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ORIGINAL ARTICLE

The role of lipid peroxidation in individuals with autism spectrum disorders

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Abstract

The role of malondialdehyde-modified low-density lipoprotein (MDA-LDL), an oxidized LDL, in the pathophysiology of autism spectrum disorder (ASD) is unclear. We studied association between MDA-LDL and behavioral symptoms in 11 individuals with ASD and 7 age -matched normal controls. Behavioral symptoms were assessed using the Aberrant Behavior Checklists (ABC). Because small sample size in this study, three measures were conducted: first, employment of adaptive Lasso for enhancing the accuracy of prediction and interpretability; second, calculation of coefficient of variation for an appropriate selection of plasma variables; and third, selection of good candidates of plasma variables. Plasma levels of MDA-LDL, eicosapentaenoic acid, docosahexaenoic acid (DHA) and DHA/arachidonic acid ratios were significantly higher, while plasma superoxide dismutase (SOD) levels were significantly lower in the ASD group than in the control group. The total ABC scores were significantly higher in the ASD group than in the control group. Multiple linear regression analysis and the adaptive Lasso revealed association of increased plasma DHA levels with the ABC total scores and increased plasma MDA-LDL levels. Such association between DHA and plasma MDA-LDL levels may contribute to behavior in individuals with ASD.

Keywords Lipid peroxidation · Autistic behavior · Malondialdehyde-modified low-density lipoprotein · Docosahexaenoic acid (DHA)

Introduction

Studying the mechanisms of action of lipid peroxidation is an important research strategy to understand the pathophysiology of autism spectrum disorder (ASD) (Meguid et al. 2011). Membrane phospholipids in the brain are highly enriched in polyunsaturated fatty acids (PUFAs), which are converted to other products including reactive aldehydes such as lipid peroxidation: production. Of reference, two main omega-6 fatty acids lipid peroxidation products: malondialdehyde (MDA) and 4-hydroxy-2-nonenal (4-HNE) have been reported (Ayala et al. 2014). MDA has been known as a useful biomarker for lipid peroxidation of omega-3 and omega-6 fatty acids (Ayala et al. 2014). However, which of n-3 or n-6 PUFAs mainly contributes to the formation of MDA is still under debate. A few studies have reported the elevation of plasma MDA in 20 children with ASD compared to age-matched 20 controls (Meguid et al. 2011), and that blood MDA levels in 45 autistic children aged 3–11 years were higher than age-matched 42 normal controls (González-Fraguela et al. 2013).

Accumulating evidence suggested a positive relationship between the antioxidant protein superoxide dismutase (SOD) and MDA in erythrocytes; for example, results from a meta-analysis revealed higher MDA levels and lower SOD levels in 3961 subjects with major depression compared to 1484 healthy controls (Jiménez-Fernández et al. 2015). Other studies found higher serum MDA levels and decreased serum SOD levels in 20 children with AD compared to 25 age-matched normal controls (Meguid et al. 2011), indicating a deficit of antioxidant defense mechanism in ASD subjects.

One of the lipid profiles, low-density lipoprotein (LDL) has been well known to play an important role in brain development. A previous study reported significantly higher blood levels of LDL in 22 adult subjects with Asperger syndrome (mean age, 40.8 ± 10.8 years) than 22 age-matched normal

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controls, suggesting abnormal cholesterol metabolism (Dziobekm et al. 2007). Thus, blood LDL levels may contribute to the pathophysiology of ASD.

Lipid peroxidation markers such as glutathione peroxidase (GSH-Px) were significantly higher in autistic subjects compared to control children (Al-Gadani et al. 2009). Serum SOD, and GSH-Px were significantly lower in autistic children compared with their controls, while MDA was significantly higher among patients than controls in children less than six years of age (Meguid et al. 2011). Additionally, the association between MDA and SOD was found in 90 children with ASD who were treated with coenzyme Q10 (Mousavinejad et al. 2018). These studies indicated a close association between lipid peroxidation markers and antioxidant enzyme SOD.

The MDA can further react with acetaldehyde to form malondialdehyde-modified low-density lipoprotein (MDA-LDL). Recent evidence indicated that MDA-LDL is a good marker of oxidative stress (Ogawa et al. 2015) and lipid peroxidation. Several studies have reported that MDA-LDL significantly predicts mild traumatic brain injury (Sharma et al. 2017). However, there are a few studies on the role of MDA-LDL in the pathophysiology of neurodegenerative diseases including ASD. This study for the first time examined the role of MDA-LDA in ASD.

Taken together, the topics being studied are the role of both lipid peroxidation marker and oxidative stress marker MDA-LDL and its relation to antioxidant SOD and major families of PUFAs s in autistic behaviors of individuals. The purpose of this study specially addressed to examine which variable including major PUFAs and MDA-LDL may predict autistic behaviors. For variable selection and regularization in order to enhance the prediction accuracy and interpretability in small sample size, we used adaptive Lasso (Wahid et al. 2017; Abdul-Razik Ismail 2015). To select reasonable variables selection and regularization, and enhance the accuracy of prediction and interpretability in small sample research, adaptive Lasso was employed in this study.

