



Original Research Article

Genetic Variation of Leptin Hormones in Hemodialysis Patients in Thi-Qar Province/ Iraq

Article History:

Name of Author:

Ali Manhal Ibrahim¹, Mohammed Ajah Aouda², Kadhim M Manhil³

Affiliation: ¹Department of chemistry, College of sciences, university of Thi-Qar, Thi-Qar, 64001, Iraq

²university of Thi-Qar, college of sciences, Department of chemistry.

³university of Thi-Qar, college of Medicine.

Corresponding Author:

Mohammed Ajah Aouda, Kadhim M Manhil

Received: 12-11-2025

Revised: 05-12-2025

Accepted: 20-12-2025

Published: 30-12-2025

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-Noncommercial-Share Alike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

Abstract: Chronic kidney disease (CKD) is a complex, progressive chronic condition that is defined by either abnormalities of kidney structure or function present for ≥ 3 months, it is end-stage renal disease (ESRD). Either markers of kidney damage or decreased glomerular filtration rate may be present. dialysis procedures stimulate protein degradation and reduce protein synthesis; these responses persist following dialysis, which might lead to loss of muscle mass. Having a specific genetic polymorphism can be a predisposing factor to developing some diseases. This study aimed to investigate the linked between Leptin gene SNP with the development of CKD. Genomic DNA from blood samples were extracted by using DNA extraction kit (Geneaid, Uk), then Leptin gene was amplified through conventional PCR and SNP was discover using restriction enzyme SmaI. The present study recorded the CC and TC genotypes at 370 positions increased non-significantly in control group, while the TC genotype increased non-significantly in dialysis patients than control group. The frequency of C allele increased non-significantly in control group than dialysis patients, while the T allele increased non-significantly in dialysis patients than control group, also recorded by odds ratio a non-significant between patients and control at p. value < 0.05 .

Keywords: Leptin gene, SNP, CKD, Dialysis, RFLP..

INTRODUCTION

The prevalence of chronic kidney disease (CKD) is increasingly becoming recognized as a global health concern as well as a critical determinant of poor health outcomes. Decreased access to health care and low socioeconomic status (SES) worsen the adverse effects of biologic or genetic predisposition to CKD¹. Glomerulonephritis (GN) are relatively rare kidney diseases with substantial morbidity and mortality, can lead to chronic kidney disease (CKD) and end stage kidney disease (ESKD)². It is typically defined as a reduction in kidney function, estimated glomerular filtration rate [eGFR] < 60 ml/min/1.73m², or markers of kidney damage, such as albuminuria,

hematuria or abnormalities detected on imaging, present for at least 3 months³. Although creatinine clearances can be calculated from urine creatinine concentration measured in a 24-hour urine collection and a concomitant serum creatinine concentration, a more practical approach in the office is to estimate GFR (estimated GFR or eGFR) from the serum creatinine concentration, using either the Cockcroft-Gault or the Modification of Diet in Renal Disease (MDRD) Study estimating equations⁴. There are many causes of CKD, including those that more common and well-researched such as older age, diabetes, Hypertension and cardiovascular disease, In addition to that accumulated uremic toxins, increased

oxidative stress, endothelial dysfunction, and mineral and bone disorders⁵. Creatinine and urea levels are increased in both males and females in chronic kidney disease⁶. Serum creatinine as well as urea are well-known indicators of GFR, although serum creatinine is the most sensitive indicator of renal dysfunction. Every time there was poor kidney function or kidney damage, the urea value increased. Kidney damage resulting from increased blood sugar levels when the urea concentration rises with the sugar level⁷.

Leptin is a peptide hormone secreted by adipose tissue that was identified by positional cloning of the ob gene responsible for the development of obesity⁸. It is a product of the ob gene and is associated with obesity because increasing adipose tissue mass leads to higher leptin levels⁹. Leptin levels increase in proportion to the body's energy reserves in the form of stored fat, and leptin thus serves as an indicator of energy availability¹⁰. Leptin then increases metabolic rate¹¹. Leptin gene is located on chromosome 7q32.1, spanning approximately 20 kb with three exons separated by two introns¹². The level of the hormone leptin increases in patients with type 2 diabetes¹³.

