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Association of insulin resistance and leptin receptor gene polymorphism in type 2 diabetes mellitus

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Abstract

Background: Type 2 diabetes mellitus (T2DM) is a chronic disease that is characterized by impaired glucose metabolism and insulin resistance. The objectives of the study were to evaluate the pattern of leptin receptor gene polymorphism Gln223Arg in T2DM and to identify its association with the serum leptin and insulin levels as well as with insulin resistance in diabetes.

Methods: In this cross-sectional study, genotyping of leptin receptor was done for Gln223Arg alleles by PCR-restriction fragment length polymorphism in 39 patients with type 2 diabetes. Serum leptin and insulin levels were assayed using enzyme linked sorbent assay in 39 cases and 45 nondiabetic controls. Insulin resistance was calculated by the homeostasis model assessment of insulin resistance (HOMA-IR) formula. Statistical analysis was performed with Graph pad Instat version 3.

Results: Hardy–Weinberg Equilibrium for the leptin receptor (*LEPR*) gene variants showed that alleles were in equilibrium. Leptin levels were insignificantly low in patients with diabetes compared to those in controls. Women in the control group showed significantly higher leptin levels ($p < 0.05$) compared with men. There was a significant difference in the serum insulin levels and insulin resistance (HOMA-IR) among patients with different genotypes ($p = 0.04$ and $p = 0.0378$, respectively).

Conclusion: Leptin receptor gene polymorphism affected glucose metabolism by altering insulin resistance and pancreatic beta cells. Thus, single-nucleotide polymorphism of *LEPR* may affect the pathogenesis of T2DM.

Keywords: Diabetes mellitus; Insulin resistance; Leptin receptor; Single-nucleotide polymorphism.

1. INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic disease that is characterized by impaired glucose metabolism owing to insulin resistance. Leptin is an important adipokine hormone, which is essential in carbohydrate metabolism and affects insulin sensitivity and, hence, diabetes mellitus.

Leptin has been reported to be involved in the pathogenesis of diabetes.[1] It has been determined that leptin reduces insulin synthesis and secretion and increases hepatic extraction of insulin. It has also been determined that leptin increases insulin sensitivity. Few studies have examined the association between leptin receptor gene polymorphism and insulin resistance.

Few studies have reported that diabetes mellitus is not associated with leptin levels,[2–4] whereas some studies have reported a significant positive association between plasma leptin levels and diabetes.[5–8] However, contradictory reports are also available.[9,10] Some studies have established the association between plasma leptin levels and diabetes mellitus only in men.[6–8] Previous studies examining the association between serum leptin levels and diabetes mellitus were restricted to specific racial/ethnic groups and were not consistent in their findings.[11]

Leptin binds with a leptin receptor (*LEPR*) that is located on pancreatic beta cells to regulate insulin secretion.[12,13] The *LEPR* gene, which is located on chromosome 1p31, spans more than 70 kb, contains 20 exons and 19 introns, and encodes for 1165 amino acids. The *Gln223Arg* gene polymorphism of the *LEPR* gene is caused by the substitution of adenine at the 668th position by guanine and mutation of the 223rd amino acid, arginine, which is replaced by glutamine. This mutation is located on the sixth exon of the protein and may sufficiently impair signal transduction to increase susceptibility to T2DM.[14] Although many studies on the relationship between the *LEPR Gln223Arg* gene polymorphism and T2DM have been conducted in the Chinese population, the reported results are contradictory. Ying et al have reported the A allele of *LEPR Gln223Arg* gene polymorphism to be associated with an increased risk of T2DM.[15] In contrast, Fang et al have determined that the G allele of *LEPR Gln223Arg* gene polymorphism is a risk factor for T2DM.[16] Shi et al have obtained similar results.[17] Insulin resistance is essential for the development of T2DM. Clinical study reports suggest that elevation of leptin levels occurs owing to the upregulation of the leptin gene owing to insulin resistance and hyperinsulinemia.[18] It has been reported that leptin affects whole-body insulin sensitivity by regulating insulin-mediated glucose metabolism by skeletal muscle as well as hepatic regulation of gluconeogenesis.[19,20] Leptin has been determined to have an inhibitory effect on insulin secretion.[21] Thus, it is justifiable to study the association of

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leptin gene polymorphism, circulating leptin levels, and insulin resistance in T2DM. The relationship between polymorphisms of leptin and its receptor with T2DM is well established.[22–24] However, few studies have been conducted in India.

