成人を対照群としたため，思春期成人期項目の判別力は幼児期項目と比べるとやや低くなったが，PDDのスクリーニングとしては満足すべき結果であった。カットオフ20点に満たなかったPDD群10名は，アスペルガー症候群7名と知的障害を伴うPDD-NOSの2名(知的水準は不明)，そして高IQのPDD-NOSの1名であった。カットオフを超えた非PDD群6名は，統合失調症4名，行為障害とてんかんの合併1名，そしてAD/HD1名(幼児期項目でもカットオフを超えた青年と同一人物)であった。このように，アスペルガー症候群やPDD-NOSについては，思春期成人期項目の得点は低くなりやすく，一部の非PDD青年成人は高得点となったので，実際の青年成人の評価に際しては，より判別力の高い幼児期回顧評価項目とともに他の情報と組み合わせることが望ましい。対照群のAD/HD青年1名は，DSM-IVの除外診断ルールにより非PDDとされたが，実際にはPARSがとらえたようにPDDの特徴を持っていたことから，臨床的にはPARS思春期成人期尺度は適切であったと思われる。

PARSはスクリーニング機能に加えて，支援計画立案に必要な情報を提供する。PDDの判別力が低かった項目は，低頻度だが存在すると日常生活への影響が大きく，極端な場合，社会生活に破壊的影響を与えるという点で，支援ニーズの総合的評価に必要と考えられる。逆に判別力が低かった思春期成人期項目には，非PDD群にもPDD群と同程度に高頻度にみられた行動も含まれた((項目56「被害念慮」と項目57「気分変動」)など)。PDDは思春期以降，PDD固有の症状に加えて2次的，3次的な症状が複合化し，複雑な臨床像となることが少なくない。PDDの判別力が劣るこれらの項目も，青年成人の複雑な臨床像を総合的に把握するうえでは重要な情報を提供すると考えられる。

本研究には限界点が複数存在する。第一に，思春期成人期の各項目について，評価者間の一致度がばらつく要因を明らかにするために，評価者および対象の人数を増やして検討する必要がある。第二に，回顧評価の幼児期項目についても評価者間信頼性を検討する必要がある。第三に，PDD群を知能水準や下位診断によって下位群に分類すると，それぞれの人数が少なくなり詳細な分析ができなかった。第四に，対照群との比較において，性比が統制されていなかったので性比の影響が検討できなかった。今後，より大きなサンプルに基づいた青年成人臨床群との比較研究を行って，以上の問題の解決と，PARS思春期成人期尺度の利点と限界点をより明確にしていく課題が残された。

本研究は，PDD思春期成人期尺度が，スクリーニングツールとして，また総合的な支援計画立案の基礎資料として，医療，教育，福祉，司法など幅広い臨床場面で用いるのに有用な尺度である可能性を示した。

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Summary

Reliability and Validity of the Pervasive Developmental Disorder(PDD)—Autism Society Japan Rating Scale(PARS)：A behavior checklist for adolescents and adults with PDDs

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A behavior checklist, the Pervasive Developmental Disorder(PDD)—Autism Society Japan Rating Scale (PARS), was developed as a screening questionnaire to determine Pervasive Developmental Disorders(PDDs) and also as a rating scale to evaluate the severity of a wide range of PDD symptoms. When assessing adoles-

cents and adults using the PARS for these purposes, 34 toddlerhood items are evaluated retrospectively and 33 items are used for current evaluation. In this study, the reliability and validity of the PARS was tested on a clinical sample of 53 adolescents and adults with PDD and 42 with non-PDD diagnoses. Interrater and internal reliability was found to be adequate. Both the 34 toddlerhood evaluation items and the 33 current evaluation items accurately discriminated PDD from non-PDD. Results suggested that the PARS may be a useful screening scale for various clinical settings.

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Increased serum levels of glutamate in adult patients with autism

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Abstract

Background: Precise mechanisms underlying the pathophysiology of autism are currently unknown. Given the major role of glutamate in brain development, we have hypothesized that glutamatergic neurotransmission plays a role in the pathophysiology of autism. In this study, we studied whether amino acids (glutamate, glutamine, glycine, D-serine, and L-serine) related to glutamatergic neurotransmission are altered in serum of adult patients with autism.
Methods: We measured serum levels of amino acids in 18 male adult patients with autism and age-matched 19 male healthy subjects using high-performance liquid chromatography.
Results: Serum levels (mean=89.2 μM, S.D.=21.5) of glutamate in the patients with autism were significantly ($t=-4.48$, $df=35$, $p<0.001$) higher than those (mean=61.1 μM, S.D.=16.5) of normal controls. In contrast, serum levels of other amino acids (glutamine, glycine, D-serine, L-serine) in the patients with autism did not differ from those of normal controls. There was a positive correlation ($r=0.523$, $p=0.026$) between serum glutamate levels and Autism Diagnostic Interview–Revised (ADI-R) social scores in patients.
Conclusions: The present study suggests that an abnormality in glutamatergic neurotransmission may play a role in the pathophysiology of autism.

