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Useful Cases of Patients With Developmental Disorders Improved by Oral Administration of LPS Derived from *Pantoea agglomerans*

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Abstract. *Background: Developmental disorders are associated with microglial dysfunction. Oral administration of lipopolysaccharide derived from Pantoea agglomerans bacteria (LPSp) leads to normalization of phagocytic activity of microglia and suppression of inflammation in mice. In this article, we report on a successful trial in which we achieved a significant improvement of symptoms in patients with developmental disorders. Patients and Methods: Five pediatric patients diagnosed with autism spectrum disorders (ASD)/attention deficit hyperactivity disorder (ADHD) who visited our clinic received either 0.75 or 1 mg/day LPSp for 6 months or more, in addition to our usual therapy regimens (detoxification therapy, nutritional therapy, and vibration therapy). A survey questionnaire was completed by the patients' parents and evaluated using the Numerical Rating Scale. Results: Behavior, verbal ability, and communication disabilities associated with ASD/ADHD improved in all patients. Conclusion: Oral administration of LPSp may represent a new treatment option in the area of developmental disorders where there is currently no treatment available.*

Developmental disorders result from a set of disabilities in body, learning, language, or behavioral fields (1). Typical developmental disorders include autism spectrum disorder (ASD), attention deficit hyperactivity disorder (ADHD), learning disabilities, and other similar disorders of brain function (1). These symptoms usually appear at a young age. Although 10% of cases have a genetic background, the underlying etiology is unknown in most patients (2). These conditions begin during development and can adversely affect daily life. Developmental disorders rarely resolve and usually last a lifetime (1).

ASD is a neurodevelopmental disorder characterized by social and communication difficulties and rigid repetitive behaviors. Another neurodevelopmental disorder, ADHD, is characterized by excessive activity and impulsiveness inappropriate to the patient's age, as well as inattention, hyperactivity, and impulsivity (1). The prevalence of these two disorders is high, with recent reports showing that ASD occurs in 1.5% of the population and ADHD in 7.2% (3-4). Studies also show that the incidence of ASD in boys is 3-4 times that in girls (5).

Treatment of ASD mainly follows the recommendations of the British Autism Society's "SPELL (Structure, Positive, Empathy, Low Arousal, Links)" philosophy, however, suitable pharmacotherapy has not yet been developed because the cause and nature of the condition are as yet unknown (6). Treatment of ADHD mainly includes nursing and pharmacotherapy. ADHD treatment focuses on improving symptoms through environmental adjustment, social life skills training, and parent training. Pharmacotherapy includes the use of central stimulants (methylphenidate) and noradrenaline reuptake inhibitors. As much as 41-78% of patients with ASD also have ADHD and when the two disorders are combined, the symptoms of autism (social disorders, impaired daily living functions) become severe (1). This combination of disorders is also more difficult to treat than ADHD alone. In addition, if adolescents do not experience any improvement in their developmental disorders, various co-morbidities such as behavioral disorders, depression, and anxiety disorders may develop (1). Therefore, early diagnosis and treatment of developmental disorders is important to prevent the occurrence of secondary psychiatric problems.

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Key Words: Autism spectrum disorder, attention deficit hyperactivity disorder, *Pantoea agglomerans* bacteria derived LPS, oral administration.

Table I. *Background of the five study patients.*

Case	Age, years	Gender	Weight (kg)	Body length (cm)	Symptom	Quantity of LPSp/day (mg)	Period of LPSp intake (months)
1	5	Male	18	120	ASD	0.75	12
2	9	Male	25	135	ADHD	1.00	7
3	7	Male	20	117	ASD	0.75	7
4	5	Female	19	106	ASD, ADHD	1.00	6
5	5	Male	17	110	ASD	0.75	6

ASD: Autism spectrum disorder; ADHD: attention deficit hyperactivity disorder.

