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Abstract

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Keywords (separated by '-') Autism spectrum disorders - Clusterin - Fatty acids - Carnitine - Neurodegeneration

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RESEARCH

Neurodegeneration in Autism: A Study of Clusterin, Very Long-Chain Fatty Acids, and Carnitine

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Abstract

The clinical identification of regression phenomena in ASD lacks specific biological or laboratory criteria and is often based on family history and highly subjective observations by clinicians. The present study aimed to investigate the potential role of plasma clusterin (CLU), very long-chain fatty acids (VLCFA), and carnitine as biomarkers of neurodegeneration in children with autism spectrum disorder (ASD) with and without regression. By exploring these biomarkers, we sought to provide insights into mitochondrial dysfunction, glial activation, and lipid metabolism, which may contribute to the pathophysiology of ASD and aid in the early diagnosis and intervention of regression phenomena in ASD. Ninety children aged 2–6 years were included: 30 with autism spectrum disorder (ASD), 30 with regressive ASD, and 30 healthy controls. Psychiatric assessments were conducted using DSM-5 criteria, CARS, ABC, RBS-R, and ASSQ scales. Regression in ASD was evaluated retrospectively using a modified ADI-R questionnaire. Fasting blood samples were collected, and plasma clusterin (CLU), VLCFA, and carnitine levels were measured. Statistical analyses were performed using MANOVA to assess the effect of group differences on dependent biochemical variables. Serum clusterin and carnitine levels showed no significant differences between groups. However, C22 VLCFA levels were significantly higher in both autism groups compared to controls ($p=0.04$), with post hoc analysis indicating the difference between the non-regressive and control groups ($p=0.02$). Serum carnitine was positively correlated with stereotypic behaviors subscale scores ($r=0.37$, $p=0.004$) and total scores ($r=0.35$, $p=0.006$) of RBS-R. Our study provides insights into the complexities of biomarker research in autism spectrum disorder (ASD), highlighting the challenges in identifying consistent biological markers for regression and non-regression phenotypes. Although no significant findings were observed, further biomarker studies are essential to distinguish possible endophenotypes, improve early diagnosis, and uncover potential therapeutic targets in ASD.

Keywords Autism spectrum disorders · Clusterin · Fatty acids · Carnitine · Neurodegeneration

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Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by difficulties in social communication and interaction, as well as restricted or repetitive behaviors (American Psychiatric Association 2013). The unclear etiology of ASD and the necessity for effective treatments have prompted biomarker studies to better clarify the pathophysiological and clinical features (Chen et al. 2021a, b; Du et al. 2023).

The prevailing understanding in the literature is that the behavioral symptoms of ASD exhibit two distinct developmental patterns: early and regressive. The early-onset pattern, characterized by delays or differences in speech and social communication within the first 12 months of life, is considered to be the more common developmental trajectory in individuals with ASD. On the other hand, some children initially exhibit a normal developmental process but then experience a significant loss of skills in the second year of life; this is known as "regression." Although there is no standard definition of regression, the central features of this condition include a loss of language and social skills, with possible regression in adaptive and motor skills as well (Boterberg et al. 2019).

The regression phenomenon in autism can occur at any age, but the average age at onset is 19.8 months (Tan et al. 2021). The exact etiology and mechanism of regression remain unclear, despite various hypotheses (Stefanatos 2008). Among these, the idea that regression observed in children with autism may be a form of early neurodegeneration is one of the most significant topics of study (Kern et al. 2013). Evidence of activated microglia, astrocytes, and neuronal loss in individuals with ASD suggests that glial activation may play a key role in the neurodegenerative processes associated with the disorder (Kern et al. 2013). In particular, astrocyte-induced neurotoxicity has been linked to lipoproteins such as APOE and APOJ (Clusterin-CLU), which are upregulated during glial activation in preclinical studies (Guttenplan et al. 2021).

CLU, also known as apolipoprotein J, is a lipoprotein expressed in almost all vertebrates and found in physiological fluids such as plasma, CSF, and urine. It is involved in cell differentiation, the regulation of cell death and survival mechanisms, oxidative stress pathways, the complement system, and lipid and cholesterol metabolism (Yuste-Checa et al. 2022). Due to its diverse functions, CLU has been widely studied in the context of neurodegeneration. For instance, clinical studies have suggested that plasma CLU may serve as a biomarker for Alzheimer's disease even before clinical symptoms appear (Haight et al. 2018). In Rett syndrome, a neurodegenerative disorder, degradation of MECP2 siRNA has been linked to increased CLU gene expression (Gibson et al. 2010).