Keywords: Amino acids; Autism; D-Serine; Glutamate; HPLC; Human serum

1. Introduction

Autism is a neuropsychiatric disorder characterized by severe and sustained impairment in social interaction, deviance in communication, and patterns of behavior and interest that are restricted, stereotyped, or both (Volkmar and Pauls, 2003). Although genetic and environmental factors are implicated in the pathophysiology of autism, the precise mechanisms underlying the pathophysiology of this disorder remain to be determined (Volkmar and Pauls, 2003; Baron-Cohen and Belmonte, 2005; Polleux and Lauder, 2004; McDougle et al., 2005).

Glutamate, the major excitatory neurotransmitter in the brain, plays a major role in brain development, affecting neuronal migration, neuronal differentiation, axon genesis, and neuronal survival (Coyle et al., 2002). Accumulating evidence suggests that abnormalities in glutamatergic neurotransmission may play a role in the pathophysiology of autism (McDougle et al., 2005). First, cDNA microarray technology has demonstrated that the glutamate neurotransmitter system is abnormal in postmortem brain samples of autism (Purcell et al., 2001). The mRNA levels of genes, including excitatory amino acid transporter 1 (EAAT 1) and AMPA-type glutamate receptor, are significantly increased in the brain of autism, suggesting abnormalities of glutamatergic neurotransmission in the pathogenesis of this disorder (Purcell et al., 2001). Genetic studies have demonstrated the involvement

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of single nucleotide polymorphisms (SNPs) in the genes encoding both metabotropic and ionotropic glutamate receptors in autism (Jamain et al., 2002; Serajee et al., 2003). Furthermore, a strong association of autism with SNPs within SLC25A12, a gene encoding the mitochondrial aspartate/glutamate carrier (AGC1), has been demonstrated, suggesting the potential etiological role of AGC1 in autism (Ramoz et al., 2004; Segurado et al., 2005). However, recent two studies using large samples did not confirm the association of SLC25A12 gene and autism, suggesting that the SLC25A12 gene is not a major contributor to genetic susceptibility of autism (Blasi et al., 2006; Rabionet et al., 2006).

Second, it has been reported that blood levels of glutamate are altered in patients with autism (Rolf et al., 1993; Moreno-Fuenmayor et al., 1996; Aldred et al., 2003). Rolf et al. (1993) have reported that plasma levels of glutamate in children (8–14-year-olds) with autism are significantly decreased compared to age-matched healthy controls. In contrast, Aldred et al. (2003) have reported that plasma levels of glutamate in patients (4–29 year-olds) with autism or Asperger's syndrome are significantly increased compared with controls. One of the reasons for such contradictory findings could be a difference in sample composition; the study by Aldred et al. (2003) incorporated a wider age range. Nonetheless, previous studies indicate alterations in the glutamatergic system expressed at the periphery level. The studies reporting on blood levels of glutamate in autistic patients present inconsistent results. Therefore, it is of great interest to examine whether levels of amino acids such as glutamate are altered in autistic patients.

Several lines of evidence suggest that D-serine, an endogenous co-agonist at the NMDA receptors, plays a role in the pathophysiology of schizophrenia, which is a neurodevelopmental disorder (Snyder and Ferris, 2000; Coyle and Tsai, 2004; Hashimoto et al., 2005a). We have previously reported that serum levels of D-serine are significantly decreased in patients with schizophrenia (Hashimoto et al., 2003; Yamada et al., 2005). However, to our knowledge, serum D-serine levels have never been investigated in relation to autism.

The purpose of the present study was, therefore, to examine whether individuals with autism have aberrant serum levels of D-serine as well as other amino acids (glutamate, glutamine, glycine, and L-serine) associated with glutamatergic neurotransmission. Furthermore, we also examined any relationship between amino acid levels and clinical symptoms in autistic patients.

2. Methods

2.1. Participants

Eighteen male autistic subjects (mean age = 21.2 years, S.D. = 2.1, range = 18–26) and age-matched 19 male healthy control subjects (mean = 22.2 years, S.D. = 2.2, range = 18–26) were included in this study (Table 1). All participants for both groups were Japanese. The autistic subjects were recruited through advocacy groups in Nagoya and Hamamatsu cities, which are located in the middle of the mainland of Japan. For the diagnosis of autism, the recruited individuals were initially assessed according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV)(American Psychiatric Association, 1994), followed by assessment using the Autism Diagnostic Interview–Revised (ADI-R)(Lord et al., 1994) by trained child psychiatrists clinicians (KJT and AS). Participants were excluded from the study, if they had a diagnosis of fragile X syndrome, epileptic seizures, obsessive–compulsive disorder, affective disorders, or any additional psychiatric or neurological diagnoses. All the autistic subjects were drug-naive or had been free of psychoactive medications for at least 6 months. Healthy controls were recruited from Hamamatsu City by advertisement. All control-group participants underwent a comprehensive assessment of medical history to eliminate individuals with any neurological or other medical disorders. The Structured Clinical Interview for the DSM-IV (SCID) was also conducted to scrutinize any personal or familial history of past or present mental illness. None of the comparison subjects initially recruited was found to fulfill these exclusion procedures. After the participants were given a complete description of the study, written informed consent was obtained from all subjects before they entered the study. This study received approval from the ethics committee of the Hamamatsu University School of Medicine and Chiba University Graduate School of Medicine.

