ORIGINAL ARTICLE

Psychiatry and Clinical Psychopharmacology 2024;34(3):221-228
DOI:10.5152/pcp.2024.24827

Genetic Variations and Serum Levels of Leptin and Ghrelin in Autism Spectrum Disorder

Özlem Nehir Yazici[1], Nilfer Şahin[2], Çilem Özdemir[3], Ercan Saruhan[4], Hatice Topal[5], Tarkan Yazıcı[6], Özge Dombaycı[7], Gülsüm Demirkan Başkaya[1], Tuba Edgünlü[8]

[1]Department of Child and Adolescent Psychiatry, Muğla Research and Training Hospital, Muğla, Türkiye; [2]Department of Child and Adolescent Psychiatry, Muğla Sıtkı Koçman University School of Medicine, Muğla, Türkiye; [3]Department of Medical Bioinformatics, Graduate School of Natural and Applied Sciences, Muğla Sıtkı Koçman University, Muğla, Türkiye; [4]Department of Medical Biochemistry, Muğla Sıtkı Koçman University School of Medicine, Muğla, Türkiye; [5]Department of Pediatrics, Muğla Sıtkı Koçman University School of Medicine, Muğla, Türkiye; [6]Department of Medical Biochemistry, Muğla Research and Training Hospital, Muğla, Türkiye; [7]Department of Child and Adolescent Psychiatry, Manisa Mental Health and Diseases Hospital, Manisa, Türkiye; [8]Department of Medical Biology, Muğla Sıtkı Koçman University School of Medicine, Muğla, Türkiye

ABSTRACT

Background: This study aims to examine leptin and ghrelin gene polymorphisms and serum levels in children with autism spectrum disorder (ASD).

Methods: The study comprised a case group of 40 children aged 2-7 diagnosed with ASD and a control group of 40 healthy children. The severity of ASD symptoms was assessed using the Childhood Autism Rating Scale and the Autism Behavior Checklist. Leptin and ghrelin gene variants were genotyped using polymerase chain reaction (PCR) restriction fragment length polymorphism (RFLP) methods. Serum ghrelin and leptin levels were measured using enzyme-linked immunosorbent assay kits.

Results: In this study, gene polymorphisms and allele frequencies were examined, and no significant difference was found ($P > .05$ for all). Our findings indicated no significant difference in leptin serum levels between the groups ($P = .584$). However, ghrelin serum levels were significantly lower in the ASD group ($P = .027$). Receiver operating curve analysis to determine the cutoff value of serum ghrelin level as a diagnostic indicator for ASD resulted in a cutoff value of 885.7 pg/mL with 42.50% sensitivity and 85% specificity ($P = .021$). No significant relationship was found between leptin and ghrelin serum levels and the severity of ASD ($P > .05$ for all).

Conclusion: Our study is the first to evaluate leptin and ghrelin gene polymorphisms in ASD. Our findings indicate no association between leptin and ghrelin gene polymorphisms and ASD. However, our study suggests that ghrelin serum levels may potentially contribute to the etiology of ASD. More research is needed to understand the role of leptin and ghrelin in ASD.

ARTICLE HISTORY

Received: January 4, 2024
Revision Requested: February 2, 2024
Last Revision Received: April 28, 2024
Accepted: April 28, 2024
Publication Date: September 25, 2024

INTRODUCTION

Autism spectrum disorder (ASD) is defined by limited and repetitive patterns of behavior, interests, or activities, as well as deficiencies in reciprocal social interaction and social communication beginning in early infancy.[1] In recent years, there has been a notable increase in the number of diagnosed cases of ASD, underscoring the urgency to comprehend the causes and underlying pathophysiology of this disorder. Various hypotheses, including genetic, neurobiological, and psychosocial factors, as well as environmental or iatrogenic causes, have been proposed to elucidate the etiology of ASD.[2,3] Hormonal dysregulation in ASD remains a prominent consideration, given the plethora of hormonal abnormalities identified in children with ASD. Some studies suggest that leptin and ghrelin, which act as hormones, may also play a role in the etiology of ASD.[4,5]

Leptin's primary role in the body is to regulate food intake and energy metabolism by exerting a negative feedback effect on the central nervous system, especially the hypothalamus. Leptin plays crucial roles in the immune system, sexual development and reproduction, hematopoiesis, the regulation of gastrointestinal functions, and circadian rhythms.[6,7] For example, leptin regulates the secretion of various inflammatory cytokines, including tumor necrosis factor alpha, interleukin (IL)-6, and IL-12, modulating inflammation and enhancing the inflammatory response.[6] Leptin is said to be affected

Corresponding author: Nilfer Şahin, e-mail: nilfersahin@mu.edu.tr

Cite this article as: Yazici ÖN, Şahin N, Özdemir Ç, et al. Genetic variations and serum levels of leptin and ghrelin in autism spectrum disorder. *Psychiatry Clin Psychopharmacol*. 2024;34(3):221-228.

by biological parameters such as circadian rhythms, and there is an interconnected relationship between circadian clock genes and leptin.[7] Nutritional and sleep problems, circadian dysregulation, and altered immune functions are reported to be expected in ASD.[8,9] It is estimated that about 90% of children with ASD experience eating problems. Some studies have suggested that the presence of feeding difficulties in early childhood may be an early sign of ASD.[10] Immunological changes may also play a role in the pathophysiology of ASD.[2,3] Studies have indicated dysfunction in the immune system of children with ASD, and some research has demonstrated elevated proinflammatory cytokine levels alongside decreased anti-inflammatory cytokine levels in these children.[4] Therefore, numerous researchers have investigated the relationship between ASD and leptin.[4,11,12] Rodrigues et al. (2014) found higher levels of leptin in children diagnosed as having ASD compared to typically developing children.[13] Similarly, a study showed higher leptin levels in ASD compared to controls.[14]

