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Reduction in peripheral expression of the TMLHE gene in Turkish youth with autism spectrum disorder

İpek Kuşcu Özücer [a], Alper Alnak [b], Hilal Akköprü [c], Zeynep Nur Karadoğan [d], Ahmet Okay Çağlayan [e,f], Saliha B. Selman [g,*], Murat Coskun [h]

[a] Private Practice, İstanbul, Türkiye
[b] Department of Child and Adolescent Psychiatry, University of Health Sciences, Bakirkoy Prof. Dr. Mazhar Osman Research and Training Hospital for Psychiatry, Neurology and Neurosurgery, İstanbul, Türkiye
[c] Department of Child and Adolescent Psychiatry, Gynecology and Pediatrics Hospital, Bingöl, Türkiye
[d] Department of Child and Adolescent Psychiatry, Erzincan Binali Yıldırım University, Mengücek Gazi Research and Training Hospital, Erzincan, Türkiye
[e] Department of Medical Genetics, School of Medicine, Dokuz Eylul University, İzmir, Türkiye
[f] Department of Molecular Medicine, Institute of Health Sciences, Dokuz Eylul University, İzmir, Türkiye
[g] Department of Psychology, Ibn Haldun University, İstanbul, Türkiye
[h] Department of Child and Adolescent Psychiatry, Istanbul University School of Medicine, İstanbul, Türkiye

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ABSTRACT

Background: Trimethyllysine Hydroxylase, Epsilon (*TMLHE*) gene mutations have been clinically associated with an increased risk of autism spectrum disorder (ASD). This study aimed to evaluate the peripheral expression profile of the *TMLHE* gene and its association with ASD phenotype in a clinical sample of youth diagnosed with ASD.

Methods: The study sample included 205 participants (ASD: $n = 100$; controls: $n = 105$, $M_{age} = 9.25$ years, $SD = 3.74$). The Childhood Autism Rating Scale and the Aberrant Behavior Checklist were administered to assess the severity of ASD and associated symptoms. Peripheral blood samples were collected from all participants, and *TMLHE* gene expression levels were analyzed using quantitative reverse transcription PCR (RT-qPCR).

Results: *TMLHE* gene expression was significantly downregulated in the ASD group compared to controls ($p < .001$). Notably, significant correlations were identified between *TMLHE* expression levels and the CARS subscales for object use ($p = .043$) and listening response ($p = .038$).

Conclusion: This study represents the first case-control investigation of peripheral *TMLHE* gene expression in ASD, revealing that *TMLHE* expression is reduced in children with ASD compared to typically developing peers. These findings contribute to a deeper understanding of the potential implications of *TMLHE* gene mutations in the etiology of ASD.

1. Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental condition typically manifesting in early childhood, characterized by persistent deficits in social interaction and communication, alongside repetitive behaviors and restricted interests (American Psychiatric Association, 2013). The etiology of ASD is multifactorial, involving a complex interplay of genetic, environmental, and neurobiological factors (Thapar and Rutter, 2021). Genetic factors are among the most significant contributors to ASD, characterized by considerable heterogeneity and complex interactions with environmental influences that affect the expression of neurodevelopmental genes (Thapar and Rutter, 2021;

Abbreviations: ASD, Autism Spectrum Disorder; TMLHE, Trimethyllysine Hydroxylase, Epsilon; TMLD, Trimethyllysine Dioxygenase; CARS, Childhood Autism Rating Scale; ABC, Aberrant Behavior Checklist; RT-qPCR, Quantitative Reverse Transcription Polymerase Chain Reaction; cDNA, Complementary DNA; SPSS, Statistical Package for the Social Sciences; RNA, Ribonucleic Acid; DNA, Deoxyribonucleic Acid; EDTA, Ethylenediaminetetraacetic Acid; SNP, Single Nucleotide Polymorphism; CNV, Copy Number Variation.