LEP, and LEPR genes are involved in the hypothalamic leptin-melanocortin regulation pathway, which is important for energy

homeostasis¹⁴. Leptin helps to regulate the hypothalamic-pituitary-gonadal axis¹⁵. A study showed no significant alterations in the serum leptin concentrations, which reflects that peripheral signals released from adipocytes are not important in this group¹⁶. Thus, a decrease in renal function is suspected to be a major cause of hyperleptinemia in CKD. Previous studies have shown that increased serum leptin levels are seen at an early stage of CKD. Moreover, serum insulin seems to be more strongly related to serum leptin rather than to GFR per se^{17,18}. Leptin plays an important role in the development of hypertension¹⁹. It regulates energy homeostasis through signal transduction in the arcuate nucleus of the hypothalamus where it interacts with NPY and AgRP, acting as an antagonist to ghrelin²⁰. Leptin suppresses food intake by counteracting the activity of neurons containing NPY and AgRP and by increasing the activity of neurons expressing α -melanocyte stimulating hormone (α -MSH)²¹. In a study of energy homeostasis in hemodialysis (HD) patients, acyl ghrelin levels were low and leptin levels were elevated²². Leptin is cleared from the circulation by the kidney by glomerular filtration followed by metabolic degradation in the renal tubules, which accounts for the elevated level of leptin in CKD²³.

prepare a 10 pmol/L concentration as work primer suspended, 10 μ L of the stock solution in 90 μ L of free ddH₂O water to reach a final volume of 100 μ L. The molecular weight markers used in this study for DNA and PCR products were from Korea. The range of the markers were (100-2000 bp).

Materials and Method

The Primers Used in the Interaction

The primers were lyophilized, dissolved in free ddH₂O to give a final concentration of 100 pmol/L as stock solution, and stored at -20 °C to

Table (1) The product names, primers used, annealing temperature and the products size for Lep genes.

Product Name	Product size (bp)	Annealing Temperature	Primer	Oligonucleotide sequence (5'-3')
Lep	242 bp	56 C	Forward	AAAGCAAAGACAGGCATAAAAA
			Reverse	TTTCCTGTAATTTTCCCGTGAG

Table (2) protocol PCR amplification

Step		Temp.	Time	Cycle
Initial Denaturation		95	5.00 min	1 time
	Denaturation	95	30 sec.	38 cycle
	Annealing	56-60	30 sec.	
	Extension	72	40 sec.	
Final Extension		72	10.00 min	1 time

Sequencing and Alignment of Genes

The amplified PCR products were sequenced at Macrogen Company (South Korea) by sanger sequencing used " dideoxynucleotides method". Bioinformatics analysis software, such as Bioedit, was used to analyze the sequencing results after receiving them from the company, revealing the polymorphisms of the studied SNPs²⁴.

When using the blast tool available in GenBank to search for the sequence of the nitrogenous bases of the LEP gene, and using the Clustal omega tool available on the Internet, comparing two different gene sequences for the CC and TT genotypes, Figure (1).

We note from the result of the alignment between the amino acids resulting from the translation of the codes for the studied leptin gene sequence that the type of mutation occurring is due to changing the base T to C at the lep

site. T173C is a missense mutation, as this mutation, which is located in the code for the amino acid (valin), changes it to the amino acid (alanine) at position 58 of the peptide chain of the leptin protein (Figure 2)

