



Analysis of Dried Blood Spot Carnitine Levels in Children Diagnosed with Autism Spectrum Disorder

Otizm Spektrum Bozukluğu Tanılı Çocuklarda Karnitin Profilinin Değerlendirilmesi

Arzu Selamioğlu¹, Tarık Yıldırım¹, Burcu Yeter², Okan Yüce³, Fatih Mete³, Meltem Erol³

¹University of Health Sciences Turkey, İstanbul Bağcılar Training and Research Hospital, Department of Pediatric Metabolic Diseases, İstanbul, Turkey

²University of Health Sciences Turkey, Ümraniye Training and Research Hospital, Department of Pediatric Genetics, İstanbul, Turkey

³University of Health Sciences Turkey, İstanbul Bağcılar Training and Research Hospital, Department of Pediatrics, İstanbul, Turkey

Abstract

Objective: This study aimed to investigate the carnitine and acylcarnitine profiles in children diagnosed with autism spectrum disorder (ASD) and compare the findings with those of healthy controls.

Method: A total of 90 participants, including 60 children with ASD and 30 age- and sex-matched healthy controls, were enrolled. Dried blood spot samples were analyzed using Tandem mass spectrometry (MS/MS) to quantify free carnitine and acylcarnitine levels. Statistical comparisons were performed between groups, and receiver operating characteristic (ROC) curve analysis was applied to assess the diagnostic performance of significantly altered metabolites.

Results: No significant differences were observed in the majority of carnitine and acylcarnitine species between the two groups. However, the level of C8:1 (octenoylcarnitine) was significantly elevated in the ASD group compared to controls ($p=0.038$). ROC analysis identified 0.05 $\mu\text{mol/L}$ as the optimal cut-off value for C8:1, although the discriminative ability was limited (area under the curve = 0.63).

Conclusion: C8:1 levels were modestly higher in children with ASD. This observation may indicate subtle alterations in fatty acid metabolism or mitochondrial function; however, given the modest effect size and the number of comparisons performed, this result

Öz

Amaç: Bu çalışmanın amacı, otizm spektrum bozukluğu (OSB) tanısı almış çocuklarda karnitin ve asilkarnitin profillerini incelemek ve elde edilen bulguları sağlıklı kontrollerle karşılaştırmaktır.

Yöntem: Toplam 90 olgu (60 OSB tanılı çocuk ile yaş ve cinsiyet açısından benzer özelliklere sahip 30 sağlıklı kontrol) çalışmaya dahil edildi. Kurutulmuş kan damlası örnekleri kullanılarak Tandem kütle spektrometrisi (MS/MS) yöntemiyle serbest karnitin ve asilkarnitin düzeyleri ölçüldü. Gruplar arasındaki istatistiksel karşılaştırmalar yapıldı ve anlamlı değişiklik gösteren metabolitler için alıcı işletim karakteristiği (ROC) eğrisi analizi uygulandı.

Bulgular: İki grup arasında çoğu karnitin ve asilkarnitin türü açısından anlamlı bir fark gözlenmedi. Ancak, C8:1 (oktenoilkarnitin) düzeyi OSB grubunda kontrol grubuna göre anlamlı derecede yüksek bulundu ($p=0,038$). ROC analizi sonucunda C8:1 için en uygun eşik değerin 0,05 $\mu\text{mol/L}$ olduğu belirlendi ve eğri altında kalan alan 0,63 olarak hesaplandı.

Sonuç: Çalışmamızda OSB'li çocuklarda C8:1 düzeyleri hafifçe daha yüksek bulundu. Bu gözlem, yağ asidi metabolizması veya mitokondriyal fonksiyondaki ince değişikliklere işaret edebilir; ancak, etki büyüklüğünün düşük olması ve analiz edilen çok sayıdaki metabolit nedeniyle, bu sonuç kesin bir biyobelirteç olarak

Address for Correspondence: Arzu Selamioğlu, University of Health Sciences Turkey, İstanbul Bağcılar Training and Research Hospital, Department of Pediatric Metabolic Diseases, İstanbul, Turkey

E-mail: arzuceyln@hotmail.com **ORCID:** orcid.org/0000-0001-7264-038X

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Abstract

should be considered a preliminary observation rather than evidence of a definitive biomarker. Larger multicenter studies with broader sampling are required to validate these results and clarify their clinical significance.

