNA extraction

Details regarding anthropometric parameters, lipid profiling and DNA extraction are described in **section 2.2.2** of chapter 2.

4.2.3 Genotyping of the polymorphisms

PCR-RFLP and ARMS methods was used for genotyping of *vaspin* gene two polymorphisms. The primers details of genotyping of SNPs of *vaspin* gene and monitoring *vaspin* transcript levels are shown in Table 4.1. 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; shown in Table 4.1) for 30 seconds and 72°C for 30 seconds, followed by final extension at 72°C for 10 minutes. The information of the REs (Thermo Fisher Scientific, Wilmington, DE, USA) and digested products are mentioned in Table 4.1. 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 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 4.1 Primers and REs used for genotyping for the studied polymorphisms and expression.

SNP/ Gene primer	Sequence (5' to 3')	Annealing temperature	Amplicon size (bp)	RE	Digested products (bp)
Vaspin	**FP:** CACACCCTAAC	67°C	309	*Mbo*I	193+116

rs77060950 G/T	TCCAAGAGCT **RP:** GAACCAGAAG CCCAAAGTCAC				
Vaspin rs2236242 A/T	**FP:** GGACCCAGGATA ACTTAC**A** **FP:** GGACCCAGGATA ACTTAC**T** **RP:** AGCCTACACTTG GACTTCA	58°C	187	_	_
Internal Control (HGH)	**FP:** CCTTCCCAACCA TTCCCTTA **RP:** TCACGGATTTCT GTTGTGTTTC	58°C	428	_	_
Vaspin transcript	**FP:** CGAGGCTGTG CACAAGG **RP:** TCTCCATGGGC AGAGTCT	58°C	220	-	-
GAPDH	**FP:** CATCACCATC TTCCAGGAGCGAG **RP:** CCTGCAAATG AGCCCCAGCCT	64°C	122	-	-

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

4.2.4 Determination of the transcript levels

Total RNA was extracted from VAT by Trizol method. RNA integrity and purity were confirmed by 1.5 % agarose gel electrophoresis/EtBr 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 transcript levels of *vaspin* and *GAPDH* (reference gene) were detected by LightCycler®480 Real-time PCR (Roche Diagnostics GmbH, Manneheim, Germany) using gene-specific primers (Eurofins, Bangalore, India) as shown in Table 4.1. The thermal cycling conditions comprised an initial activation step at 95°C for 10 min, followed by 45 cycles of denaturation (95°C for 10 sec.), annealing (60-69°C for 10 sec; shown in Table 4.1), and amplification (72°C for 10 sec.). The fluorescence data collection was accomplished during the extension step. At the end of the

amplification phase, a melt curve analysis was carried out to validate the specificity of the products formed. The PCR cycle at which PCR amplification begins its exponential phase was considered as the crossing point (Cp) or cycle threshold (Ct). The ΔCt or ΔCp value was obtained as a difference between the Ct of *vaspin* gene and Ct of *GAPDH* gene. The difference among the two ΔCt values (ΔCt Controls and ΔCt patients) was considered as ΔΔCt to attain the value of fold expression ($2^{-\Delta\Delta Ct}$).

4.2.5 Determination of plasma vaspin protein levels

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

4.2.6 Statistical analyses

The clinical characteristics (metabolic and lipid profile, transcript and 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 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 allele 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).

4.3 Results: Baseline Characteristics

The clinical parameters varied significantly between recruited controls and T2D patients (Table 2.1) for the study. Clinical parameters varied significantly between T2D controls and patients recruited for the transcript levels analysis (Table 4.2). Patients had significantly higher FBG (p=0.0408) and HbA1c (p=0.05) compared to controls. However, other parameters were not significant between the controls and patients.

Table 4.2 Clinical characteristics of the controls and T2D patients.