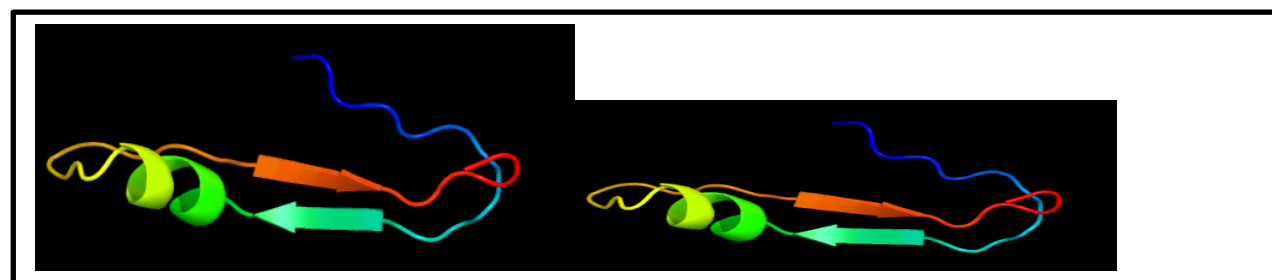
amino acid	I	W	E	L	I	S	V	H	I	K	M	K	S	S	K	F	M	F
							L											
TT	1																	
TTATTTGGGA	ACTGATAAGTGTCCATATTAAATGAAATCTTCAAAATTTATGTTCTCT	60																
CC	1																	
TTATTTGGGA	ACTGATAAGTGTCCATATTAAATGAAATCTTCAAAATTTATGTTCTCT	85																
amino acid	I	W	E	L	I	S	V	H	I	K	M	K	S	S	K	F	M	F
							L											

Fig (1) Multiple Alignment (CDs Coding Sequence) between TT & CC polymorphisms for Lep. Gene

With regard to drawing the three-dimensional shape of the peptide chain of the leptin gene and the region studied in the current study, I used the Phyre2 Server tool on the website for the purpose of converting from the amino acid chain as shown in Figure (2) to the PDB file format (Protein Data Bank) for the purpose of drawing the three-dimensional shape of the peptide chain. For the leptin gene and for the region studied in the current study, we obtained a PDB file and used the RasMol program to draw the protein. When comparing the three-dimensional shapes of the three structures (TT & TC) and (CC), the shape did not show any clear difference between the two models, Figure (3), and this may be due to The amino acids (valine) and (alanine) have the same chemical properties, or we guess that both acids are given the same importance through their similar wrapping in both forms. This gives the impression that there are functional differences between these two types of protein, based on the well-known general rule: "The function of the protein depends on "its shape".

The amino acid sequences of the resulting forms of the studied region of the leptin gene were in the International Gene Bank (NCBI), and we obtained independent accession numbers for our current study, which are BET20313 and BET20314, in addition to the sequences of the genotypes of the LEP gene in the International Gene Bank (NCBI), and we obtained independent accession numbers, and the following table shows That table (4):-

BET20313.1 (TT, TC)	TGIKNYKSIIWELISVHIKMKSSKFMFLCHGSSRSLCSGSLTSCNISALRETEAGGS	VQP
		60
BET20314.1 (CC)	TGIKNYKSIIWELISVHIKMKSSKFMFLCHGSSRSLCSGSLTSCNISALRETEAGGS	AQP
		60



BET20314.1 (CC)

BET20313.1 (TT, TC)

Genotype and Allele Frequency of Leptin 173.T>C Gene in Dialysis Patients and Control Group

The present study recorded the CC and TC genotypes at 370 position increased non-significantly in control group, while the TC genotype increased non-significantly in dialysis patients than control group. The frequency of C allele increased non-significantly in control group than dialysis patients, while the T allele increased non-significantly in dialysis patients than control group, also recorded by odds ratio a non-significant between patients and control at p. value < 0.05, as in Table 5.

Table 5: Genotype and allele frequency of Leptin 173.T>C gene in dialysis patients and control group

Gene	Genotype	Dialysis P No. 100		Control No. 50		CalX ² and p. value	OR 95%CI
		No.	%	No.	%		
Leptin 173.T>C	CC	4	8.89	6	13.33	CalX ² 3.21 0.200	Non-OR
	TC	26	57.78	29	64.44		
	TT	15	33.33	10	22.22		
	Allele	Dialysis P		Control		CalX ²	OR 95%CI
Leptin 173.T>C	C	30	42.25	35	47.30	CalX ² 0.50	0.87 (0.46-1.4)
	T	41	57.75	39	52.70	0.477	