In recent years, it has been reported that inflammatory cytokines, oxidative stress markers, and microglia are abnormal in animal models of ASD developmental disorders, and the involvement of microglia has attracted particular attention (7-9). Microglia are brain tissue macrophages which are activated upon detecting neuropathy at the time of brain pathology or injury. It has also been reported that microglial dysfunction during development may be a cause of developmental disorders. Rett syndrome develops when a mutation occurs in the methyl-CpG-binding protein 2 (*MEPC2*) gene present on the X-chromosome, resulting in developmental disorders mainly involving the nervous system, such as autism (10). The phagocytic activity of microglia was reduced in the brains of mice of this disease model, and transplantation of phagocytic microglia improved their symptoms. These observations indicate the possibility that certain disorders in the action of microglia may destabilize higher functional activities of the brain in developmental disorders. It may therefore be possible to take measures to treat microglial dysfunction and reduce the symptoms of developmental disorders (10). One of these measures is the oral administration of liposaccharide (LPS), which has been shown to improve the function of brain macrophages.

LPS is a substance composed of lipids and polysaccharides and is a component of the outer membrane of the cell wall of Gram-negative bacteria. Physiological effects of LPS are expressed *via* toll-like receptor 4 present on the cell surface of the host cell (11). The plant symbiotic Gram-negative bacteria *Pantoea agglomerans* has been shown to be a useful microorganism that coexists with plants supplying them with nitrogen and phosphorus (12, 13). To date, various treatments have been tested in the clinical setting using LPS derived from *P. agglomerans* (LPSp) (14, 15). Inagawa *et al*. reported that they isolated LPSp from bacteria coexisting with wheat and that oral administration of LPSp increased the ability of macrophages to eliminate foreign substances *in vivo* (16). The group have also reported that the transdermal/oral administration of LPSp can improve the pathology of various chronic diseases (14, 15, 17-21). Among them, administration of LPSp to mice as a model of Alzheimer's disease (caused by amyloid β accumulated in the brain) led to suppression of amyloid β accumulation and reduction in cognitive decline compared to control mice (17). Our clinic reported that treatment of 16 patients with malignant tumors using LPSp improved the symptoms of 10 patients, and three of them were completely cured (11).

Therefore, it was hypothesized that oral administration of LPSp would result in the normalization of the effects of microglia and improve developmental disorders. In this article, we report that our clinic was successful in treating five patients with developmental disorders by oral administration of LPSp in addition to our usual treatment regimens of detoxification, nutritional therapy, and vibration therapy.

Patients and Methods

Patients. Five pediatric patients (5 to 9 years) with ASD with and without ADHD who visited our clinic from 2017 January to 2019 September. Of the five patients, three had ASD, one had ADHD, and one had ASD with ADHD complications.

Treatment. There are three usual therapies at our clinic. The first is detoxification, which used the following lactic acid bacteria for 3 months: Lactobacillus species (*L. rhamnosus*, *L. casei*, *L. paracasei*, *L. gasseri*, and *L. salivarius*) at 5×10^9 units/day, and *Bidifobacterium* species (*B. bifidum, B. longum, B. breve, B. infantis* and *B. lactis*) 5×10^9 units/day, (Klaire Labs. Japan Ltd., Kanagawa, Japan). At the same time, patients received the following as nutritional therapy: 50 mg/day calcium, 50 mg/day magnesium, 134 mg/day vitamin E, 133 μg/day folate, lactic acid bacteria, vitamin B mix: (30 mg/day vitamin B1, 30 μg/day biotin, 30 mg/day vitamin B2, 300 μg/day folic acid, 30 mg/day niacin, 30 μg/day vitamin B12, 30 mg/day pantothenic acid, 15 mg/day phosphatidylcholine, 30 mg/day vitamin B6, and 15 mg/day inositol) (all from Healthy-Pass. Inc., Shizuoka, Japan). However, case 2 did not take nutritional therapy.