Parameters	**Controls (Mean ± SD)**		**Patients (Mean ± SD)**	***p* value**
	(n =22)		(n =20)	
Age (years)	40.75 ± 11.5		51.42 ± 13.6	
Sex: Male	8		9	-
Female	14		11	-
FBG (mg/dL)	104.80 ± 4.2		200.40 ± 34.4	**0.0408**
HbA1c (%)	6.3 ± 0.3		8.3 ± 0.9	**0.05**
BMI (Kg/m^2)	41.88 ± 5.6		40.04 ± 8.8	0.3334
Total Cholesterol	144.4 ± 44.6		159.80 ± 47.1	0.3334
(mg/dL)	95.57 ± 62.13		229.0 ± 72.34	0.0677
Triglycerides (mg/dL)	59.28 ± 12.98		41.46 ± 9.39	0.0867
HDL (mg/dL)	81.53 ± 22.18		74.05 ± 22.17	0.1485
LDL (mg/dL)				

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

4.3.1 Genetic analysis of the *vaspin* polymorphisms:

The *vaspin* SNPs were genotyped using PCR-RFLP, as shown in Figure 4.1, indicating the genotypes obtained. The genotype and allele frequencies of the explored *vaspin* polymorphisms are summarized in Table 4.3. The distribution of genotype frequencies for all the polymorphisms investigated was consistent with Hardy-Weinberg in both patient and control groups ($p>0.05$). *Vaspin* rs77060950 G/T was not associated with T2D ($p>0.05$) and was hence stopped after a preliminary assessment. *Vaspin* rs2236242 A/T was related to T2D risk. The AT genotype was associated with increased risk for T2D with an Odds Ratio (OR) of 1.432, while the mutant homozygous TT genotype increased the risk by 3.087-fold. The mutant allele 'T' of rs2236242 was associated with the risk of T2D having an OR of 1.649.

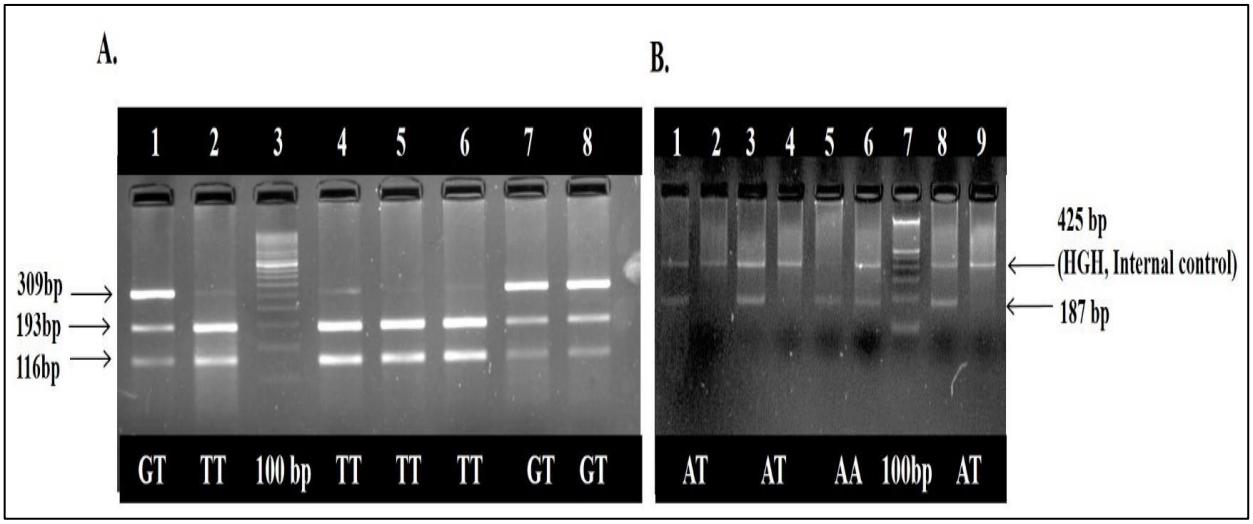

Figure 4.1 Representative gel image of *vaspin* SNPs. A) PCR-RFLP analysis of *vaspin* rs77060950 G/T polymorphism on 3.5% agarose gel: lanes 1, 7 and 8 show heterozygous (GT) genotypes; lanes 2, 4, 5 and 6 show homozygous (TT) genotypes; AA genotype was not observed. Lane 3 shows a 100bp DNA ladder. B) ARMS-PCR analysis of *vaspin* rs2236242 A/T polymorphism on 3.5% agarose gel. Internal control (Human Growth Hormone [HGH]) was 425 bp. 187-bp amplicon for the A/T allele was observed. Lane 1 had A target primer, and lane 2 had T target primer. Lanes 1-2, 3-4 and 8-9 show homozygous genotypes (AA), while lanes 5-6 show heterozygous AT genotype. Lane 7 shows a 100bp DNA ladder.