Materials and methods

Participants

Subjects in this study comprised a total of 18 young, physically healthy individuals. Diagnoses were performed based on the DSM-5 criteria and were additionally confirmed by the Autism Diagnostic Interview-Revised (ADI-R).

As shown in Table 1, among the 18 individuals, 11 had an independent clinical diagnosis of ASD (8 males and 3 females, mean age: 12.3 ± 5.4 years old, age range: 6–21 years old), and the remaining 7 were normal healthy controls (4 males and 3 females, mean age: 10.0 ± 4.1 years old, age range: 5–21 years old). The 11 individuals with ASD and the 7 normal controls were matched with respect to feeding habits, age and full intelligent quotient (IQ) scores (Table 1). These individuals had the core symptoms of the DSM-5 diagnostic criteria for ASD without any abnormal neurological symptoms. The 7 normal controls were considered to be physically and mentally healthy based on initial physical and mental screening tests. At the initial screening, physical (blood pressure and heart rate) and clinical laboratory examinations (hematology and plasma chemistry, including plasma fatty acids, cholesterol and triglycerides) were performed on all 18 participants. These participants did not have any abnormalities in their physical examinations and laboratory findings. The IQ of the individuals was estimated using the Wechsler Intelligence Scale for children and adolescents aged 6–16 years old (Wechsler intelligence scale for children-III, WISC-III) or the respective scale for adults (Wechsler Adult Intelligence Scale, WAIS-R) (Table 1). None of the ASD or control individuals had any history of neurological conditions, Attention-Deficit Hyperactivity Disorder or learning disorders.

This study was performed with the approval of the Ethics Committee of the Fujimoto Medical Clinic in Kobe City, Japan. Written informed consent was obtained from the participants and/or their parents.

Cautions for dealing with a small sample size

Small sample sizes in this study can be limitations of a study design that can be affect the interpretation as they can affect the interpretation of the effectiveness of a course; therefore an approproate anakysis schould be considered (Morgan 2017). There may be three measures for cautions in interpreting the small sample data; first, the outcome-adaptive lasso for selecting appropriate covariates for inclusion in propensity score models to account for confounding bias and maintaining statistical efficiency (Shortreed and Ertefaie 2017). To identify the most effective variables on plasma levels of MDL-LDL, DHA, eicosapentaenoic acid (EPA), and arachidonic acid (ARA), omega 3/omega 6 ratio and SOD levels, a modified least absolute shrinkage and selection operator (LASSO), called adaptive Lasso was used; second, standard deviation (SD) are used interchangeably to express the variability because SD estimates the variability among sample observations (Curran-Everett 2008), and precise summarization of small sample data (Nature Neuroscience, Rights and Permissions. 8, 123, 2005; https://doi.org/10.1038/nn0205-123). Of reference, coefficient of variation (CV, %), which is defined as the SD divided by mean (SD/mean values, %) (Packiasabapathy et al. 2020) is widely used to measure the relative variation of a random variable to assess and compare the performance of analytical techniques/equipments (Albert and Zhang 2010). The CVs of PUFAs were 24.3–28.9% (Song et al. 2016) have been reported in blood PUFAs; and third, identification of interesting target variables has been reported to select good candidates for small sample size

Table 1 Subject characteristics and plasma levels of PUFAS, MDA-LDL and antioxidant proteins, and the ABC subscale scores in the 11 individuals with ASD and 7 normal controls

Variables	ASD ($n = 11$)	Controls ($n = 7$)	U	p value
Age (Year	12.3 ± 5.4	10.0 ± 4.1	26.50	0.29
Sex (male/female)	3/8	4/3	$\chi^2 = 0.47$	0.49
Scores of Autism Diagnostic Interview-Revised				
Domain A (social)	13.5 ± 4.3	N/A		
Domain B (communication)	10.2 ± 4.4	N/A		
Domain C (stereotyped)	3.7 ± 3.7	N/A		
Plasma biomarkers levels				
Cp[a] (mg/dl)	26.45 ± 6.74	24.29 ± 7.25	33.00	0.66
Tf[b] (mg/dl)	276.64 ± 44.15	262.29 ± 25.75	31.0	0.54
MDA-LDL[c] (mg/dl)	93.36 ± 18.54	71.29 ± 17.87	15.00	0.035*
SOD[d] (U/ml)	2.45 ± 0.39	5.69 ± 4.64	9.00	0.006**
Total scores of the ABC[e]	56.82 ± 22.31	0.71 ± 1.25	0.00	0.000***

Values are mean ± SD

[a] Cp: ceruloplasmin; [b] Tf: transferrin; [c] MDL-LDL low density: malondialdehyde modified low-density lipoprotein; [d] SOD: superoxide dismutase; [e] ABC, Aberrant Behaviour Checklist

* $p < 0.05$. ** $p < 0.001$, *** $p < 0.001$, versus normal controls (Mann-Whitney U test)

[abcd] Subject characteristics

studies (Lichou et al. 2019). These three measures may be useful for taking precautions for interpreting the data of the small sample size in this study.