CLU, one of the most prevalent lipoproteins in the brain, plays an essential role in lipid transport within the central nervous system, which is crucial for neuronal health (Yuste-Checa et al. 2022). While its involvement in neurodegeneration is well-established, it remains unclear whether examining glial lipid metabolism alongside CLU could reveal how this lipoprotein contributes to neurodegenerative processes and serves as a disease biomarker. Notably, adipokines and lipid metabolism pathways have also been implicated in ASD (Chen et al. 2024). A previous biomarker study showed that a common metabolic phenotype is present in autism and that this phenotype is characterized by high plasma levels of lipids containing very long-chain fatty acids (VLCFA) as a result of impaired mitochondrial dysfunction (Pastural et al. 2009). L-carnitine, one of the metabolites that play an important role in the functioning of the central nervous system, especially in the mitochondrial metabolism of fatty acids, is required for the transport of long-chain fatty acids from the cytosol to the mitochondrial matrix where β-oxidation occurs (Kępka et al. 2021). Alterations in carnitine metabolism along with abnormal fatty acid metabolism have been documented in ASD, and an association between carnitine and autism has been demonstrated (Demarquoy and Demarquoy 2019; Kępka et al. 2021). Carnitine levels are significantly reduced in individuals with autism (Rossignol and Frye 2012), and the L-carnitine profile may be a potential biomarker of mitochondrial dysfunction in autism (Frye et al. 2013).

In this study, we examined the plasma levels of CLU, which is involved in various physiological and pathological processes, particularly neurodegeneration. Although it has been proposed as a potential biomarker for neurodegenerative diseases, its association with ASD has not been explored previously. Our goal is to investigate CLU levels in relation to neurodegeneration in patients with ASD with and without regression, as well as in healthy children. Our primary hypothesis was that plasma CLU levels are higher in ASD patients with regression than in ASD patients without regression and healthy children. To support our hypothesis of lipid-mediated toxicity, we evaluated CLU and plasma VLCFA and carnitine levels. These three parameters—clusterin, VLCFA, and carnitine—are being evaluated together to understand their roles in mitochondrial dysfunction, glial activation, and neurodegeneration pathways, which may contribute to the development of autism. Ultimately, we aim to identify a potential biomarker for early diagnosis and intervention for ASD.

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Method and Materials

Participants

A total number of 90 children aged 2–6 years were included in the study. The ASD group comprised 30 children diagnosed with ASD based on DSM-5 criteria, without any additional psychiatric diagnoses apart from developmental delay or intellectual disability. Another 30 children with regressive ASD were selected using a questionnaire assessing the presence of regression history. The control group consisted of 30 healthy children with no psychiatric diagnoses.

Procedure

After obtaining ethical approval, children underwent a detailed psychiatric examination, with sociodemographic information and medical history collected. The inclusion criteria covered the absence of any psychiatric or medical diseases, such as epilepsy and inflammatory conditions, allergies, and the non-use of any medications or supplements, as well as not adhering to any specific diet. The children with medical conditions or any mental/physical limitations affecting participation were not included in the study. The participants diagnosed with ASD based on DSM-5 criteria received a standardized developmental or intelligence test. The Childhood Autism Rating Scale (CARS) was used to assess symptom severity, while parents completed the Autism Behavior Checklist and Repetitive Behavior Scale (RBS-R). Clinicians conducted a parent interview using a modified ADI-R questionnaire to assess regression retrospectively among the children diagnosed with ASD. In the control group, parents completed the Autism Spectrum Screening Questionnaire (ASSQ) to evaluate the social skills of children. The other scales—CARS, ABC, and RBS-R—were not used in the control group. After psychiatric evaluations and parental consent, blood samples were collected from all children.