Leptin can improve insulin resistance or conversely it may induce insulin resistance. Leptin and insulin have been reported to have antagonistic actions. Thus, the expression of leptin may considerably affect the pathogenesis of diabetes. There are not many studies that establish the role of leptin and polymorphism of its genes in the pathogenesis of T2DM.

2. METHODS

2.1. Study design

This prospective cross-sectional study was conducted at the Justice KS Hegde Charitable Hospital, Mangalore, India. A total of 39 T2DM patients, diagnosed according to American Diabetic Association 2018 criteria, were recruited as cases. The majority of patients were on oral hypoglycemic agents such as second-generation sulfonylureas (glibenclamide/glimepiride), metformin, and thiazolidinediones (pioglitazone). A total of 45 nondiabetes patients, not suffering from any major illnesses, who attend hospital for regular checkups were used as controls.

Patients with diabetes with complications or other associated comorbidities (eg, hypertension, obesity, hyperlipidemia, and obstructive sleep apnea) were excluded.

Institutional ethics committee approval was obtained (INST.EC/EC/027/2019-20), and written informed consent was taken from patients.

Patients who fulfilled the study criteria were recruited. A total of 5 mL of venous blood samples, including 2-mL ethylene diamine tetra acetic acid (EDTA) sample for genotyping and 3-mL plain sample for assay of serum fasting insulin and leptin, were obtained.

2.2. Laboratory investigations

Serum leptin and fasting insulin levels were estimated using commercially available enzyme linked sorbent assay kits using blood samples collected in plain vials.

Insulin resistance was calculated by homeostasis model assessment (HOMA).

Homeostasis model assessment of insulin resistance (HOMA-IR) = fasting glucose × fasting insulin/22.5; insulin level is expressed in µIU/L; glucose level is expressed in mmol/L.

2.3. Genetic study

2.3.1. Blood sample collection for genetic analysis

A total of 3 mL of venous blood samples was collected in an EDTA (2%) vial. EDTA blood was used for DNA extraction and genotyping. Genomic DNA from whole blood was isolated using the phenol–chloroform extraction and ethanol precipitation method. The quality of DNA was checked and quantified.

2.3.2. Amplification and genotyping of gene polymorphism

Genotyping of all genes was confirmed by PCR-restriction fragment length polymorphism (RFLP).

2.3.3. Amplification and genotyping of the leptin receptor

PCR was performed using forward and reverse primers that are suitable for leptin receptor Gln223Arg alleles. The final product was digested with restriction enzyme, MspI. The reaction mixtures were electrophoresed on 2% agarose gel and visualized by ethidium bromide staining. The details of primers and restriction enzymes used are shown in Table 1.

2.4. Statistical analysis

Statistical analysis was performed using Graphpad Instat, version 3. Hardy–Weinberg Equilibrium (HWE) for the *LEPR* gene variant between cases was performed, and a comparison of the distribution of allele frequencies between different variants was performed using the chi-square test.

Student's unpaired "t" test was used to compare leptin levels in cases and controls. One-way analysis of variance (ANOVA) was used to compare leptin levels between different genotypic variants. Pearson's correlation was used to identify the correlation between leptin levels and fasting blood glucose values. The Kruskal–Walis test followed by post hoc Dunn's test was used to compare insulin and insulin resistance in individuals with diabetes of different genotypes because those parameters had skewed distribution. The "p" value <0.05 was regarded as statistically significant.

3. RESULTS

The distribution of genotypes and alleles of *LEPR* (rs1137101) gene variants in T2DM patients is shown in Table 2. None of the genotype frequency distributions for rs1137101 variants significantly deviated from HWE in T2DM cases ($p > 0.05$), which suggested that alleles were in equilibrium (Fig. 1A, 1B).