Keywords: Acylcarnitine, autism spectrum disorder, C8:1 carnitine, carnitine, octenoylcarnitine

Öz

değil, öncül ve dikkatle yorumlanması gereken bir sonuç olarak değerlendirilmelidir. Bu sonuçların doğrulanması ve klinik öneminin netleştirilmesi için daha geniş örneklem içeren çok merkezli çalışmalara ihtiyaç vardır.

Anahtar kelimeler: Asilkarnitin, C8:1 karnitin, karnitin, oktenoilkarnitin, otizm spektrum bozukluğu

Introduction

Autism spectrum disorder (ASD) is a multifaceted neurodevelopmental disorder that usually manifests in early childhood, primarily through difficulties in social interaction, challenges in communication, and the occurrence of repetitive and restricted behaviors (1). Over the past two decades, the prevalence of ASD has notably increased. According to the 2023 surveillance data from the Centers for Disease Control and Prevention, ASD affects approximately 27.6 per 1,000 children aged 8 years in the United States, which equates to nearly 1 in every 36 children (2). In Turkey, a population-based study including 6.712 children aged 16-36 months reported an ASD prevalence of 1 in 117 (3). Although the underlying mechanisms of ASD remain incompletely understood, current evidence suggests a multifactorial origin involving genetic, epigenetic, environmental, and potentially metabolic components (2).

Inherited metabolic diseases (IMDs), also known as inborn errors of metabolism (IEM), represent a heterogeneous group of genetic disorders resulting from alterations in various biochemical pathways. A wide spectrum of IMDs—including disorders of amino acid metabolism (e.g., phenylketonuria, homocystinuria, maple syrup urine disease), organic acidurias (e.g., propionic aciduria, glutaric aciduria type 1), neurotransmitter disorders, purine metabolism defects (e.g., Lesch-Nyhan syndrome), creatine deficiency syndromes, disorders of cobalamin and folate metabolism, lysosomal storage disorders such as mucopolysaccharidosis type III, and bile acid synthesis defects—have been reported to present with autistic features. Some of these conditions are amenable to treatment, highlighting the clinical relevance of their identification. However, there is no established cause–effect relationship between ASD and IMDs; rather, autistic features in these conditions are typically observed in the context of intellectual disability and broader neurodevelopmental impairment. Therefore, neurometabolic diseases presenting with intellectual disability may meet the diagnostic criteria

for ASD, and genetic consultation is recommended for all children presenting with autistic features (4,5).

Carnitine, a crucial nutrient largely acquired through dietary sources, especially from animal-based foods, plays a key role in mitochondrial energy production and fatty acid metabolism. In the human body, carnitine is present as free carnitine and acylcarnitine. Free carnitine facilitates the transport of long-chain fatty acids into the mitochondria, where they are subjected to β -oxidation to generate energy. Acylcarnitines serve as intermediates in the metabolic processing of fatty acids and organic acids (6). In individuals with ASD, carnitine profiles are of special interest, as disturbances may point to mitochondrial abnormalities or irregularities in fatty acid metabolism. These metabolic imbalances may be relevant to the pathophysiology of ASD (7).

This study was designed to evaluate the carnitine profiles in children with ASD using tandem mass spectrometry (MS/MS) and to compare the results with those of healthy children matched for age and sex. The objective was to uncover metabolic deviations that might provide new insights into the biological basis of ASD.

Materials and Methods

Study Design and Participants

This retrospective, cross-sectional study was conducted in accordance with the ethical principles outlined in the latest revision of the Declaration of Helsinki and was approved by the Ethics Committee of the University of Health Sciences Turkey, İstanbul Bağcılar Training and Research Hospital (decision no: 2024/10/04/082, date: 22.10.2024). Written informed consent was obtained from the parents or legal guardians of all participants. The study population consisted of pediatric patients aged between 18 months and 18 years who were diagnosed with ASD based on the criteria of the diagnostic and statistical manual of mental disorders-fifth edition, by a board-

