Biochemistry and Molecular Biology
2022; 7(3): 54-60
http://www.sciencepublishinggroup.com/j/bmb
doi: 10.11648/j.bmb.20220703.11
ISSN: 2575-5064 (Print); ISSN: 2575-5048 (Online)

Association of Leptin Receptor Gene Polymorphisms with Blood Glucose Concentration and Obesity

Mo Mo Than*, Yan Naing Soe, Zaw Myo Lwin, Ye Wint Kyaw, Kyaw Thet Paing, Khine Kyaw Oo, Phyo Thaw Htun, Aung Lin Oo, Min Thein, Ye Myat Kyaw, Zaw Min Htut

Department of Biochemistry, Defence Services Medical Academy, Yangon, Myanmar

Email address:

momomoekyaw@gmail.com (Mo Mo Than), dr.yannaisoe@gmail.com (Y. N. Soe), zawmyolwin13sept@gmail.com (Z. M. Lwin), yewintkyaw4384@gmail.com (Ye W. Kyaw), alarm.1500@gmail.com (K. T. Paing), khinekyawoo@yahoo.com (K. K. Oo), pthawtun@gmail.com (P. T. Htun), aunglinoo.al8@gmail.com (A. L. Oo), kominthein@gmail.com (M. Thein), yemyatkyawdsmrc@gmail.com (Ye M. Kyaw), drhtutgyi77@gmail.com (Z. M. Htut)

*Corresponding author

To cite this article:

Mo Mo Than, Yan Naing Soe, Zaw Myo Lwin, Ye Wint Kyaw, Kyaw Thet Paing, Khine Kyaw Oo, Phyo Thaw Htun, Aung Lin Oo, Min Thein, Ye Myat Kyaw, Zaw Min Htut. Association of Leptin Receptor Gene Polymorphisms with Blood Glucose Concentration and Obesity. *Biochemistry and Molecular Biology*. Vol. 7, No. 3, 2022, pp. 54-60. doi: 10.11648/j.bmb.20220703.11

Received: June 22, 2022; **Accepted**: July 9, 2022; **Published**: July 18, 2022

Abstract: Obesity has become a global epidemic and is a known risk factor for several adverse health outcomes. Single Nucleotide Polymorphism (SNP) in leptin receptor genes become interesting candidates as susceptibility genes for obesity and glucose homeostasis. The present study intended to explore the genetic analysis of *LEPR* gene K109, Q223R, and K656N polymorphisms and their relation to obesity and fasting plasma glucose (FPG) concentration in the Myanmar population. One hundred and fifty diagnosed obese subjects and 150 healthy non-obese controls were included. Fasting plasma glucose (FPG) was measured and *LEPR* gene K109R, Q223R, and K656N polymorphisms were detected by DNA analysis. Data were analyzed by chi-square and one-way ANOVA tests. Each genotype frequency distribution of *LEPR* gene (K109R, Q223R, and K656N) polymorphisms was not associated with obesity ($p > 0.05$), as well as each allele frequency distribution also similar outcome ($p > 0.05$). FPG levels of the study population showed no significant differences between each genotype of *LEPR* gene polymorphisms ($p > 0.05$). The K109R, Q223R, and K656N polymorphisms of the *LEPR* gene were not linked to obesity or FPG levels in the population of Myanmar, according to our findings. Therefore, it does not seem that these polymorphisms have an equivalently significant role for the people of Myanmar. To completely understand the unique genetic variables that predispose to obesity in humans, an ongoing study of diverse obesity phenotypes and related gene mutations is necessary as our understanding of the genes causing obesity increases as a result of new findings.

Keywords: Obesity, Fasting Plasma Glucose, *LEPR* Gene K109R SNP, *LEPR* Gene Q223R SNP, *LEPR* Gene K656N SNP