Serum leptin levels were insignificantly low in patients with diabetes compared to those in controls, as shown in Table 3. There was no significant gender difference in leptin levels among patients with diabetes; whereas in the control group, serum leptin levels were significantly higher in women compared to those in men (Table 4).

One-way ANOVA test results showed that there was no significant difference in leptin levels between different variants, $p = 0.4356$ (Fig. 2). Odds ratio was calculated for leptin levels among AG+GG variants and AA genotypic variants. Leptin levels were 1.2 times higher among AG+GG genotypic variants compared to those in AA genotypes (Table 5).

Pearson's correlation showed an insignificant negative correlation between fasting blood sugar (FBS) and serum leptin levels, r = – 0.190 and $p = 0.286$.

In addition, it was observed that there was a significant difference in serum insulin levels, HOMA-IR, HOMA-1%B, and

Table 1

Information about PCR-RFLP for gene *LEPR*

SNP	Location (base change)	Forward primer, reverse primer	PCR program (35 cycles)	Length (bp)	Restriction enzyme, incubation temperature	Allele: RFLP fragment size
LEPR (rs1137101)	Exon 6 (A > G)	5′-AAACTCAACGACACT CTCCTT-3′ 5′-TGAACTGACATTAGA GGTGAC-3′	93°C, 45′, 57°C, 30′, 72°C, 30′	80	MspI, 37°C	Allele A:80 Allele G:59 + 21

LEPR = leptin receptorl; RFLP = restriction fragment length polymorphism; SNP = single-nucleotide polymorphism.

Table 2

HWE for the *LEPR* gene

Gene variant	Frequency (%)	Chi-square value
AA	11 (28%)	0.8434
AG	22 (56%)	
GG	06 (16%)	

AA = Homozygous dominant
•Expected—12.41.
•Observed—11.
AG = Heterozygous
•Expected—19.18.
•Observed—22.
GG = Homozygous recessive
•Expected—7.41.
•Observed—6.
Frequency range: "A"' allele—0.564; "G" allele—0.436.
HWE = Hardy–Weinberg Equilibrium; *LEPR* = leptin receptor.

Table 3

Comparison of parameters in patients with diabetes and nondiabetic patients

Parameter	T2DM (n = 39)	Nondiabetes patients (n = 45)	*p*
Age (y)	55.85 ± 2.39	53.06 ± 2.35	0.066
BMI (kg/m^2)	22.23 ± 1.87	21.22 ± 1.24	0.678
Blood pressure:			
Systolic	126.2 ± 2.6	124.4 ± 2.4	0.122
Diastolic	79.7 ± 3.4	78.3 ± 2.8	0.54
Lipid profile:			
Cholesterol (mg/dL)	169.04 ± 27.36	164.68 ± 15.61	0.42
Triglyceride (mg/dL)	136.7 ± 22.34	135.22 ± 17.18	0.5
HDL (mg/dL)	39.66 ± 10.17	52.02 ± 5.5	0.07
LDL (mg/dL)	91.53 ± 16.32	90.16 ± 12.4	0.072
FBS (mg/dL)	164.81 ± 9.25	92.2 ± 3.83	0.0001*
Leptin (ng/mL)	30.41 ± 3.29	34.37 ± 4.28	0.46

**p* highly significant.
FBS = fasting blood sugar; HDL = high density lipoprotein; LDL = low density lipoprotein; T2DM = type 2 diabetes mellitus.

Table 4

Gender differences in serum leptin levels

Subjects	Leptin levels (ng/mL)		*p*
	Men	Women	
T2DM cases (29,10)	29.96 ± 3.89	35.98 ± 7.7	0.45
Nondiabetes patients (32,13)	28.76 ± 4.6	48.17 ± 8.67	0.039*

**p* value significant.
T2DM = type 2 diabetes mellitus.

HOMA B cell between three different allelic variants of *LEPR* gene, as shown in Table 6.

It was observed that serum insulin levels were significantly higher in patients with AA genotype compared to those in patients with AG. HOMA-IR, HOMA-1%B, and HOMA B cells were very significantly high in AA genotypes as compared to AG genotypes. These parameters were significantly high in AG genotypes compared to those in GG genotypes, as shown in Table 7.