* Corresponding author.
E-mail addresses: ipekkuscuozucer@gmail.com (İ.K. Özücer), alperalnak@gmail.com (A. Alnak), hilalakkopru@hotmail.com (H. Akköprü), zeynepnur.gulle@gmail.com (Z.N. Karadoğan), ahmetokay.caglayan@deu.edu.tr (A.O. Çağlayan), salihabselman@gmail.com (S.B. Selman), drmc78@gmail.com (M. Coskun).

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Bhandari et al., 2020). Over recent years, various candidate gene studies have aimed to identify genetic variants that increase susceptibility to ASD, shedding light on specific genes involved in neurodevelopment (Bhandari et al., 2020).

One such gene is Trimethyllysine Hydroxylase, Epsilon (*TMLHE*), located on the Xq28 locus, which encodes the enzyme trimethyllysine dioxygenase (TMLD) (Nava et al., 2012a). TMLD catalyzes the first step in the carnitine biosynthesis pathway by converting trimethyllysine (TML) into hydroxymethyllysine (Vaz et al., 2001). L-carnitine is integral to the central nervous system's functioning and plays a critical role in mitochondrial fatty acid metabolism. Altered carnitine metabolism has been documented in patients with ASD, indicating potential disruptions in metabolic pathways relevant to ASD pathology (Kępka et al., 2021). L-carnitine facilitates long-chain fatty acid oxidation in the brain, supports acetylcholine synthesis as an acyl group donor, enhances the expression of growth-associated protein-43, prevents apoptosis and neuronal damage, and improves neurotransmission. Collectively, these properties underscore its neuroprotective, neuromodulatory, and neurotrophic functions.

Clinical evidence has suggested an association between *TMLHE* mutations and an increased risk of ASD. A rare mutation involving the deletion of exon 2 in the *TMLHE* gene has been identified in individuals with ASD, and *TMLHE* deficiency was found to be 2.82 times more prevalent in male-male multiplex autism families compared to controls (Celestino-Soper et al., 2011; Celestino-Soper et al., 2012a). Moreover, six of seven affected male siblings in these families also exhibited this deletion, indicating that *TMLHE* deficiency may contribute to ASD risk, albeit with low penetrance (2–4 %) (Celestino-Soper et al., 2012a). Other studies have similarly identified ASD-related mutations in the *TMLHE* gene, further substantiating its potential role (Nava et al., 2012a). For instance, a case report described a 4-year-old boy with ASD and neurodevelopmental regression who carried a 2-bp deletion (c.961_962del) in exon 6 of the *TMLHE* gene. Remarkably, carnitine supplementation halted the regression, and the child began achieving developmental milestones, suggesting a clinical relevance of carnitine metabolism in ASD (Ziats et al., 2015). While case reports highlight the potential significance of *TMLHE* mutations, broader population-based studies are essential to establish consistent patterns and understand incomplete penetrance.

Evaluating the transcriptional activity of genes with known mutations provides insight into why certain genetic alterations yield variable phenotypic outcomes. Peripheral gene-expression profiling complements genomic testing by capturing downstream dysregulation when causal variants are heterogeneous and by reflecting regulatory and environmental influences not apparent in DNA sequence data. Indeed, transcriptomic meta-analyses have shown that expression profiling may distinguish individuals with ASD from neurotypical controls more effectively than sequencing alone, highlighting its hypothesis-generating value (Voineagu, 2012; Akköprü et al., 2023; Alnak et al., 2021).

While rare *TMLHE* variants and deficiencies have been associated with ASD and, in case reports, with clinical response to carnitine (Nava et al., 2012a; Celestino-Soper et al., 2011; Celestino-Soper et al., 2012a; Ziats et al., 2015), the peripheral expression of this gene remains largely unexplored, representing a critical gap in the literature. To address this, we assessed *TMLHE* mRNA levels in peripheral blood from youth with ASD compared to typically developing controls. Establishing whether expression differences exist adds functional context to genetic observations and to carnitine-pathway hypotheses in ASD (Kępka et al., 2021; Voineagu, 2012). Such findings, if replicated alongside functional and genetic data, may inform the development of accessible biomarkers and metabolism-targeted therapeutic strategies.