Ghrelin, on the other hand, is the best-known orexigenic hormone. In addition to its appetite-regulating effects, ghrelin increases growth hormone secretion, reduces somatostatin secretion, lowers mean arterial pressure, and increases cardiac output by reducing systemic vascular resistance through vasodilation.[15,16] Ghrelin has different effects on different parts of the brain. Its main effect is to promote nutrition, but it also affects higher cognitive functions, sleep regulation, and motor and sensory functions.[17] Difficulties in executive functions, feeding problems, sleep problems, obesity, and sensory differences are common in cases with ASD.[9,18,19] Ghrelin targets the hippocampus, a crucial area for memory and learning, and is involved in hippocampal synaptogenesis. Abnormal synaptogenesis in the hippocampus has been observed in ASD cases.[20,21] Various studies have investigated the connection between ASD and ghrelin, yielding differing results. A study conducted in 2014 with children aged 3-8 years suggested that ghrelin serum levels were lower in children diagnosed as having ASD compared to the control group.[5] In another study, ghrelin levels were found to be higher in cases with ASD than in the control group.[22]

Despite studies reporting abnormalities in serum leptin and ghrelin levels, to our knowledge, no studies have examined serum levels and leptin and ghrelin gene polymorphisms in young children with ASD. However, studies have examined other psychiatric disorders' leptin and ghrelin gene polymorphisms. In these studies, leptin gene polymorphism has been associated with alcohol use, nicotine use, anxiety,[23] and suicidal behavior.[24] In contrast, ghrelin gene polymorphism has been suggested to be linked with depressive disorder[25] and eating disorders.[26] When the literature is examined, it is found that there are few studies examining leptin and ghrelin gene variations in other neurodevelopmental disorders, and the relevant studies only focus on attention deficit hyperactivity disorder (ADHD). These studies predominantly concentrate on ghrelin gene polymorphism, with no studies found regarding leptin gene polymorphism. One of these studies showed that ghrelin-deficient zebrafish exhibited ADHD-like behaviors such as hyperactivity, inattention, impaired learning and memory, and impulsivity, as well as dysfunction in the dopaminergic system.[27] In another study, it was demonstrated that depleted ghrelin levels in zebrafish resulted in behaviors resembling ADHD.[28]

This study evaluated leptin and ghrelin gene polymorphisms and serum levels in ASD patients and their relationship with ASD severity to elucidate the potential contributions of leptin and ghrelin to ASD etiology. Elucidation of the genetic basis and serum levels of leptin and ghrelin in ASD patients and their association with ASD severity will advance understanding of hormonal dysregulation in ASD.

MAIN POINTS

- According to the results of this study, ghrelin serum levels were significantly lower in the ASD group, and ghrelin serum levels were identified as an independent risk factor for ASD.
- The cutoff value for serum ghrelin level as a diagnostic indicator for ASD was 885.7 pg/mL, with 42.50% sensitivity and 85% specificity.
- The leptin and ghrelin gene polymorphisms revealed no significant differences between the groups.

MATERIAL AND METHODS

The study comprised 40 children (ranging from 2 to 7 years old) diagnosed as having ASD and an equal number of children without ASD, matched for age and sex. The diagnosis of ASD was established by a child psychiatrist based on clinical features and the criteria outlined in the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5). Additionally, the absence of other psychiatric disorders was verified using the Schedule for Affective Disorders and Schizophrenia for School-Age Children–Present and Lifetime Version—DSM-5 (K-SADS-PL-DSM-5–T), which was adapted into Turkish in November 2016.[29,30]

The severity of ASD in children was assessed using the Childhood Autism Rating Scale (CARS) score and the Autism Behavior Checklist (ABC) score. Childhood Autism Rating Scale, developed by Schopler and colleagues in 1971, is used for the assessment of children over two years of age suspected of having ASD and for differentiating autistic children from those with other developmental disorders.[31] The scale consists of 15 items, each representing a subscale. Turkish validity and reliability studies were conducted by Sucuoğlu and colleagues in 1996, and by İncekaş and colleagues in 2016.[32,33] The Cronbach's alpha value for the scale's total score was determined to be 0.95.

Autism Behavior Checklist, developed by Krug[34] and colleagues in 1993, comprises 57 items covering emotional, relationship-building, body and object usage, language skills, social components, and self-care skills, including 5 subscales. The Turkish validity and reliability study was conducted by Yılmaz and colleagues in 2007.[35] The Cronbach's alpha value for the scale's total score was reported to be 0.92.

Demographic and clinical characteristics, including gender, age, parental age, parental education, and details related to prenatal, perinatal, and postnatal periods, were documented. The study's inclusion criteria encompassed the non-use of medication or psychoactive drugs, the absence of accompanying psychiatric disorders, and the lack of neurological, genetic, metabolic, endocrine, or infectious diseases. The study excluded individuals with severe head trauma and those with inadequate sampling.