1. Introduction

Obesity may be caused by a complex mechanism of genetics, socioeconomic, metabolic factors, and lifestyle variations [1]. Our understanding of the mechanisms governing energy balance and body weight has considerably improved as a result of the role of leptin and its receptor [2]. Leptin is a 167 amino acid protein that is secreted primarily from white adipose tissue into the bloodstream and can cross the blood-brain barrier [3]. Brown adipose tissue (BAT), mammary gland, placenta, skeletal muscle, stomach, and pituitary gland, all secrete leptin, but their relative contribution to total circulating leptin levels is minimal [4], leptin levels normally correspond with total body fat content apart from fasting period [5]. Although the circulating leptin hormone decreases appetite, obese people often have higher levels of circulating leptin than people of normal weight due to their higher body fat percentage [6]. Similar to how insulin resistance manifests in type 2 diabetes, these obese people exhibit resistance to leptin, with increased levels failing to regulate their appetite and body weight. Alterations to leptin receptor signaling, particularly in the arcuate nucleus of the hypothalamus, are a significant factor in leptin resistance; however, leptin receptor deficiency or significant changes to

the leptin receptor itself.

Type I cytokine receptors, which are a distinct class of cytokine receptors, include leptin receptors (LEPR) [7]. Six different types of leptin hormone receptors, known as ObRa–ObRf or LepRa–LepRf, interact by leptin hormone which is encoded by a single gene, *LEPR* [8]. The leptin receptor (*LEPR*) gene is located at chromosome 1p 31.3 between the anonymous microsatellite markers D1S515 and D1S198. The genomic structure of the human leptin receptor gene spans over 168 kilobase pairs and includes 20 exons. The first 2 exons are non-coding and are capable of altering several secondary structures whereas exon 3 to 17 encode extracellular cytokine motifs of LEPR, and the transmembrane LEPR region was encoded by exon 18. Exon 19 and 20 encode the intracellular domain of the receptor [9] (Figure 1). Single nucleotide changes of the leptin gene (resulting in a truncated protein) or the leptin receptor (*LEPR*) gene (resulting in premature termination of the intracellular domain) are responsible for the morbid obesity seen in mouse models of obesity (*ob*/*ob* and *db*/*db* mice, respectively) as well as in human obesity [10-13]. Several studies have shown that in most obese individuals, leptin levels are either normal or higher than in normal individuals. These results indicate that in obese individuals there may be leptin resistance. This resistance is supposed to play a role in the development of obesity because the excess amount of leptin cannot perform its role in controlling food intake, due to the presence of leptin receptor resistance [14]. In the pathogenesis of human obesity, it has been suggested that genetic changes at the leptin receptor locus and/or post-receptor abnormalities may be important [15].

Leptin regulates insulin secretion and its action through leptin receptors, developing peripheral insulin sensitivity and glucose absorption. The LEP and LEPR are critical in glucose metabolism. Leptin resistance occurs in obesity because the concentration of leptin is comparatively high and roughly equal to the extent of fat mass in the body. Leptin resistance disrupts the interaction between leptin and peripheral tissues that express leptin receptor protein, such as pancreatic beta-cells, which further contribute to the onset of type 2 diabetes mellitus and insulin resistance. Leptin receptor gene (*LEPR*) mutations promote the development of shortened receptors, which have been linked to type 2 diabetes and obesity in humans and rats [16].

Leptin receptor resistance may be due to mutations in the leptin receptor (*LEPR*) gene. The human *LEPR* gene has been found to have some common polymorphisms, and many populations have evaluated their possible links with obesity. An example of these polymorphisms was the single nucleotide substitutions which cause a single amino acid substitution, three of the common polymorphisms are Lys109Arg (K109R), Gln223Arg (Q223R), and Lys656Asn (K656N) (Figure 1) [17].