Table 4.3 Genotype and allele frequencies distribution of *vaspin* polymorphisms in controls and T2D patients.

SNP	Genotype /Allele	Controls	Patients	*p* for Association	OR	95% CI
(rs77060950) *Vaspin* Intron I G/T		n=250	n=250			
	GG	201 (80.40)	180 (72.00)	R	-	
	GT	45 (18.00)	61 (24.40)	0.0606[a]	1.514	0.9764 - 1.582
	TT	4 (1.60)	9 (3.60)	0.1187 [a]	2.513	0.7543 - 3.897
	G	447 (0.89)	421 (0.84)			
	T	53 (0.11)	79 (0.16)	0.0824[b]	1.376	0.9588 - 1.9
(rs2236242) *Vaspin* Intron IV A//T		n=500	n=478			
	AA	259 (51.80)	187 (39.12)	R	-	-
	AT	206 (41.20)	213 (44.56)	**0.0095**[a]	1.432	1.095 to 1.873
	TT	35 (7.00)	78 16.32)	**0.0001**[a]	3.087	1.986 to 4.767

	A	199 (0.40)	175 (0.37)			
	T	301 (0.60)	291 (0.63)	**0.0001**[b]	1.649	1.363 to 1.995

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.

4.3.2 Haplotype analysis of the *vaspin* polymorphisms:

The haplotypes estimated frequencies for rs77060950 G/T and rs2236242 A/T did not differ (global $p=7.36x10^{-6}$). Yet, GT ($p=2.46x10^{-6}$) haplotypes were associated with T2D risk, while GA and TA haplotypes were more frequent in the control population (Table 4.3).

Table 4.4 Distribution of haplotype frequencies of *vaspin* in controls and T2D patients.

Haplotype	Patients (Freq. %; n=500)	Controls (Freq. %; n=478)	*p* for Association	$p_{(global)}$	Odds Ratio [95%CI]
GA	511.60 (57)	632.24 (65)	**0.0053**	$7.36x10^{-6}$	0.714 [0.590~0.864]
GT	312.40 (35)	243.76 (25)	**$2.46x10^{-6}$**		1.619 [1.324~1.980]
TA	46.40 (5.2)	72.76 (7.5)	**0.0441**		0.678 [0.464~0.992]
TT	25.60 (2.8)	23.24 (2.5)	-		-

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

4.3.3 Linkage Disequilibrium analysis of the *vaspin* polymorphisms:

The LD analysis showed that the polymorphisms of *vaspin* (rs77060950 G/T and rs2236242 A/T) were in no linkage disequilibrium (D'=0.10; r^2= 0.001) (Figure 4.2).

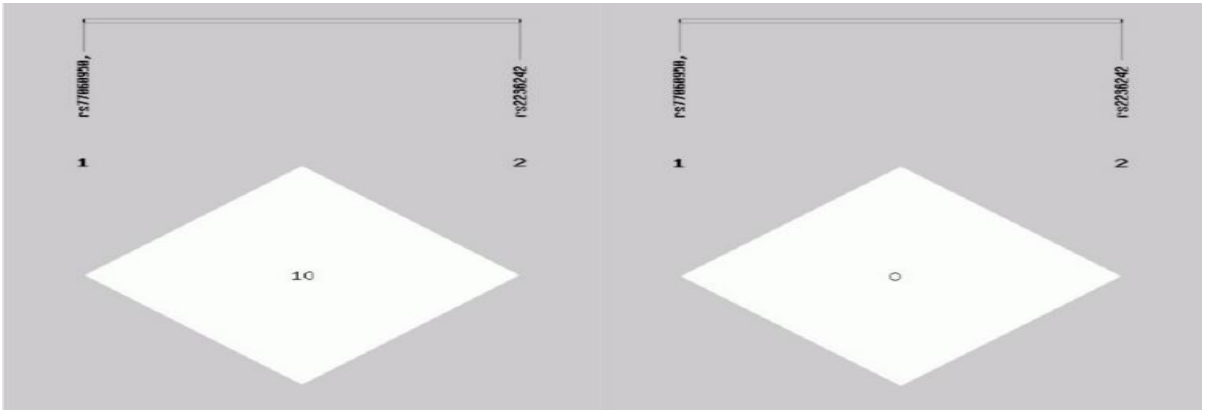

Figure 4.2 Linkage disequilibrium block: LD block with respect to *Vaspin* rs77060950 G/T and rs2236242 A/T polymorphisms in Gujarat population.