2. Method

2.1. Participants

The participants in this study were enrolled from the Child and Adolescent Psychiatry outpatient clinic at Istanbul University Faculty of Medicine, Istanbul, Turkey, between June and December 2017. Ethical approval was granted by the Institutional Review Board of the Istanbul University Faculty of Medicine (IRB Protocol: 2017/748). Written informed consent was obtained from parents, and assent was provided by the children where appropriate.

Sample size estimation was based on the assumption that a minimum 30 % difference in expression levels between the case and control groups would be statistically significant with a 95 % confidence level and 90 % statistical power. Under these parameters, the required minimum sample size was calculated as 82 participants per group. To ensure adequate power and account for potential attrition, the study was designed to include 105 individuals in the case group and 105 individuals in the control group.

The study included 100 youth with autism spectrum disorder (ASD) and 105 typically developing controls. Participants with ASD were eligible for inclusion if they met the following criteria: (a) a confirmed ASD diagnosis according to DSM-5 criteria; (b) absence of severe or profound intellectual disability; (c) no significant visual or hearing impairment; and (d) no known genetic, metabolic, or progressive neurological disorder. These exclusions were applied to reduce the likelihood of enrolling individuals with syndromic ASD or other metabolic or structural brain pathologies. The presence of severe or profound intellectual disability was determined through standardized psychometric testing and clinical evaluation.

The control group were recruited from a general pediatric outpatient clinic. Controls were individually matched to ASD participants on age and sex and were required to have no history of psychiatric diagnoses, no current psychiatric symptoms, and no significant medical conditions, including acute infections, at the time of sampling. Written informed consent was obtained from parents or legal guardians of all participants.

Measures.

Childhood Autism Rating Scale (CARS).

The Childhood Autism Rating Scale (CARS) is a clinician-rated instrument validated for assessing ASD symptom severity and differential diagnosis within neurodevelopmental populations (Schopler et al., 1980). The Turkish version, validated by İncekaş Gassaloğlu et al. (2016), demonstrated high reliability with a Cronbach's alpha of 0.95 and a cut-off score of 30, effectively differentiating ASD from intellectual disabilities without ASD ($p < .001$) (İncekaş Gassaloğlu et al., 2016). The scale has been applied in various studies, supporting its reliability and relevance for research in diverse settings (Akköprü et al., 2023; Alnak et al., 2021; Guldiken et al., 2023; Karadogan et al., 2023; Gedik et al., 2023).

Aberrant Behavior Checklist (ABC).

The Aberrant Behavior Checklist (ABC) is a parent-report instrument quantifies clinically significant maladaptive behaviors (Aman et al., 1985). The Turkish version, validated for reliability and validity, consists of 46 items distributed across five subscales: Hyperactivity/Noncompliance, Lethargy/Social Withdrawal, Stereotypic Behavior, Self-injurious Behavior, and Other Behaviors (Karabekiroglu and Aman, 2009). Each item is rated on a 4-point scale from 0 (no problem) to 3 (severe problem). The Turkish version has been validated and utilized in several clinical studies, confirming its applicability in research (Akköprü et al., 2023; Alnak et al., 2021; Karabekiroglu and Aman, 2009).

Quantitative Reverse Transcription PCR (RT-qPCR) Study.

Blood samples were collected by nurses trained in pediatric phlebotomy procedures. A total of 5–8 cc of blood was drawn from the peripheral vein into EDTA-containing tubes. To minimize RNA degradation, samples were stored at +4 °C and delivered to the laboratory for processing within 24 h.

Total RNA was extracted from peripheral venous blood samples using the Hybrid-RTM kit (GeneAll, Seoul, South Korea; catalog no. 315–150). RNA quantity and quality were assessed using the Qubit 2 Fluorometer (Thermo Fisher Scientific Inc., Wilmington, DE, USA). Complementary DNA (cDNA) synthesis was performed with 1 μg of RNA using the Ipsogen® cDNA Synthesis Kit (Qiagen GmbH, Hilden, Germany; catalog no. 679923), following the manufacturers protocol.