Assessment of behavioral symptoms

The Aberrant Behavior Checklist (ABC) was used to assess the behavioral symptoms. ABC is an extensive assessment that captures a wide variety of behavioral problems (Karabekiroglu and Aman 2009). This test also appears to be capable of discriminating between several syndromes, such as disruptive behavior disorders and the behavioral symptoms of ASD (Karabekiroglu and Aman 2009).

Assays of plasma levels of PUFAs, Cp, SOD and Tf

Blood sampling procedures

Whole-blood samples were collected in the EDTA tubes by venipuncture after five hours of fasting, and immediately placed on ice in a refrigerator. Regarding fasting time, a previous review report on fasting in studies of triglycerides considered 9–12 h fasting (Simundic et al. 2014). Another study reported that fasting for 8 h before assay of serum triglyceride may be sufficient (Pongsuthana and Tivatunsakul 2016). While, recent lipid studies commented that except for triglyceride, non-fasting lipid parameters are useful when screening children for dyslipidemia (Kubo et al. 2017), and that non-fasting blood samples can be routinely used for the assessment of plasma lipid profiles (Nordestgaard et al. 2016). Thus, five hours of fasting time in this study may be reasonable.

The serum was obtained by centrifugation for 20 min at 3000 x g at room temperature (22 °C). The samples were frozen at −80 °C until analysis. The specialists at SRL, Inc. (Tokyo, Japan) measured the plasma levels of PUFAs, and antioxidant protein such as ceruloplasmin (Cp), transferrin (Tf), SOD and MDA-LDL.

Plasma levels of PUFAs

The fatty acid composition of the PUFA fraction was determined as previously described (Yui et al. 2017). In summary, the total lipids were extracted from the plasma according to previously described method. After transmethylation with HCl-methanol, the PUFA composition was analyzed using gas chromatography (GC2010 Shimazu Co., Japan). A total of 24 long-chain fatty acids were identified. The plasma levels of the fatty acids were expressed as the mean ± SD weight (percentage) of the total fatty acids.

Plasma levels of SOD

Human plasma was assayed using SOD Assay Kit (Takara Bio, Tokyo) according to the cytochrome c method. The plasma SOD levels are expressed as units per milliliter.

Plasma levels of MDA-LDL

Venous blood sampling was performed during the fasting state. An enzyme-linked immunosorbent assay used. The plasma samples were enzyme-linked immunosorbent assay method. The detection limit was 6.3 U/l, and intra- and inter-assay coefficients were < 5.6 and < 9.4% respectively (Kitano et al. 2004).

Controlling for dietary intake

Plasma levels of PUFAS and MDA-LDL may be affected by prior dietary intake. In this study, all 18 participants received the "Japanese Food Guide" (Ministry of Health Labour and Welfare and Ministry of Agriculture Forestry and Fishers), which outlines the daily intake guide of nutrients and food based on the "Overview of Dietary Reference Intake for Japanese.

Statistical analyses

As the data were not normally distributed, the non-parametric Mann-Whitney U test for multiple comparisons was used to determine the significant differences between the ASD and control groups. Multiple regression analysis was used to confirm the relationship between the plasma oxidative stress-related biomarkers and the other variables (two subject groups and the ABC total scores) (Table 2). To identify the most effective variables on plasma levels of MDL-LDL, DHA, EPA, and ARA, omega 3/omega 6 ratio and SOD levels as a measure for cautions in interpreting the small sample data, an adaptive Lasso was used for statistical analysis on these plasma variables. The adaptive lasso is a model selection method shown to be both consistent in variable selection and essential in identifying important variables (Wahid et al. 2017) in small sample size (Abdul-Razik Ismail 2015).

Results

Results of the solution for dealing with a small sample size in this study

Adaptive LASSO identified the most effective variables on plasma levels of MDL-LDL, DHA, SOD concentrations, and the results were similar to those of a multiple regression analysis. Therefore, the statistical analyses applied in this study were proven as suitable procedures. The mean CVs of plasma lipid peroxidation - related variables such as DHA, EPA and MDL-LDL levels and plasma DHA/ARA ratio were 29.93% in the ASD group and 33.25% in the control group. Finally, we selected possibly sensitive lipid peroxidation markers as candidates for small sample size studies (Lichou et al. 2019).