3.1. Data availability statement

Individual de-identified data (eg, the concentration of leptin, insulin, genetic pattern, and other laboratory investigations) can be shared, with the permission of the funding agency.

4. DISCUSSION

Our study results indicate the absence of significant association between leptin receptor gene polymorphism and serum leptin levels in people with diabetes. Few studies have analyzed the association of leptin receptor gene polymorphism and leptin levels in T2DM. However, there are studies that have separately investigated the *LEPR* gene polymorphism and leptin levels in T2DM.

A study by Ying et al which was conducted on the Chinese population, investigated the association between *LEPR* Gln223Arg gene polymorphism and T2DM.[15] The abovementioned study has reported that the "A" allele of *LEPR* Gln223Arg gene polymorphism is associated with an increased risk of T2DM.[15] In contrast, Fang et al have determined that the G allele of *LEPR* Gln223Arg gene polymorphism is a greater risk factor for T2DM.[16] Shi et al have also reported similar results.[17]

Meta-analysis by Li et al has shown that single-nucleotide polymorphism of leptin receptor gene, *LEPR* Gln223Arg, was associated with an increased risk of T2DM in the Chinese population. The abovementioned study has suggested that Chinese carriers of the G allele of *LEPR* Gln223Arg gene polymorphism may be more susceptible to T2DM than the general population.[25] Our results suggest that individuals with "AA" genotypes may be associated with diabetes because they have hyperinsulinemia and insulin resistance (Tables 6 and 7).

Our study showed insignificantly low leptin levels among patients with diabetes, without gender differences (Table 3 and 4). However, nondiabetic women had significantly higher leptin levels compared to men (Table 4). Patients with diabetes with the GG variant of *LEPR* had the highest leptin levels, and AG variant had the lowest levels, although they were statistically insignificant (Fig. 2).

The reports on leptin levels in patients with diabetes are inconclusive; some studies report lower serum leptin in patients with diabetes.[26–29] However, there are reports that suggest higher serum leptin in patients with diabetes.[30–32]

Relatively few studies have examined the putative association between leptin and diabetes, and their results are inconsistent. Some studies have reported that there is no association between plasma leptin levels and diabetes,[2–4] whereas other studies have reported a significant positive association between plasma leptin levels and diabetes.[1,6–8] However, two studies have reported an inverse relationship between the two groups.[9,10] Few studies have reported that the association between plasma leptin and diabetes mellitus is observed only in men and not in women.[6–8]

An insignificant association was observed between leptin levels and fasting blood glucose. Contradictory reports are available, which suggest that a significant positive correlation is found between serum leptin and FBS.[33] The above mentioned study also suggests that serum leptin levels are significantly ($p < 0.05$) higher in diabetics compared to those in diabetics.

Type 2 diabetes is characterized by insulin resistance, which is positively associated with hyperleptinemia.[34] Insulin induces dose-dependent leptin synthesis.[35] Hence, in the presence of insulin resistance and chronic hyperinsulinemia, patients with type 2 diabetes are expected to exhibit hyperleptinemia.

In our study, significant differences in insulin levels, HOMA-IR, HOMA1-%B cell, and HOMA-B cell were observed in different genotypes (ie, AA, AG, and GG) among patients with diabetes. Insulin levels, HOMA-IR, HOMA1-%B cell, and HOMA-B cell were significantly higher in patients with diabetes with AA allele compared to those with AG. HOMA1-%B cell and HOMA-B cell were significantly higher in patients with diabetes with AG compared to those with GG alleles. HOMA β-cell is a calculated variable, which indicates insulin activity. It is a marker of basal insulin secretion of pancreatic β-cells.[36]