4.3.4 The analysis of the association of the *vaspin* polymorphisms with the metabolic profile:

Vaspin rs77060950 G/T did not show any association with metabolic parameters (p>0.05) (Table 4.5). Further, *vaspin* rs2236242 TT genotype was associated with increased FBG (p=0.0001), BMI (p=0.0001) and TG (p=0.0065) but was not associated with other parameters (Table 4.5).

Table 4.5 Genotype-phenotype association analysis of *vaspin* 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
***Vaspin* Intron I G/T (rs77060950)**							
TT	128.4±2.2	25.7±0.2	154.9±3.2	166.6±1.4	106.4±1.2	41.7±0.7	41.1±0.8
AT	116.7±5.2	25.7±0.2	156.7±7.9	169.5±2.9	106.3±1.9	37.8±2.6	37.8±3.2
AA	122.4±1.9	25.6±1.1	151.4±18.9	153.1±7.9	101.3±5.4	39.3±1.3	40.1±1.4
p value	0.0867	0.2056	0.8876	0.0739	0.1669	0.2559	0.1934
***Vaspin* Intron 4 A/T (rs2236242)**							
TT	120.3+22.3	24.9±2.3	132±27.2	167.6±37.15	101.1±82.90	40.1±12.2	39.8±11.7
TG	129.2+11.3	26.4±1.0	135.8±21.3	165.3±33.85	97.88±91.16	41.1±11.9	41.1±11.3
GG	**134.4+7.2**	**27.5±0.8**	**161.0±11.2**	166.2±37.24	96.4±76.58	38.6±10.9	37.6±13.1
p value	**<0.0001**	**<0.05**	**0.0063**	0.8984	0.8911	0.3213	0.2112

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

4.3.5 *Vaspin* transcript levels and their association with *vaspin* polymorphisms, and a correlation with metabolic profile:

After normalization with *GAPDH* expression, a 2.26-fold (p=0.028) decrease in the expression of *vaspin* transcript levels was observed in T2D patients by $2^{-\Delta\Delta Cp}$ analysis (Figure 4.3A). However, there was no association between *vaspin* transcript levels and their polymorphisms (p>0.05) (Figure 4.3B). Spearman's correlation analysis showed no correlation between *vaspin* transcript levels and metabolic profile (r^2=0, p>0.05) (Table 4.6).

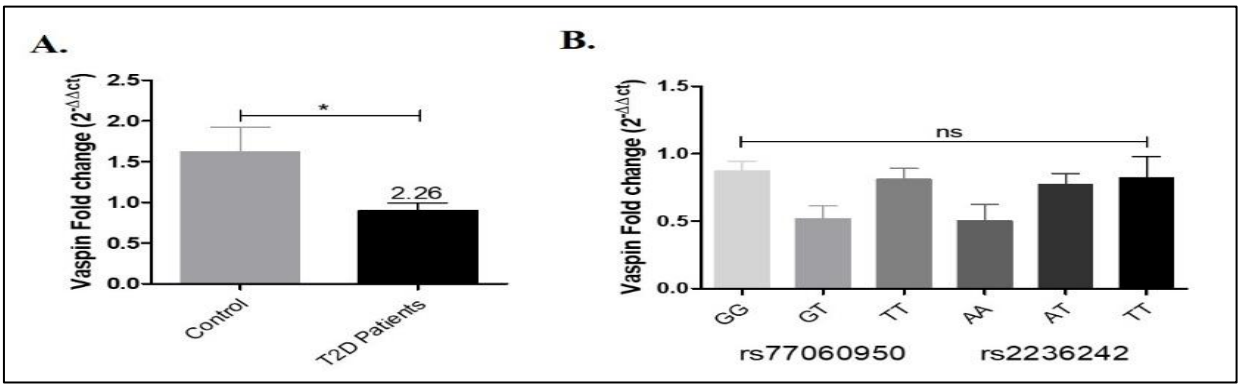

Figure 4.3 A) Relative fold change of *vaspin* transcript levels in adipose tissue of controls and patients. T2D patients showed 2.26-fold (p=0.028) decrease in *vaspin* transcript levels as

