

Original Research Article

Genetic study of the -243A>G GAD2 promoter polymorphism and its correlation with obesity

Ibrahim Jafri^{1*}, Ghadi Alsharif^{2,3}, Mohamed El-Sharnouby¹, Basem H Elesawy⁴, Osama M Mehanna^{5,6}, Ahmed ME El-Sharnouby⁷, Ahmad El Askary⁸

¹Department of Biotechnology, College of Science, Taif University, Taif, ²Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, King Saud Bin Abdulaziz University for Health Sciences, Jeddah, ³Department of Biomedical Research, King Abdullah International Medical Research Center, Jeddah, ⁴Department of Pathology, College of Medicine, Taif University, Taif, Saudi Arabia, ⁵Department of Medical Physiology, Faculty of Medicine, Al-Azhar University, Damietta, ⁶Department of Medical Physiology, Faculty of Medicine, Horus University, Damietta, ⁷Medical Student, College of Medicine, Modern University for Technology and Information, Cairo, Egypt, ⁸Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Taif University, Taif, Saudi Arabia

*For correspondence: **Email:** dr587@yahoo.com

Sent for review: 2 June 2025

Revised accepted: 15 May 2026

Abstract

Purpose: To investigate the link between obesity and the -243A>G polymorphism in the glutamate decarboxylase 2 GAD2 gene.

Method: This study involved 20 obese participants (which constituted the study group) from Al Azhar University Hospital, recruited between January 2024 and January 2025, and a control group of 20 healthy participants. -243A>G GAD2 polymorphism was identified in the peripheral blood of both study and control groups using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique.

Result: This study found that control group had significantly higher frequencies of the AA genotype and "A" allele compared to study group ($p < 0.01$). Furthermore, frequencies of the AG and GG genotypes, in addition to the "G" allele, were significantly higher in study group compared to control group ($p < 0.01$).

Conclusion: The study revealed a significant link between the -243A>G polymorphism and obesity.

Keywords: Gene, Glutamate decarboxylases, Obesity, -243A>G Polymorphism

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

Tropical Journal of Pharmaceutical Research is indexed by Scopus, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Obesity is defined as too much or inappropriate collection of fat or adipose tissue in the body (body mass index (BMI) > 30). It has developed over the past several decades as an outbreak, with an ongoing rise in incidence rate. Global age-standardized incidence of obesity has risen from 8.8 % in 1990 to 18.5 % in 2022 among women and from 4.8 % in 1990 to 14 % in 2022

among males, leading to 504 million females and 374 males suffering from obesity globally [1].

Obesity is a long-lasting, complicated, multifactorial metabolic disorder caused by a combination of inherited, biological, behavioral, socio-cultural, and financial variables. It presents multiple dangers related to health [2]. Obese people are more probable to need hospitalization and have a three-year lower standard duration of

life alongside. Extreme obesity could decrease lifespan by 8 to 10 years. In addition, obese individuals are more likely to suffer from anxiety and depressive disorders, have fewer job opportunities, and face additional stigma and prejudice [3]. Glutamate decarboxylases (GADs) convert glutamate to γ -aminobutyric acid (GABA), the primary inhibitory neurotransmitter in the central nervous systems (CNS). GAD1 and GAD2 sequences are greatly equivalent to each other, and they possess an identical intron-exon structure, suggesting that they originated together [4].

Several studies have linked serious obesity to the -243A>G polymorphism (rs2236418) in the GAD2 promoter zone [5,6]. GAD2 gene polymorphisms have been linked to obesity in children, birth weight, and overeating [6]. The G allele of the -243A>G polymorphism has been shown to be modestly related to decreased body mass index and fasting blood glucose levels [7]. There have been conflicting findings with regard to the relationship of GAD2 gene single nucleotide polymorphisms SNPs with obesity. Furthermore, several investigations including European and American communities found no relationship of GAD2 gene with obesity [8,9]. Obesity in the north Indian population showed a strong correlation with the -243 A>G SNP and the combined haplotypes of the GAD-2 gene [10]. This study therefore investigated the association between -243A>G polymorphism of the GAD2 gene and obesity.

METHODS

Design

This case-control study was conducted at the Physiology Department, College of Medicine, Al Azhar University. The study involved 20 obese patients from Al Azhar University Hospitals, recruited between January 2024 and January 2025 which constituted the study group, and a control group of 20 healthy participants. The study population comprised of a study group (obese; $n = 20$) with $\text{BMI} \geq 30.0 \text{ kg/m}^2$ and a control group (healthy; $n = 20$) with $\text{BMI} < 25.0 \text{ kg/m}^2$ with same age range.