DISCUSSION

The clinical outcomes of CKD depend on various factors, including metabolic disorders. Leptin, a peptide hormone, produced in adipose tissues, plays an important role in regulating food consumption and energy expenditure. Leptin also influences the immune system and hematopoiesis. Increased leptin status is observed in CKD, leptin deficiency attenuates the immune response in nephritis. Conversely, leptin inhibits the development of obesity, which is closely associated glomerular disorder. Now, the precise role of leptin in CKD remains elusive. This review will give an integrated understanding of the potential role of leptin and its interactions with other signal molecules in CKD²⁵. Recent studies have drawn attention to the role of leptin (LEP) and leptin receptor (LEPR) gene polymorphisms in the pathogenesis of T2DM²⁶. A few studies have reported on how to identify leptin gene mutation(s)²⁷. developed a gel-free SNP detection for leptin mutation²⁸. In this method, the annealing of the oligonucleotide complementary to the SNP allele led to the cleavage of the oligonucleotide by a cleavage enzyme. The released 5' arm fragment then served as an invader, leading to a secondary cleavage reaction to release a fluorescent signal from fluorescence resonance energy transfer (FRET) cassettes²⁹. In this study the dominant genotype was TC in both dialysis patients and control group without significant difference p. value 0.20, also, the dominant allele was T in both dialysis patients and control group without significant difference p. value 0.477. the Egyptian study of Abd El-Aziz et al³⁰, not recorded association between LEP gene polymorphism with occurrence of both kidney failure and CVD, while the of Ragin et al³¹, showed an association between LEP gene polymorphism and disorder of glucose metabolism and CVD. In addition, Wu and Sun³², showed a positive association between leptin gene polymorphism and occurrence of CVD. This study suggested that the occurrence of diabetes, as well as heart disease, is a gateway to kidney failure in the long

term. Leptin polymorphisms activate ATP-sensitive potassium channels and cause beta-cell hyperpolarization, which reduces intracellular calcium concentration and inhibits insulin secretion in post-renal transplant patients³³. Leptin SNPs rs1137100 and rs1137101 were associated with the risk of obesity, diabetes type 2, gestational diabetes and cardiovascular diseases in various populations³⁴. Also, the study of Dziedziczko et al.³⁵ showed that the polymorphisms in genes involved in leptin and adiponectin function modify long-term outcomes in kidney transplantation. the linking between leptin-related gene polymorphisms to PTDM is not unprecedented. the study of Romanowski et al³⁶, have shown in 323 renal transplant recipients that the rs2167270 SNP of the LEP gene, coding for the leptin protein, was also associated with increased risk of PTDM.

Conclusion

In this study the dominant genotype was TC in both dialysis patients and control group without significant difference p. value 0.20, also, the dominant allele was T in both dialysis patients and control group without significant difference p. value 0.477, not recorded association between LEP gene polymorphism with occurrence of both kidney failure .Further studies are needed to explore the potential mechanisms by which SNP rs2167270 of the LEP gene modulates predisposition to CKD.

REFERENCES

1. Nelson, M. L., Buchanan-Pearl, K. A. R., Oribhabor, G. I., Khokale, R. V., Cancarevic, I., & Khokale, R. (2020). Survival of the fittest: Addressing the disparities in the burden of chronic kidney disease. *Cureus*, 12(7).
2. AlYousef, A., AlSahow, A., AlHelal, B., Alqallaf, A., Abdallah, E., Abdellatif, M., ... & Elmahalawy, R. (2020). Glomerulonephritis histopathological pattern change. *BMC nephrology*, 21(1), 1-7.
3. Kalantar, K. A. M., Jafar, T., Nitsch, D.,