Table 1
Clinical characteristics of 18 adult patients with autism

Characteristics	Mean±S.D. (range)
Age at onset (years)	3.72±1.07 (1–5)
Duration of illness (years)	17.5±2.23 (14–22)
ADI-R	
A. Social	22.11±4.96 (14–29)
B. Communication	15.44±4.84 (6–21)
C. Stereotype	5.22±1.77 (3–10)
Y-BOCS	11.28±5.39 (2–26)
Obsession	6.44±3.13 (1–14)
Compulsion	4.94±3.62 (0–14)
AQ-Aggression	50.56±12.3 (34–69)
Theory of Mind—Faux Pas Test	23.44±8.16 (6–34)
IQ	
Full-scale IQ	96.83±20.33 (62–140)
Verbal IQ	95.11±19.87 (53–131)
Performance IQ	100.4±18.4 (75–137)

ADI-R: Autism Diagnostic Interview–Revised, Y-BOCS: Yale–Brown Obsessive–Compulsive Scale, AQ: Aggression Questionnaire, IQ: Intellectual Quotient.

2.2. Psychological measures

ADI-R is a semi-specially formulated structured psychiatric interview with a parent, especially a mother, which is administered to the parent. It is used to confirm diagnosis and also to evaluate the core symptoms of autism. ADI-R is based on three separate scores. Score A quantifies impairment in social interaction (the range of score: 0–32), score B quantifies impairment in communication (the range of score: 0–26), and score C quantifies restricted, repetitive, and stereotyped patterns of behavior and interests (the range of score: 0–16). Higher scores on each indicate worse performance.

Obsessional/repetitive behavior was rated using the Yale-Brown Obsessive–Compulsive Scale (Y-BOCS)(Goodman

et al., 1989a,b); additional aggression symptoms were also assessed using the Aggression Questionnaire (AQ)(Buss and Perry, 1992). We used a Faux Pas Test to evaluate the function of "Theory of Mind" (*mentalizing*) (Baron-Cohen et al., 1999; Stone et al., 2003). The performance of individuals with autism on the Faux Pas Test is an experimental demonstration of their theory-of-mind deficit at a higher level. There were 40 points possible for Faux Pas-related questions about 10 stories (range: 0–40, 1 point for each question).

2.3. Amino acid measures

Serum samples of autistic patients and normal comparison subjects were collected from 11:00–12:00 a.m., and stored at −80 °C until assay. Measurement of amino acids levels was carried out according to the methods described in previous publications (Hashimoto et al., 2003, 2005b; Yamada et al., 2005). The serum levels of glutamate, glutamine, and glycine were measured according to the method using a high-performance liquid chromatography (HPLC) system, as reported previously (Hashimoto et al., 2005b). D- and L-serine measurements were made by the established method (Fukushima et al., 2004) using a column-switching HPLC system. Briefly, 20 μl of human serum was homogenized in 180 μl of methanol (HPLC grade). The homogenates were centrifuged at 4500×*g* for 10 min, and 20 μl of supernatant was evaporated to dryness at 40 °C. To the residue, 20 μl of H_2O (HPLC grade), 20 μL of 0.1 M borate buffer (pH 8.0), and 60 μL of 50 mM 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F; Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan) in CH_3CN (HPLC grade) were added. The reaction mixture was then heated at 60 °C for 1 min and immediately supplemented with 100 μL of H_2O/CH_3CN (90/10) containing 0.1% trifluoroacetic acid (TFA) to stop the reaction. Ten microliters of the resultant solution was injected into the HPLC system, as