Table 2 Results of the multiple linear regression

Model	Model R2[a]	Model P value	Coefficients		
			B[b]	Beta	*p* value
EPA	0.997	0.000**			
SOD[c]			0.018 ± 0.008	0.117	0.055
MDA-LDL[d]			0.002 ± 0.001	0.065	0.092
ABC[e] total score			0.001 ± 0.001	0.094	0.182
Group (1 = ASD, 2 = control)			0.132 ± 0.061	0.133	0.073
DHA	0.995	0.000**			
SOD			−0.035 ± 0.024	- 0.102	0.198
MDA-LDL			- 0.004 ± 0.002	- 0.068	0.159
ABC[e] total score			- 0.002 ± 0.003	- 0.054	0.550
Group (1 = ASD, 2 = control)			- 0.228 ± 0.191	- 0.105	0.279
DHA/ARA	0.985	0.000**			
SOD			- 0.001 ± 0.008	0.019	0.900
MDA-LDL			0.000 ± 0.001	0.057	0.540
ABC total score			0.000 ± 0.001	- 0.039	0.813
Group (1 = ASD, 2 = control)			0.008 ± 0.059	0.024	0.895

[a] R2: R-squared values; [b] B: Unstandardized coefficients; [c] SOD: Superoxide dismutase

[d] MDL-LDL: malondialdehyde-modified low-density lipoprotein; [e] ABC: Aberrant Behavior Checklist

[cde] The multiple linear regression

Study population

There was no statistically significant difference be- tween the two groups with respect to age ($p = 0.29$) and sex ($p = 0.49$). The 11 individuals with ASD were characterized by restricted, repetitive and stereotyped patterns of behavior, ($n = 8$), irritability and crying ($n = 3$). Their mean total ABC score was 58.5 ± 19.6 (Table 1). Earlier studies reported a total ABC score of 60.14 for children and adolescents with moderate to severe ASD (Singh et al. 2014). Thus, the patients in this study suffered from moderate behavioral autistic symptoms.

Plasma levels of lipid peroxidation related biomarker

There was a significant increase in plasma MDA-LDL levels ($p = 0.034$) and a significant decrease in plasma SOD levels ($p = 0.006$) in the 11 individuals with ASD compared to the 7 normal controls. Plasma EPA and DHA levels and the ratio of plasma DHA/ARA were significantly higher in the ASD group than in the control group (Table 1).

Predictor variables: the multiple linear regression analysis

The multiple linear regression analysis demonstrated that the plasma levels of EPA ($R^2 = 0.997$, $p = 0.000$) and DHA ($R^2 = 0.995$, $p = 0.000$), and plasma ratios of DHA/ARA ($R^2 = 0.985$, $p = 0.000$) and EPA/ARA ($R^2 = 0.978$, $p = 0.000$) were significantly associated with adjustment in the variables, SOD and the total ABC scores in the two subject groups (Table 3). These findings indicated that the plasma EPA and DHA levels equally allowed for the prediction of these variables in the two groups. The use of the plasma SOD levels as the dependent variable showed a trend towards significant contribution to the plasma EPA levels (unstandardized coefficients, $B = 0.18 \pm 0.08$, $\beta = 0.117$, $p = 0.055$).) (Table 2). As the plasma EPA and DHA levels equally fit the models that distinguished the ASD group from the control group.

Results of adaptive Lasso

The adaptive Lasso was used to enhance the prediction accuracy and interpretability of the statistical model in small sample size (Wahid et al. 2017; Abdul-Razik Ismail 2015). For the ABC total scores, plasma DHA levels (coefficient, 176.68) and plasma DHA/ARA ratio (coefficient, −166.368) were selected by the adaptive Lasso (Table 3). Plasma EPA levels were not selected as appropriate covariates for inclusion in propensity score models. For plasma MDA-LDL levels, plasma levels of DHA (coefficient, −79.89) and EPA (coefficient, 70.72) were selected (Table 3). Collectively, these statistical findings indicated that plasma DHA levels were more significantly associated with total ABC scores and plasma MDA-LDL levels.

Discussion

Because small sample sizes, this study employed three types of measures: first, the adaptive lasso was used to identify the most effective variables on plasma levels of MDL-LDL, DHA, eicosapentaenoic acid (EPA), and arachidonic acid (ARA), omega 3/omega 6 ratio and SOD levels; second, CV was well known to be useful in examining the effect of adjustment for variable cluster size (Eldridge et al. 2006); and third, identification of useful targets for reducing target variables were selected for small size study (Lichou et al. 2019). Importantly, as the CVs of PUFAs were 24.3–28.9% in 305 men and women, aged 18–96 years, with immunodeficiency virus (HIV), human herpesvirus and sarcoma (Song et al.