In addition, LPSp (Macrophi Inc., Kagawa, Japan) was used at 0.75 or 1.0 mg/day (Table I) in combination with our standard treatment regimens. When the patient's condition improved, LPS was halted. Before and after treatment, behavior, verbal ability, and communication were evaluated using the Numerical Rating Scale (NRS) (Figure 1). Brain inflammation, intestinal inflammation, ASD and ADHD were measured at the initial examination and after the

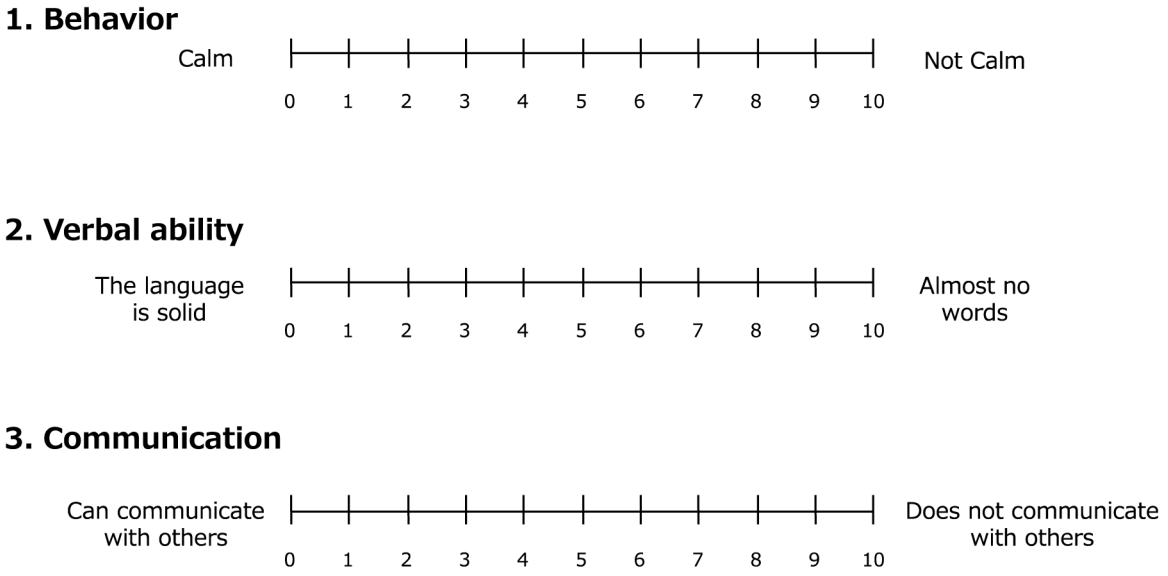

Figure 1. *Numerical Rating Scale for evaluation of study patients.*

end of administration using frequency measuring equipment (Rayocomp PS 1000 Polar; Layonex, Lennestadt Germany) that can be used noninvasively even in infants.

Frequency measuring equipment for diagnosis. The Rayocomp PS 1000 Polar is a frequency measuring instrument made by Layonex. It was fundamentally developed in 1976 by Paul Schmidt, a German physicist, who discovered that each cell and tissue vibrates at an individual frequency, which changes depending on its status. A treatment device that induces resonance at these individual frequencies using electromagnetic vibration was developed and related treatment methods were established. Various therapy programs have been developed based on individual vibration data from tens of thousands of measurements and are being applied clinically and in research (18). Bioresonance measurements are full-body scans, in which the patient is examined while seated. At this study, during a whole-body scan, brain inflammation in the patient's head, intestinal inflammation in the intestine, systemic ASD status, and ADHD status were measured by bioresonance machine. Measurements using Rayocomp PS 1000 Polar were digitized and used as bioresonance indices (very good, 1; good, 2; mild, 3; moderate, 4; severe; 5; extreme, 6) for evaluation. The lower the index, the closer the tissue is to its natural healthy state.

NRS. The NRS is a type of Visual Analog Scale used in surveys as a means of evaluating sensation on a linear scale. In recent years, it has been widely used, mainly in the medical and educational fields, and its usefulness has been well-documented. Changes in behavior, verbal ability and communication were evaluated using an NRS subjective assessment (Figure 1). The measure of behavior was calmness. Verbal ability was measured in terms of the ability to transmit words, and communication was measured as the ability to interact with others. Each NRS consists of a 100 mm straight line with "Calm" "The language is solid" "Can communicate with others" respectively at the left end and "Not calm" "Almost no words" and "Does not communicate with others" at the right end respectively. Parents were asked to place a mark or tick along the line at the point that they felt was most appropriate to describe the patient's condition. The higher the number on the scale, the more severe the symptom. In this study, NRS evaluation was performed by the parent of the pediatric patient before and after treatment, and the numerical values were recorded.