The research control group comprised 40 healthy participants aged 2-7, matched for age and gender, who visited our hospital for routine clinical health examinations. A clinician examined all control group participants, and children without any chronic physical illnesses were included in the study. A child psychiatrist assessed the control group participants using the K-SADS-PL-DSM-5-T to identify the presence of psychiatric disorders, and individuals diagnosed with psychiatric disorders were excluded from the study. Additionally, individuals using any medications were not included in the control group.

The study received approval from the Local Ethics Committee of Muğla Sıtkı Koçman University Faculty of Medicine (Approval No: 9/IV, Date: May 11, 2022) and was conducted by the principles of the Helsinki Declaration. Detailed informed consent was obtained from the parents of all participating children.

Procedure

For both the case and control groups, a 2 mL blood sample was taken from individuals in the sample group who had fasted for 8 hours between 08:30 AM and 10:00 AM. The blood samples were collected in 5 mL centrifuge tubes containing 2% ethylenediaminetetraacetic acid (EDTA) and stored at −20°C until DNA isolation to determine leptin-ghrelin gene polymorphisms.

To determine serum levels, 2 mL blood samples were taken from individuals in the sample group and placed in gel biochemistry tubes with yellow caps. Subsequently, the collected samples were centrifuged at 3500 rpm for 10 minutes in a NÜVE NF1200R refrigerated centrifuge. After centrifugation, serum samples were separated and stored in Eppendorf tubes at −20°C until the analysis day.

Genotype Analysis

A 2 mL volume of venous blood was drawn into vacuum tubes containing sodium/potassium EDTA. The Hibrigen Blood DNA Isolation Kit (MG-KDNA-02-250; Hibrigen Biotechnology R&D Industry and Trade Inc., Gebze, Kocaeli, Türkiye) was employed to extract DNA from the blood samples. Polymorphisms rs34911341 in the Ghrelin gene and rs7799039 in the Leptin gene were determined using the polymerase chain reaction (PCR)-restriction fragment length polymorphism method. Polymerase chain reaction was performed using an automatic thermal cycler (SimpliAmp Thermal Cycler, Thermo Fisher Scientific, USA) with 100 ng DNA in a 25 µL volume of 2X Taq Master Mix (MG-TAQMX-01-80; Hibrigen Biotechnology R&D Industry).

The proper restriction enzymes, SacI for rs34911341 and HhaI for rs7799039, were used to digest the PCR products.

Polymerase chain reaction products and the products obtained after digestion were visualized, and alleles were detected using 3% agarose gel electrophoresis. For this purpose, 4.2 g of agarose was weighed on a precision scale, dissolved in 140 ml of 1X TBE in the microwave, and then poured into the gel tank after adding EtBr to achieve a 0.5 µg/mL concentration. After the gel solidified, 2 µL of loading buffer and 10 µL of the sample were loaded into each well. A 100 bp marker was used to determine the sizes of the samples, and after running at 120 V, the samples were visualized on a UV transilluminator. Subsequently, genotyping was carried out by 2 independent observers.

Biochemical Analysis

Measurement of Serum Leptin and Ghrelin Levels: An enzyme-linked immunosorbent assay kit with the "double-antibody sandwich" method was used to determine the levels of serum Leptin and Ghrelin (respectively, Wuhan USCN Business Co. Ltd; product no: SEA084Hu and Wuhan USCN Business Co. Ltd; Product no: CEA991Hu).

Statistical Analysis

Statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS) version 20.0 (IBM SPSS Corp.; Armonk, NY, USA). The normality of distribution in the groups was assessed using the Kolmo gorov-Smirnov/Shapiro-Wilk test. It was determined that the ABC scale scores and ghrelin serum levels did not exhibit a normal distribution. Descriptive statistics: mean ± standard deviation were expressed for variables with a normal distribution, median (minimum-maximum) for variables with a non-normal distribution, and frequency (n) and percentage (%) for nominal variables. In intergroup comparisons, the independent samples *t*-test was used for normally distributed variables, while the Mann-Whitney *U*-test was applied for those not exhibiting normal distribution. The Pearson chi-square and Fisher-Freeman-Halton tests were utilized to determine if there were differences in categorical variables.

Spearman and Pearson correlation coefficients were performed to assess the relationship between the severity of ASD, as determined by scale scores, and leptin and ghrelin serum levels. The univariate binary logistic regression

analysis was conducted to evaluate the impact of ghrelin serum levels on ASD. The receiver operating curve (ROC) analysis determined the cutoff value for ghrelin serum levels as a diagnostic indicator for ASD. Youden's J Index criterion defined the optimal cutoff score. In the analyses, a significance level of $P < .05$.

RESULTS

The study comprised 80 individuals. In the ASD group (n = 40), the mean age was 3.95 ± 1.46 years, with 29 (79.5%) males. In the control group (n = 40), the mean age was 4.42 ± 1.61 years, with 23 (57.5%) males. No significant differences were observed between the groups regarding age, gender, height, weight, or body mass index (BMI) percentiles (all $P > .05$). Sociodemographic characteristics are detailed in Table 1.

Regarding leptin gene rs7799039 polymorphism, genotypic frequencies were determined. In the ASD group, 9 individuals (22.5%) had the AA genotype, 21 individuals (52.5%) had the GA genotype, and 10 individuals (25%) had the GG genotype. In the control group, 9 individuals (22.5%) had the AA genotype, 22 individuals (55%) had the GA genotype, and 9 individuals (22.5%) had the GG genotype. However, the difference in the genotypic frequencies of leptin gene rs7799039 polymorphism between the groups was insignificant ($P = .963$). When examining allele frequencies, in the ASD group, 39 individuals (48.75%) had the A allele, and 41 individuals (51.25%) had the G allele. In the control group, 40 individuals (50%) had the A allele, and 40 individuals (50%) had the G allele. Again, the difference was not statistically significant ($P = .874$).