After 8 h of fasting, 5 ml of venous sample was collected into one blood tube containing ethylenediaminetetraacetic acid (K2EDTA) by two nurses in the presence of the caregiver and transferred to the lab within 1 h. Fatty acids were esterified and analyzed using EDTA plasma. Dried blood spots impregnated on Guthrie paper from the tube were used for acylcarnitine evaluation on the Thermo TSQ Quantis LC–MS/MS device with the RECIPE Amino Acids and Acylcarnitines (München/Germany) kit. To measure CLU and VLCFA levels, the remaining plasma sample was centrifuged at 2500 g for 10 min and stored at − 80 °C for batch analysis. Plasma CLU concentrations were measured using the USCN kit (USCN Life Science Inc., Cloud Clone Corp) the enzyme-linked immunosorbent assay (ELISA; DCLU00, R&D System) method which was carried out under the distributor's instructions. Absorbance was measured at 450 nm wavelength using an ELISA reader. Intra- and inter-assay coefficient variation is < 10%. VLCFA levels were measured by gas chromatography on a Thermo Trace 1300 GC/MS (ISQ 7000) using a single ion monitoring mode (Jaspers et al. 2020).

Ethical approval was obtained from the Ankara University Human Research Ethical Committee for the current study following the Declaration of Helsinki. Informed consent was obtained from all parents.

Clinical Measurements

Sociodemographic Data Form

The clinician filled out the form that included questions related to sociodemographic characteristics, perinatal features, developmental stages and sensory sensitivities of the child, types of education, medication use, and medical/psychiatric illness history.

Childhood Autism Rating Scale (CARS)

This is a well-established, valid, and frequently used scale for assessing ASD severity. It is filled out by the clinician based on family interviews and clinical observation. CARS that was developed by Schopler et al. (1980) provides clinical data for grading autism. Turkish adaptation was conducted by Sucuoğlu et al. (1996). According to the validity and reliability analysis conducted by İncekaş Gassaloğlu et al. (2016), the Cronbach alpha value was found to be 0.95, and the cut-off score for autism diagnosis was reported to be 29.5.

Autism Behavior Checklist (ABC)

The scale was developed by Krug et al. (1993). A Turkish validity and reliability study was conducted (Irmak et al. 2007). It consists of 5 domains and 57 items that assess sensory, body and object use, relationship, social-self-care skills, and language skills. The lowest score was 0, and the highest score was 159. The Turkish cut-off score of the scale was 39, and Cronbach's alpha was 0.92.

Repetitive Behavior Scale-Revised (RBS-R)

The scale was developed by Bodfish et al. (2000) to assess the severity of repetitive behaviors. The RBS-R consists of six subscales, each targeting different types of behaviors: stereotyped behaviors (6 items), self-injurious behaviors (8

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items), compulsive behaviors (8 items), ritualistic behaviors (6 items), sameness behaviors (11 items), and restricted interests (4 items), totaling 43 items. This scale is completed through interviews with parents or directly by parents themselves. Items are rated on a 4-point scale (0, behavior not present; 1, mild; 2, moderate; 3, severe). Higher total scores indicate greater severity of repetitive behaviors. The Turkish reliability analyses conducted by Ökcün Akçamuş et al. (2019) reported that internal consistency coefficients of the total score and subscales range from 0.73 to 0.94.

Autism Spectrum Screening Questionnaire (ASSQ)

The Likert scale is a 3-point Likert scale consisting of 27 items developed in Sweden. The items of the scale were designed to assess social interaction and communication problems, restricted and repetitive behaviors, and related problems such as motor clumsiness and motor and/or vocal tics (Ehlers et al. 1999). The validity and reliability of the scale were examined (Köse et al. 2017). The score range was 0–54, and the optimal cutoff score for distinguishing clinically diagnosed ASD cases was found to be 16 (Köse et al. 2017). In the current study, it was fulfilled by the parents of the children in the control group to evaluate autism symptoms.

Assessment of Regression

Regression in ASD lacks a universally accepted definition, complicating study comparisons (Boterberg et al. 2019). The Autism Diagnostic Interview-Revised (ADI-R), a semi-structured interview for caregivers, is commonly used to document regression (Pearson et al. 2018). According to the ADI-R, regression is coded when a child demonstrates a specific skill for at least 3 months, followed by a loss of that skill lasting at least 3 months (Lord et al. 1994). In this study, the researchers developed a questionnaire based on a comprehensive literature review to establish a more objective definition of regression and to assess the presence of regression retrospectively.

Statistical Analysis

Statistical analyses were performed using the SPSS software 30.0.0. A one-way ANOVA indicated that 24 participants were needed in each group to achieve 80% power at a significance level of 0.05. To account for dropout rates, we planned to recruit 90 participants across 3 groups, with 30 in each group.