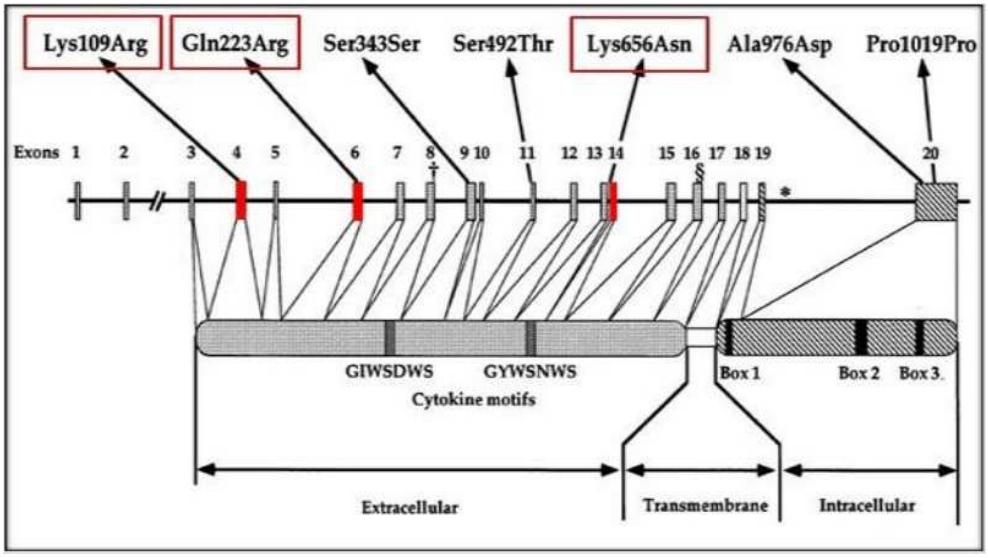

Figure 1. *Schematic representation of the leptin receptor gene (upper panel) and its protein structure (lower panel) [9].*

The human *LEPR* gene has been found to contain both common polymorphisms and unusual mutations, and studies in various populations have investigated the potential links between these polymorphisms and obesity. Even though two linkage or association studies discovered strong correlations between various body weights and *LEPR* gene polymorphisms [18-20], some studies observed the opposite results [21-23]. The different populations may be one cause of contradictory findings among some studies related to this topic.

Restriction fragment length polymorphisms (RFLP) were used to identify amino acid substitutions in exons 4, 6, and 14 of the *LEPR* gene (K109R, Q223R, and K656N polymorphisms, respectively) to test for associations with blood glucose concentration and body weight variables from a genetically homogeneous Myanmar population. The present study evaluated the potential contributions of these variables to the variability of obesity, an analysis that found that these variables may have a role in the above conflict. It was expected to highlight the high-risk genotypes for leptin

resistance individuals with obesity and impaired glucose concentration.

2. Materials and Method

This case-control study was conducted in the Common Research Laboratory of the Defence Services Medical Academy. The research participants have included the Mingalardon Cantonment Area of Yangon from January 2019 to June 2020. The obese participants were recruited by the WHO classification of BMI [24]. This study included 150 obese people (both sexes) and 150 non-obese volunteers who were age and sex-matched. The inclusion criteria for obese subjects were 18 to 60 years of age and BMI ≥ 30 kg/m^2 and for non-obese participants were BMI < 30 kg/m^2. The potential participants with a history of heart disease, liver disease, kidney disease, severe acute illness, diabetes mellitus (DM) were excluded from the study. After being informed of the purpose and procedure of the study, written informed consent was obtained from those who agreed to participate in this study. The study protocol was approved by the Ethical Review Committee of the Institute. Then, the history taking, physical examination, anthropometric measurement, and collecting blood specimens were done from both groups of participants.

The six mL of 8-hr overnight fasting venous blood sample was collected. In this total 6 mL of the blood sample, 3 mL was collected in the sodium fluoride (NaF) tube for plasma glucose estimation and 3 mL was collected in EDTA tube for DNA analysis. Plasma was separated from NaF tube by centrifuged with 2000 rpm for 2 minutes within 3 hours and stored at -20°C for estimation of plasma glucose in batches. EDTA tube blood was also stored at -20°C for DNA analysis in batches. Fasting Plasma Glucose (FPG) was analyzed using hexokinase enzymatic endpoint method by Roche Cobas C 111, Switzerland.

For DNA analysis, DNA was isolated by the phenol-chloroform method. DNA was amplified using the following oligonucleotide primers (Macrogen, USA) by polymerase chain reaction (PCR); for *LEPR* gene K109R SNP: (Forward primer: 5′-TTTCCACTGTTGCTTTCGGA-3′) (Reverse primer: 5′- AAACTAAAGAATTTACTGTTGAAACAAA TGGC-3′); for *LEPR* gene Q223R SNP: (Forward primer: 5′-AAACTCAACGACACTCT CCTT-3′) (Reverse primer: 5′-TGAACTGACATTAGAGGTGAC-3′), for *LEPR* gene K656N SNP: (Forward primer: 5′-ACAACTTGTCATTTTGCAGTTCCTA-3′) (Reverse primer: 5′-CCAAAGTAAA GTGACATTTTTCGC-3′).