Ethical considerations

The study was approved by the Ethics Committee of Al Azhar University (approval no. 47-12/2023) and complied to the Declaration of Helsinki [11]. Names of the participants were obscured and supplanted with code numbers, and informed consent was obtained to participate in the study.

Inclusion criteria

Obese patients with $\text{BMI} \geq 30.0 \text{ kg/m}^2$ [12], male and female, age between 20 - 50 years

Exclusion criteria

Participants with type 1 diabetes mellitus, endocrine diseases such as hypothyroidism, Graves' disease, neurological diseases such as seizures, epilepsy, ataxia, and psychiatric disorders such as depression, bipolar disorders, and schizophrenia.

Evaluation of parameters/indices

Medical and family history, full clinical examination including weight, height, body mass index (BMI), waist circumference (WC) and blood pressure was obtained.

Body mass index (BMI)

Body mass index (BMI) was calculated as $\text{weight/height}^2 \text{ (kg/m}^2\text{)}$. Based on WHO criteria, underweight was identified as $\text{BMI} < 18.5 \text{ kg/m}^2$, overweight ($\text{BMI } 25.0\text{-}29.9 \text{ kg/m}^2$), and obese ($\text{BMI} \geq 30.0 \text{ kg/m}^2$) [12].

Blood pressure

After 10 min of sitting quietly, blood pressure was measured to the closest 2 mmHg using a mercury sphygmomanometer with the arm supported at heart level. Three readings at 1min intervals were taken. Average of the last two reading was taken and recorded.

Waist circumference

The waist circumference was measured using an inelastic measuring instrument with an accuracy of 0.1 cm, directly on the skin.

Biochemical parameters

An automatic analyzer (Toshiba TBA 120FR; Toshiba Medical Systems Co., Ltd., Tokyo, Japan) was used to measure fasting plasma glucose and serum total cholesterol, triglycerides (TG), and HDL cholesterol (HDL-C), as well as LDL cholesterol (LDL-C). HbA1c was determined using automatic analysers (HLC-723 G7 HPLC systems, Tosoh Corporation, Tokyo, Japan).

Polymorphism

Identification of -243A>G GAD2 polymorphism in peripheral blood was done using PCR-RFLP

technique. Blood samples (2 mL) were collected in a sterile K3 EDTA vacutainer and stored at -20 °C to detect -243A>G polymorphism of the GAD2 gene. Repetition of freezing and thawing were prevented.

Genomic DNA extraction

The DNA was extracted from whole blood using The Thermo Scientific™ GeneJET™ Whole Blood Genomic DNA Purification Mini Kit (Thermo Fisher Scientific Baltics, Lithuania cat. NO. K0781) [4]

Polymerase chain reaction (PCR) amplification

The extracted DNA was amplified by PCR using a thermal cycler (QIA amplifier 96, Germany). Applied Biosystems were used to prepare the test primers; (Applied Biosystems, Waltham, Massachusetts, USA.) The forward primer was 5'-CCTCATTCATCCCCACTG-3', while the reverse primer was 5'-CACGCAGGAACAGAAAACG -3'. The reaction mixture included 10 µL of DNA extract, 1 µL of forward and reverse primers, 0.5 µL of nuclease-free water, as well as 12.5 µL of ready-to-use hot start master mix (Thermo Scientific, USA), which included hot start *Thermus aquaticus* (Taq) DNA polymerase, optimized hot start PCR buffer, Mg²⁺, and dNTPs [4]. The PCR conditions consisted of a 4 min activation step at 94 °C, 35 cycles of denaturation at 95 °C for 35 s, annealing at 60 °C for 30 s, and extension at 72 °C for 60 s, with the last extension occurring at 72 °C for 10 min [4].

Restriction fragment length polymorphism (RFLP)

The PCR products were digested using the restriction enzyme specific for the recognition site (DraI; Fast Digest, New England Biolabs Inc. Ipswich, MA, USA). The various elements were delicately mixed, turned and incubated at 37 °C for 20 min. The reaction mixture and 100 bp DNA ladder were loaded onto a 2 % agarose gel. The DNA fragments were separated using an electrophoretic system at 100 volts for 30 min (Clever Scientific Limited) [4].