certified child psychiatrist (1). All diagnoses were further confirmed by an independent public health committee composed of psychiatrists responsible for determining special educational needs. Patients were evaluated at the Pediatric Metabolism Outpatient Department of the University of Health Sciences Turkey, İstanbul Bağcılar Training and Research Hospital between October 2023 and November 2024. The ASD group included 60 children receiving routine follow-up and treatment for autism. The control group comprised 30 age- and sex-matched children. MS/MS acylcarnitine profiling was performed in children who were referred to the pediatric metabolism outpatient clinic because of non-specific clinical concerns, including mild gastrointestinal complaints, nutritional concerns, or evaluation for suspected inherited metabolic disorders. Following comprehensive clinical, biochemical, and metabolic evaluation, only children with completely normal findings and no evidence of metabolic, psychiatric, neurological, or chronic systemic disease were included as healthy controls. Although this approach allowed comprehensive metabolic assessment and minimized the likelihood of including children with unrecognized metabolic disorders that could have influenced acylcarnitine profiles, recruitment of controls from a referral population rather than the general community may have introduced selection bias, affected the comparability between the study groups, and limited the generalizability of the findings. All control participants also underwent clinical evaluation by a pediatrician to exclude subclinical signs of autism. Exclusion criteria included the presence of any comorbid psychiatric disorder, chronic systemic disease, use of medications affecting carnitine metabolism, absence of parental consent, or failure to meet the specified age range. In addition, patients with a previously diagnosed inherited metabolic or genetic disorder were excluded from the study. Patients who had received probiotic supplementation within the last 3 months were also excluded to minimize potential confounding effects on carnitine metabolism.

Data Collection

Demographic variables (age, sex), presence of parental consanguinity, anthropometric measurements [weight, height, body mass index (BMI) in standard deviation scores (SDSs)], and laboratory findings were retrospectively retrieved from electronic medical records and recorded in a structured Excel file. Serum lipid profiles, including total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triglycerides, were measured using the Hitachi 7600-020 automated biochemical analyzer.

Reference ranges for lipid parameters were defined according to the NHLBI pediatric guidelines as follows: Total cholesterol <170 mg/dL, LDL-C <110 mg/dL, HDL-C $\geq$ 40 mg/dL, and triglycerides <75 mg/dL for children aged 0-9 years and <90 mg/dL for those aged 10-19 years (8). Dried blood spot (DBS) carnitine profiles were determined via MS/MS. DBS samples were collected in a non-fasting state during morning outpatient visits. Children with recent infections, acute illness, or medication use affecting carnitine metabolism were excluded. Reference ranges and cut-off values for acylcarnitine profiles were based on previously published tandem mass spectrometry validation studies (9).

Statistical Analysis

Descriptive data were summarized using appropriate measures, including mean, standard deviation, median, minimum, maximum, frequency, and percentages. The normality of continuous variables was evaluated using both the Kolmogorov-Smirnov and Shapiro-Wilk tests. For comparisons involving independent quantitative data, the independent samples t-test was applied when a normal distribution was present. In contrast, the Mann-Whitney U test was utilized for variables that did not meet the normality assumption. Since all acylcarnitine variables were non-normally distributed, comparisons of acylcarnitine parameters between the ASD and control groups were performed exclusively using the Mann-Whitney U test. To analyze relationships between categorical variables, the chi-square (χ^2) test was used. Receiver operating characteristic (ROC) curve analysis was conducted to assess the diagnostic performance of C8:1 octenoylcarnitine levels in distinguishing children with ASD from controls. The area under the curve (AUC) was used to measure discrimination ability, and the optimal threshold value was determined based on Youden's index. All statistical computations were carried out using IBM SPSS Statistics for Windows, version 28.0 (IBM Corp., Armonk, NY, USA).

Results

This study enrolled 90 Turkish children, 60 with confirmed ASD and 30 age- and sex-matched controls, after parental consent was obtained. In the control group (n=30), the mean age was 6.5 ± 2.7 years, with a median of 6.3 years. Socio-demographic data of the children with ASD and control groups are shown in Table 1. In the ASD group (n=60), the mean age was 7.2 ± 3.4 years, with a median of 6.4 years. The age distribution did not differ significantly between the groups (p=0.421). Regarding sex distribution,

Table 1. Characteristics of the autism spectrum disorder group and control groups