Statistical analysis. All results are expressed as the mean±SD. All results are expressed as the mean±SD. When the difference based on one-way analysis of variance (ANOVA) was significant, Student's *t*-test was used for unpaired comparisons using GraphPad Prism for Windows (GraphPad Software, Inc., San Diego, CA, USA). Results were considered significant at $p<0.05$.

Results

Improvements in behavior, verbal ability, and communication after oral administration of LPSp. Case 1 was a pediatric patient with ASD who received LPSp at 0.75 mg/day for 12 months in combination with conventional therapy (Table I). As a result, behavior improved from 7 to 0, verbal ability improved from 10 to 0, and communication improved from 10 to 0. The patient returned to their regular class from the support class (Figure 2).

In case 2, a pediatric patient with ADHD received LPSp at 1 mg/day for 7 months in combination with conventional therapy (Table I). As a result, behavior improved from 8 to 2, and communication improved from 6 to 1 (Figure 2).

In case 3, a pediatric patient with ASD received LPSp at 0.75 mg/day for 7 months in combination with conventional therapy (Table I). As a result, behavior improved from 8.5 to

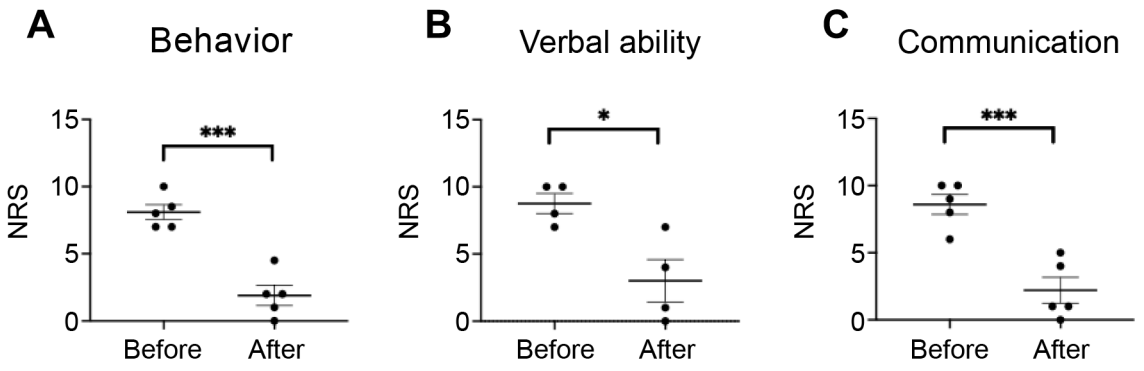

Figure 2. *The Numerical Rating Scale (NRS) scores for behavior (A), verbal ability (B) and communication (C) before and after treatment. The data are presented as the means±SD and were obtained from 4-5 patients per group. Significantly different at: *p<0.05 and ***p<0.001.*

4.5, verbal ability improved from 7 to 4, and communication improved from 8 to 4. This patient also returned to regular class from the support class (Figure 2).

Case 4 was a pediatric patient with ASD and ADHD complications who was given 1 mg/day LPSp for 6 months in combination with conventional therapy (Table I). As a result, behavior improved from 10 to 2, verbal ability improved from 10 to 7, and communication improved from 10 to 5 (Figure 2).

Case 5, a pediatric patient with ASD, received LPSp at 0.75 mg/day for 6 months in combination with conventional therapy (Table I). As a result, behavior improved from 7 to 1, verbal ability improved from 8 to 1, and communication improved from 9 to 1 (Figure 2).

As shown in Figure 2, the average NRS results of these five pediatric patients show that behavior, and verbal and communication abilities significantly improved after treatment.

The bioresonance results also show that brain inflammation, intestinal inflammation, ASD and ADHD were significantly improved after treatment (Figure 3).