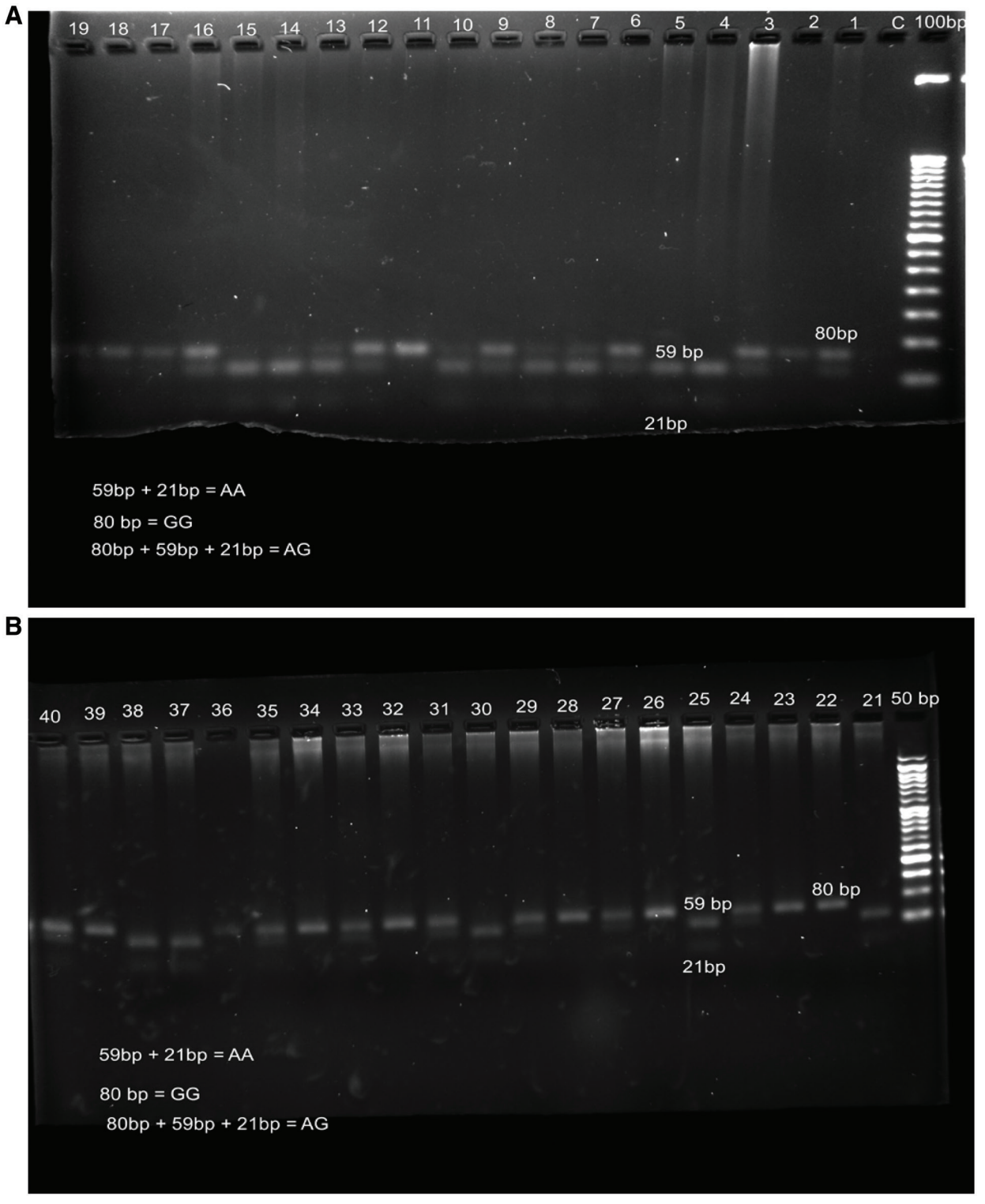

Fig. 1 A, Image of PCR-RFLP of T2DM—cases 1–20. B, Image of PCR-RFLP of T2DM—cases 21–40. RFLP = restriction fragment length polymorphism; T2DM = type 2 diabetes mellitus.

Leptin acts through its receptor, *LEPR*. Single-nucleotide polymorphism of *LEPR* may result in altered leptin functions. Normally, leptin decreases insulin synthesis and its secretion by B cells of the pancreas. In addition, it increases insulin extraction and reduces insulin delivery. Leptin mediates inhibitory feedback during insulin secretion. Our study clearly shows that single-nucleotide polymorphism of the *LEPR* gene resulted in variation in leptin levels among different genotypes. Although the difference in leptin levels is insignificant, it resulted in significant differences in insulin, HOMA-IR, HOMA1-%B cell, and HOMA-B cell in patients with diabetes with different genotypes.