Variable	Control group (n=30)		Autism spectrum disorder group (n=60)		p (test)
	Mean $\pm$ SD/n-%	Median	Mean $\pm$ SD/n-%	Median	
Age (years)	6.5 $\pm$ 2.7	6.3	7.2 $\pm$ 3.4	6.4	0.421 (^m)
Sex-female	5 /16.7%		9/15.0%		0.837 (χ^2)
Sex-male	25/83.3%		51/85.0%		
Weight SDS	-0.32 $\pm$ 0.81	-0.42	0.05 $\pm$ 1.48	-0.03	0.143 (^l)
Height SDS	-0.17 $\pm$ 0.85	-0.14	-0.01 $\pm$ 1.13	-0.03	0.519 (^l)
BMI SDS	-0.31 $\pm$ 1.11	-0.45	0.03 $\pm$ 1.56	-0.24	0.299 (^l)
Consanguinity-no	21/70.0%		41/68.3%		0.872 (χ^2)
Consanguinity-yes	9/30.0%		19/31.7%		
Consanguinity-1 cousin	6/66.7%		8/42.1%		0.225 (χ^2)
Consanguinity-other	3/33.3%		11/57.9%		
Total cholesterol (mg/dL)	158.1 $\pm$ 35.3	153.5	155.9 $\pm$ 25.0	153.0	0.733 (^l)
LDL cholesterol (mg/dL)	88.3 $\pm$ 31.4	81.5	79.4 $\pm$ 23.3	76.0	0.141 (^l)
HDL cholesterol (mg/dL)	53.3 $\pm$ 12.0	52.5	55.3 $\pm$ 20.4	52.0	0.577 (^l)
Triglyceride (mg/dL)	84.6 $\pm$ 34.6	79.0	109.0 $\pm$ 90.4	75.5	0.922 (^m)

^m: Mann-Whitney U test, χ^2 : Chi-square test, ^l: Independent samples t-test, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, BMI: Body mass index, SDS: Standard deviation score, SD: Standard deviation
Reference ranges for lipid parameters were based on NHLBI pediatric guidelines: total cholesterol <170 mg/dL, LDL-C <110 mg/dL, HDL-C $\geq$ 40 mg/dL, triglycerides <75 mg/dL (0-9 years) and <90 mg/dL (10-19 years) (8)

16.7% (n=5) of the control group were female and 83.3% (n=25) were male, whereas in the ASD group, 15.0% (n=9) were female and 85.0% (n=51) were male. There was no statistically significant difference in sex distribution between the groups (p=0.837).

Anthropometric measurements showed that the mean weight SDS was -0.32 $\pm$ 0.81 in the control group and 0.05 $\pm$ 1.48 in the ASD group (p=0.143). The mean height SDS was -0.17 $\pm$ 0.85 in the control group and -0.01 $\pm$ 1.13 in the ASD group (p=0.519). The mean BMI SDS was -0.31 $\pm$ 1.11 in the control group and 0.03 $\pm$ 1.56 in the ASD group (p=0.299). None of these differences were statistically significant.

Consanguinity was reported in 30.0% (n=9) of the control group and in 31.7% (n=19) of the ASD group. There was no significant difference in the presence of consanguinity between groups (p=0.872). Among those with consanguinity, 66.7% (n=6) of the control group and 42.1% (n=8) of the ASD group reported a cousin relationship, while 33.3% (n=3) of the control group and 57.9% (n=11) of the ASD group reported distant relationships. The degree of consanguinity did not differ significantly between groups (p=0.225).

Biochemical analyses showed that mean total cholesterol levels were 158.1 $\pm$ 35.3 mg/dL in the control group and 155.9 $\pm$ 25.0 mg/dL in the ASD group (p=0.733). LDL levels were 88.3 $\pm$ 31.4 mg/dL in the control group and 79.4 $\pm$ 23.3 mg/dL in the ASD group (p=0.141). HDL levels were

53.3 $\pm$ 12.0 mg/dL in the control group and 55.3 $\pm$ 20.4 mg/dL in the ASD group (p=0.577). Triglyceride levels were 84.6 $\pm$ 34.6 mg/dL in the control group and 109.0 $\pm$ 90.4 mg/dL in the ASD group (p=0.922). None of these parameters showed statistically significant differences between the groups.

Age and sex distributions were comparable between the ASD and control groups, with no statistically significant differences detected (p>0.05). Similarly, there were no significant differences in weight SDS, height SDS, and BMI SDS values between the two groups (p>0.05). The presence of consanguinity and the degree of consanguinity did not differ significantly between the control and ASD groups (p>0.05). The groups were comparable in terms of total cholesterol, LDL, HDL, and triglyceride levels, with no significant statistical differences observed (p>0.05) (Table 1).