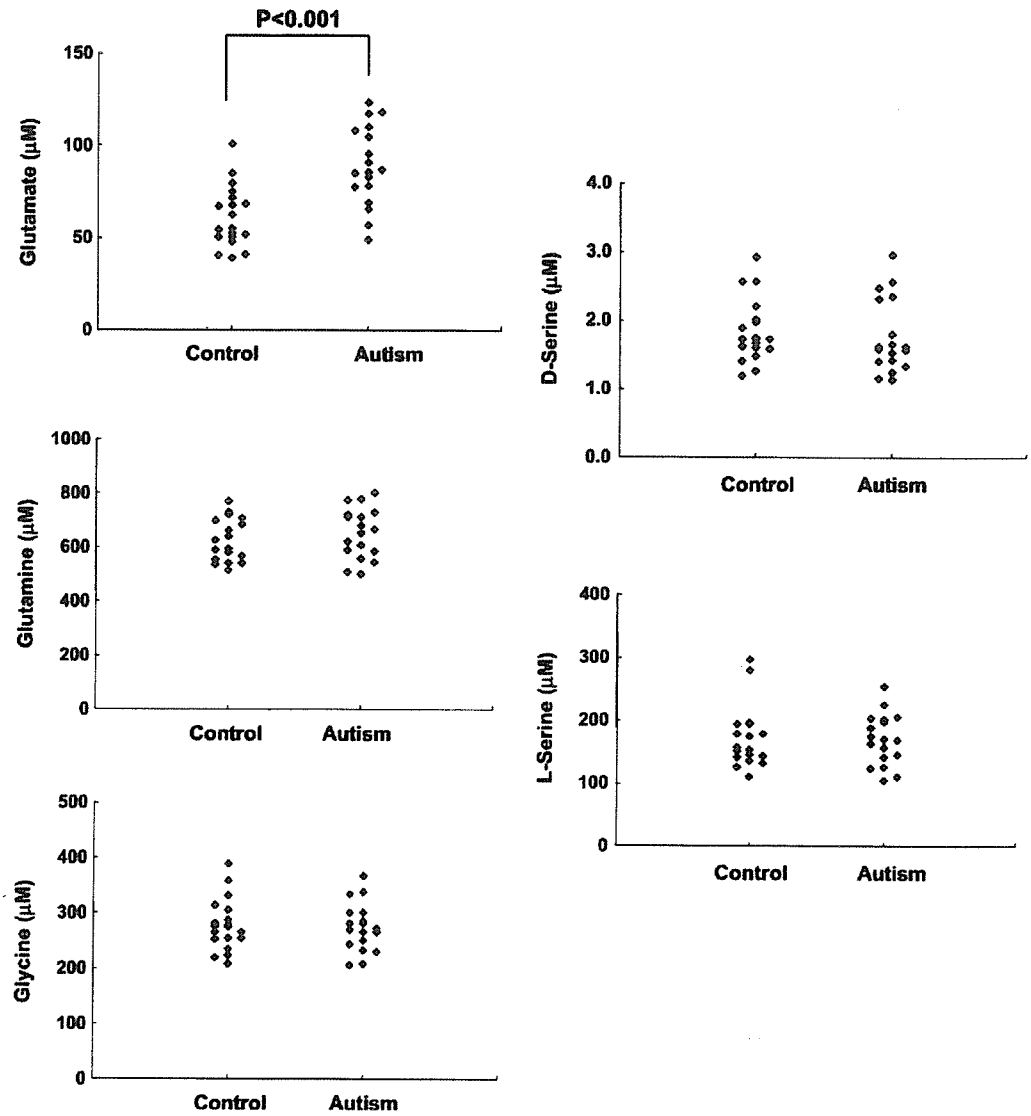

Fig. 1. Serum levels of amino acids in normal controls and autistic patients. Serum levels of glutamate in autistic patients were significantly higher than those of normal controls. In contrast, the levels of other amino acids (glutamine, glycine, d-serine, and L-serine) were not altered between two groups.

reported previously (Hashimoto et al., 2003, 2005b; Yamada et al., 2005).

2.4. Statistical analysis

The data were presented as the mean ± standard deviation (S.D.). Since all clinical and amino acids measures had an approximate standard normal distribution, we used an unpaired Student's *t*-test to compare the measures between patients and comparison subjects. The relationships between amino acid levels and clinical variables among patients with autism were evaluated by computing Pearson's correlation coefficients. We used a conservative α level of 0.01 for statistical significance in view of the number of variables examined. Additionally, we calculated the effect size (Cohen's *d*) for variables with a significant group mean difference. A *p* value of less than 0.01 was considered to be statistically significant.

3. Results

The serum levels (mean = 89.2 μM, S.D. = 21.5) of glutamate in the patients with autism were significantly ($t = -4.48$, $df = 35$, $p < 0.001$) higher than those (mean = 61.1 μM, S.D. = 16.5) of normal controls (Fig. 1); the Cohen's *d* for the mean difference was 1.52. When the analysis was repeated for never-medicated patients only ($n = 13$) compared with controls, the difference remained highly significant ($t = -4.14$, $df = 30$, $p < 0.001$: Cohen's $d = 1.54$). In contrast, the serum levels of other amino acids such as glutamine, glycine, D-serine, or L-serine in patients did not differ significantly from those of normal controls (Fig. 1). Furthermore, we found a positive correlation between L-serine and glutamate in controls ($r = 0.633$, $p = 0.04$), but not patients ($r = 0.294$, $p = 0.237$). Moreover, we found a positive correlation between D-serine (or L-serine) and glycine in patients (D-serine: $r = 0.641$, $p = 0.004$, L-serine: $r = 0.683$, $p = 0.002$), but not controls (D-serine: $r = 0.141$, $p = 0.564$, L-serine: $r = 0.284$, $p = 0.239$).

We then examined the correlations between serum glutamate levels and clinical variables among patients with autism. There was a relatively high positive correlation ($r = 0.523$, $p = 0.026$) between the glutamate levels and social subscores as assessed by ADI-R, although this fell outside our stringent level of significance (Fig. 2). There were no marked or significant correlations between serum glutamate levels and other clinical symptoms studied, including the Faux Pas Test of "Theory of Mind" or Y-BOCS scores. Additionally, we examined any correlations between the serum levels of other four amino acids and clinical variables, but no correlations were evident.