Descriptive statistics were reported as mean ± standard deviation for homogenous variables and as median and interquartile range for non-homogenous variables, whereas the categorical variables were reported as number and frequency (percentage). Clusterin, carnitine, and VLCFA levels served as dependent variables, while the groups—regressive autism, non-regressive autism, and control—were the independent variables. For categorical data, the chi-square or Fisher's exact test was employed, while continuous data were analyzed using Student's *t*-test or Mann–Whitney *U* test, depending on group number and test assumptions. One-way ANOVA or Kruskal–Wallis test was utilized to compare the three groups. Levene's test was conducted to assess the homogeneity of variances. If significant differences were found, pairwise post hoc comparisons were performed using the Tukey honestly significant difference (HSD) test for homogeneous variances and the Tamhane T2 test for non-homogeneous variances. The Mann–Whitney *U* test was performed to test the significance of pairwise differences using Bonferroni correction to adjust for multiple comparisons. An overall 5% type I error level was used to infer statistical significance.

The Spearman test was used to calculate the correlation coefficients. The Bonferroni correction was used to adjust for multiple correlations. Multivariate analysis of variance (MANOVA) was used to assess the effect of group differences on dependent biochemical variables. Before performing MANOVA, assumptions of multivariate normality, homogeneity of variance–covariance matrices (Box's M test), and the absence of multicollinearity were assessed. Significant MANOVA results were followed by post hoc analyses, including univariate ANOVAs and pairwise comparisons with appropriate corrections (Tukey's HSD) to identify specific group differences for each biochemical variable.

Results

The mean age of children was 46.68 ± 10.46 months for children diagnosed with non-regressive autism, 45.80 ± 15.29 months for children with regressive autism and 49.56 ± 14.19 months for controls. Seventeen (18.8%) children were female, and 83 (81.2%) were male. The male/female ratio was 4.8. There were no statistically significant differences between the groups regarding age ($p = 0.53$) and gender ($p = 0.62$) distribution. The age and sociodemographic data of the children are presented in Table 1.

The CARS subscale scores of the regressive and non-regressive ASD groups were evaluated, and the nonverbal communication subscale scores were found to be significantly higher in the regressive autism group ($p = 0.049$) compared to the non-regressive group. The other CARS subscale scores did not significantly differ between the groups.

When the ABC scores and RBS-R subscale scores of the regressive and non-regressive ASD groups were compared, only the "compulsive behaviors" subscale score was

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significantly higher in the non-regressive group ($p=0.005$). No statistically significant difference in other RBS-R subscales and ABC scores was found between the groups.

In the control group, the ASSQ total score reflecting autism symptoms was 4.86 ± 3.41 on average, below the cutoff score for all children, as expected.

According to the comparison of serum clusterin and carnitine levels between the three groups, no statistically significant difference was found ($p=0.46$, $p=0.60$). When serum VLCFA levels were analyzed, C22 levels were found to be significantly higher in the autism groups with and without regression compared to the control group ($p=0.04$); no statistically significant difference was found in phytanic acid ($p=0.96$), pristanic acid ($p=0.45$), C24 ($p=0.40$), and C26 ($p=0.16$) levels. According to the post hoc analyses (Tukey test) performed to understand which groups this difference was due to, the difference in C22 was found between the non-regressive and control groups ($p=0.01$). The comparison of serum CLU, VLCFA, and carnitine levels between the regressive autism, non-regressive autism, and control groups are presented in Table 2. The graphics of the levels of those parameters (serum CLU, VLCFA, and carnitine) in all three groups are shown in Fig. 1.

When comparing the control group and all children with ASD, regardless of the presence of regression, serum C22 ($p=0.04$) and C26 levels ($p=0.03$) were found to be

Table 1 Sociodemographic data of the groups

	Non-regressive ($n=30$)		Regressive ($n=30$)		Controls ($n=30$)		p
	M/n	SD/%	M/n	SD/%	M/n	SD/%	
Age (months)	46.68	10.46	45.8	15.29	49.56	14.19	0.53[a]
Gender							0.62[b]
Girl	5	16.7	4	20	8	26.7	
Boy	25	83.3	26	80	22	73.3	
Socioeconomic level							0.61[b]
Low	8	29.6	11	40.7	8	29.6	
Medium	15	36.6	13	31.7	13	31.7	
High	4	21.1	6	31.6	9	47.7	