No statistically significant differences were found between the control and ASD groups in terms of C0 free carnitine, C2 acetylcarnitine, C3 propionylcarnitine, C4 butyrylcarnitine, C5:1 tiglylcarnitine, C5 isovalerylcarnitine, C6 hexanoylcarnitine, C5OH 3OH isovalerylcarnitine, C8 octanoylcarnitine, C10:1 decenoylcarnitine, C10 decanoylcarnitine, C5DC glutaryl carnitine, C12 dodecanoylcarnitine, and C6DC adipoylcarnitine levels (p>0.05). However, C8:1 octenoylcarnitine was significantly higher in the ASD group compared to the control

Table 2. Comparison of acylcarnitine and carnitine derivatives between groups

Variable	Control group (n=30) Mean ± SD	Median	Autism spectrum disorder group (n=60) Mean ± SD	Median	p-value
C0 Free Carnitine	25.6±7.2	24.0	25.5±7.4	24.4	0.884
C2 Acetylcarnitine	14.6±11.8	11.1	11.4±6.3	10.1	0.176
C3 Propionylcarnitine	1.20±0.42	1.20	1.12±0.40	1.09	0.364
C4 Butyrylcarnitine	0.13±0.09	0.11	0.12±0.07	0.10	0.993
C5:1 Tiglylcarnitine	0.07±0.04	0.07	0.09±0.03	0.08	0.058
C5 Isovalerylcarnitine	0.08±0.04	0.07	0.08±0.04	0.07	0.983
C6 Hexanoylcarnitine	0.03±0.02	0.03	0.03±0.02	0.03	0.807
C5OH 3OH Isovalerylcarnitine	0.13±0.08	0.10	0.12±0.06	0.11	0.833
C8:1 Octenoylcarnitine	0.04±0.02	0.03	0.05±0.03	0.05	**0.038**
C8 Octanoylcarnitine	0.07±0.04	0.06	0.08±0.05	0.06	0.325
C10:1 Decenoylcarnitine	0.02±0.01	0.02	0.03±0.02	0.02	0.339
C10 Decanoylcarnitine	0.08±0.05	0.07	0.10±0.06	0.09	0.236
C5DC Glutaryl carnitine	0.06±0.03	0.06	0.06±0.02	0.06	0.317
C12 Dodecanoylcarnitine	0.05±0.03	0.04	0.05±0.02	0.04	0.903
C6DC Adipoylcarnitine	0.02±0.02	0.02	0.03±0.03	0.02	0.692
C14:2	0.02±0.02	0.02	0.03±0.04	0.02	0.568
C14:1	0.03±0.02	0.03	0.04±0.02	0.04	0.417
C14 Myristoylcarnitine	0.06±0.03	0.05	0.05±0.02	0.05	0.765
C4DC Methylmalonylcarnitine	0.19±0.06	0.17	0.19±0.05	0.19	0.523
C16:1 Palmitoleoylcarnitine	0.03±0.02	0.02	0.03±0.02	0.03	0.287
C16 Palmitoylcarnitine	0.80±0.27	0.73	0.72±0.25	0.69	0.255
C18:2 Linoleoylcarnitine	0.04±0.02	0.04	0.03±0.02	0.03	0.076
C18:1 Oleoylcarnitine	0.08±0.04	0.06	0.07±0.02	0.06	0.588
C18 Stearoylcarnitine	0.58±0.26	0.56	0.51±0.24	0.50	0.154
C18:1OH 3OH Oleoylcarnitine	0.00±0.00	0.00	0.01±0.02	0.00	0.498
C4OH	0.05±0.02	0.04	0.05±0.03	0.04	0.972
C3DC	0.04±0.04	0.03	0.05±0.04	0.03	0.917
C16OH	0.01±0.01	0.01	0.01±0.02	0.01	0.695
C3/C0	0.05±0.01	0.04	0.05±0.02	0.04	0.809
C3/C2	0.10±0.04	0.11	0.11±0.05	0.11	0.458
C8/C10	0.87±0.33	0.76	0.85±0.50	0.75	0.398

All comparisons of acylcarnitine parameters were performed using the Mann-Whitney U test. Reference ranges and cut-off values for acylcarnitine profiles were based on previously published tandem mass spectrometry validation studies (9).
SD: Standard deviation

group ($p < 0.05$). Additionally, there were no significant differences between the groups in terms of C14:2, C14:1, C14 myristoylcarnitine, C4DC methylmalonylcarnitine, C16:1 palmitoleoylcarnitine, C16 palmitoylcarnitine, C18:2 linoleoylcarnitine, C18:1 oleoylcarnitine, C18 stearoylcarnitine, C18:1OH 3OH oleoylcarnitine, C4OH, C3DC, C16OH, C3/C0, C3/C2, and C8/C10 ratios ($p > 0.05$) (Table 2). ROC curve analysis showed that a C8:1 octenoylcarnitine level above $0.05 \mu\text{mol/L}$ may differentiate children with ASD from healthy controls with a moderate

discriminatory ability ($\text{AUC} = 0.63$). Therefore, the clinical significance of this finding remains uncertain (Figure 1).