For ghrelin gene rs34911341 polymorphism, genotypic frequencies were examined, revealing that in the ASD group, 36 individuals (90%) had the GA genotype, and 4 individuals (10%) had the GG genotype. In the control group, 5 individuals (12.5%) had the AA genotype, 33 individuals (82.5%) had the GA genotype, and 2 individuals (5%) had the GG genotype. Similar to the leptin gene, the difference between the groups was insignificant ($P = .061$). Examining allele frequencies, in the ASD group, 36 individuals (45%) had the A allele, and 44 individuals (55%) had the G allele. In the control group, 43 individuals (53.75%) had the A allele, and 37 individuals (46.25%) had the G allele; the difference was not significant ($P = .268$) (Table 2).

Table 1. Sociodemographic Characteristics of the Autism Spectrum Disorder and Control Groups

	ASD (Mean ± SD) or n (%)	Control (Mean ± SD) or n (%)	*P*
Age (years)	3.95 ± 1.46	4.42 ± 1.61	.173
Gender (n %) Male Female	 29 (72.5) 11 (27.5)	 23 (57.5) 17 (42.5)	.160
BMI percentile (kg/m²)	52.39 ± 30.98	39.59 ± 28.29	.057

ASD, autism spectrum disorder; BMI, body mass index.

Table 2. Genotype and Allele Frequencies of Leptin and Ghrelin Gene Polymorphisms in Children with Autism Spectrum Disorder

			ASD n (%) (n = 40)	Control n (%) (n = 40)	*P*
Leptin rs7799039	Genotype	AA	9 (22.5)	9 (22.5)	.963
		GA	21 (52.5)	22 (55)	
		GG	10 (25)	9 (22.5)	
	Allele	A	39 (48.75)	40 (50)	.874
		G	41 (51.25)	40(50)	
Ghrelin rs34911341	Genotype	AA	0 (0)	5 (12.5)	.061
		GA	36 (90)	33 (82.5)	
		GG	4 (10)	2 (5)	
	Allele	A	36 (45)	43 (53.75)	.268
		G	44(55)	37 (46.25)	

ASD, autism spectrum disorder.

In the analysis of serum levels between the groups, there was no significant difference in leptin levels. In contrast, ghrelin serum levels were statistically significantly lower in the ASD group ($P = .584$, $P = .027$, respectively) (Table 3).

When the cases in the ASD group were examined in terms of the relationship between leptin and ghrelin serum levels and the severity of ASD, no significant correlation was found between ABC and CARS scores and leptin and ghrelin serum levels ($P > .05$) (Table 4).

Receiver operating characteristic curve analysis conducted to determine the cutoff value for ghrelin serum level as a diagnostic marker for ASD resulted in a cutoff value of 885.7 pg/mL with 42.50% sensitivity and 85% specificity

Table 3. Comparison of Children in the Autism Spectrum Disorder and Control Groups in Terms of Serum Leptin and Ghrelin Levels

	ASD	Control	*P*
Serum leptin level (ng/mL) Mean ± SD	1.98 ± 1.56	1.79 ± 1.55	.584
Serum ghrelin level (pg/mL) Median (min-max)	898.35 (577.8-932.5)	917.4 (857.9-933.7)	**.027**

ASD, autism spectrum disorder; SD, standard deviation.
Bold values: $P < 0.05$ were considered significant.

Table 4. Relationship Between the Severity of Autism Behavior Checklist and Serum Levels of Leptin and Ghrelin in Autism Behavior Checklist Group

	Leptin Serum Levels		Ghrelin Serum Levels	
	r	*P*	*r*	*P*
ABC	−0.83	0.612	−0.068	.675
CARS	−0.144	0.375	−0.097	.552

ABC, Autism Behavior hecklist; CARS, Childhood Autism Rating Scale.

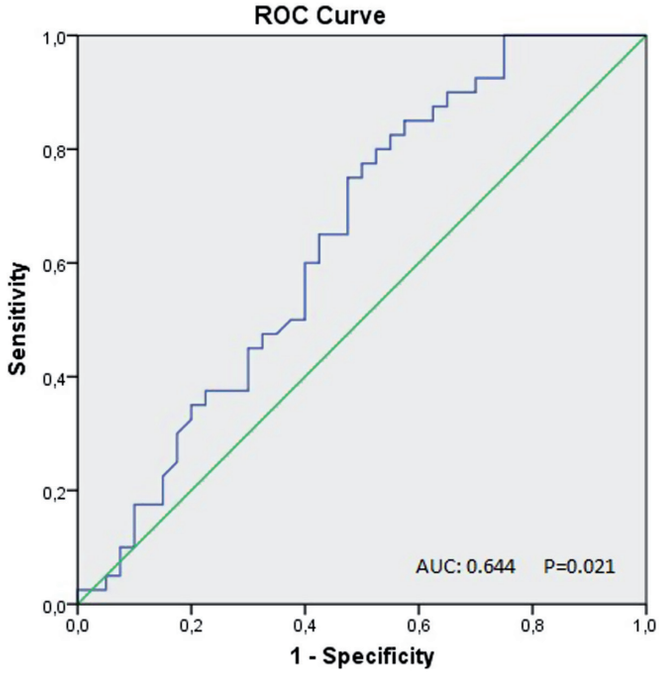

Figure 1. Ghrelin receiver operating characteristic curve curve: The area under the curve was determined as 0.644 for ghrelin with a cutoff value of 885.7 pg/mL.