Specific primer sets for *TMLHE* were designed using Primer-BLAST (5′ to 3′ sequences: forward 5′-CTCTAAGACTCACCAGCGCA-3′, reverse 5′-TCTACCGATGGAACTTGGGC-3′). PCR amplification was carried out in 20 μL reaction volumes containing 10 μL Real time PCR Master Mix with EVAGREEN (GenMark, Turkey), 1 μL of primer mix (10 pmol), 3 μL double-distilled water, and 5 μL of sample DNA. Reactions were performed on a CFX96 Real-time PCR system (Bio-Rad) under the following conditions: initial denaturation at 95 °C for 15 min, followed by 50 cycles of 95 °C for 15 s and 60 °C for 60 s.

For data normalization, three reference genes, ABL-1, CUL1, and ZNF207, were utilized, selected for their stable expression across various tissues and validation in previous studies. The use of at least two reference genes is recommended for accurate normalization, ensuring robust and reliable data. The specificity of PCR products was confirmed through melting curve analysis. Gene expression fold changes were calculated using the $2 - \Delta\Delta CT$ method, with the $-\Delta\Delta CT$ value representing the logarithmic expression of the target gene, based on a logarithmic scale with a base of 2.

Demographic Information Form.

A demographic form was used to collect participants' sociodemographic and clinical characteristics, including mother and father age at birth.

Analysis Plan.

Statistical analyses were conducted using SPSS version 21.0 (IBM Corp., Armonk, NY, USA) and R version 3.4.0 (R Foundation for Statistical Computing, Vienna, Austria). Descriptive statistics were reported as means and standard deviations. Normality was evaluated (Kolmogorov-Smirnov). Between-group comparisons used *t*-tests when assumptions held and Wilcoxon rank-sum otherwise. A *p*-value of less than 0.05 was considered indicative of statistical significance.

3. Results

The sociodemographic and clinical characteristics of the participants are summarized in Table 1. The study sample included 205 participants, with 100 youth in the case group (M = 9.22 years, SD = 3.62) and 105 in the control group (M = 9.27 years, SD = 3.86). Gender distribution was similar across both groups (87/13 male/female in the case group and 91/14 in the control group, $\chi^2 = 0.005$, $p = .944$). The average maternal age at birth was 28.3 years (SD = 6.38) for the case group and 27.1 years (SD = 5.68) for the control group, with no significant difference ($t = 200$, $p = .171$). Similarly, the mean paternal age at birth was 32.1 years (SD = 6.46) for the case group and 30.5 years (SD = 5.51) for the control group, showing no significant difference ($t = 192.98$, $p = .081$). Consanguineous marriage was more prevalent in the ASD group (26 %) compared to the control group (13 %) ($p = .02$). Table 2 presents the clinical characteristics of the ASD group, including CARS total scores and ABC subscale and total scores.

TMLHE gene expression was detected in all participants from both the study group (ASD) and the control group, albeit at varying levels. The expression of three reference genes (ABL-1, CUL1, and ZNF207), chosen for their stability as housekeeping genes, was confirmed across all samples, ensuring the reliability of the normalization process. The results indicated that the expression of the *TMLHE* gene was significantly downregulated in the ASD group compared to the control group ($p < .001$). Detailed results are provided in Table 3, and the distribution of *TMLHE* expression levels is illustrated as a boxplot in Fig. 1.

To further investigate the association between *TMLHE* gene expression and ASD symptomatology, correlation analyses were conducted using the CARS total and subscale scores, as well as the ABC total and subscale scores (see Table 4). The analysis revealed significant correlations specifically between *TMLHE* expression levels and the CARS subscales for object use ($p = .043$) and listening response ($p = .038$), suggesting that variations in *TMLHE* expression may be associated with these specific aspects of ASD behavior. No significant correlations were observed between *TMLHE* expression and the total or other subscale scores of the CARS and ABC. The CARS scores were correlated with the total ABC scores ($r = 0.49$, $p < .001$) as well as with the subscales of ABC ($p < .001$).