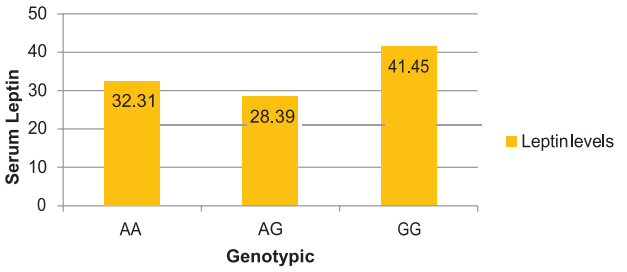

Fig. 2 Comparison of leptin levels between different genotypic variants.

Table 5

Odds ratio for genotypic variants and leptin levels

Leptin levels (ng/mL)	AG+GG variants	AA variants
High leptin	19	7
Normal leptin	9	4

Table 6

Comparison of parameters in different genotypes

Parameter	AA	AG	GG	*p*
Leptin (ng/mL)	32.31 ± 24.87	28.39 ± 41.45	41.45 ± 23.73	0.43
Insulin (μIU/L)	35.8 ± 42.9	11.2 ± 11.9	24.4 ± 24.6	0.04*
HOMA-IR	16.96 ± 18.77	3.6 ± 3.8	7.7 ± 6.9	0.018*
HOMA-1%B	181.39 ± 168.2	40.5 ± 60.75	157.5 ± 196.9	0.0078**
HOMA B cell	161.9 ± 152.38	36.05 ± 53.43	134.07 ± 164.65	0.007**

*p significant.
**p very significant.
HOMA-IR = homeostasis model assessment of insulin resistance.

Table 7

Post hoc test results for the comparison of parameters in different genotypes

Parameter	AA vs. AG	AG vs. GG	AA vs. GG
Insulin (μIU/L)	0.016*	0.069	0.56
HOMA-IR	0.0083**	0.38	0.38
HOMA-1%B	0.0045**	0.013*	0.06
HOMA B cell	0.0047**	0.0138*	0.97

*p highly significant.
**p very highly significant.
HOMA-IR = homeostasis model assessment of insulin resistance.

Previous studies have shown that intra-cerebrovascular infusion of leptin improves hyperglycemia, hyperglucagonemia, hyperketonemia, and polyurethaning, which are caused by insulin deficiency in mice.[37] The activation of JAK-STAT, PI3K, and AMPK pathway, which overlap with those of insulin, contribute to the leptin-mediated decrease in insulin resistance.[38]

Leptin is positively correlated with insulin resistance both in individuals with diabetes and normal individuals.[39,40] Hypothalamic insulin and leptin signaling are essential for the regulation of glucose homeostasis and the development of insulin resistance.[41] In addition, leptin is essential for the pathophysiology of insulin resistance related to obesity.

It has been shown that the direct action of insulin and leptin on proopiomelanocortin neurons is required to maintain normal glucose homeostasis.[42] Thus, single-nucleotide polymorphism of leptin receptor gene results in altered leptin levels, which in turn alters insulin sensitivity. High HOMA-IR, HOMA1-%B cell, and HOMA-B cell in patients with diabetes can be attributed to altered leptin levels owing to the single-nucleotide polymorphism of *LEPR*.

The limitation of this study may be the use of single rather than multiple measurements of serum leptin (which are known to follow a circadian pattern) and the lack of detailed measurements of body fat and distribution.[43]

In conclusion, because leptin receptor gene polymorphism in T2DM has not been previously studied in India, this study sheds light on the pattern of polymorphism of *LEPR* gene in the Indian population. Our study did not show a direct association between T2DM and *LEPR* gene polymorphism. However, this study demonstrated hyperinsulinemia and insulin resistance in patients with "AA" genotypes of *LEPR*, which indicated a possible role of *LEPR* gene polymorphism and pathogenesis of diabetes mellitus.

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