The *LEPR* gene PCR products were digested; with *Hae*III endonuclease (Thermo Fisher Scientific, USA) for *LEPR* gene K109R SNP detection; with *Msp*I endonuclease (Thermo Fisher Scientific, USA) for *LEPR* gene Q223R SNP detection, and; with *Bst*UI endonuclease (Thermo Fisher Scientific, USA) for *LEPR* gene K656N SNP detection overnight at 37°C respectively. The digested products were separated by 5% agarose gel electrophoresis. The lengths of each digested DNA fragment were determined by comparing the migration of a standard DNA marker (Thermo Fisher Scientific, USA). After electrophoresis, the separated bands of DNA were visualized under UV light, and genotypes were determined by comparing the migration of 50 bp (base pairs) DNA step ladder.

The descriptive characteristics of the group variables were expressed as mean ± standard deviation. Statistical analysis was carried out using SPSS statistical software program (version 20.2, SPSS, Chicago, USA). The allelic and genotypic frequencies were calculated by the direct gene counting method and a chi-square (χ^2) test was used for comparing the allele and genotype frequencies between groups. The level of significance was defined at a value of $p < 0.05$.

3. Results

The descriptive statistics for the different phenotypic variables in this study for males and females are shown in Table 1. The mean age of obese group was 46.88 ± 8.09 years and that of control group was 44.39 ± 9.92 years. The obese male was 49% and the obese female was 51% and 57% and 43% in non-obese group. The demographic variables and biochemical parameters were statistically significantly different ($p < 0.05$) between the two groups. The present study was in line with the Hardy-Weinberg principle on the genotype and allele frequencies of *LEPR* gene in this population. The study population remained constant from generation to generation in the absence of other evolutionary influences (Table 2). A statistically significant association was not found between each genotype frequencies distribution of *LEPR* gene polymorphisms and obesity ($p > 0.05$) and also no significant relationship between each allele frequencies distribution and obesity ($p > 0.05$) (Table 3). Each genotype frequencies distribution of polymorphisms and FPG was not statistically significant relationship too ($p > 0.05$) (Table 4). In the comparison between normal and impaired FPG of different genotypes of *LEPR* gene polymorphisms, no statistically significant relation between FPG and different genotypes ($p > 0.05$) (Table 5) was noted.

Table 1. *Demographic measures and Biochemical values.*

Characteristics	Obese (n = 150)	Non-obese (n = 150)	Statistic	
	Mean± SD	Mean± SD	*t*	*p*-value
Age (years)	46.88±8.09	44.39±9.92	0.22	0.820
BMI (kg/m^2) *	32.48±3.03	23.63±3.30	22.48	0.001
FPG (mg/dl) *	114.51±21.54	85.27±25.86	9.22	0.001

* $p < 0.05$.

Table 2. *Hardy-Weinberg equilibrium (HWE) of the genotype frequency distribution of LEPR gene polymorphisms in the study population.*

***LEPR* gene polymorphisms**	**Genotypes**	**Expected (n = 300)**	**Observed (n = 300)**	**HWE**	
				χ^2	***p*-value**
K109R	AA	49 (16.4%)	42 (14.0%)	2.91	0.23
	AG	145 (48.2%)	158 (52.7%)		
	GG	106 (35.4%)	100 (33.3%)		
Q223R	AA	12 (3.8%)	5 (1.7%)	4.66	0.1
	AG	95 (31.5%)	108 (36.0%)		
	GG	193 (64.6%)	187 (62.3%)		
K656N	GG	269 (89.9%)	268 (89.3%)	0.76	0.68
	GC	30 (9.9%)	32 (10.7%)		
	CC	1 (0.3%)	0 (0.0%)		

χ^2 = Chi square test, $p < 0.05$ = Significant.