4. Discussion

The present study evaluated *TMLHE* gene expression levels in youth with autism spectrum disorder (ASD) compared to an age- and gender-matched control group, revealing significantly lower expression in the ASD group. The *TMLHE* gene encodes an enzyme integral to the initial step of carnitine biosynthesis, a pathway essential for central nervous system function due to its neuroprotective, neuromodulatory, and neurotrophic roles (Vaz et al., 2001; Kępka et al., 2021). Reduced expression of *TMLHE*, and consequently altered carnitine metabolism, may play a contributory role in the pathogenesis of ASD (Kępka et al., 2021; Celestino-Soper et al., 2011; Celestino-Soper et al., 2012a; Ziats et al., 2015). This aligns with prior findings linking *TMLHE* gene mutations to heightened ASD risk (Celestino-Soper et al., 2011; Celestino-Soper et al., 2012a; Ziats et al., 2015).

Reduced expression of the *TMLHE* gene, which encodes the mitochondrial enzyme trimethyllysine hydroxylase epsilon, may play a mechanistic role in ASD by disrupting carnitine biosynthesis. Carnitine is essential for the transport of long-chain fatty acids into mitochondria for β-oxidation, thereby supporting neuronal energy metabolism. Deficiency of *TMLHE* activity could impair this pathway, leading to decreased energy availability in neurons. Such metabolic disruption aligns with prior evidence indicating that mitochondrial dysfunction is frequently observed in individuals with ASD (Rossignol and Frye, 2012).

In addition to its role in energy homeostasis, carnitine contributes to neuronal maturation, myelination, and synaptic function. Insufficient carnitine levels may therefore compromise neurodevelopmental processes, potentially exacerbating ASD-related phenotypes. Furthermore, disturbances in carnitine metabolism have been associated with

Table 1
Sociodemographic characteristics of the participants.

	Case Group (n = 100)	Control Group (n = 105)	χ^2/t	p
Gender (male/female) (n)	87/13	91/14	0.005	0.94
Age (years)	9.22 (3.62)	9.27 (3.86)	0.107	0.91
Mother's age at birth (years)	28.34 (6.38)	27.17 (5.68)	200	0.17
Father's age at birth (years)	32.06 (6.46)	30.56 (5.51)	192.9	0.08

Note. Values are Mean (SD) unless otherwise indicated.

Table 2
Clinical characteristics of the ASD group.

Measure	Mean (SD)
CARS Total Score	42.19 (4.51)
ABC Hyperactivity	20.50 (9.05)
ABC Lethargy	21.12 (9.89)
ABC Stereotypic behaviors	6.49 (4.82)
ABC Injurious behaviors	1.59 (2.30)
ABC Other behaviors	6.15 (3.05)
ABC Total Score	55.85 (23.41)

Note. Values are Mean (SD). CARS = Childhood Autism Rating Scale; ABC = Aberrant Behavior Checklist.

Table 3
t-test results of *TMLHE* expression levels.

Gene	Case Group	Control Group	FC (log2(H/K))	t-test *p*-value
TMLHE	2.74383542	8.65501767	−1.65734281	0.0000009440593

Note. Values are Mean.

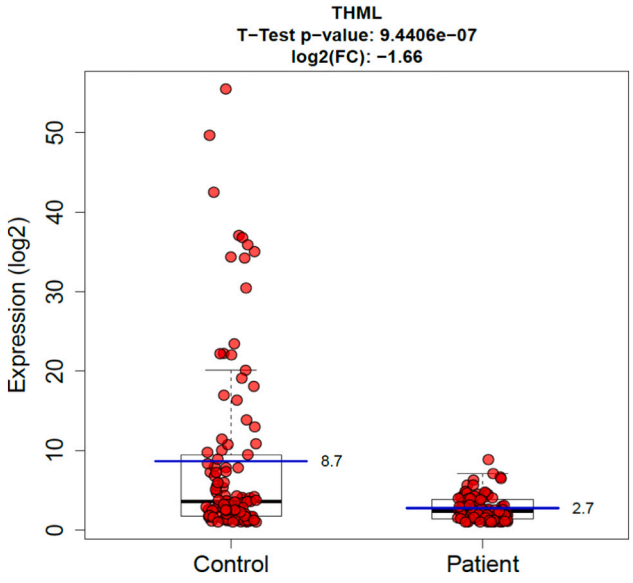

Fig. 1. Boxplot chart showing the association of *TMLHE* expression data between control and patient samples.