estimated by the $2^{-\Delta\Delta Cp}$ method (Controls n=22; T2D patients n=20). **B) Association of *vaspin* polymorphisms with their transcript levels.** *Vaspin* polymorphisms showed no association with their transcript levels (p>0.05).

Table 4.6 Correlation analysis of *vaspin* transcript levels with the metabolic profile.

Parameters	r^2		*p value*
BMI (Kg/m^2)	0.4135		0.6583
FBG (mg/dL)	-0.3989		0.7500
TG (mg/dL)	-0.5676		0.7500
TC (mg/dL)	-0.0909		0.7568
HDL (mg/dL): Male Female	0.7700 0.7500		0.9881 0.9091
LDL (mg/dL)	0.6515		0.0789

p>0.05, non-significant. n=20

4.3.6 Plasma vaspin protein levels and their association with *vaspin* polymorphisms and metabolic profile:

Reduced plasma vaspin protein levels were observed (p=0.0001) in T2D patients (Figure 4.4A). Further, no association was observed between vaspin protein levels and *vaspin* polymorphisms (p>0.05) (Figure 4.4B). Spearman's correlation analysis showed a negative correlation between plasma vaspin protein levels and BMI (p=0.0307) and FBG (p=0.0006) and no correlation with the lipid profile (p>0.05) (Table 4.7).

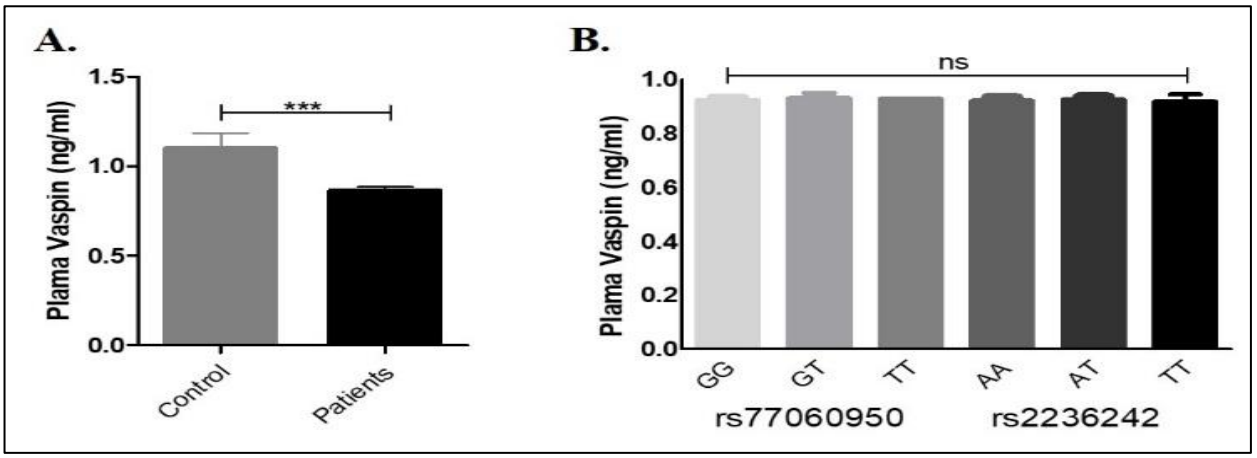

Figure 4.4 Plasma vaspin levels in A) controls vs patients. Our results showed a significant reduction in plasma vaspin protein levels in T2D patients (p<0.0001) (Controls n=40; T2D patients n=40). **C) Association of *vaspin* polymorphisms with plasma vaspin protein levels.** V*aspin* polymorphisms showed no association with vaspin protein levels (p>0.05).

Table 4.7 Correlation analysis of plasma vaspin with the metabolic profile.