Table 3. *Genotype frequency distribution of LEPR gene polymorphisms in the study population.*

***LEPR* gene polymorphisms**		**Obese (n = 150)**	**Non-obese (n = 150)**	**Statistic**	
				χ^2	***p*-value**
K109R	Genotypes			1.31	0.52
	AA	16 (10.7%)	26 (17.3%)		
	AG	85 (56.7%)	73 (48.7%)		
	GG	49 (32.7%)	51 (34.0%)		
	Alleles			0.04	0.84
	A	117 (39.0%)	125 (41.7%)		
	G	183 (61.0%)	175 (58.3.0%)		
Q223R	Genotypes			2.25	0.32
	AA	3 (2.0%)	2 (1.3%)		
	AG	60 (40.0%)	48 (32.0%)		
	GG	87 (58.0%)	100 (66.7%)		
	Alleles			1.92	0.16
	A	66 (22.0%)	52 (17.3%)		
	G	234 (78.0%)	248 (82.7%)		
K656N	Genotypes			2.75	0.09
	GG	129 (86.0%)	139 (92.7%)		
	GC	21 (14.0%)	11 (7.3%)		
	Alleles			2.59	0.11
	G	279 (93.0%)	289 (96.3%)		
	C	21 (7.0%)	11 (3.7%)		

χ^2 = Chi square test, $p < 0.05$ = Significant.

Table 4. *Comparison of different genotypes of LEPR gene polymorphisms according to FPG.*

LEPR gene polymorphisms	**Fasting Plasma Glucose level (mg/dl) (Mean ± SD)**			**Statistic**	
	Wild	**Heterozygous**	**Mutant**	**F**	**p-value**
K109R	AA (n = 42) 99.12 ± 29.28	AG (n = 158) 99.79 ± 26.48	GG (n = 100) 102.37 ± 29.74	0.032	0.969
Q223R	AA (n = 5) 100.17 ± 25.79	AG (n = 108) 99.41 ± 26.49	GG (n = 187) 100.15 ± 28.85	0.024	0.976
K656N	GG (n = 268) 98.96 ± 27.38	GC (n = 32) 107.68 ± 31.37	CC (n = 0) -	2.807	0.095

$p < 0.05$ = Significant.

Table 5. *Association between different genotypes of LEPR gene polymorphisms and FPG.*

***LEPR* gene polymorphisms**	**Genotypes**	**Normal FPG**	**Impaired FPG**	**Statistic**	
				χ^2	***p*-value**
K109R		(n = 243)	(n = 57)	3.43	0.48
	AA	37 (15.3%)	2 (3.1%)		
	AG	128 (52.6%)	34 (59.2%)		
	GG	78 (32.1%)	21 (37.4%)		

LEPR gene polymorphisms	Genotypes	Normal FPG	Impaired FPG	Statistic	
				χ^2	*p*-value
Q223R		(n = 245)	(n = 55)	2.1	0.35
	AA	4 (1.6%)	3 (4.5%)		
	AG	83 (33.9%)	21 (38.6%)		
	GG	158 (64.6%)	31 (56.7%)		
K656N		(n = 243)	(n = 57)	0.48	0.49
	GG	216 (88.8%)	53 (93.5%)		
	GC	27 (11.0%)	4 (7.4%)		

χ^2 = Chi square test, $p < 0.05$ = Significant.

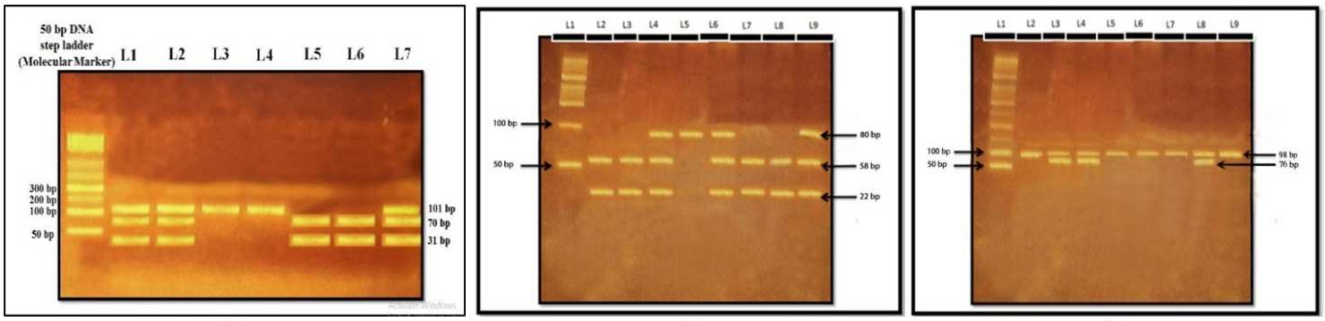

Figure 2. *Electrophoresis patterns of Genotyping result for (A) LEPR gene K109R SNP, (B) LEPR gene Q223R SNP and, (C) LEPR gene K656N SNP.*