Correlation of bioresonance and NRS scores. Five patients were evaluated for brain inflammation, intestinal inflammation, and ASD/ADHD symptoms by bioresonance testing before and after treatment (Figure 3). The correlations between these values and the results of the NRS for behavior, and verbal ability and communication were analyzed. The correlation between brain inflammation by bioresonance and behavior, verbal and communication by NRS were each 0.879, 0.801 and 0.920, respectively (Figure 4A). The correlation coefficient between intestinal inflammation by bioresonance and behavior, verbal ability and communication by NRS were each 0.920, 0.802 and 0.879, respectively (Figure 4B). The correlation coefficient between results of ASD by bioresonance and behavior, verbal ability and communication by NRS were 0.766, 0.564 and 0.767, respectively (Figure 4C). The correlation coefficient between results of ADHD by bioresonance and behavior, and communication by NRS were 0.14 and 0.625 (Figure 4D). Brain inflammation and intestinal inflammation were strongly positive correlated with ASD but not with ADHD.

Discussion

Developmental disorders arise in early childhood. They affect social, communicative and cognitive development, and the prevalence of these disorders has increased in recent years (23). As yet, no appropriate treatment exists for developmental disabilities and we have only a limited understanding of the nature and etiologies of developmental disabilities in children, which represent a serious social problem. If developmental disorders are improved in early childhood, secondary disorders, which frequently arise in puberty, may be prevented.

Five children with ASD with/without ADHD were treated with LPSp for 6 to 12 months in addition to the usual treatment at our clinic (Figure 2). Results of the NRS evaluation showed improvements in behavior, verbal ability, and communication scores of all five patients after LPSp treatment. Improvements in their bioresonance results showed a strong correlation with the NRS results and the patients' conditions were improved (Figure 4). In addition, two of the primary-school-aged patients were able to return to normal classes indicating the usefulness of oral LPSp treatment (case 1 and case 3).

The mechanism by which oral administration of LPSp might improve developmental disorders is as yet unknown. However, microglial activation by oral administration of LPSp has been reported. Kobayashi *et al*. confirmed that microglia were stimulated in vitro by a trace amount of LPS which enhanced phagocytosis of amyloid β1-42 (24). Kobayashi *et al*. also