Parameters	r^2	*p value*
BMI (Kg/m^2)	-0.2514	**0.0307**
FBG (mg/dl)	-0.4695	**0.0307**
Triglycerides (mg/dl)	-0.0971	0.4765
Total Cholesterol (mg/dl)	-0.0743	0.5659
HDL (mg/dl): Male Female	-0.5191 -0.4673	0.6881 0.1589

LDL (mg/dl)	-0.0561	0.7080

p>0.05, non-significant. n=40

4.4 Discussion

Adipocytes and beta-cell dysfunction are the trademarks of T2D pathophysiology, and abundant factors contribute towards it, the most prominent ones being obesity and genetic predisposition [Breitfeld *et al.,* 2013]. A few studies have evaluated polymorphisms of adipokines with T2D susceptibility in the Indian population. Our results reveal no association between Vaspin rs77060950 G/T with either T2D risk or any other parameters. Similar results have been documented in the German population [Breitfeld *et al.,* 2013]. *Vaspin* intronic polymorphism rs2236242 A/T is significantly associated with T2D. This polymorphism was validated in different populations in respect of different diseases/disorders (Table 4.8).

Table 4.8 Association of *vaspin* rs2236242 A/T with various disorders.

Sr.No.	Associated with	Population	References
1.	T2D	German, Chinese	Kempf *et al.*, 2010; Li *et al.*, 2019
2.	CAD	Chinese	Li *et al.*, 2013
3.	Obesity	Egyptian	Ghany *et al.*, 2017
4.	Metabolic Syndrome	Egyptian	Mehanna *et al.*, 2016
5.	Metabolic Syndrome	Iranian	Kohan *et al.*, 2014
6.	PCOS	Iranian	Hashemi *et al.*, 2012
7.	ESRD	Iranian	Nomani *et al.*, 2018

The TT genotype exhibited a 3.087-fold increased risk for T2D. We report for the first time that the mutant T allele predisposes an individual towards the risk of T2D, unlike in other populations. Kempf *et al.* [Kempf *et al.,* 2010] have stated an association of rs2236242 towards the risk of T2D; however, the functional significances of rs2236242 polymorphism remains unexplored. A possible effect on the stability of mRNA or splicing efficiency of the transcribed product is hypothesized [Kempf *et al.,* 2010]

Association analysis reveals a significant association of *vaspin* rs2236242 TT genotype with metabolic risk factors manifested by elevated BMI, FBG and TG levels in T2D patients. Other populations have shown similar results [Mehanna *et al.*, 2016; Ghany *et al.*, 2017]. Assessment of VAT *vaspin* transcript and plasma protein levels revealed a significant reduction in their levels in T2D patients, which was also observed in other populations [Kobat *et al.,* 2012; Zhang *et al.,* 2013]. Several research studies present the notion that the compensatory ability of vaspin secretion gradually declines with the severity of diabetes or the onset of cardiovascular diseases resulting in decreased vaspin levels [Hida *et al.,* 2005; Breitfeld *et al.,* 2013].

Obesity is a chronic low-grade inflammation that regulates the levels of pro-and anti-inflammatory adipokines by macrophage polarization [Mancuso *et al.,* 2016]. Resistin is