[a]Independent sample *t*-test, [b]chi-square test

M mean, *SD* standard deviation

Table 2 The comparison of CLU, VLCFA, and carnitine levels between the regressive autism, non-regressive autism, and control groups

	Non-regressive ($n=30$)		Regressive ($n=30$)		Controls ($n=30$)		p
	M/Md	SD/IQR	M/Md	SD/IQR	M/Md	SD/IQR	
Clusterin	25.9	53.2	15.5	7.65	14.3	5.26	0.46[a]
Phytanic acid	0.77	0.51	0.86	0.65	0.76	0.63	0.96[b]
Pristanic acid	0.22	0.21	0.2	0.1	0.22	0.12	0.45[b]
C22	66.2	22.09	51.9	33.9	49.4	16.5	**0.04**[b,c]
C24	53.73	14.37	50.25	30.62	46.27	16.71	0.40[b]
C26	0.59	0.23	0.57	0.24	0.47	0.21	0.26[a]
Carnitine	24.9	6.5	23.7	4.8	23.6	4.8	0.60[a]

[a]One-way ANOVA. [b]Kruskal–Wallis test. [c]Pairwise comparisons: non-regressive group > controls ($p=0.012$)

M mean, *Md* median, *IQR* interquartile range, *SD* standard deviation

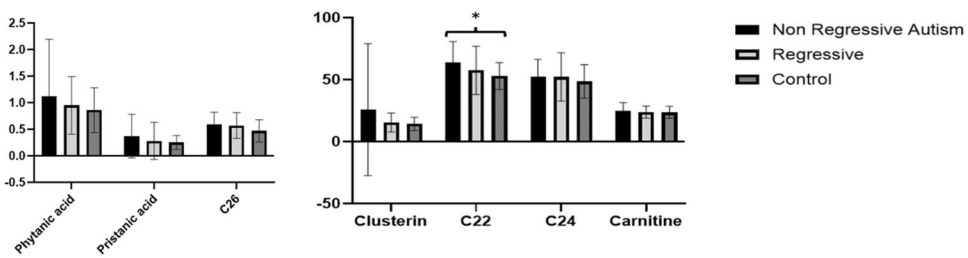


Fig. 1 The comparison of biochemical parameters across regressive autism, non-regressive autism, and control groups

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significantly higher in the ASD group. The comparison of CLU, VLCFA, and carnitine levels between autism and control groups is shown in Table 3. The graphics of the CLU, VLCFA, and carnitine levels in autism and control groups are shown in Fig. 2.

The correlations between biochemical parameters (CLU, VLCFA, and carnitine) and CARS scores of the autism group including regressive and non-regressive patients were analysed by using Bonferroni correction for correlation analyses and subscales of CARS. $P < 0.05/10$ was considered statistically significant. According to the analysis, serum CLU and C24 levels were significantly correlated with CARS listening response subscale scores (Table 4).

We also analyzed the correlations between biochemical parameters and RBS-R scores in autism group (including both regressive and non-regressive patients). We used Bonferroni correction for correlation analyses and 7 subscales of RBS-R, and $p < 0.05/7$ was considered statistically significant. It was found that serum carnitine levels were positively correlated with the "Stereotypic Behaviors" ($r = 0.37$, $p = 0.004$) and total scores ($r = 0.35$, $p = 0.006$) of RBS-R.

Table 3 The comparison of CLU, VLCFA, and carnitine levels between autism and control groups

	Autism (regressive and non-regressive) group ($n = 60$)		Control group ($n = 30$)		p
	M/Md	SD/IQR	M/Md	SD/IQR	
Clusterin	15.9	6.9	14.3	5.26	0.61[a]
Phytanic acid	1.04	0.85	0.86	0.42	0.78[a]
Pristanic acid	0.33	0.38	0.25	0.13	0.34[a]
C22	60.96	18.42	52.9	10.9	**0.04[a]**
C24	52.69	16.84	48.6	13.5	0.22[a]
C26	0.58	0.23	0.47	0.21	**0.03[a]**
Carnitine	24.37	5.75	22.74	7.79	0.56[b]

[a]Mann–Whitney *U* test, [b]independent sample *t*-test

M mean, *Md* median, *IQR* interquartile range, *SD* standard deviation

No significant correlation was observed between serum clusterin and VLCFA levels and RBS-R scores. Additionally, regarding the correlation between ABC scores and serum CLU, VLCFA, and carnitine levels, there was no statistical association between these parameters.