- Percovic, V., & Brendon, N. (2021). Preserving kidney function in people with chronic kidney disease (submitted). Proofs currently state 'Chronic kidney disease'. The Lancet.
4. Ndrepepa, G., Holdenrieder, S., Neumann, F. J., Lahu, S., Cassese, S., Joner, M., ... & Kastrati, A. (2021). Prognostic value of glomerular function estimated by Cockcroft-Gault creatinine clearance, MDRD-4, CKD-EPI and European Kidney Function Consortium equations in patients with acute coronary syndromes. *Clinica Chimica Acta*, 523, 106-113.
5. Ishigami, J., & Matsushita, K. (2019). Clinical epidemiology of infectious disease among patients with chronic kidney disease. *Clinical and experimental nephrology*, 23, 437-447.
6. Yosuf, L. M. (2022). Study the correlation between thyroid disorder and chronic kidney failure. *University of Thi-Qar Journal of Science*, 9(2), 59-65.
7. Al-Fayyadh, M. S. M., Ismael, K. H., & Al-Saady, A. J. (2023). Study of insulin resistance, cortisol hormone and some biochemical parameters in Iraqi type 2 diabetic patients. *University of Thi-Qar Journal of Science*, 10(2), 68-72.
8. Beltowski, J. (2006). Role of leptin in blood pressure regulation and arterial hypertension. *Journal of hypertension*, 24(5), 789-801.
9. Al-garawi, N. A. H. D., Suhail, A. A., Kareem, H. A., & Bustani, G. S. (2022). Study of Lipid Profile and Leptin hormone and Adiponectin hormone hypertensive patients in Najaf Governorate. *Revista Electronica de Veterinaria*, 45-51.
10. Nedergaard, J., Fischer, A. W., & Cannon, B. (2023). Leptin as an antitorpor hormone: an explanation for the increased metabolic efficiency and cold sensitivity of ob/ob mice?. *Physiological and Biochemical Zoology*, 96(1), 30-39.
11. Fischer, A. W., Cannon, B., & Nedergaard, J. (2020). Leptin: is it thermogenic?. *Endocrine reviews*, 41(2), 232-260.
12. Ishigami, J., & Matsushita, K. (2019). Clinical epidemiology of infectious disease among patients with chronic kidney disease. *Clinical and experimental nephrology*, 23, 437-447.
13. Mahdi, Q. I., Thuwaini, M. M., & Abbas, H. J. (2023). Evaluation of leptin hormone and Tumor necrosis factor levels in patients with type two diabetes mellites. *University of Thi-Qar Journal of Science*, 10(1 (SI)).
- 14.
15. Raskiliene, A., Smalinskiene, A., Kriaucioniene, V., Lesauskaite, V., & Petkeviciene, J. (2021). Associations of MC4R, LEP, and LEPR polymorphisms with obesity-related parameters in childhood and adulthood. *Genes*, 12(6), 949.
16. Salem, A. M. (2021). Variation of leptin during Menstrual cycle and its relation to the hypothalamic-pituitary-gonadal (HPG) axis: A systematic review. *International Journal of Women's Health*, 445-458.
17. Carter, S., Caron, A., Richard, D., & Picard, F. (2013). Role of leptin resistance in the development of obesity in older patients. *Clinical interventions in aging*, 829-844.
18. Fouque D, Geelen G, Bernhard J, et al: Hyperleptinemia is mediated by insulin but not by renal function in pre-dialysis and kidney transplant patients. *J Am Soc Nephrol* 12:202, 2001 (abstr).
19. Stenvinkel P, Heimbürger O, Lonnqvist F: Serum leptin concentrations correlate to plasma insulin concentrations independent of body fat content in chronic renal failure. *Nephrol Dial Transplant* 12:1321-1325, 1997
20. AUDA, M. A., & KHALAF, D. (2020). Biochemical Study of Leptin and Associated with Hypertension Patients in Iraq. *International Journal of Pharmaceutical Research* (09752366).
21. Vohra, M. S., Benchoula, K., Serpell, C. J., & Hwa, W. E. (2022). AgRP/NPY and POMC neurons in the arcuate nucleus and their potential role in treatment of obesity. *European journal of pharmacology*, 915, 174611.
22. Lee, N. J., Qi, Y., Enriquez, R. F., Clarke, I., Ip, C. K., Wee, N., ... & Herzog, H. (2020). Energy partitioning between fat and bone mass is controlled via a hypothalamic leptin/NPY relay. *International Journal of Obesity*, 44(10), 2149-2164.
23. Suneja, M., Murry, D. J., Stokes, J. B., & Lim, V. S. (2011). Hormonal regulation of energy-protein homeostasis in hemodialysis patients: an anorexigenic profile that may predispose to adverse cardiovascular outcomes. *American Journal of Physiology-Endocrinology and Metabolism*, 300(1), E55-E64.
24. Cumin F, Baum HP, De Gasparo M, Levens N (1997) Leptin is cleared from the circulation primarily by the kidney. *Int J obes Relat Metab Disord* 21:495-504.
25. Kim, E. Y., Chin, H. M., Park, S. M., Jeon, H. M., Chung, W. C., Paik, C. N., & Jun, K. H. (2012). Susceptibility of gastric cancer according to leptin and leptin receptor gene polymorphisms in Korea. *Journal of the Korean Surgical Society*, 83(1), 7-13.
26. Mao, S., Fang, L., Liu, F., Jiang, S., Wu, L., & Zhang, J. (2018). Leptin and chronic kidney diseases. *Journal of Receptors and Signal Transduction*, 38(2), 89-94.
27. Ashraf, R., Khan, M. S., Lone, S. S., Bhat, M. H., Rashid, S., Majid, S., & Bashir, H. (2022). Implication of Leptin and leptin receptor gene