4. Discussion

In this study, we investigated the effects of three common exonic polymorphisms in the *LEPR* gene, namely the K109R, Q223R, and K656N polymorphisms, on obesity and blood glucose concentration in the Myanmar population. According to the calculation of our result data, there was no significant association between obesity and each genotype frequency distribution of *LEPR* gene polymorphisms ($p > 0.05$), as well as each allele frequency distribution ($p > 0.05$). The findings of our study revealed that *LEPR* gene polymorphisms were neither related to obesity nor FPG levels in the Myanmar population. Our findings agreed with the studies of some ethnic populations such as Malaysian [21], Indian [22], Japanese [18], and Korean [25] populations. Nonetheless, the results of our study were inconsistent with the reports of some studies on the Brazilian [19], Romanian [26], and Caucasian [20] populations. These discrepancies reports help to deduce that the Oriental population has similar reports rather than the Western population related to these polymorphisms.

All three polymorphisms are linked to amino acid changes in the LEPR's extracellular region and may have functional implications. In exon 4 of *LEPR* gene K109R SNP, an A to G transition of codon 109 (AAG to AGG) may cause the changing amino acid lysine (Lys/K) into arginine (Arg/R) of the leptin receptor protein. Similarly, glutamine (Gln/Q) is changed to arginine (Arg/R) in the receptor due to A to G (CAG to CGG) transition of codon 223 in the exon 6 region of *LEPR* gene Q223R SNP. The K656N SNP is located in exon 14 of *LEPR* gene. Lysine amino acid (Lys/K) is changed to asparagine amino acid (Asn/N) because of a transition of G to C (AAG to AAC) in codon 656 of *LEPR* gene in this SNP [17].

Since obesity was a multifactorial major global epidemic problem, this difference could be a result of the interplay between various risk factors (both genetic and environmental), which very often provide a variety of results in different ethnicities populations. Although this alteration produces amino acid changes and hence may have functional consequences, its alteration in functionality is not evidence clear. There was no evidence of the possible functional implications of nucleotide alterations in *LEPR* gene, yet it was likely that these variants serve as genetic markers for nearby functional variants which are in linkage disequilibrium with our selected SNPs [27]. So, in our study population, there is no complex heterozygotic process including these three SNPs that predispose to obesity. The report of Matsuoka and coworkers' study suggests that a missense mutation in one allele of the *LEPR* gene may cause obesity in combination with an unidentified and/or unstudied modification in the other allele. However, our investigation did not rule out any other potential polymorphisms [18].

The mechanism for the regulation of blood glucose level is a complex mechanism with multiple hormones that acts on multiple organs. Leptin hormone is one of the hormones involved in the regulation of blood glucose levels. Our study explored the relationship of *LEPR* gene SNPs with blood glucose level via the action of leptin hormone on its receptor. As the result, the mean FPG levels of the study population showed no significant differences between each genotype of *LEPR* gene polymorphisms ($p > 0.05$). The finding of our study elucidated that our interested SNPs of *LEPR* gene were not associated with the regulation of blood glucose concentration in the Myanmar population. The finding of our study was in agreement with the meta-analysis study of Yunzhong Yang and Tianhua Niu from Tulane University School Medicine, USA [28].

The potential limitation of our study was not a large sample size but the sample size of our study was a minimum number of requirements and it was enough number to conduct the study. The next limitation was that we could not explore leptin (*LEP*) gene polymorphisms and the leptin hormone concentration and consequently, we could not suppose that the abnormality of leptin hormone production may be or not in the case of leptin resistance related to obesity. The other genotype and allelic variations in coding and non-coding sequences of the *LEPR* gene have also been reported, some of which result in silent variations or represent rare mutations [17, 18, 29]. These were not selected in our study because they were unlikely to have any functional consequences and limitations of resources available in our condition. Further studies should be performed on long-term outcome data and other SNP in the leptin gene and leptin receptor gene which would help assess the utility of the genetic marker in risk stratification.