All patients, including those with elevated C8:1 levels, were clinically evaluated, and no new diagnosis of an underlying metabolic or genetic disorder was established during the study period.

Discussion

In this study, the analysis of DBS carnitine profiles in children diagnosed with ASD revealed no significant

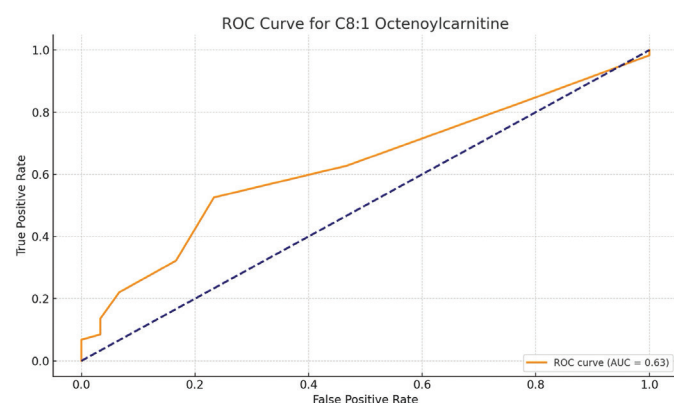


Figure 1. ROC curve of C8:1 octenoylcarnitine
ROC: Receiver operating characteristic, AUC: Area under the curve

alterations in most carnitine and acylcarnitine derivatives when compared to healthy controls, except for a modest elevation in C8:1 octenoylcarnitine. Additionally, no new syndromic diagnosis (such as Fragile X syndrome, Rett syndrome, or Smith-Lemli-Opitz syndrome) was identified during the study period. Therefore, the observed biochemical findings are more likely to reflect metabolic characteristics associated with ASD rather than known syndromic conditions.

Sex-based differences in ASD are well known. While the male-to-female diagnostic ratio is often cited as 4:1, evidence from a comprehensive meta-analysis by Loomes et al. (10), encompassing more than 13 million participants from 54 studies, indicates that this figure likely overestimates the actual sex difference. In high-quality studies and those using active case-ascertainment methods, the male-to-female ratio was found to be closer to 3.32:1 and 3.25:1, respectively. These findings indicate that while some females with ASD may be underdiagnosed, a true biological predisposition in males is also plausible. Proposed explanations include the “extreme male brain” theory, differential hormonal influences, and sex-linked genetic vulnerability (10).

Nonetheless, diagnostic processes themselves may also contribute to the observed imbalance. Girls with ASD often engage in compensatory behaviors such as camouflaging, display less stereotypical restricted interests, and may be influenced by gender-related expectations, all of which can obscure symptom detection. Moreover, studies indicate that the male-to-female ratio appears lower among individuals with intellectual disability, while higher-functioning females are more likely to remain undiagnosed (10). In our study, the proportion of males was consistent

with previous literature; however, it is essential to interpret this finding within the context of both diagnostic limitations and biological variability. Future research should integrate both neurobiological mechanisms and gender-sensitive diagnostic strategies to improve the recognition and understanding of ASD across sexes.

Parental consanguinity is a known risk factor in numerous inherited neurodevelopmental disorders; however, its role in ASD is still debated. In our study, the prevalence of consanguinity was 31.7% among ASD cases and 30.0% in controls, showing no statistically significant difference. This finding is consistent with the results of Alshaban et al. (11), who conducted a large, population-based case-control study in Qatar. Their analysis, based on a representative sample of 891 children, also showed no clear association between consanguinity and ASD risk (adjusted odds ratio =1.065; 95% confidence interval: 0.751-1.509; $p>0.05$). Similarly, a recent genomic analysis by Al-Sarraj et al. (12) showed that although parental consanguinity was common in multiplex families with ASD, it was not independently associated with the presence of pathogenic variants or with an increased risk of ASD across the cohort. Instead, their findings underscored the complexity of ASD inheritance, showing that while some affected siblings shared recessive mutations, many such variants also occurred in families without consanguinity.