Table 3 Results of adaptive Lasso

Intercept	Estimate	SE	*p* value	95% confidence Interval Lower bound	Upper bound
ABC total scores					
DHA[a]	176.69	96.86	0.073	−17.08	370.45
DHA/ARA	−166.37	79.85	0.037	−322.87	−9.87
ARA[b]	−104.16	87.86	0.039	−203.71	−5.035
SOD[c]	−33.26	18.94	0.079	−70.37	3.87
MDA-LDL[d]					
DHA	−79.89	113.92	0.483	−506.86	−250.94
EPA[e]	70.73	28.58	0.013	14.72	126.73
SOD[f]	−47.32	12,33	0.001	−71.50	−23.15
Tf[g]	30.10	20.67	0.080	−4.43	76.61

[a] DHA: docosapentaenoic acid; [b] ARA: Arachidonic acid; [c] SOD: Superoxide dismutase; [d] low-density: malondialdehyde-modified low-density lopoprotein; [e] EPA: Eicosapentaenoic acid; [f] SOD; [g] Tf: Transferrin

2016), the CVs of plasma variables in this study may indicate an appropriate variable selection. These three measures may contribute to taking cautions need by interpreting the small sample size data in this study.

The multiple linear regression analysis and the adaptive Lasso identified plasma DHA levels that equally fit the models that distinguished the ASD group from the control group. We conducted a control experiment for the dietary intake of all 20 participants.

The present findings suggested that plasma DHA levels were the most useful predictors for autistic behavior. Moreover, for ABC total scores, the adaptive Lasso selected serum DHA levels. Thus, plasma DHA levels may be associated with autistic behaviors. Of reference, a review article studied the association between n-3/n-6 PUFA ratio and the risk of breast cancer through PubMed, Cochrane Library and EMBASE database on relevant English-language studies concluded that a higher ratio of n-3/n-6 PUFA was associated with a lower risk of breast cancer due to lowered serum phospholipids (Yang et al. 2014). We have already reported that a higher plasma n-3/n-6 ratio may be related to lower plasma levels of ARA-related signaling mediator such as ceruloplasmin, and thus may contribute to the pathophysiology of ASD (Yui et al. 2017). These previous studies indicated the beneficial effects of a higher ratio of n-3/n-6 PUFA as reflected by increased DHA levels in human health or inflammatory outcomes.

In this study, plasma DHA levels have more important effects on autistic behaviors compared to plasma EPA levels or plasma EPA/ARA ratio. These potential DHA effects on the neuronal function may depend on the following neuronal membrane properties (Dyall 2015): a) DHA had a much greater capacity of accumulating into sphingomyelin/cholesterol-rich lipid rafts than EPA, inducing a much greater effect on cell signaling; b) DHA is more favorably converted into phosphatidylserine increasing neuronal survival species in the brain; c) DHA has a unique and indispensable role in neuronal membranes, whereas, the role for EPA is only as a precursor to the eicosanoids and related peroxy-fatty acids; d) DHA also promotes synaptogenesis and synaptic expression of synapsin and glutamate receptors, producing more positive effects on neuronal plasticity. Collectively, DHA has more important roles in neuronal function including signaling and neuronal survival compared to EPA. Furthermore, a recent review article indicated that high DHA fish oils have more excellent effects on various human health conditions including the brain and visual function, inflammation and immune function (Ghasemi Fard et al. 2019). Thus, more potential effects of DHA on the neuronal function might contribute to autistic behaviors. These previous findings may support the present findings that plasma DHA levels a have more potent influence on autistic behaviors.

DHA is a major component of the brain and a lipid essential for its structure and function by providing a lipid domain and has an important role in signaling and lipid mediator production. DHA is most susceptible to oxidation among the PUFAs (Luchi et al. 2018). Therefore, plasma DHA levels might have a more potent influence on autistic behaviors. DHA changed the oxidant/antioxidant balance such as increased SOD and decreased MDA in the rat brain tissue (Avramovic et al. 2016) have been reported. Drawing these findings together, increased MDA-LDL levels may be closely related to decreased plasma SOD levels. Therefore, plasma DHA levels primarily and preferentially influence autistic behaviors.

There is a trend toward significant correlation in plasma levels between MDA-LDL and SOD. MDA is the end product of lipid peroxidation in the cell membrane, and its content can reflect the extent of lipid peroxidation. Plasma MDA levels were consistently increased in children with ASD, while plasma SOD levels decreased, increased, or remained unchanged (Altun et al. 2018). Thus, the present finding that elevated serum levels of MDA-LDL are associated with decreased serum levels of SOD may be reasonable.

MDA has been found to weaken cerebral function during carbonyl stress (Itokawa et al. 2018) and MDA treatment in vitro reduced cortical neuronal viability and induced cellular apoptosis and necrosis simultaneously (Cheng et al. 2011). However, MDA is considered as an oxidative stress marker and a fraction of metabolite of n-3 PUFAS. Therefore, plasma MDA-LDL might have a little effect on autistic behaviors in individuals with ASD.