5. Conclusion

Our results support the idea that in a Myanmar community, the *LEPR* gene variants K109R, Q223R, and K656N are not associated with obesity or FPG levels. Therefore, it does not seem that these polymorphisms have an equivalently significant role for the people of Myanmar. To completely understand the unique genetic variables that predispose to obesity in humans, an ongoing study of diverse obesity phenotypes and related gene mutations is necessary as our understanding of the genes causing obesity increases as a result of new findings.

Conflict of Interest

The authors declared that there was no conflict of interest regarding the publication of this paper.

Acknowledgements

We would like to sincerely thank the Academic Board of Defence Services Medical Academy, Yangon, Myanmar for the grant to conduct this informative research study.

References

[1] Mantzoros, C. S. (1999). The role of leptin in human obesity and disease: a review of current evidence. *Annals of internal medicine*, *130* (8), 671-680.

[2] Friedman, J. M., & Halaas, J. L. (1998). Leptin and the regulation of body weight in mammals. *Nature*, *395* (6704), 763-770.

[3] Burguera, B., Couce, M. E., Curran, G. L., Jensen, M. D., Lloyd, R. V., Cleary, M. P., & Poduslo, J. F. (2000). Obesity is associated with a decreased leptin transport across the blood-brain barrier in rats. *Diabetes*, *49* (7), 1219-1223.

[4] Odle, A. K., Haney, A., Allensworth-James, M., Akhter, N., & Childs, G. V. (2014). Adipocyte versus pituitary leptin in the regulation of pituitary hormones: somatotropes develop normally in the absence of circulating leptin. *Endocrinology*, *155* (11), 4316-4328.

[5] Ahrén, B., Larsson, H., Wilhelmsson, C., Näsman, B., & Olsson, T. (1997). Regulation of circulating leptin in humans. *Endocrine*, *7* (1), 1-8.

[6] Considine, R. V., Sinha, M. K., Heiman, M. L., Kriauciunas, A., Stephens, T. W., Nyce, M. R.,... & Caro, J. F. (1996). Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *New England Journal of Medicine*, *334* (5), 292-295.

[7] Cirillo, D., Rachiglio, A. M., La Montagna, R., Giordano, A., & Normanno, N. (2008). Leptin signaling in breast cancer: an overview. *Journal of cellular biochemistry*, *105* (4), 956-964.

[8] Lee, S., Lee, A., Kweon, O. K., & Kim, W. H. (2016). Presence and distribution of leptin receptor in the canine gallbladder.

[9] Bruce Thompson, D., Ravussin, E., Bennett, P. H., & Bogardus, C. (1997). Structure and sequence variation at the human leptin receptor gene in lean and obese Pima Indians. *Human molecular genetics*, *6* (5), 675-679.

[10] Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L., & Friedman, J. M. (1994). Positional cloning of the mouse obese gene and its human homologue. *Nature*, *372* (6505), 425-432.

[11] Chen, H., Charlat, O., Tartaglia, L. A., Woolf, E. A., Weng, X., Ellis, S. J.,... & Morgenstern, J. P. (1996). Evidence that the diabetes gene encodes the leptin receptor: identification of a mutation in the leptin receptor gene in db/db mice. *Cell*, *84* (3), 491-495.

[12] Clement, K., Vaisse, C., Lahlou, N., Cabrol, S., Pelloux, V., Cassuto, D.,... & Guy-Grand, B. (1998). A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature*, *392* (6674), 398-401.

[13] Montague, C. T., Farooqi, I. S., Whitehead, J. P., Soos, M. A., Rau, H., Wareham, N. J.,... & O'Rahilly, S. (1997). Congenital leptin deficiency is associated with severe early-onset obesity in humans. *Nature*, *387* (6636), 903-908.

[14] Oksanen, L., Kaprio, J., Mustajoki, P., & Kontula, K. (1998). A common pentanucleotide polymorphism of the 3′-untranslated part of the leptin receptor gene generates a putative stem-loop motif in the mRNA and is associated with serum insulin levels in obese individuals. *International journal of obesity*, *22* (7), 634-640.

[15] D'souza, A. M., Neumann, U. H., Glavas, M. M., & Kieffer, T. J. (2017). The glucoregulatory actions of leptin. Molecular Metabolism, 6 (9), 1052–1065.