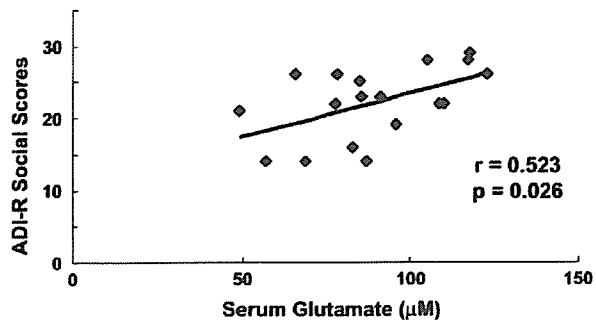

Fig. 2. Correlation between serum glutamate levels and ADI-R social scores in autistic patients. There was a positive correlation ($r = 0.523$, $p = 0.026$) between serum glutamate levels and ADI-R social scores in autistic patients.

4. Discussion

The major findings of the present study are that serum levels of glutamate in adult patients with autism are significantly higher than those of normal healthy controls, and that there is a positive correlation ($r = 0.523$, $p = 0.026$) between serum glutamate levels and ADI-R social scores in patients, although this result fell outside our stringent level of significance. To our knowledge, this is the first report demonstrating the increased serum levels of glutamate in adult male patients with autism. Our data showing higher glutamate levels in autistic patients is consistent with a previous report (Aldred et al., 2003). However, our findings are inconsistent with a previous report (Rolf et al., 1993). It seems that methodological differences (e.g., time of sample collection, serum vs plasma, age of subjects) may be involved in this discrepancy although the reasons underlying this discrepancy are currently unknown. It has been reported that levels of glutamate in human blood are positively correlated with CSF levels of glutamate in humans (McGale et al., 1977; Alfredsson et al., 1988). Therefore, it is likely that increased levels of glutamate may occur in the brains of autistic patients. In this study, we also found a positive correlation between L-serine and glutamate in controls ($r = 0.633$, $p = 0.04$), but not patients ($r = 0.294$, $p = 0.237$). These findings suggest that synthetic/metabolic pathways of L-serine and glutamate may be impaired in the autism although a further study using a large sample will be necessary.

Several reports have demonstrated that patients with autism are at greater risk for developing seizure disorders, particularly in adolescence (Volkmar and Pauls, 2003; Volkmar and Nelson, 1990; Tuchman and Rapin, 2002). It is well known that glutamate plays a role in the initiation and spread of seizure activity, and that it also plays a critical role in epileptogenesis (Meldrum, 1994). A number of antagonists for NMDA or non-NMDA receptors show potent protective effects in a variety of animal models of epilepsy. Furthermore, there have been several clinical reports demonstrating that the mood stabilizer valproic acid is effective in autistic patients with or without clinical seizures but with epileptiform abnormalities on electroencephalography (Tuchman and Rapin, 2002; Plioplys, 1994; Hollander et al., 2001). Valproic acid exerts neuroprotective effects against glutamate-induced excitotoxicity (Manji and Lenox, 2000; Chuang, 2004). In addition, postmortem brain studies have shown a variety of abnormalities, including a decreased number of neurons and reduced dendritic arborization in areas of the limbic system such as the amygdala, hippocampus, septum, and anterior cingulate cortex (Kemper and Bauman, 1998; Palmen et al., 2004). Taken together, these results suggest that increased glutamate levels may be implicated in the high rates of seizure disorder in autism, although further studies of the role of

glutamate in the high rates of seizure in autism are required for investigation of its pathological role in autism.

In the present study, we found no change in D-serine levels in autistic patients, inconsistent with the results of schizophrenia (Hashimoto et al., 2003; Yamada et al., 2005), suggesting that D-serine may not play a role in the pathophysiology of adult patients with autism. However, it has been suggested that D-serine plays an important role in neuronal migration (Kim et al., 2005), suggesting that D-serine serves as a co-agonist for the NMDA receptor-dependent cell migration at the development stage. Therefore, it may be of interest to examine serum D-serine levels in children with autism.

One may raise the question as to whether the higher levels of serum glutamate observed in this study of adult patients with autism reflect a persistent abnormal function that is invariably present at an earlier stage, i.e., in childhood. It could be that factors related to the illness course rather than the process of the development of the disorder itself may be pertinent to our observation. However, there was no correlation between the duration of the disorder and the serum glutamate levels in our sample ($r=-0.018$, $p=0.94$), implicating that glutamate dysfunction may occur in the early stage and be maintained through adulthood. Furthermore, it is of interest to measure serum glutamate levels in children with and without autism in order to determine the role of glutamate as a serological marker in children who will go on to develop an autistic disorder.

Accumulating evidence suggest that abnormality of inflammatory events may be implicated in the pathophysiology of autism (Licinio et al., 2002; Cohly and Panja, 2005). It has been reported that excessive production of pro-inflammatory cytokine tumor necrosis factor-α (TNF-α) with stimulation of endotoxin lipopolysaccharide is shown in children with autism spectrum disorders (Jyonouchi et al., 2002, 2005a,b). Interestingly, it has been demonstrated that patients with Rett syndrome had high cerebrospinal fluid glutamate levels (Riikonen, 2003), and that levels of TNF-α are increased with glutamate (McNearney et al., 2004). Taken together, it is likely that increased glutamate levels may contribute to raised levels of TNF-α although we did not measure serum TNF-α levels in these patients. Further investigation measuring levels of glutamate and cytokines including TNF-α will be necessary to unravel the role of glutamate/cytokines imbalance in autism.