Growth patterns in children with ASD have shown substantial heterogeneity across studies, reflecting a complex interplay between genetic, metabolic, and behavioral factors. In our study, no statistically significant differences were observed between the ASD and control groups in terms of weight SDS, height SDS, or BMI SDS values. These results contrast with a recent systematic review by Molani-Gol et al. (13), which analyzed 23 studies and reported that most infants later diagnosed with ASD exhibited accelerated weight gain (in 90.47% of included studies) and increased height (in 76.92%) during the first year of life. The authors proposed that early overgrowth might reflect underlying neurodevelopmental dysregulation, possibly linked to genetic or hormonal mechanisms. Furthermore, they suggested that these growth patterns might serve as early predictive biomarkers for ASD risk, especially when combined with behavioral or developmental screening tools. However, the authors also emphasized substantial heterogeneity across studies, with some reporting no differences or even reduced growth in ASD compared to typically developing peers. Such variability may be influenced by developmental subtypes,

age at evaluation, and external factors such as nutrition (13).

Cholesterol is an unsaturated alcohol that plays a vital role in neurodevelopment by contributing to synaptogenesis, myelination, and membrane fluidity.

It is also a key structural component of neuronal membranes, axons, dendrites, and synaptic vesicles, where its availability directly influences neuronal signaling and connectivity. Although our study did not reveal statistically significant differences in total cholesterol, LDL, HDL, or triglyceride levels between the ASD and control groups, alterations in cholesterol metabolism have often been observed in individuals with ASD, especially regarding hypocholesterolemia in both syndromic and non-syndromic cases. A recent review by Lin et al. (14) emphasized that patients with ASD or associated genetic conditions—such as Fragile X syndrome, Rett syndrome, and Smith-Lemli-Opitz syndrome—often exhibit disrupted cholesterol homeostasis, potentially contributing to the neurobiological mechanisms underlying autism. These findings highlight the need for larger-scale, mechanistic studies that explore both systemic and central cholesterol regulation in individuals with ASD, including assessments of intracellular lipid pathways and CNS-specific cholesterol dynamics.

IEMs have been reported to present with autistic features more frequently than in the general population. Lipiński (4) reported that targeted metabolic screening in ASD populations revealed IMDs in up to 12.4% of cases. Among the metabolic abnormalities implicated in ASD, carnitine-related disorders are of particular interest due to the essential role of carnitine in mitochondrial β -oxidation, energy production, and central nervous system development. In our study, no statistically significant differences were observed in free carnitine (C0) concentrations between the ASD and control groups except for a statistically significant elevation in C8:1 octenoylcarnitine. Nevertheless, isolated case reports and small-scale studies indicate that abnormalities in carnitine metabolism may be present in a subset of individuals with ASD. For instance, Cakar and Yilmazbas (5) retrospectively evaluated 179 children diagnosed with ASD and identified IEMs in 3.3% of cases, including a patient with 3-methylcrotonyl-CoA carboxylase (3-MCC) deficiency. This patient exhibited markedly reduced serum C0 and elevated C5-OH levels, and demonstrated clinical improvement in language and behavior following supplementation with biotin and carnitine (5).

In our study, although most DBS carnitine and acylcarnitine profiles did not show significant differences between children with ASD and healthy controls, C8:1 was found to be modestly elevated in the ASD group. However, this difference should be interpreted with caution. Multiple acylcarnitines were evaluated, and testing numerous metabolites increases the risk of type I error. No formal correction for multiple comparisons was applied because this study was designed as an exploratory analysis. Therefore, the observed difference in C8:1 should be interpreted with caution. Although an elevated level of C8:1 has been reported in some studies alongside other acylcarnitine abnormalities, it is not considered a specific biomarker for any particular metabolic disorder. The presence of increased C8:1 carnitine might reflect mild alterations in mitochondrial β -oxidation or fatty acid metabolism, yet its clinical significance is not yet fully understood in the absence of consistent metabolic or genetic findings.

This finding aligns with the results of Chakkera et al. (15), who reported increased C8:1 levels and decreased concentrations of several other acylcarnitines—including C4, C5, and C26—in children with ASD, particularly those with developmental regression. While their study involved a broader targeted metabolomic profile including amino acids and purines, both datasets raise the possibility that alterations in fatty acid oxidation and mitochondrial β -oxidation may be present in a subset of individuals with ASD. The observation of elevated C8:1 octenoylcarnitine in both studies may suggest a potentially interesting metabolic signal warranting further investigation. However, given that multiple metabolites were analyzed and only C8:1 reached nominal statistical significance, the possibility of a chance finding cannot be excluded. Therefore, this observation should be considered exploratory and requires confirmation in larger independent cohorts.