[16] Bains, V., Kaur, H., & Badaruddoza, B. (2020). Association analysis of polymorphisms in LEP (rs7799039 and rs2167270) and LEPR (rs1137101) gene towards the development of type 2 diabetes in North Indian Punjabi population. *Gene*, *754*, 144846.

[17] Gotoda, T., Manning, B. S., Goldstone, A. P., Imrie, H., Evans, A. L., Strosberg, A. D.,... & Aitman, T. J. (1997). Leptin receptor gene variation and obesity: lack of association in a white British male population. *Human molecular genetics*, *6* (6), 869-876.

[18] Matsuoka, N., Ogawa, Y., Hosoda, K., Matsuda, J., Masuzaki, H., Miyawaki, T.,... & Nakao, K. (1997). Human leptin receptor gene in obese Japanese subjects: evidence against either obesity-causing mutations or association of sequence variants with obesity. *Diabetologia*, *40* (10), 1204-1210.

[19] Oliveira, R. D., Cerda, A., Genvigir, F. D. V., Sampaio, M. F., Armaganijan, D., Bernik, M. M. S.,... & Hirata, R. D. C. (2013). Leptin receptor gene polymorphisms are associated with adiposity and metabolic alterations in Brazilian individuals. *Arquivos Brasileiros de endocrinologia & metabologia*, *57*, 677-684.

[20] Masuo, K., Straznicky, N. E., Lambert, G. W., Katsuya, T., Sugimoto, K., Rakugi, H.,... & Esler, M. D. (2008). Leptin-receptor polymorphisms relate to obesity through blunted leptin-mediated sympathetic nerve activation in a Caucasian male population. *Hypertension Research*, *31* (6), 1093-1100.

[21] Fan, S. H., & Say, Y. H. (2014). Leptin and leptin receptor gene polymorphisms and their association with plasma leptin levels and obesity in a multi-ethnic Malaysian suburban population. *Journal of physiological anthropology*, *33* (1), 1-10.

[22] Murugesan, D., Arunachalam, T., Ramamurthy, V., & Subramanian, S. (2010). Association of polymorphisms in leptin receptor gene with obesity and type 2 diabetes in the local population of Coimbatore. *Indian journal of human genetics*, *16* (2), 72.

[23] Pyrzak, B., Wisniewska, A., Kucharska, A., Wasik, M., & Demkow, U. (2009). No association of LEPR Gln223Arg polymorphism with leptin, obesity or metabolic disturbances in children. *European journal of medical research*, *14* (4), 1-4.

[24] R. C. Weisell. (2002). Body mass index as an indicator of obesity, *Asia Pac. J. Clin. Nutr.*, vol. 11, pp. S681–S684.

[25] Kim, J. Y., Lee, H. B., Lim, S. H., Lee, B. W., Baik, H. H., Kim, Y. O.,... & Chung, J. H. (2011). Association between polymorphisms of leptin receptor and hypercholesterolemia, hypertension, and obesity in Korean population. *Stress*, *19* (2), 155-163.

[26] Mărginean, C. O., Mărginean, C., Voidăzan, S., Meliţ, L., Crauciuc, A., Duicu, C., & Bănescu, C. (2016). Correlations between leptin gene polymorphisms 223 A/G, 1019 G/A, 492 G/C, 976 C/A, and anthropometrical and biochemical parameters in children with obesity: a prospective case-control study in a Romanian population—the nutrichild study. *Medicine*, *95* (12).

[27] Lakka, T. A., Rankinen, T., Weisnagel, S. J., Chagnon, Y. C., Lakka, H. M., Ukkola, O.,... & Bouchard, C. (2004). Leptin and leptin receptor gene polymorphisms and changes in glucose homeostasis in response to regular exercise in nondiabetic individuals: the HERITAGE family study. *Diabetes*, *53* (6), 1603-1608.

[28] Yang, Y., & Niu, T. (2018). A meta-analysis of associations of LEPR Q223R and K109R polymorphisms with Type 2 diabetes risk. *PloS one*, *13* (1), e0189366.

[29] Chung, W. K., Power-Kehoe, L., Chua, M., Chu, F., Aronne, L., Huma, Z.,... & Leibel, R. L. (1997). Exonic and intronic sequence variation in the human leptin receptor gene (LEPR).