We conducted MANOVA analyses to evaluate the biochemical variables across the different groups. Our findings indicated a significant difference among the groups, particularly concerning C22. Subsequent post hoc analyses revealed that this difference was observed between the non-regressive autism group and the control group, as illustrated in Fig. 3 ($p = 0.02$).

Discussion

This study aimed to evaluate CLU, VLCFA, and carnitine levels as potential biomarkers associated with ASD and explore their relationship with regression. We hypothesized that elevated plasma CLU levels in children with regressive autism compared to those without regression and healthy controls, but our findings did not support this. No statistically significant differences were observed in serum CLU levels across the three groups, suggesting CLU is not a distinguishing biomarker for regression.

Evaluation of Findings Associated with CLU Levels

Despite fundamental differences between neurodegenerative disorders and ASD, recent literature increasingly highlights important shared features and potential common pathomechanisms (Jesko et al. 2020). Given that regression in autism has been suggested to be associated with neurodegeneration (Kern et al. 2013), our study specifically examined this relationship within the regressive phenotype, alongside controls. While CLU is a well-established marker in neurodegenerative diseases such as Alzheimer's (AD) and Parkinson's disease (PD) (Haight et al. 2018; Sasaki et al. 2002), the lack of association in our findings suggests its role in ASD regression may be more complex than previously thought. This outcome may have been influenced by the retrospective

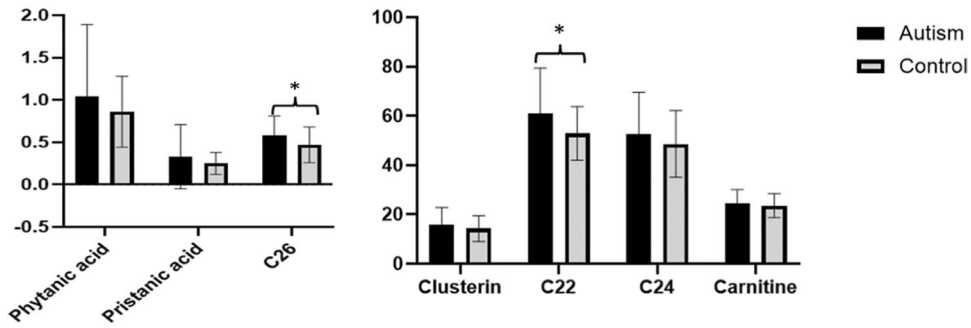


Fig. 2 The comparison of biochemical parameters across autism (regressive and non-regressive) and control groups

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Table 4 The correlations of serum clusterin, VLCFA, and carnitine levels with CARS scores

		CARS Relationship to people	CARS Imitation	CARS Emotional response	CARS Body use	CARS Visual response	CARS Listening response	CARS Fear- nervousness	CARS Verbal communication	CARS Nonverbal communication	CARS Total
Clusterin	*r*	−0.05	−0.08	0.02	0.03	0.29	**0.351***	−0.026	−0.117	−0.009	0.072
	p	0.70	0.54	0.88	0.82	0.02	**0.006**	0.846	0.379	0.944	0.585
Phytanic acid	*r*	0.05	0.14	0.27	0.22	0.21	0.261	0.196	0.258	0.253	0.316
	p	0.62	0.28	0.03	0.08	010	0.04	0.133	0.046	0.051	0.014
Pristanic acid	*r*	−0.02	−0.12	0.18	0.18	0.22	−0.02	0.259	0.09	−0.104	0.113
	p	0.82	0.34	0.15	0.15	0.86	0.86	0.045	0.492	0.430	0.390
C22	*r*	0.09	0.22	0.26	−0.03	0.008	0.26	0.298	0.227	0.233	0.283
	p	0.47	0.08	0.04	0.79	0.95	0.04	0.021	0.081	0.074	0.028
C24	*r*	0.15	0.26	0.27	0.15	0.168	**0.400***	0.165	0.251	0.332	0.322
	p	0.23	0.04	0.03	0.23	0.198	**0.002**	0.207	0.053	0.009	0.012
C26	*r*	0.12	0.21	0.18	0.04	0.19	0.324	0.117	0.177	0.247	0.203
	p	0.32	0.10	0.15	0.76	0.12	0.011	0.372	0.176	0.057	0.119
Carnitine	*r*	0.07	0.15	0.13	0.26	0.09	−0.081	−0.059	0.131	0.086	0.126
	p	0.55	0.23	0.30	0.04	0.47	0.539	0.653	0.320	0.515	0.337