- Variations in type 2 diabetes mellitus: a case-control study. *Journal of Endocrinology and Metabolism*, 12(1), 19-31.
28. Yupanqui-Lozno, H., Bastarrachea, R. A., Yupanqui-Velazco, M. E., Alvarez-Jaramillo, M., Medina-Méndez, E., Giraldo-Peña, A. P., ... & Celis-Regalado, L. G. (2019). Congenital leptin deficiency and leptin gene missense mutation found in two Colombian sisters with severe obesity. *Genes*, 10(5), 342.
 29. Chen, J., Xu, X., Dalhaimer, P., & Zhao, L. (2022). Tetra-Primer Amplification-Refractory Mutation System (ARMS)—PCR for Genotyping Mouse Leptin Gene Mutation. *Animals*, 12(19), 2680.
 30. Mohanrao, M. D., Senthilvel, S., Reddy, Y. R., Kumar, C. A., & Kadirvel, P. (2023). Amplifluor-Based SNP Genotyping. In *Plant Genotyping: Methods and Protocols* (pp. 191-200). New York, NY: Springer US.
 31. Abd El-Aziz T A, Mohamed R H, Mohamed R H and Pasha H. F. (2012). Leptin, leptin gene and leptin receptor gene polymorphism in heart failure with preserved ejection fraction. *Heart and vessels*, 27, 271-279.
 32. Ragin C, Dallal C, Okobia M, Modugno F, Chen J, Garte S and Taioli, E. (2009, February). Leptin levels and leptin receptor polymorphism frequency in healthy populations. In *Infectious Agents and Cancer* (Vol. 4, No. 1, pp. 1-6). BioMed Central.
 33. Wu L and Sun, D. (2017). Leptin receptor gene polymorphism and the risk of cardiovascular disease: A systemic review and meta-analysis. *International journal of environmental research and public health*, 14(4), 375.
 34. Cochrane V and Shyng, S. L. (2019). Leptin-induced trafficking of KATP channels: a mechanism to regulate pancreatic β -cell excitability and insulin secretion. *International journal of molecular sciences*, 20(11), 2660.
 35. Illangasekera Y A, Kumarasiri P V R, Fernando D J and Dalton C F. (2020). Association of the leptin receptor Q223R (rs1137101) polymorphism with obesity measures in Sri Lankans. *BMC research notes*, 13(1), 1-4.
 36. Dziedziejko V, Safranow K, Kijko-Nowak M, Malinowski D, Domanski and Pawlik, A. (2023). Leptin receptor gene polymorphisms in kidney transplant patients with post-transplant diabetes mellitus treated with tacrolimus. *International Immunopharmacology*, 124, 110989.
 37. Romanowski M, Dziedziejko V, Maciejewska-Karłowska A, Sawczuk M, Safranow K, Domanski L and Pawlik A. (2015). Adiponectin and leptin gene polymorphisms in patients with post-transplant diabetes mellitus. *Pharmacogenomics*, 16(11), 1243-1252.