Table 4
Correlations between *TMLHE* expression and CARS Scores in ASD group.

	r	*p**
Object Use	0.203	**0.043**
Listening Response	0.207	**0.038**
Total CARS Score	0.027	0.788

Note. *Spearman test; CARS: Childhood Autism Rating Scale.

increased oxidative stress, another factor widely implicated in ASD pathogenesis (Celestino-Soper et al., 2012b; Giulivi et al., 2010). Consistent with this model, rare *TMLHE* variants and *TMLHE* deficiency have been linked to ASD risk and clinical response to carnitine in case reports (Nava et al., 2012a; Celestino-Soper et al., 2011; Celestino-Soper et al., 2012a; Ziats et al., 2015), while brain transcriptomic data indicate *TMLHE* is expressed across fetal and adult regions relevant to neurodevelopment (Celestino-Soper et al., 2012b). Taken together, our observation of lower peripheral *TMLHE* expression in ASD is compatible with a plausible mitochondrial–metabolic vulnerability contributing to ASD-related phenotypes (Voineagu, 2012; Giulivi et al., 2010) (Fig. 2).

It is also noteworthy that *TMLHE* is located on the X chromosome, suggesting that reduced expression or loss-of-function variants may exert stronger effects in males, who already show a higher prevalence of ASD. This genetic context provides a plausible explanation for sex differences in ASD susceptibility (Nava et al., 2012b). Taken together, our findings highlight the possibility that *TMLHE* dysregulation, through impaired carnitine metabolism, may contribute to the biological heterogeneity of ASD. These results extend existing literature linking *TMLHE* mutations and carnitine deficiency to neurodevelopmental disorders (Longo et al., 2016; Sahakyan et al., 2020) and underscore the need for future studies incorporating functional assays of carnitine metabolism and mitochondrial activity.