Reading of electrophoretic separation

The GAD2 gene has two fragments produced by the A allele, while the G allele produces only one undigested band due to lack of a restriction region. Genotyping was based on band length of the restriction fragment (Figure 1). homozygous wild (AA) genotype (2 bands at 98 and 105 bp), heterozygous (AG) genotype (3 bands at 98, 105

and 203 bp), and homozygous mutant (GG) genotype (a single undigested fragment at 203 bp).



Figure 1: 2 % agarose gel electrophoresis of GAD2 gene -243 A>G polymorphism genotypes

Statistical analyses

Data were analyzed using the statistical package for social sciences, version 23.0 (SPSS Inc., Chicago, Illinois, USA). Quantitative data were presented as mean $\pm$ standard deviation (SD) and ranges when their distribution was parametric (normal) while non-normally distributed variables (non-parametric data) were presented as median with inter-quartile range (IQR). Also, qualitative variables were presented as frequency and percentages. Inferential statistics was done using student t- test and Chi-square. $P < 0.05$ was considered statistically significant.

RESULTS

Clinical data

There was significant difference in BMI, waist circumference, genotype and allele frequency between study and control groups ($p < 0.05$; Table 1).

Distribution of GAD2 gene-243A>G polymorphism

The GAD2 gene -243A>G polymorphism genotypes did not show any statistically significant variation between control and study groups in relation to age, gender, HTN, DM, hypercholesterolemia, hypertriglyceridemia, low HDL-C, and elevated LDL-C ($p < 0.05$; Table 2).

Table 1: Clinical data

Parameter		Study group (n=20)	Control group (n=20)	P- value
Age (years)	Mean ± SD	39.09±3.44	37.90±2.27	0.865
Gender	Females (N, %)	9(45)	7(35)	0.768
	Males (N, %)	11(55)	13(65)	
BMI (kg/m ²)	Mean ± SD	22.14±1.92	35.43±13.17	< 0.01
Waist Circumference (cm)	Females	114.36±26.94	73.25±8.12	< 0.01
	Males	131.56±25.18	95.20±11.43	
Hypertension (HTN)	No (N, %)	11(55)	14(70)	0.24
	Yes (N, %)	9(45)	6(30)	
Diabetes mellitus (DM)	No (N, %)	15(75)	18(90)	0.13
	Yes (N, %)	5(25)	2(10)	
Lipid profile	Hypercholesterolemia (%)	6 (30)	5(25)	0.3
	Hypertriglyceridemia (%)	7 (35)	4(20)	
	Low HDL-C (%)	4(20)	3(15)	
	Low HDL-C (%)	5(25)	3(15)	
Genotype	AA	1(5)	15(75)	< 0.01
	AG	5(25)	3(15)	
	GG	14(70)	2(10)	
Allele frequency	A	3(15)	17(85)	< 0.01
	G	17(85)	3(15)	

Total cholesterol (mg/dl); Normal (<200), High (≥200); Triglycerides (mg/dl); Normal (<150), High (≥150); HDL-C (mg/dl); Normal 40-60; LDL-C (mg/dl); Normal (<100), High (≥100)

Table 2: Distribution of GAD2 gene -243A>G polymorphism with clinical parameters

Parameter (n = 40)	Unit	AA (n = 16)	AG (n = 8)	GG (n = 16)	P-value
Age (Years)	Mean ± SD	37.43±13.17	39.53±16.84	39.24±9.09	0.791
Gender	Females	7 (43.8)	3 (37.5)	6 (37.5)	0.671
	Males	9 (56.3)	5 (62.5)	10 (62.5)	
Hypertension (HTN; n = 15)	N (%)	6 (37.5)	0 (0)	9 (56.3)	0.453
Diabetes mellitus (DM; n = 7)	N (%)	2 (12.5)	2 (25)	3 (18.8)	0.426
Hypercholesterolemia (n = 11)	N (%)	6 (37.5)	0 (0)	5 (31.3)	0.209
Hypertriglyceridemia (n = 11)	N (%)	4 (25)	1 (12.5)	6 (37.5)	0.315
Low HDL-C (n = 7)	N (%)	4 (25)	0 (0)	3 (18.8)	0.145
Elevated LDL-C (n = 8)	N (%)	4 (25)	0 (0)	4 (25)	0.161

DISCUSSION

Obesity is a worldwide health pandemic that has gradually increased throughout the last fifty years [13,14]. Results of the present study showed that mean age of the study group was higher compared to control group which agrees with the findings of Al-Zubaidi *et al* [15] and disagrees with Hassanin *et al* [16]. Gender distribution revealed that 55 % of obese patients were males compared to 65 % in the control group. This finding agreed with Al-Zubaidi *et al* [15] and Hassanin *et al* [16] and disagrees with Liu *et al* [17] who reported that obese patients were more likely to be women. Furthermore, an Indian study showed that obese group had a greater proportion of females compared to non-obese group [18].