CARS Childhood Autism Rating Scale, *VLCFA* very long-chain fatty acids. *$p \leq 0.05/10$

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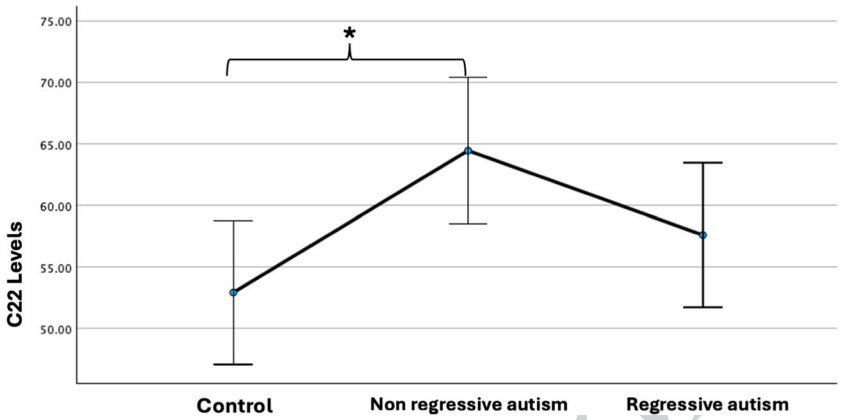


Fig. 3 Results of MANOVA analyses for C22 levels of groups

nature of our regression assessment. Studies with prospective follow-up data may provide more clarity in this regard. Furthermore, the lack of a clear definition of regression and limited objective evaluations pose additional challenges. Further research evaluating CLU and other parameters associated with neurodegeneration in ASD etiopathogenesis in a larger sample is warranted (Rodríguez-Rivera et al. 2021; Athanas et al. 2015).

Evaluation of Findings Associated with VLCFA Levels

While neurodegenerative processes have been suggested as factors in regressive ASD (Kern et al. 2013), etiological factors in regression may extend beyond neurodegeneration. Impaired mitochondrial energy metabolism has been proposed as a key element in the pathogenesis of ASD (Frye et al. 2013; Rossignol and Frye 2012). Additionally, a study comparing fatty acid compositions of cell polar lipids in non-regressive and regressive autism suggested that significant loss of fatty acids during cold storage may indicate increased phospholipid metabolism in the regressive group (Bell et al. 2004). Recent research has also linked lipids and RBP4 to regression in ASD (Chen et al. 2021a, b), while metabolic and dietary changes have been associated with the regressive form (El-Ansary et al. 2011; Goldani et al. 2014).

Our analysis revealed that serum levels of C22 and C26 were significantly higher in the autism group compared to controls, but no differences were found between regressive and non-regressive groups. Significantly higher C22 and C26 levels in the ASD group align with previous studies suggesting that altered lipid metabolism plays a role in ASD pathophysiology (Frye et al. 2013; Demarquoy and Demarquoy 2019). Elevated VLCFA levels and mitochondrial dysfunction have been highlighted as important metabolic phenotypes of ASD (Pastural et al. 2009; Doria et al. 2019). A recent study compared the plasmatic metabolomic profiling of children with regressive and non-regressive autism and reported subtle significant differences; however, several alterations in lipid, amino acid, and nicotinamide metabolism were presented in ASD compared with controls. Furthermore, alterations in the arginine and glutamate pathways were associated with the regression phenomenon (Rangel-Huerta et al. 2019). However, studies investigating these relationships are still insufficient.