(P=.021; area under the curve=0.644, SE_{AUC}= 0.062, 95% confidence interval - 0.529-0.748) (Figure 1).

A statistically significant binary logistic regression model exists between ghrelin serum level and a group containing ASD and control individuals (P=.002). In this model, ghrelin serum levels were identified as an independent risk factor for ASD (P=.011). As serum ghrelin level increases, ASD risk decreases by 0.980 times. In addition, there is a statistically significant binary logistic regression model between ghrelin serum level (categorized by cutoff value) and a group containing ASD and control individuals (P=.006). The ASD risk of patients with ghrelin levels ≤ 885.7 is 4.188 times higher than those >885.7 (Table 5).

DISCUSSION

In this study comparing leptin and ghrelin gene polymorphisms and serum levels between young individuals with ASD and healthy controls, no significant differences were observed in leptin and ghrelin gene polymorphisms and allele frequencies between the groups. Ghrelin serum levels were significantly lower in the ASD group compared to the control group, and ghrelin serum levels were identified as an independent risk factor for ASD.

Table 5. Effects of Ghrelin Serum Levels on Autism Spectrum Disorder

	Odds Ratio (95% CI)	*P*
Ghrelin serum levels	0.977 (0.960-0.995)	**.011**
Ghrelin serum levels (Ref: >885.7)	4.188 (1.436-12.218)	**.009**

Bold values: P < 0.05 were considered significant

This study did not show differences in serum leptin levels in children with ASD compared to healthy controls. In contrast, studies have shown that children with ASD have higher serum leptin levels than healthy controls.[11,22] A cohort study in 2018 reported an association between elevated serum leptin levels measured before the diagnosis of ASD in early childhood and an increased risk of ASD.[12] Leptin plays crucial roles in sexual development and reproduction, hematopoiesis, the regulation of gastrointestinal functions, and immune system function.[6] While leptin plays critical roles in various systems in the body, its potential involvement in the pathophysiology of psychiatric disorders has also been suggested. Evidence of the relationship between leptin and psychopathology is often derived from studies examining the association between psychiatric disorders such as bipolar affective disorder, major depressive disorder, panic disorder, and obesity.[36] Administering leptin to individuals with low leptin levels increases gray matter concentration in areas responsible for attention, emotion, and motivation, such as the anterior cingulate gyrus, inferior parietal lobe, and cerebellum.[37]

Various theories have been proposed to elucidate the potential role of leptin in ASD. Studies have indicated increased proinflammatory cytokine levels and decreased anti-inflammatory cytokine levels, leading to immune function impairment in children diagnosed with ASD. Leptin has been reported to share structural and functional similarities with specific cytokines, such as IL-6 and IL-12.[38] Considering its role in the differentiation of TH1 cells and the sustained inflammation through increased cytokine production, leptin may be regarded as a proinflammatory cytokine.[39] In line with this information, changes in leptin serum levels may occur in children diagnosed with ASD due to potential immune function disturbances, leading to dysregulation of the immune response.

The effects of leptin on regulating food intake and energy metabolism are well-known. Feeding problems are more frequently experienced in children diagnosed as having ASD compared to typically developing children.[9] These associated findings suggest that developmental differences in leptin levels in children with ASD might lead to the emergence of feeding problems or that differences in leptin serum levels may occur in ASD children due to frequent feeding problems observed in this population, compared to typically developing children.

A literature review indicates that no study has investigated the relationship between leptin gene polymorphism and ASD. Leptin gene polymorphisms have been associated with various diseases,[40,41] but studies examining their relationship with psychiatric disorders are limited. In a study investigating the association between leptin gene rs3828942 polymorphism and generalized anxiety disorder,

a gender-dependent relationship was found, with the A allele being associated with anxiety disorders in women. Additionally, men carrying the A allele had lower leptin serum levels.[42] The polymorphisms rs4731426 and rs7799039 in the leptin gene are effective in weight gain associated with lithium use in major depressive disorder.[43] However, in our study, no significant differences were found in leptin gene polymorphisms between the ASD and control groups. To our knowledge, our study is essential as the first to evaluate leptin gene polymorphism in ASD patients.

In this study, ghrelin serum levels were significantly lower in the ASD group. Our study's results are noteworthy as one of the rare studies indicating low levels of ghrelin in children with ASD. Additionally, the ROC curve analysis conducted to determine the diagnostic indicator potential of ghrelin serum levels in ASD represents initial steps toward the potential use of this indicator.

In a study by Al-Zaid et al[5] in 2014, ghrelin serum levels were found to be lower in children with ASD compared to the control group. Yamashita et al[44] conducted a study in 2019 involving 20 children with ASD and 20 children without, where venous blood cultures were obtained. They reported that a subgroup of ASD patients with inflammation and immune dysfunction showed positive therapeutic benefits following ghrelin administration.[44] Another recent study reported higher ghrelin serum levels in an ASD group compared to the control group.[22] Although there was no significant difference in BMI percentile between the 2 groups in our study, the BMI percentiles of the ASD group were higher than those of the control group. This near-significant difference might have affected ghrelin serum levels, which is considered a study limitation.