Our findings have led us to the hypothesis that hyperglutamatergic neurotransmission in the brain may contribute to the pathophysiology of autism. We will attempt to confirm our hypothesis in further studies, particularly through the use of high-resolution magnetic resonance spectroscopy, which can directly measure the levels of glutamate in the brain of autism.

5. Conclusion

In conclusion, the present study suggests that abnormalities in glutamatergic neurotransmission may play a role in the pathophysiology of autism. In the future, we hope to gain a more complete understanding of glutamatergic neurotransmission in the pathophysiology of autism in order to provide new perspectives on treating autism.

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Short communication

Reduced serum levels of brain-derived neurotrophic factor in adult male patients with autism

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Abstract

Background: The precise mechanisms underlying the pathophysiology of autism are currently unknown. Given the key role of brain-derived neurotrophic factor (BDNF) in brain development, we hypothesized that BDNF may play a role in the pathophysiology of autism. In this study, we studied whether serum levels of BDNF are altered in patients with autism.
Methods: We measured serum levels of BDNF in 18 adult male patients with autism and 18 age-matched healthy male control subjects.
Results: The serum levels of BDNF in patients with autism (25.6±2.15 ng/ml (mean±S.D.)) were significantly ($z=-4.42$, $p<0.001$) lower than those of normal controls (61.6±10.9 ng/ml (mean±S.D.)). Nevertheless, we found no correlations between BDNF levels and clinical variables in autistic patients.
Conclusions: This study suggests that reduced BDNF levels may play a role in the pathophysiology of autism.

Keywords: Autism; Brain-derived neurotrophic factor; Neurodevelopmental disorder; Serum

1. Introduction

Autism is a developmental disorder characterized by severe and sustained impairment in social interaction, deviance in communication, and patterns of behavior and interest that are restricted or stereotyped, or both. The precise mechanisms underlying the pathophysiology of this disorder remain to be determined (Volkmar and Pauls, 2003; Baron-Cohen and Belmonte, 2005; McDougle et al., 2005).

Multiple lines of evidence suggest that brain-derived neurotrophic factor (BDNF) plays a critical role in brain development, and that it might play a role in the pathophysiology of psychiatric diseases, including mood disorders and schizophrenia (Hashimoto et al., 2004; Angelucci et al., 2005; Berton and Nestler, 2006). Nelson et al. (2001) initially reported higher BDNF levels in archived samples (dried blood spot) of neonatal blood obtained from children with autism compared with normal controls based on data obtained by recycling immunoaffinity chromatography (RIC; a single-antibody system). However, further analysis using Luminex technology (a double-antibody system) did not confirm this reduction in BDNF in autism (Nelson et al., 2006). Furthermore, Miyazaki et al. (2004) reported that serum BDNF levels in patients with autism were higher than those in normal controls; however, the age range (3- to 27-year-olds) of their patients was too large compared with that of normal controls (22- to 24-year-olds). Interestingly, it has been reported that serum BDNF levels in rats (Karege et al., 2002) and healthy human subjects (Nelson et al., 2006) are markedly altered by age. Given these data,

Abbreviations: ADI-R, Autism Diagnostic Interview–Revised; BDNF, brain-derived neurotrophic factor; IQ, intellectual quotient; Y-BOCS, Yale–Brown Obsessive–Compulsive Scale.

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it is of interest to determine whether serum BDNF levels differ between patient and control groups within a small age range. The purpose of the present study, therefore, was to examine whether serum levels of BDNF are altered in adult autistic patients.

2. Materials and methods

2.1. Subjects

Eighteen male autistic subjects (21.2±2.1 years (mean±S.D.); 18–26 years (range)) and 18 age-matched healthy male control subjects (22.2±2.2 years (mean±S.D.); 18–26 years (range)) were enrolled in the present study (Table 1). All participants in both groups were Japanese. The autistic subjects were recruited through advocacy groups in the cities of Nagoya and Hamamatsu, which are located in the middle of mainland Japan. For the diagnosis of autism, the recruited individuals were initially assessed according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) (American Psychiatric Association, 1994), followed by further assessment using the Autism Diagnostic Interview–Revised (ADI-R) (Lord et al., 1994). Potential participants were excluded from the study if they had a diagnosis of fragile X syndrome, epileptic seizures, obsessive–compulsive disorder, affective disorders or any additional psychiatric or neurological conditions. All autistic subjects were drug-naive or had been free of psychoactive medications for at least 6 months. This study was approved by the Ethics Committees of the Hamamatsu University School of Medicine and Chiba University Graduate School of Medicine. The control participants were given a complete description of the study, and their written informed consent was obtained before they entered the study.