Our findings partially contrast with those of Aslan et al. (7), who analyzed 23 ASD cases using LC-MS/MS and reported significantly lower levels of several carnitine species—including C0, C2, C4-OH, C5, C5-OH, C6, C16, and C18—alongside a significant elevation in C18:1. Similarly, Barone et al. (16) reported increased levels of short-, medium- (including C8:1), and long-chain acylcarnitines in ASD patients, accompanied by evidence of β -oxidation dysfunction in fibroblast assays. In contrast, Lv et al. (6) reported that blood levels of free carnitine, glutaryl carnitine, octyl carnitine, twenty-four carbonyl carnitine, and carnosyl carnitine were significantly lower in the ASD group compared to typically developing controls.

Based on their findings, glutaryl carnitine and carnosyl carnitine were proposed as potential biomarkers for the diagnosis of ASD. The divergence among these studies may stem from differences in sample size, age range, inclusion criteria, analytical platforms, or regional and dietary factors influencing carnitine metabolism. Moreover, Burger et al. (17) described elevated C8:1 levels in ASD children with specific genetic mutations, suggesting that C8:1 accumulation may be restricted to certain molecular subtypes.

Study Limitations

This study has several limitations. First, its retrospective, cross-sectional, and single-center design may restrict the generalizability of the findings. Additionally, although the use of a metabolism clinic-based control group enabled detailed clinical and biochemical evaluation, it may have introduced selection bias, as these participants were recruited from a referral population rather than the general community. Furthermore, DBS samples were collected during routine morning visits in a non-fasting state. While non-fasting sampling is acceptable for MS/MS-based acylcarnitine analysis, postprandial variability may lead to minor fluctuations in some short- and medium-chain metabolites. These methodological factors should be taken into account when interpreting the results and highlight the need for future multicenter studies across diverse populations using population-based controls and standardized sampling conditions. The study was not designed to evaluate the predictive value of biochemical parameters for genetic diagnoses. Additionally, other disease-specific biochemical markers were not assessed, which may be relevant in certain metabolic or genetic conditions.

Medication use may represent a potential confounding factor in studies evaluating carnitine metabolism. In the present study, patients receiving medications known to affect carnitine metabolism were excluded to minimize this potential bias. Finally, no formal correction for multiple comparisons was applied despite the evaluation of multiple acylcarnitine metabolites. Therefore, the observed association between C8:1 and ASD should be considered exploratory and requires confirmation in larger independent cohorts.

Conclusion

In this study, we evaluated the carnitine and acylcarnitine profiles of children with ASD using MS/MS. Although

most carnitine species did not differ significantly between the ASD and control groups, C8:1 levels were found to be slightly higher in the ASD group. However, given that multiple carnitine and acylcarnitine metabolites were analyzed and only C8:1 reached nominal statistical significance, the possibility of a chance finding cannot be excluded. Furthermore, given its limited discriminatory performance (AUC =0.63), this observation should be considered exploratory and interpreted cautiously rather than as evidence of biomarker utility. Larger multicenter studies are needed to validate this observation, clarify the underlying mechanisms, and determine the clinical significance of acylcarnitine alterations in ASD.

Ethics

Ethics Committee Approval: This retrospective, cross-sectional study was conducted in accordance with the ethical principles outlined in the latest revision of the Declaration of Helsinki and was approved by the Ethics Committee of the University of Health Sciences Turkey, İstanbul Bağcılar Training and Research Hospital (decision no: 2024/10/04/082, date: 22.10.2024).

Informed Consent: Written informed consent was obtained from the parents or legal guardians of all participants.

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Footnotes

Authorship Contributions

Surgical and Medical Practices: A.S., T.Y., B.Y., O.Y., F.M., M.E., Concept: A.S., T.Y., B.Y., Design: A.S., T.Y., B.Y., O.Y., F.M., M.E., Data Collection or Processing: A.S., O.Y., F.M., M.E., Analysis or Interpretation: A.S., T.Y., B.Y., Literature Search: A.S., T.Y., B.Y., Writing: A.S.

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