Ghrelin plays essential roles in various systems in the body, and it is suggested that ghrelin may have a role in the pathophysiology of psychiatric disorders. Studies indicate a strong relationship between eating behavior and impulsivity. Anderberg et al[45] suggested that, based on ghrelin's appetite-stimulating effect, it could also increase impulsivity. After injecting ghrelin into the lateral ventricles of rats and conducting impulsivity tests, they reported an increase in impulsive behaviors. Studies suggest that ghrelin may have a role in the pathophysiology of anxiety disorders, depression, and eating disorders.[46-48] Various theories have been proposed in attempts to elucidate the potential role of ghrelin in ASD. Ghrelin is known to play a role in obesity, nutrition, sleep, sensory processing, and executive functions, which are among the difficulties known to accompany individuals with ASD frequently. It is suggested that ghrelin regulates executive functions by primarily targeting the hippocampus, an essential region in the central nervous system for memory and learning. This region plays a role in ASD. Ghrelin, particularly within the hippocampal region, has been demonstrated to play a significant role in synaptogenesis. Abnormal synaptogenesis in this area has been shown in ASD.[20,21] Ghrelin has anti-apoptotic and proliferative effects in the central nervous system, especially without glucose or oxygen. Therefore, ghrelin is thought to protect against reactive oxygen radicals associated with ASD in the hypothalamus.[49,50] Additionally, ghrelin is believed to protect cortical neurons in children with ASD from apoptosis triggered by increased levels of the amino acid glutamate.[5] While numerous clinical observations and hormone studies have hinted at the potential involvement of ghrelin, a neuroprotective hormone, in ASD, there is a scarcity of studies exploring the specific relationship between ghrelin and ASD.

Upon reviewing the literature, no studies investigating the relationship between ghrelin gene polymorphism and ASD were found. Limited studies examining the association between ghrelin gene polymorphism and psychiatric disorders have drawn attention. In a study exploring Leu72Met polymorphism of the ghrelin gene, it was suggested that the gene polymorphism might play a role in depressive disorders. However, it might not have a significant role in the etiology of panic disorder.[25] Furthermore, in an animal study, it was shown that ghrelin-depleted zebrafish exhibit ADHD-like behaviors.[28]

Our study stands out as the first to assess ghrelin gene polymorphism in ASD cases. Although our genetic polymorphism analysis findings do not indicate a relationship between ghrelin gene polymorphism and ASD, these results need to be supported by larger sample sizes and independent studies.

We identified several limitations, including the small sample size and the cross-sectional design. Additionally, there was a near-statistically significant difference in BMI percentiles between the case and control groups of children. Another limitation was the need for more investigation into other gene polymorphisms associated with leptin and ghrelin genes in other neurodevelopmental disorders. The study's strengths include the exclusion of cases involving medical treatment and additional diagnoses. Furthermore, to our knowledge, this study is the first to examine the relationship between ASD and leptin and ghrelin gene polymorphisms.

In conclusion, our study comparing children with ASD to healthy controls revealed no significant differences in leptin and ghrelin gene polymorphisms and allele frequencies between the groups. Ghrelin serum levels were significantly lower in the ASD group compared to the control group, and ghrelin serum levels were identified as an independent risk factor for ASD. Receiver operating characteristic curve analysis aimed at determining the cutoff value for ghrelin serum level as a diagnostic indicator resulted in a cutoff value of 885.7 pg/mL with 42.50% sensitivity and 85% specificity. These findings contribute further evidence supporting the potential role of ghrelin in the pathophysiology of ASD. However,

additional data are needed to comprehend the possible role of polymorphisms in the leptin and ghrelin genes and whether these parameters can serve as hormonal markers for childhood ASD.

Ethics Committee Approval: This study was approved by the Local Ethics Committee of Muğla Sıtkı Koçman University Faculty of Medicine (Approval No: 9/IV; Date: May 11, 2022).

Informed Consent: Informed consent was obtained from the parents of all participants who agreed to take part in the study.

Peer-review: Externally peer-reviewed

Author Contributions: Concept – N.Ş., Ö.N.Y.; Design – N.Ş., Ö.N.Y.; Supervision – N.Ş.; Resources – E.S., T.Y., Ç.Ö., T.E.; Materials – E.S., T.Y., Ç.Ö., T.E.; Data Collection and/or Processing – Ö.N.Y., Ö.D., G.D.B, H.T., Ç.Ö, T.Y., E.S., T.E.; Analysis and/or Interpretation – N.Ş., Ö.N.Y.; Literature Search – N.Ş., Ö.N.Y.; Writing – Ö.N.Y., N.Ş.; Critical Review – N.Ş., T.E.

Declaration of Interests: The authors have no conflicts of interest to declare.

Funding: The authors declare that this study received no financial support.