Healthy controls were recruited from Hamamatsu City by advertisement. All control group participants underwent a comprehensive assessment of medical history to eliminate individuals with any neurological or other medical disorders. The Structured Clinical Interview for the DSM-IV (SCID) was also conducted in order to determine the existence of any personal or familial history of past or present mental illness. None of the control subjects initially recruited was found to fulfill these exclusion criteria.

Table 1
Clinical characteristics of 18 adult patients with autism

Characteristics	Mean±S.D. (range)
Age at onset, year	3.72±(1–5)
Duration of illness, year	17.5±2.23 (14–22)
ADI-R	
Social	22.11±4.96 (14–29)
Communication	15.44±4.84 (6–21)
Stereotype	5.22±1.77 (3–10)
Y-BOCS	11.28±5.39 (2–26)
Obsession	6.44±3.13 (1–14)
Compulsion	4.94±3.62 (0–14)
AQ–Aggression	50.56±12.3 (34–69)
Theory of Mind–Faux Pas Test	23.44±8.16 (6–34)
IQ	
Full scale IQ	96.83±20.33 (62–140)
Verbal IQ	95.11±19.87 (53–131)
Performance IQ	100.4±18.4 (75–137)

ADI-R: Autism Diagnostic Interview–Revised, Y-BOCS: Yale–Brown Obsessive–Compulsive Scale, AQ: Aggression Questionnaire, IQ: intellectual quotient.

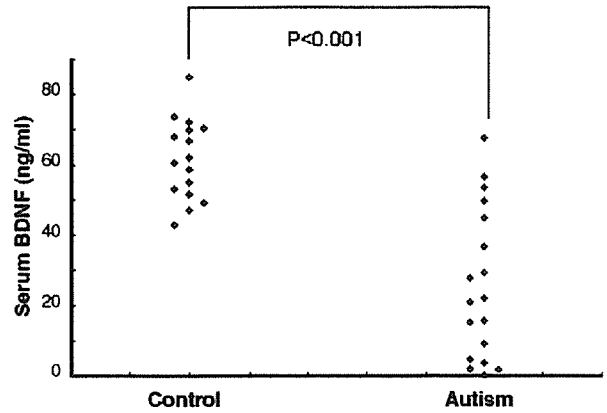

Fig. 1. The serum levels of BDNF in normal controls and autistic patients. The serum levels of BDNF in autistic patients ($n=18$) were significantly ($z=-4.42$, $p<0.001$, Mann–Whitney U-test) lower than those of normal controls ($n=18$).

2.2. Measurement of serum BDNF levels

Serum samples of autistic patients and normal comparison subjects were collected between 11:00 a.m. and noon, and were stored at −80 °C until assay. Serum BDNF levels were measured using a BDNF Emax Immuno Assay System (Promega Corporation, Madison, WI, USA). To minimize the assay variance, serum BDNF levels were measured in all subjects on the same day.

2.3. Data analysis

The data were presented as mean±S.D. The data were analyzed by Mann–Whitney U-test. The relationships between BDNF levels and clinical variables among patients with autism were evaluated by Spearman correlations. A p value of less than 0.05 was considered to be statistically significant.

3. Results

Serum levels of BDNF in patients with autism (25.6±2.15 ng/ml (mean±S.D.)) were significantly ($z=-4.42$, $p<0.001$) lower than those of age-matched healthy controls (61.6±10.9 ng/ml (mean±S.D.)) (Fig. 1). We also examined correlations between serum BDNF levels and clinical variables among patients with autism, finding no marked or significant correlations; the tested clinical variables were age of onset, duration of illness, ADI-R scores, Yale–Brown Obsessive–Compulsive Scale (Y-BOCS) scores, aggression, Theory of Mind assessment and Intellectual Quotient (IQ) (Table 1).

4. Discussion

In the present study, we found that serum BDNF levels in adult patients with autism were significantly lower than those of age-matched normal controls. To the best of our knowledge, this is the

first report demonstrating decreased serum levels of BDNF in adult autistic patients, however, our data is inconsistent with previous reports (Miyazaki et al., 2004; Connolly et al., 2006). One possible reason for this discrepancy may be the difference in the age range of the subjects: 3 to 27 years (Miyazaki's study) and 5.9±3.9 years (Connolly's study) vs. 18 to 26 years in the present study. This may be significant since BDNF levels in the blood of rats (Karege et al., 2002) and healthy human subjects (Nelson et al., 2006) have been found to be significantly affected by age. Furthermore, serum BDNF levels of normal controls in our study were higher than those of other reports (Shimizu et al., 2003; Lommatzsch et al., 2005). At present, the reasons for this discrepancy are unknown. Recently, Lommatzsch et al. (2005) demonstrated a negative correlation between plasma BDNF levels and age. Therefore, a possibility for this discrepancy may be the difference in the age range of the subjects. Another possibility for this discrepancy may be the difference of methodological differences (e.g., time of sample collection, preparation of serum). Furthermore, there is a positive correlation between serum BDNF levels and cortical BDNF levels that continues from early maturation throughout the aging process (Karege et al., 2002). Taken together, it is likely that decreased levels of BDNF occur in the brain of autistic patients.