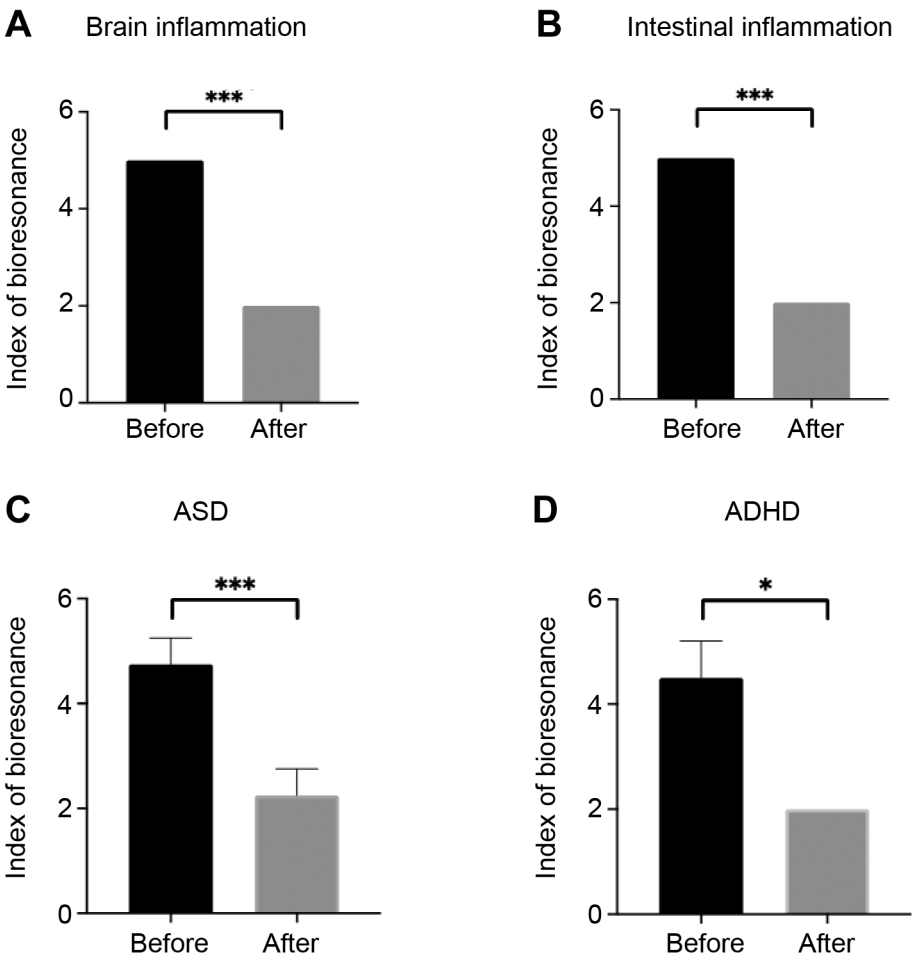

Figure 3. *Index of bioresonance for brain inflammation (A), intestinal inflammation (B), autism spectrum disorder (ASD) (C) and attention deficit hyperactivity disorder (ADHD) (D) before and after treatment. The data are presented as the means±SD and were obtained from 2-5 patients per group. Significantly different at: *p<0.05 and ***p<0.001.*

administered LPSp to mice in a model of Alzheimer's before disease onset to activate microglia (18). These results imply that LPS may activate microglia, making it useful for prevention of brain diseases. Furthermore, Derecki *et al*. hypothesized that developmental disorders were caused by microglia dysfunction. They transplanted microglia with phagocytic activity into a mouse model of Rett's syndrome, which is a type of autism, and reported that the symptoms of the developmental disorder were improved (10). This suggests that increasing the phagocytic ability of microglia may improve the symptoms of autism. The results of the current study suggest that oral administration of LPSp to pediatric patients with ASD/ADHD normalized the phagocytic action of microglia, resulting in the observed improved symptoms of developmental disorders.

Children with ASD often complain of gastrointestinal symptoms other than cerebral dysfunctions. The intestinal flora is involved in immune regulation, drug pharmacokinetics, detoxification, nutrition, metabolism, and interact with the central nervous system (25, 26), presumably through neural, endocrine, and immune pathways leading to the host's brain, affecting both function and behavior (27-29). Intestinal flora has also been reported to contribute to the development of the host's brain. Germ-free mice showed increased gut flora and reduced anxiety compared to specific-pathogen-free mice (30). Sterile mice also showed significant social impairments, such as reduced social preference and preference for novelty (31). This may suggest that gastrointestinal disorders associated with an abnormal composition of intestinal flora may be linked to ASD (32). Finegold *et al*. reported that children with ASD differ in gut microbiota when compared to their non-ASD siblings and non-siblings (33). Oral administration of LPSp has been reported to improve intestinal flora. Disturbance of the intestinal flora is associated with arteriosclerosis and