The current study findings showed that study group had significantly higher BMI compared to control group ($p < 0.05$), which is in tandem with the findings of Al-Zubaidi *et al* [15]. Also, study group showed significantly higher waist

circumference (WC) compared to control group ($p < 0.01$) which is in tandem with the findings of Fitri *et al* [19] in Indonesia. Furthermore, majority of the study group had HTN which agrees with the study of Hennessey *et al* [20]. There was no significant difference in hypercholesterolemia, hypertriglyceridemia, low HDL-C, and increased LDL-C between study and control groups. In contrast, the study group showed lower HDL cholesterol levels compared to the control group. Another investigation revealed that obese participants had higher baseline levels of triglycerides, total cholesterol, and low-density lipoprotein compared to non-obese patients ($p < 0.05$) [21].

Results of the current study showed that frequencies of AA genotype and the A allele were significantly higher in control group compared to study group, while frequencies of the genotypes (AG) and (GG), and the G allele, were significantly higher in study group compared to ($p < 0.01$). These results are in agreement with Boutin *et al* [5] and Prakash *et al*

[10] which revealed that the existence of G allele leads to increased GAD2 promoter activity and affinity for transcription factors, resulting in an increased GABA pool in the hypothalamus. The investigators discovered that obese patients with homozygous (GG) were more probable to overeat and have greater susceptibility to nutrition sensorial excitement, potentially leading to reduced ability to regulate eating habits. However, these findings disagreed with Swarbrick *et al* [22], and Hunt *et al* [23] which revealed no significant difference in genotypes and alleles of the GAD2 gene between obese and non-obese groups. The GAD2 gene -243A>G polymorphism genotypes showed no significant difference in age, gender HTN, DM, hypercholesterolemia and lipid profile ($p > 0.05$) between the obese patients and control group which is in contrast to an earlier study [10].

Limitations of the study

This study utilized a small sample size which may affect generalizability of the results. Multicentre studies involving larger sample sizes are required to support the findings.

CONCLUSION

Obesity shows significant link with -243A>G polymorphism with significantly higher frequencies of AG, and GG genotypes as well as the G allele.

DECLARATIONS

Acknowledgement/Funding

The authors extend their appreciation to Taif University, Saudi Arabia, for supporting this research. This research was funded by Taif University, Saudi Arabia (TU-DSPP-2024-205).

Ethical approval

The study was approved by the Ethics Committee of Al Azhar University (approval no. 47-12/2023).

Use of Artificial intelligence/Large language models

The authors confirm that AI-based tools were used solely for language editing and manuscript polishing purposes (e.g., grammar, phrasing, and clarity). No AI tools were used to generate content, interpret data, or influence study design, results, or conclusions. All intellectual contributions, study analyses, and interpretations

reported in the manuscript are the original work of the authors.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

REFERENCES

1. NCD Risk Factor Collaboration (NCD-RisC). Worldwide trends in underweight and obesity from 1990 to 2022: a pooled analysis of 3663 population-representative studies with 222 million children, adolescents, and adults. *Lancet* 2024; 403(10431): 1027–1050.
2. Janez A, Muzurovic E, Bogdanski P, Czupryniak L, Fabryova L, Fras Z, et al. Modern management of cardiometabolic continuum: from overweight/obesity to prediabetes/type 2 diabetes mellitus. Recommendations from the Eastern and Southern Europe diabetes and obesity Expert Group. *Diabetes Ther* 2024; 15(9): 1865-1892.
3. Patoulas D, Koufakis T, Ruža I, El-Tanani M, Rizzo M. Therapeutic advances in obesity: How real-world evidence impacts affordability beyond standard of care. *Pragmat Obs Res* 2024; 15: 139-149.
4. Grone B, Maruska K. Three Distinct Glutamate Decarboxylase Genes in Vertebrates. *Sci Rep* 2016; 6: 30507
5. Boutin P, Dina C, Vasseur F, Dubois S, Corset L, Séron K, et al. GAD2 on chromosome 10p12 is a candidate gene for human obesity. *PLoS Biol* 2003; 1: E68.
6. Meyre D, Boutin P, Tounian A, Deweirder M, Aout M, Jouret B, et al. Is glutamate decarboxylase 2 (GAD2) a genetic link between low birth weight and subsequent development of obesity in children? *J Clin Endocrinol Metab* 2005; 90: 2384–2390
7. Boesgaard TW, Castella SI, Andersen G, Albrechtsen A, Sparsø T, Borch-Johnsen K, et al. A -243A-->G polymorphism upstream of the gene encoding GAD65 associates with lower levels of body mass index and glycaemia in a population-based sample of 5857 middle-aged White subjects. *Diabet Med* 2007; 24: 702–706.
8. Groves CJ, Zeggini E, Walker M, Hitman GA, Levy JC, O'Rahilly S, et al. Significant linkage of BMI to