In this study, plasma SOD levels in the ASD group were significantly lower than in the control groups. The increase of serum DHA levels as well as serum DHA/ARA ratio might counteract serum SOD levels, which might reduce serum SOD levels. SOD did not afford any protection against the loss of barrier integrity induced by DHA (Roig-Pérez et al. 2014), and played a primary role in mediating the behavioral plasticity (Horspool and Chang 2018). Particularly, SOD plays a key role in the nervous system to mediate pathogen avoidance behavior in response to the internal state of redox homeostasis and avoidance behavior against dangerous situations (Horspool and Chang 2018). These observations suggest that lowered plasma SOD levels might induce neuronal deficit related to autistic behavior in the autism group. However, further precise studies are needed.

Our study had some limitations: the small sample size may increase the likelihood of a false null hypothesis (type II and type I errors), which skews the results and decreases the power of the study. Previous studies have demonstrated that the adaptive Lasso used in this study is very competitive in terms of variable selection, estimation accuracy, and high efficiency when small sample sizes are used (Wahid et al. 2017; Abdul-Razik Ismail 2015). Using adaptive Lasso in statistical analyses may improve the reproducibility and sensitivity of the findings and minimize the likelihood of type II as well as type I errors due to small sample sizes. Additionally, the results from the linear regression analysis and adaptive Lasso were

consistent with previous reports, which indicated two important aspects of lipid peroxidation: the association between increased plasma MDA-LDL levels and decreased plasma SOD levels (Meguid et al. 2011; Jiménez-Fernández et al. 2015) and the role of plasma DHA levels (Dyall 2015; Luchi et al. 2018; Ghasemi Fard et al. 2019). Although the sample size is small, this paper contributes to growing evidence that plasma DHA levels may play important roles in lipid peroxidation and consequently in autistic behavior. However, further studies with larger sample sizes are needed.

In conclusion, the present findings might provide useful information that increases in plasma DHA levels might increase plasma MDA-LDL levels, which counteracts plasma SOD levels, thus reducing plasma SOD levels. This neurobiological phenomenon may induce neuronal deficit related to autistic behavior in individuals with ASD. Three measures for small sample size may contribute to taking cautions need by interpreting the small sample size data in this study.

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Compliance with ethical standards

Conflict of interest The authors have no conflict of interests concerning the materials or methods used in the present study or findings presented in this study.

Informed consent Informed consent was obtained by all participants in this study. This study was performed with the approval of the Ethics Committee of the Fujimoto Medical Clinic in Kobe City, Japan. Written informed consent was obtained from the patients or their parents.

Ethical responsibilities of authors

- The manuscript was not be submitted to more than one journal for simultaneous consideration.
- The submitted work was original and should not have been published elsewhere in any form or language (partially or in full).
- A single study was not be split up into several parts to increase the quantity of submissions and submitted to various journals or to one journal over time (i.e. 'salami-slicing/publishing').
- Results were presented clearly, honestly, and without fabrication, falsification or inappropriate data manipulation (including image based manipulation). Authors adhered to discipline-specific rules for acquiring, selecting and processing data.
- No data, text, or theories by others are presented as if they were the author's own ('plagiarism').
- This research do not misapply to pose a threat to public health or national security.
- We avoided untrue statements about an entity (who can be an individual person or a company) or descriptions of their behavior or actions that could potentially be seen as personal attacks or allegations about that person.
- Research that did not misapply to pose a threat to public health or national security.
- The corresponding author, and the order of authors are all correct at submission. We understood that change to authorship cannot be made after acceptance of a manuscript.

Authorship clarified All authors adhere to the guidelines for authorship that are applicable in their specific research field and the following guidelines:

1) Kunio Yui developed the study project.
2) All authors whose names appear on the submission.
3) Kunio Yui, George Imataka and Hitomi Sasaki made substantial contributions to the conception, design of the work, the acquisition, analysis, or interpretation of data; Hitomi Sasaki and or Ryoichi Shiroki reviewed the paper. All authors drafted the work or revised it critically for important intellectual content and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Data transparency All authors made sure that all data and materials as well as software application or custom code support their published claims and comply with field standards. All authors have agreed that journals may have individual policies on (sharing) research data in concordance with disciplinary norms and expectations.

References

Abdul-Razik Ismail E (2015) Behavior of lasso quantile regression with small sample sizes. J Multidiscip Eng Sci Technol 2:388–394 ISNN, 3159–0040

Albert A, Zhang L (2010) A novel definition of the multivariate coefficient of variation. Biom J 52:667–675. https://doi.org/10.1002/bimj.201000030

Al-Gadani Y, El-Ansary A, Attas O, Al-Ayadhi L (2009) Metabolic biomarkers related to oxidative stress and antioxidant status in Saudi autistic children. Clin Biochem 42:1032–1040. https://doi.org/10.1016/j.clinbiochem.2009.03.011