REFERENCES

1. American Psychiatric Association and American Psychiatric Association DSM-5 Task Force. *Diagnostic and Statistical Manual of Mental Disorders: DSM-5*. Washington, DC: American Psychiatric Association; 2013.
2. Herbert MR. Contributions of the environment and environmentally vulnerable physiology to autism spectrum disorders. *Curr Opin Neurol*. 2010;23(2):103-110. [CrossRef]
3. Onore C, Careaga M, Ashwood P. The role of immune dysfunction in the pathophysiology of autism. *Brain Behav Immun*. 2012;26(3):383-392. [CrossRef]
4. Blardi P, de Lalla A, Ceccatelli L, Vanessa G, Auteri A, Hayek J. Variations of plasma leptin and adiponectin levels in autistic patients. *Neurosci Lett*. 2010;479(1):54-57. [CrossRef]
5. Al-Zaid FS, Alhader AA, Al-Ayadhi LY. Altered ghrelin levels in boys with autism: A novel finding associated with hormonal dysregulation. *Sci Rep*. 2014;26(4):647.
6. Fantuzzi G, Faggioni R. Leptin in the regulation of immunity, inflammation, hematopoiesis. *J Leukoc Biol*. 2000;68(4):437-446. [CrossRef]
7. Ansarin A, Mahdavi AM, Javadivala Z, Shanehbandi D, Zarredar H, Ansarin K. The cross-talk between leptin and circadian rhythm signaling proteins in physiological processes: A systematic review. *Mol Biol Rep*. 2023;50(12):10427-10443. [CrossRef]
8. Al Lihabi A. A literature review of sleep problems and neurodevelopment disorders. *Front Psychiatry*. 2023;14:1122344. [CrossRef]
9. Micai M, Fatta LM, Gila L, et al. Prevalence of co-occurring conditions in children and adults with autism spectrum disorder: A systematic review and meta-analysis. *Neurosci Biobehav Rev*. 2023;155:105436. [CrossRef]
10. Volkert VM, Vaz PCM. Recent studies on feeding problems in children with autism. *J Appl Behav Anal*. 2010;43(1):155-159. [CrossRef]
11. Ashwood P, Kwong C, Hansen R, et al. Brief report: plasma leptin levels are elevated in autism: Association with early onset phenotype? *J Autism Dev Disord*. 2008;38(1):169-175. [CrossRef]
12. Raghavan R, Zuckerman B, Hong X, et al. Fetal and infancy growth pattern, cord and early childhood plasma leptin, and development of autism spectrum disorder in the Boston Birth Cohort. *Autism Res*. 2018;11(10):1416-1431. [CrossRef]
13. Rodrigues DH, Rocha NP, Sousa LFC, Barbosa IG, Kummer A, Teixeira AL. Changes in adipokine levels in autism spectrum disorders. *Neuropsychobiology*. 2014;69(1):6-10. [CrossRef]
14. Essa MM, Braidy N, Al-Sharbati MM, Al-Farsi YM, Ali A, Waly MI, et al. Elevated plasma leptin levels in autistic children of Sultanate of Oman. *Int J Biol Med Res*. 2011;2(3):803-805.
15. Nakai Y, Hosoda H, Nin K, et al. Plasma levels of active form of ghrelin during oral glucose tolerance test in patients with anorexia nervosa. *Eur J Endocrinol*. 2003;149(1):R1-R3. [CrossRef]
16. Wiley KE, Davenport AP. Comparison of vasodilators in human internal mammary artery: Ghrelin is a potent physiological antagonist of endothelin-1. *Br J Pharmacol*. 2002;136(8):1146-1152. [CrossRef]
17. Akalu Y, Molla MD, Dessie G, Ayelign B. Physiological effect of ghrelin on body systems. *Int J Endocrinol*. 2020;2020:1385138. [CrossRef]
18. Braconnier ML, Siper PM. Neuropsychological assessment in autism spectrum disorder. *Curr Psychiatry Rep*. 2021;23(10):63. [CrossRef]
19. Huebner R, Dunn W. Introduction and basic concepts. In: *Autism: A Sensorimotor Approach Manag*. Gaithersburgh: Aspen publishers; 2001:3-40.
20. Kim C, Kim S, Park S. Neurogenic effects of ghrelin on the hippocampus. *Int J Mol Sci*. 2017;18(3):588. [CrossRef]
21. Barón-Mendoza I, Del Moral-Sánchez I, Martínez-Marcial M, García O, Garzón-Cortés D, González-Arenas A. Dendritic complexity in prefrontal cortex and hippocampus of the autistic-like mice C58/J. *Neurosci Lett*. 2019;703:149-155. [CrossRef]
22. Çelikkol Sadıç Ç, Bilgiç A, Kılınç İ, Oflaz MB, Baysal T. Evaluation of appetite-regulating hormones in young children with autism spectrum disorder. *J Autism Dev Disord*. 2021;51(2):632-643. [CrossRef]
23. Browning BD, Schwandt ML, Farokhnia M, Deschaine SL, Hodgkinson CA, Leggio L. Leptin gene and leptin receptor gene polymorphisms in alcohol use disorder: Findings related to psychopathology. *Front Psychiatry*. 2021;12:723059. [CrossRef]
24. Acikel SB, Eroglu C, Ugras Dikmen A, Kurar E. The association between leptin receptor polymorphism and suicidal behaviour in depressed adolescents. *Int J Psychiatry Clin Pract*. 2020;24(2):120-126. [CrossRef]
25. Nakashima K, Akiyoshi J, Hatano K, et al. Ghrelin gene polymorphism is associated with depression, but not panic disorder. *Psychiatr Genet*. 2008;18(5):257. [CrossRef]
26. Ando T. Ghrelin gene variants and eating disorders. *Vitam Horm*. 2013;92:107-123. [CrossRef]