- chromosome 10p in the U.K. population and evaluation of GAD2 as a positional candidate. *Diabetes* 2006; 55: 1884–1889.
9. Hunt SC, Xin Y, Wu LL, Hopkins PN, Adams TD. Lack of association of glutamate decarboxylase 2 gene polymorphisms with severe obesity in Utah. *Obesity* 2006; 14: 650–655.
 10. Prakash J, Mittal B, Awasthi S, Srivastava N. Association of the -243A>G, +61450C>A polymorphisms of the glutamate decarboxylase 2 (GAD2) gene with obesity and insulin level in North Indian Population. *Iran J Public Health* 2016; 45(4): 460-468.
 11. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA* 2013; 310: 2191-2194
 12. Pasco JA, Holloway KL, Dobbins AG, Kotowicz MA, Williams LJ, Brennan SL. Body mass index and measures of body fat for defining obesity and underweight: a cross-sectional, population-based study. *BMC Obe* 2014; 1: 9.
 13. Akinkuotu AC, Hamilton JK, Birken C, Toulany A, Strom M, Noseworthy R, et al. Evolution and outcomes of a Canadian Pediatric bariatric surgery program. *J Pediatr Surg* 2019; 54(5): 1049-1053.
 14. Kozlov AI. Carbohydrate-related nutritional and genetic risks of obesity for indigenous northerners. *Vopr Pitan* 2019; 88(1): 5-16.
 15. Al-Zubaidi AM, Alzobydi AH, Alsareii SA, Al-Shahrani A, Alzaman N, Kassim S. Body mass index and *Helicobacter pylori* among obese and non-obese patients in Najran, Saudi Arabia: A case-control study. *Int J Environ Res Public Health* 2018; 15(11): 2586.
 16. Hassanin A, Hassanein M, Lanier GM, Sadaka M, Rifaat M, Sanhoury M. Prevalence of obesity and its association with cardiometabolic risk factors, heart failure phenotype and mortality among patients hospitalized for heart failure in Egypt. *Egypt Heart J* 2022; 74(1): 1.
 17. Liu Q, Major B, Le-Rademacher J, Al-Kali AA, Alkhateeb H, Begna K, et al. The Impact of Obesity on the Outcomes of Adult Patients with Acute Lymphoblastic Leukemia - A Single Center Retrospective Study. *Blood Lymphat Cancer* 2021; 11: 1-9.
 18. Gupta RD, Tamanna N, Siddika N, Haider SS, Apu EH, Haider MR. Obesity and abdominal obesity in Indian population: Findings from a Nationally representative study of 698,286 Participants. *Epidemiologia (Basel)* 2023; 4(2): 163-172
 19. Fitri NK, Sari DK, Lipoeto NI. Anthropometric and body composition analysis in obese and non-obese subjects in three major cities in Indonesia: A cross-sectional study. *Hum Nutr Metab* 2024; 37: 200271.
 20. Hennessey DB, Bolton EM, Thomas AZ, Manecksha RP, Lynch TH. The effect of obesity and increased waist circumference on the outcome of laparoscopic nephrectomy. *Adv Urol* 2017; 2017: 3941727.
 21. Zuza EP, Barroso EM, Fabricio M, Carrareto AL, Toledo BE, R Pires J. Lipid profile and high-sensitivity C-reactive protein levels in obese and non-obese subjects undergoing non-surgical periodontal therapy. *J Oral Sci* 2016; 58(3): 423-430.
 22. Swarbrick MM, Waldenmaier B, Pennacchio LA, Lind DL, Cavazos MM, Geller F, et al. Lack of support for the association between GAD2 polymorphisms and severe human obesity. *PLoS Biol* 2005; 3(9): e315.
 23. Hunt SC, Xin Y, Wu LL, Hopkins PN, Adams TD. Lack of association of glutamate decarboxylase 2 gene polymorphisms with severe obesity in Utah. *Obesity (Silver Spring)* 2006; 14(4): 650-655.