Altun H, Şahin N, Kurutaş EB, Karaaslan U, Sevgen FH, Fındıklı E (2018) Assessment of malondialdehyde levels, superoxide dismutase, and catalase activity in children with autism spectrum disorders. Psychiatry Clin Psychopharmacol 28:408–415. https://doi.org/10.1080/24750573.2018.1470360

Avramovic N, DragutinovicV KD, Colovic M, Trbovic A, de Luka S, Milovanovic I, Popovic T (2016) The effects of omega 3 fatty acid supplementation on brain tissue oxidative status in aged Wistar rats. Hippokratia 16(3):241–245

Ayala A, Muñoz MF, Argüelles S (2014) Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxidative Med Cell Longev 2014:360438–360431. https://doi.org/10.1155/2014/360438

Cheng J, Wang F, Yu DF, Wu PF, Chen J (2011) The cytotoxic mechanism of malondialdehyde and protective effect of carnosine via protein cross-linking/mitochondrial dysfunction/reactive oxygen species/MAPK pathway in neurons. Eur J Pharmacol 650:650184–650194. https://doi.org/10.1016/j.ejphar.2010.09.033

Curran-Everett D (2008) Explorations in statistics: standard deviations and standard errors. Adv Physiol Educ 32(3):203–208. https://doi.org/10.1152/advan.90123.2008

Dyall SC (2015) Long-chain omega-3 fatty acids and the brain: a review of the independent and shared effects of EPA, DPA and DHA. Front Aging Neurosci 7:52. https://doi.org/10.3389/fnagi.2015.00052

Dziobekm I, Gold SM, Wolf OT, Convit A (2007) Hypercholesterolemia in Asperger syndrome: independence from lifestyle, obsessive-compulsive behavior, and social anxiety. Psychiatry Res 149(1–3):321–324

Eldridge SM, Ashby D, Kerry S (2006) Sample size for cluster randomized trials: effect of coefficient of variation of cluster size and analysis method. Int J Epidemiol 35:1292–1300

Ghasemi Fard S, Wang F, Sinclair AJ, Elliott G, Turchini GM (2019) How does high DHA fish oil affect health? A systematic review of evidence. Crit Rev Food Sci Nutr 59:1684–1727. https://doi.org/10.1080/10408398.2018.1425978

González-Fraguela ME, Hung MD, Vera H, Maragoto C, Noris E, Blanco L, Galvizu R, Robindon M (2013) Oxidative stress markers in children with autism spectrum disorders. Br J Med Med Res 3:307–317

Horspool AM, Chang HC (2018) Neuron-specific regulation of superoxide dismutase amid pathogen-induced gut dysbiosis. Redox Biol 17:377–385. https://doi.org/10.1016/j.redox.2018.05.007

Itokawa M, Miyashita M, Arai M, DanT, Takahashi K, Tokunaga T et al (2018) Pyridoxamine: A novel treatment for schizophrenia with enhanced carbonyl stress. Psychiatry Clin Neurosci 72:35–44. https://doi.org/10.1111/pcn.12613

Jiménez-Fernández S, Gurpegui M, Díaz-Atienza F, Pérez-Costillas L, Gerstenberg M, Correll CU (2015) Oxidative stress and antioxidant parameters in patients with major depressive disorder compared to healthy controls before and after antidepressant treatment: results from a meta-analysis. J Clin Psychiatry 76:1658–1667. https://doi.org/10.4088/JCP.14r09179

Karabekiroglu K, Aman MG (2009) Validity of the aberrant behavior checklist in a clinical sample of toddlers. Child Psychiatry Hum Dev 40:99–110. https://doi.org/10.1007/s10578-008-0108-7

Kitano S, Kanno T, Maekawa M, Sakiurabayashi I, Kotani K, Hisatomi H (2004) Improved method for the immunological detection of malondialdehyde-modified low-density lipoproteins in human serum. Anal Chim Acta 509:229–235

Kubo T, Takahashi K, Furujo M, Hyodo Y, Tsuchiya H, Hattori M, Fujinaga S, Urayama K (2017) Usefulness of non-fasting lipid parameters in children. J Pediatr Endocrinol Metab 30:77–83. https://doi.org/10.1515/jpem-2016-0271

Lichou F, Orazio S, Dulucq S, Etienne G, Longy M, Hubert C, Groppi A, Monnereau A, Mahon FX, Turcq B (2019) Novel analytical methods to interpret large sequencing data from small sample sizes. Hum Genomics 13:41. https://doi.org/10.1186/s40246-019-0235-1

Luchi K, Ema M, Suzuki M, Yokoyama C, Hisatomi H (2018) Oxidized unsaturated fatty acids induce apoptotic cell death in cultured cells. Mol Med Rep 19:2767–2773. https://doi.org/10.3892/mmr.2019.9940

Meguid N, Dardir AA, Abdel-Raouf ER, Hashish A (2011) Evaluation of oxidative stress in autism: defective antioxidant enzymes and increased lipid peroxidation. Biol Trace Elem Res 143:58–65. https://doi.org/10.1007/s12011-010-8840-9