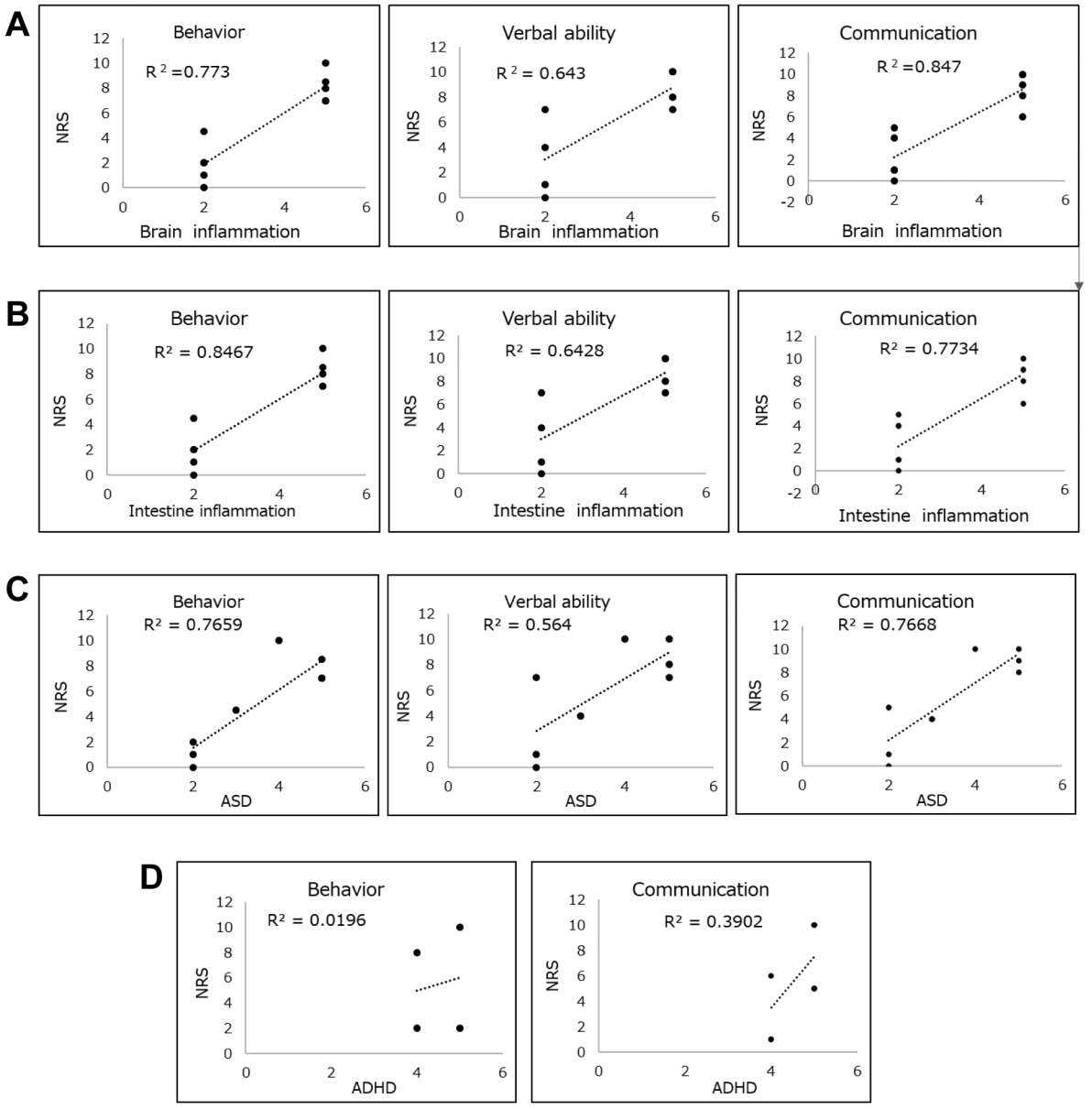

Figure 4. *Correlation of the bioresonance index and Numerical Rating Scale (NRS) score. The index of bioresonance and NRS score were analyzed in a distribution chart with an approximate curve. A: Brain inflammation; B: Intestinal inflammation; C: autism spectrum disorders (ASD); D: attention deficit hyperactivity disorder (ADHD) (n=2-5).*

hypertension, and the ingestion of LPS has been shown to normalize the intestinal flora and prevent arteriosclerosis and hypertension (19, 20). It is possible that oral administration of LPSp may have reduced the disturbance of intestinal bacteria in these pediatric patients.

The results of this trial provide some hope that LPSp administration to pediatric patients with developmental disorders may offer a new treatment avenue in areas of developmental disorder where no treatment is currently available.

Conflicts of Interest

The Authors have no financial conflicts of interest.

Authors' Contributions

A.M. and H. I. were responsible for the study concept and design; A.M. was responsible for acquisition of data; T. N. and R. Z. were responsible for analysis and interpretation of data; A.M. and R. Z. was responsible for drafting the article; and H. I. was responsible for study supervision.

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