Gene expression studies for ASD can use both brain and peripheral

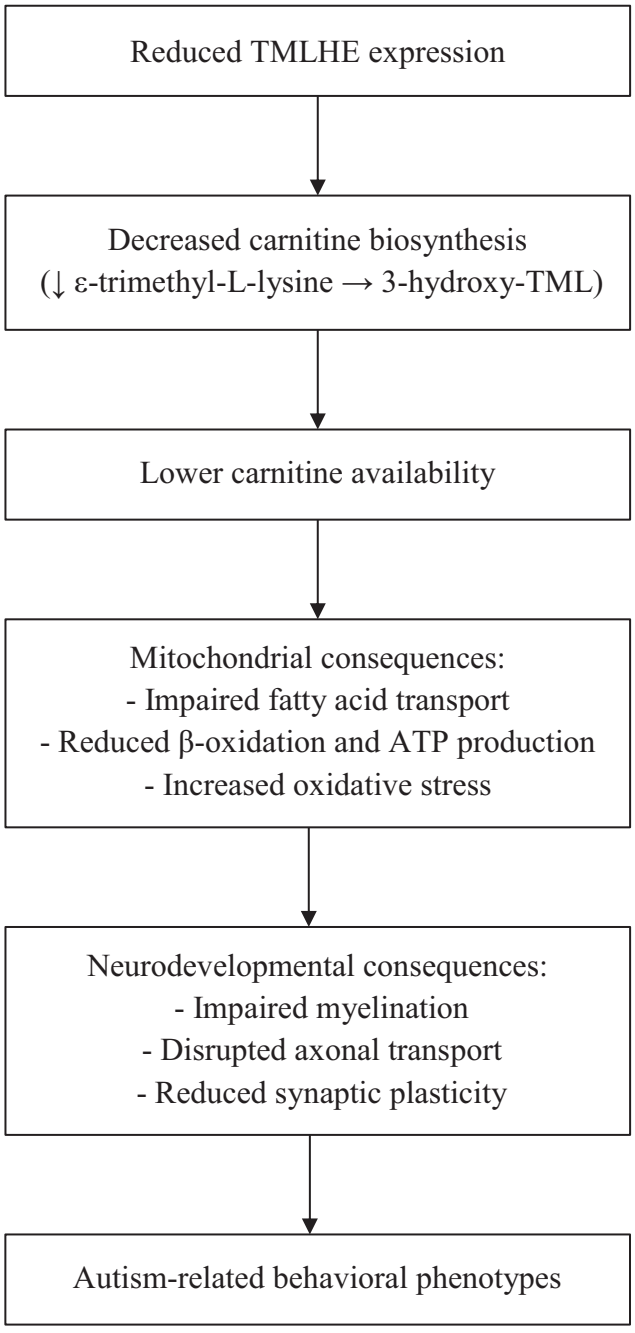

Fig. 2. Detailed mechanistic pathway linking reduced *TMLHE* expression to ASD
Footnote: Reduced *TMLHE* expression decreases carnitine biosynthesis (ε-trimethyl-L-lysine → 3-hydroxy-TML) (Vaz et al., 2001), leading to lower carnitine availability (Vaz et al., 2001; Kępka et al., 2021). This can impair mitochondrial long-chain fatty-acid transport, reduce β-oxidation and ATP production, and increase oxidative-stress susceptibility (Kępka et al., 2021). Consequent neurodevelopmental effects, impaired myelination, disrupted axonal transport, reduced synaptic plasticity, may follow (Kępka et al., 2021), contributing to ASD-related phenotypes, consistent with links between *TMLHE* variants/deficiency and ASD (and case-report responses to carnitine) (Nava et al., 2012a; Celestino-Soper et al., 2011; Celestino-Soper et al., 2012a; Ziats et al., 2015) and with *TMLHE* expression in fetal/adult brain regions (Celestino-Soper et al., 2012b); mouse data provide interpretive caution (Giulivi et al., 2010).

tissues, though practical and ethical limitations often restrict direct brain analysis (Voineagu, 2012; Akköprü et al., 2023; Alnak et al., 2021). Peripheral tissue analysis provides an indirect but informative approximation of the neural transcriptome and has been instrumental in identifying potential ASD biomarkers (Voineagu, 2012). While findings can be inconsistent, certain cases show alignment between peripheral and brain expression. For instance, peripheral expression of the adenosine A2A (ADORA2A) gene is increased, while that of the mono-ADP ribosylhydrolase 2 (MACROD2) gene is reduced in young individuals with ASD (Akköprü et al., 2023; Alnak et al., 2021). Notably, ITGB2 (integrin, beta 2) shows consistent upregulation in both brain tissue and peripheral blood, exemplifying congruent expression (Voineagu, 2012). Therefore, peripheral gene studies hold promise for advancing ASD genetics research and identifying biomarkers. However, assessing gene expression in the directly affected tissue is crucial for establishing causal links and understanding underlying mechanisms. Our finding of reduced *TMLHE* expression in peripheral blood supports the role of such studies in complementing genetic analyses like SNP genotyping and CNV studies, enhancing the understanding of ASD-related functional changes (Voineagu, 2012; Akköprü et al., 2023; Alnak et al., 2021; Rossignol and Frye, 2012).

The significance of *TMLHE* expression is further suggested by its extensive presence in both adult and fetal brain tissues, particularly the cerebrum and cerebellum (Celestino-Soper et al., 2012b). Given the gene's vital role in early developmental periods, understanding its expression profile could elucidate its potential contribution to neurodevelopmental disorders. In our study, although the *TMLHE* gene was expressed in all participants, its reduced expression in the ASD group indicates a potential biomarker that warrants further exploration. This result is particularly relevant considering the implications of altered carnitine metabolism in ASD pathophysiology (Kępka et al., 2021; Celestino-Soper et al., 2011; Celestino-Soper et al., 2012a; Ziats et al., 2015).