Morgan CJ (2017) Use of proper statistical techniques for research studies with small samples. Am J Phys Lung Cell Mol Phys 313:L873–L877. https://doi.org/10.1152/ajplung.00238.2017

Mousavinejad E, Ghaffari MA, Riahi F, Hajmohammadi M, Tiznobeyk Z, Mousavinejad M (2018) Coenzyme Q_{10} supplementation reduces oxidative stress and decreases antioxidant enzyme activity in children with autism spectrum disorders. Psychiatry Res 265:62–69. https://doi.org/10.1016/j.psychres.2018.03.061

Nordestgaard BG, Langsted A, Mora S, Kolovou G, Baum H, Bruckert E, Watts GF, Sypniewska G, Wiklund O, Borén J, Chapman MJ, Cobbaert C, Descamps OS, von Eckardstein A, Kamstrup PR, Pulkki K, Kronenberg F, Remaley AT, Rifai N, Ros E, Langlois M, European Atherosclerosis Society (EAS) and the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) Joint Consensus Initiative (2016) Fasting is not routinely required for determination of a lipid profile: clinical and laboratory implications including flagging at desirable concentration cutpoints-a joint consensus statement from the European atherosclerosis society and European Federation of Clinical Chemistry and Laboratory Medicine. Clin Chem 62:930–946. https://doi.org/10.1373/clinchem.2016.258897

Ogawa K, Tanaka T, Nagoshi T, Sekiyama H, Arase S, Minai K, Ogawa T, Yoshimura M (2015) Increase in the oxidised low-density lipoprotein level by smoking and the possible inhibitory effect of statin therapy in patients with cardiovascular disease: a retrospective study. BMJ Open. https://doi.org/10.1136/bmjopen-2014-005455

Packiasabapathy S, Prasad V, Rangasamy V, Popok D, Xu X, Novack V, Subramaniam B (2020) Cardiac surgical outcome prediction by blood pressure variability indices Poincaré plot and coefficient of variation: a retrospective study. BMC Anesthesiol 20:56. https://doi.org/10.1186/s12871-020-00972-5

Pongsuthana S, Tivatunsakul N (2016) Optimal fasting time before measurement of serum triglyceride levels in healthy volunteers. J Med Assoc Thail 99(Suppl 2):S42–S46

Roig-Pérez S, Guardiola F, Moretó M, Ferrer R (2014) Lipid peroxidation induced by DHA enrichment modifies paracellular permeability in Caco-2 cells: protective role of taurine. J Lipid Res 45(8):1418–1428

Sharma R, Rosenberg A, Bennett ER, Laskowitz DT, Acheson SK (2017) A blood-based biomarker panel to risk-stratify mild traumatic brain injury. PLoS One 12:e0173798. https://doi.org/10.1371/journal.pone.0173798

Shortreed SM, Ertefaie A (2017) Outcome-adaptive lasso: variable selection for causal inference. Biometrics 73:1111–1122. https://doi.org/10.1111/biom.12679

Simundic AM, Cornes M, Grankvist K, Lippi G, Nybo M (2014) Standardization of collection requirements for fasting samples: for the Working Group on Preanalytical Phase (WG-PA) of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM). Clin Chim Acta 432:33–337. https://doi.org/10.1016/j.cca.2013.11.008

Singh K, Connors SL, Macklin EA, Smith KD, Fahey JW, Talalay P, Zimmerman AW (2014) Sulforaphane treatment of autism spectrum disorder (ASD). Proc Natl Acad Sci U S A 111:15550–11555. https://doi.org/10.1073/pnas.1416940111

Song X, Schenk JM, Diep P, Murphy RA, Harris TB, Eiriksdottir G, Gudnason V, Casper C, Lampe JW, Neuhouser ML (2016) Measurement of circulating phospholipid fatty acids: association between relative weight percentage and absolute concentrations. J Am Coll Nutr 35:647–656

Wahid A, Khan DM, Hussain I (2017) Robust adaptive lasso method for parameter's estimation and variable selection in high-dimensional sparse models. PLoS One 12(8):e0183518. https://doi.org/10.1371/journal.pone.0183518 eCollection 2017

Yang B, Ren XL, Fu YQ, Gao JL, Li D (2014) Ratio of n-3/n-6 PUFAs and risk of breast cancer: a meta-analysis of 274135 adult females from 11 independent prospective studies. BMC Cancer 4:105. https://doi.org/10.1186/1471-2407-14-105

Yui K, Imataka G, Kawasak Y, Yamada H (2017) Increased ω-3 polyunsaturated fatty acid/arachidonic acid ratios and upregulation of signaling mediator in individuals with autism spectrum disorders. Life Sci 145:205–212. https://doi.org/10.1016/j.lfs.2015.12.039