Evaluation of Findings Associated with Carnitine Levels

Recent studies have identified several biomarkers related to mitochondrial dysfunction in ASD, notably reduced free carnitine levels, which have been documented in a significant proportion of individuals with autism (Frye et al. 2024; Demarquoy and Demarquoy 2019; Kępka et al. 2021). This suggests that the L-carnitine profile may serve as a potential biomarker for mitochondrial dysfunction in autism (Frye et al. 2013). In the current study, however, no significant differences in carnitine levels were observed between the regressive and non-regressive autism and control groups. While low plasma carnitine levels have been reported in autism, these findings are not systematic (Demarquoy and Demarquoy 2019). Some studies indicate that reduced carnitine levels are observed in certain patients with ASD, but this is not consistent across all individuals (Bankaitis and Xie 2019). For example, decreased carnitine levels have been documented in up to 66% of patients with ASD (Mostafa et al. 2005). In another study, it was found that 36% of participants had normal carnitine levels, while the others showed levels either above or below the expected range (Filipek et al. 2004). Additionally, low serum carnitine levels detected in early childhood may normalize over time for some individuals with ASD (Beaudet 2017). Thus, follow-up studies that include early measurements of carnitine levels will be crucial for understanding its role in this population.

Assessment of the Findings Related to the Associations Between Scale Scores and Blood Parameters

In the current study, the correlations between scale scores and levels of CLU, VLCFA, and carnitine were investigated.

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We found that carnitine levels correlated with stereotypic behaviors subscale and total RBS-R scores. This finding partially aligns with previous studies that indicated increased serum L-carnitine levels are associated with reductions in specific autistic symptoms (Kępka et al. 2021) and significant improvements in CARS scores (Fahmy et al. 2013). However, additional research is needed to explore the links between biological parameters and clinical features based on pathophysiological pathways.

Another finding of the current study was the significant positive correlation between CARS listening response subscale and serum CLU and C24. Previous studies have suggested that abnormalities in lipid metabolism may contribute to partial hearing loss by reducing vascular diameter in cochlear tissue (Chandrasekhar et al. 2019; Lee et al. 2020). This may affect various auditory processes, including cochlear mechanics and sensory transduction (Thomas et al. 2014). Despite mixed findings, hearing loss is reportedly more prevalent in the ASD population than in the general population (Tas et al. 2007), although some studies indicate a lack of conclusive evidence supporting this increased risk (Roper et al. 2003). Consequently, audiological assessments are recommended to diagnose hearing impairments as early as possible in individuals with co-existing hearing loss and ASD. In our study, participants had no known hearing defects, and most had undergone prior audiologic examinations; however, undetected or unrecognized hearing defects may still exist.

Strengths and Limitations

Our study highlights that while previous research has investigated CLU levels in other neurodegenerative diseases (Harold et al. 2009; Lambert et al. 2009; Gibson et al. 2010), the specific relationship between CLU and ASD, as well as its potential as a biomarker for ASD, has not been previously explored. This represents one of the strengths of our study. However, limitations must also be acknowledged. Our results may not be generalizable due to the relatively small sample size and cross-sectional nature of the research. Despite the ethical challenges of measuring the CLU and other parameters from brain tissue, the role of blood–brain cross-talk is vital for understanding ASD pathophysiology (Chen et al. 2023). Thus, using peripheral blood parameters to assess changes in the central nervous system is a limitation of this study. Furthermore, the absence of an objective method for defining regression, along with evaluations based on parental reports and clinician observations, may introduce bias.

Regarding methodology, the assessment tools utilized, such as CARS and the Autism Behavior Checklist, effectively captured behavioral severity and provided a nuanced understanding of autism symptoms. Nevertheless, the lack of significant findings related to CLU and carnitine levels indicates the need for further investigation, potentially involving larger sample sizes or longitudinal studies to monitor these biomarkers over time.

Conclusions

Considering that CLU levels in children with ASD have not been previously investigated, our findings, indicating that the regression phenomenon is not linked to the CLU levels, may partially contribute to the literature. However, replicated studies with larger sample groups are needed to evaluate the accuracy of the findings to understand the pathophysiology of ASD and develop effective intervention strategies.

Author Contributions E.Y., O.D., B.K., H.H.O, B.N.B.K.: Conceptualization, methodology, analysis, and writing original draft preparation. O.D.: Biochemical analysis. B.K., H.H.O., B.N.B.K.: Data curation. E.Y., M.C.U., D.B.O.: Reviewing and editing

Data Availability No datasets were generated or analysed during the current study.

Declarations

Ethical Approval Ethics approval was obtained from the Human Research Ethics Committee of Ankara University Faculty of Medicine (approval number: i05-294–23).

Informed Consent Verbal and written informed consent was obtained from all children and their parents. This study complies with the Declaration of Helsinki.

Competing Interests The authors declare no competing interests.

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