Correlational analyses in this study revealed significant associations between *TMLHE* expression levels and specific subscales of the Childhood Autism Rating Scale (CARS), notably object use and listening response. However, no significant association was observed between *TMLHE* expression and total ASD severity. This nuanced finding may indicate that while *TMLHE* expression may be associated with certain domains of ASD-related behaviors, it is not uniformly associated with broader symptom severity. This differentiation could reflect the gene's involvement in discrete neurodevelopmental pathways or symptom clusters. Importantly, a recent animal model study reported that *TMLHE* knockout in mice did not induce an ASD phenotype, despite markedly low carnitine and gamma-butyrobetaine levels, suggesting the complexity of translating genetic findings across species and underscoring the importance of human studies (Giulivi et al., 2010).

Several limitations of the present study should be acknowledged. First, the sensitivity analysis indicated that the study was sufficiently powered to detect moderate group differences but may have lacked power to detect smaller effects. Second, although the control group was matched on age and sex, other factors that may influence gene expression, such as diet, fasting status, supplementation, and medication use, were not systematically recorded. Because recruitment occurred in 2017, prior to routine collection of these variables, they could not be controlled for and remain a limitation. Future studies should incorporate standardized assessment of these factors to improve interpretability. Third, clinical assessments such as the CARS and ABC were administered only to the ASD group. This was an intentional design choice, as both instruments are validated for assessing ASD severity and maladaptive behaviors in clinical populations rather than in typically developing controls; nevertheless, this should also be considered a limitation.

Fourth, our analyses focused on expression levels of *TMLHE* but did not include variant-level data such as single nucleotide polymorphisms (SNPs) or copy number variants (CNVs). Because expression and genetic variation data are complementary, integrating both will be essential to fully understand the contribution of *TMLHE* to ASD. Fifth, the generalizability of our findings is limited to the Turkish sample studied here; replication across independent cohorts with different ethnic and geographic backgrounds, ideally using harmonized protocols, will be important for establishing external validity. Finally, our study measured only mRNA levels, which do not necessarily reflect functional enzyme activity or downstream metabolic consequences. Incorporating functional assays, such as peripheral blood mononuclear cell (PBMC)-based TMLD enzyme activity, plasma free and acyl-carnitine profiling, and fibroblast-based functional models, would provide critical complementary evidence. Taken together, these limitations highlight the need for future multi-level studies that integrate genetic, transcriptional, metabolic, and functional assays in diverse populations. Despite these constraints, our findings contribute novel evidence of reduced peripheral *TMLHE* expression in ASD, providing a foundation for further mechanistic and translational work.

5. Conclusion

In conclusion, this study contributes novel insights by documenting downregulated *TMLHE* expression in peripheral blood among youth with ASD. The potential implications for understanding ASD pathophysiology and developing new diagnostic or therapeutic strategies are noteworthy. These findings are preliminary and hypothesis-generating, indicating reduced peripheral *TMLHE* expression in ASD and motivating replication with genetic and functional assays before any biomarker inferences.

CRediT authorship contribution statement

İpek Kuşcu Özücer: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Alper Alnak:** Conceptualization, Data curation, Formal analysis, Project administration, Writing – review & editing. **Hilal Akköprü:** Conceptualization, Data curation, Formal analysis, Project administration, Writing – review & editing. **Zeynep Nur Karadoğan:** Conceptualization, Data curation, Formal analysis, Project administration, Writing – original draft, Writing – review & editing. **Ahmet Okay Çağlayan:** Conceptualization, Supervision, Validation, Writing – review & editing. **Saliha B. Selman:** Formal analysis, Validation, Visualization, Writing – original draft, Writing – review & editing. **Murat Coskun:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Project administration, Resources, Validation, Writing – original draft, Writing – review & editing.

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Declaration of competing interest

Authors have nothing to declare.

Data availability

The data that support the findings of this study are available on request from the corresponding author. The current study was not preregistered.

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