27. Shi X, Guan K, Peng X, et al. Ghrelin modulates dopaminergic neuron formation and attention deficit hyperactivity disorder-like behaviors: from animals to human models. *Brain Behav Immun*. 2021;94:327-337. [CrossRef]
28. Guan K, Shan C, Guo A, Gao X, Li X. Ghrelin regulates hyperactivity-like behaviors via growth hormone signaling pathway in zebrafish (Danio rerio). *Front Endocrinol (Lausanne)*. 2023;14:1163263. [CrossRef]
29. Kaufman J, Birmaher B, Axelson D, et al. K-SADS-PL DSM-5. Pittsburgh: Western Psychiatric Institute and Clinic; 2016.
30. Ünal F, Öktem F, Çetin Çuhadaroğlu F, et al. Reliability and Validity of the Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version, DSM-5 November 2016-Turkish Adaptation (K-SADS-PL-DSM-5-T). *Turk Psikiyatri Derg*. 2019;30(1): 42-50.
31. Schopler E, Reichler RJ, DeVellis RF, Daly K. Toward objective classification of childhood autism: Childhood Autism Rating Scale (CARS). *J Autism Dev Disord*. 1980;10(1):91-103. [CrossRef]
32. Sucuoğlu B, Öktem F, Gökler B. Otistik çocukların değerlendirilmesinde kullanılan ölçeklere ilişkin bir çalışma. *Psikiyatr Psikhol Psikofarmakol*. 1996;4: 21-117.
33. İncekas Gassaloglu S, Baykara B, Avcil S, et al. Çocukluk Otizmi Derecelendirme Ölçeği Türkçe Formunun geçerlik ve güvenilirlik çalışması. *Türk Psikiyatr Derg*. 2016; 27:1-10.
34. Krug DA, Arick JR, Almond PA. *Autism Screening Instrument for Educational Planning*. 2nd ed. Austin, TX: Pro-ed Inc; 1993.
35. Yilmaz-Irmak T, Tekinsav-Sutcu WS, Aydin A, et al. Otizm Davranış Kontrol Listesi'nin (ODKL) geçerlik ve güvenirliğinin incelenmesi (An investigation of validity and reliability of Autism Behavior Checklist (ODKL)). *Turk J Child Adolesc Ment Health*. 2007;14(1):13-23.
36. Simon GE, Von Korff M, Saunders K, et al. Association between obesity and psychiatric disorders in the US adult population. *Arch Gen Psychiatry*. 2006;63(7):824-830. [CrossRef]
37. Matochik JA, London ED, Yildiz BO, et al. Effect of leptin replacement on brain structure in genetically leptin-deficient adults. *J Clin Endocrinol Metab*. 2005;90(5):2851-2854. [CrossRef]
38. Ashwood P, Anthony A, Torrente F, Wakefield AJ. Spontaneous mucosal lymphocyte cytokine profiles in children with autism and gastrointestinal symptoms: Mucosal immune activation and reduced counter regulatory interleukin-10. *J Clin Immunol*. 2004;24(6):664-673. [CrossRef]
39. Matarese G, Moschos S, Mantzoros CS. Leptin in immunology. *J Immunol*. 2005;174(6):3137-3142. [CrossRef]
40. Shetty SS, Suchetha Kumari NS, Hegde P, Roopashree PG, Suhasini PC. Leptin gene polymorphism Rs7799039; G2548A, metabolic and oxidative stress markers in polycystic ovarian syndrome. *J King Saud Univ Sci*. 2022; 34(6):102222. [CrossRef]
41. Menezes CA, Alves Junior ER, Costa GNO, et al. Genetic polymorphisms and plasma concentrations of leptin (rs7799039) and adiponectin (rs17300539) are associated with obesity in children and adolescents. *Rev Paul Pediatr*. 2022;40:e2021030. [CrossRef]
42. Salerno PSV, Bastos CR, Peres A, et al. Leptin polymorphism rs3828942: risk for anxiety disorders? *Eur Arch Psychiatry Clin Neurosci*. 2021;271(6):1141-1148. [CrossRef]
43. Bopp SK, Heilbronner U, Schlattmann P, et al. Leptin gene polymorphisms are associated with weight gain during lithium augmentation in patients with major depression. *Eur Neuropsychopharmacol*. 2019;29(2):211-221. [CrossRef]
44. Yamashita Y, Makinodan M, Toritsuka M, et al. Anti-inflammatory effect of ghrelin in lymphoblastoid cell lines from children with autism spectrum disorder. *Front Psychiatry*. 2019;10:152. [CrossRef]
45. Anderberg RH, Hansson C, Fenander M, et al. The stomach-derived hormone ghrelin increases impulsive behavior. *Neuropsychopharmacology*. 2016;41(5):1199-1209. [CrossRef]
46. Ozmen S, Şeker A, Demirci E. Ghrelin and leptin levels in children with anxiety disorders. *J Pediatr Endocrinol Metab*. 2019;32(10):1043-1047. [CrossRef]
47. Wittekind DA, Kluge M. Ghrelin in psychiatric disorders - A review. *Psychoneuroendocrinology*. 2015;52:176-194. [CrossRef]
48. Tolle V, Kadem M, Bluet-Pajot MT, et al. Balance in ghrelin and leptin plasma levels in anorexia nervosa patients and constitutionally thin women. *J Clin Endocrinol Metab*. 2003;88(1):109-116. [CrossRef]
49. Sato T, Fukue Y, Teranishi H, Yoshida Y, Kojima M. Molecular forms of hypothalamic ghrelin and its regulation by fasting and 2-deoxy-d-glucose administration. *Endocrinology*. 2005;146(6):2510-2516. [CrossRef]
50. Bai J, Yang F, Dong L, Zheng Y. Ghrelin protects human lens epithelial cells against oxidative stress-induced damage. *Oxid Med Cell Longev*. 2017;2017:1910450. [CrossRef]