reported to get elevated in T2D conditions [Santilli *et al.,* 2016]. Resistin induces pro-inflammatory effects by enhancing cAMP-mediated activation of PKA and NF-κB-mediated transcription of various inflammatory adipokines, i.e. IL-1β and TNF-α [Lee *et al.,* 2014]. Similar results were obtained in the studied population, indicating an imbalance of adipokines in the form of increased levels of TNF-α [Patel *et al.,* 2019], resistin [Rathwa *et al.,* 2019] and IL1-β [Patel *et al.,* 2016] in T2D patients. Such stimulation of the NF-κB pathway and amplified production of pro-inflammatory adipokines also bring about a decline in the levels of anti-inflammatory adipokines (adiponectin and omentin-1) in T2D patients [Palit *et al.,* 2020; Rathwa *et al.,* 2019]. A similar observation for the anti-inflammatory cytokines (apelin, IL-10 etc.) has been made by other researchers [Zou *et al.,* 2008]. Furthermore, there is the possible involvement of angiotensin convertase enzyme (ACE) I/D polymorphisms in the same population [Dwivedi *et al.,* 2008]. The ACE 'D' allele is related to increased angiotensin II [Alsafar *et al.,* 2015], which may further diminish the adiponectin levels. Additionally, circadian rhythm also regulates metabolic processes of adipose tissue [Gómez-Santos et al., 2009; Johnston *et al.,* 2012] which are mediated by melatonin's action on VAT receptors or via the sympathetic nervous system [de Farias et al., 2015; Vriend *et al.,* 2015]. Decreased plasma melatonin levels are observed in T2D patients [Patel *et al.,* 2018]. Apparently, in this context, the reduced levels of the anti-inflammatory adipokine-vaspin seen in T2D patients could be a direct or indirect consequence of reduced melatonin levels. A summary depicting the possible role of *vaspin* polymorphisms and their altered transcript and protein levels in obesity and dyslipidemia associated with T2D is shown in Figure 4.5. This is the first study ascribing an association between *vaspin* rs2236242 A/T polymorphism and its plasma protein levels with metabolic parameters of the targeted population. Our findings are suggestive of ethnic differences being one of the essential contributors to the progression of T2D. Thus, our results open new avenues to understand the role of vaspin in obesity induced T2D.

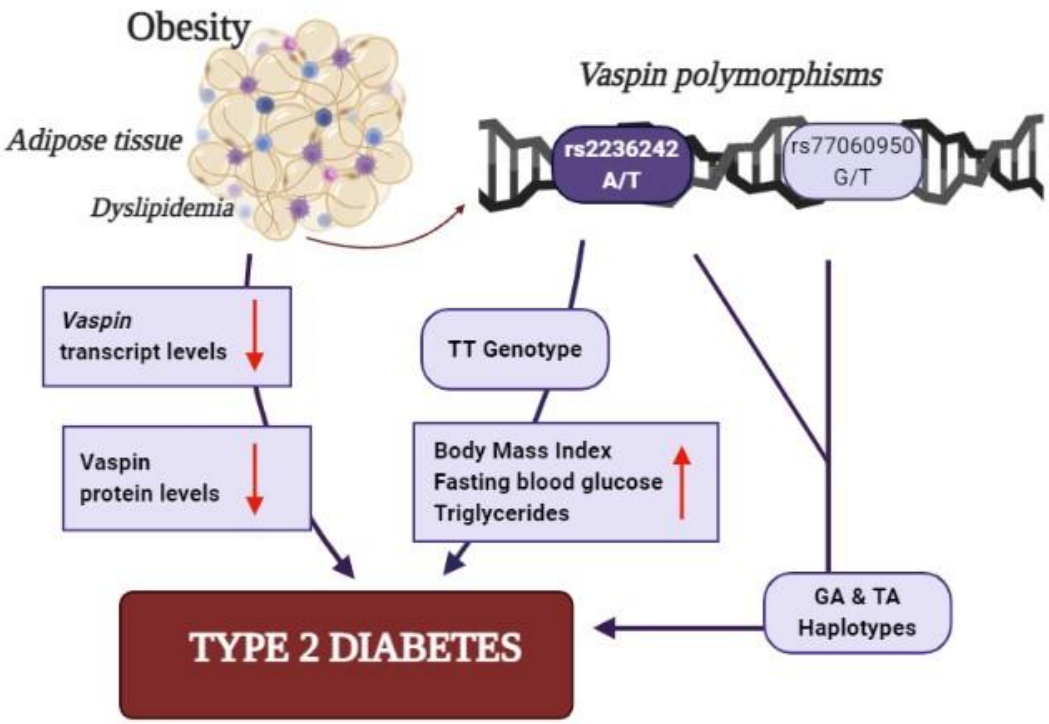

Figure 4.5. The role of vaspin in obesity-induced T2D. *Vaspin* rs2236242 A/T is associated with the risk of T2D; homozygous TT genotype increases the risk of T2D by 3.087-fold. GA and TA haplotypes are related to the risk of T2D. *Vaspin* rs2274907 TT genotype is associated with the metabolic profile (FBG, BMI and TG). The reduced *vaspin* transcript and plasma vaspin protein levels further contribute to T2D pathophysiology.

References

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