Accumulating evidence suggest the role of immune system in the pathophysiology of autism (Belmonte et al., 2004; Cohly and Panja 2005). Recently, it has been reported that IgG and IgM BDNF autoantibodies were elevated in children with autism (Connolly et al., 2006). Furthermore, BDNF is also produced by activated T-cells, B-cells, and monocytes (Kerschensteiner et al., 1999). Based on the role of immune system in the pathophysiology of autism, these findings suggest the unrecognized interaction between the immune system and BDNF in autism. Further studies underlying the role of BDNF in immune system will be necessary to examine the role of BDNF in the pathophysiology of autism.

It has been reported that social isolation (8 weeks) selectively reduced the BDNF levels in rat hippocampus whereas plasma corticosterone levels were not altered (Scaccianoce et al., 2006), suggesting that BDNF levels is responsive to psychological state (Hashimoto et al., 2004). Furthermore, we reported that serum BDNF levels in drug nave patients with major depressive disorders recovered to basal levels after antidepressant treatment (Shimizu et al., 2003). Therefore, it may be important to take the psychological states in subjects into consideration to unravel the role of BDNF in the pathophysiology of autism.

Given the critical role of BDNF in brain development, our findings lead us to the hypothesis that decreased levels of BDNF in the brain may contribute to the pathophysiology of autism. It is therefore of great interest to measure serum BDNF levels in children with and without autism in order to determine the role of BDNF as a serological marker in children who will go on to develop an autistic disorder.

5. Conclusions

The present study suggests that serum BDNF may be a biological marker for autism, and that reduced BDNF levels might play a role in the pathophysiology of this disorder. In the future, we hope to gain a more complete understanding of the role of the BDNF–TrkB pathway in the pathophysiology of autism in order to provide new perspectives on its treatment.

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Decreased serum levels of transforming growth factor-β1 in patients with autism

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Abstract

Background: The neurobiological basis for autism remains poorly understood. Given the key role of transforming growth factor-β1 (TGF-β1) in brain development, we hypothesized that TGF-β1 plays a role in the pathophysiology of autism. In this study, we studied whether serum levels of TGF-β1 are altered in patients with autism.
Methods: We measured serum levels of TGF-β1 in 19 male adult patients with autism and 21 age-matched male healthy subjects using enzyme-linked immunosorbent assay (ELISA).
Results: The serum levels (7.34±5.21 ng/mL (mean±S.D.)) of TGF-β1 in the patients with autism were significantly ($z=-5.106$, $p<0.001$) lower than those (14.48±1.64 ng/mL (mean±S.D.)) of normal controls. However, there were no marked or significant correlations between serum TGF-β1 levels and other clinical variables, including Autism Diagnostic Interview-Revised (ADI-R) scores, Yale-Brown Obsessive–Compulsive Scale (Y-BOCS), aggression, Theory of Mind, and Intellectual Quotient (IQ) in patients.
Conclusions: These findings suggest that decreased levels of TGF-β1 may be implicated in the pathophysiology of autism.

Keywords: Autism; Growth factor; Human serum; Neurodevelopmental disorder

1. Introduction

Autism is a neurodevelopmental disorder resulting in pervasive abnormalities in social interaction and communication, repetitive behaviors and restricted interests. However, the precise mechanisms underlying the pathophysiology of this disorder remain to be determined (Volkmar and Pauls, 2003; Baron-Cohen and Belmonte, 2005; Levitt et al., 2004).

Transforming growth factor betas (TGF-βs) are known as multifunctional growth factors, which participate in the regulation of key events of development, disease, and tissue repair (Böttner et al., 2000; Buisson et al., 2003; Gomes et al., 2005). TGF-β family is represented by a small group of multiple functional cytokines, consisting of three isoforms: TGF-β1, TGF-β2 and TGF-β3. Immunohistochemical and *in situ* hybridization studies have provided evidence for the widespread distribution of immunoreactive TGF-β2 and TGF-β3 and sites of their synthesis in the developing and adult central nervous system (CNS) and peripheral nervous system (PNS). These aspects appear to be of importance: (1) the virtual ubiquity of

Abbreviations: ADI-R, Autism Diagnostic Interview-Revised; AQ, Aggression questionnaire; BBB, blood–brain barrier; BDNF, brain-derived neurotrophic factor; CNS, central nervous system; CSF, cerebrospinal fluid; ELISA, enzyme-linked immunosorbent assay; IQ, intellectual quotient; PNS, peripheral nervous system; S.D., standard deviation; TGF-β1, transforming growth factor-β1; Y-BOCS, Yale-Brown Obsessive–Compulsive Scale.

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TGF-β in all areas of the CNS as well as in the PNS, (2) consistent coexpression of TGF-β2 and TGF-β3 in neurons, astroglia, and Schwann cells, and (3) the almost complete lack or low levels, respectively, of TGF-β1 in the unlesioned nervous system (Böttner et al., 2000). Within the brain, these three isoforms are produced by both glial and neuronal cells (Gomes et al., 2005). Among these isoforms, TGF-β1 is a potent immunosuppressive cytokine that can be expressed by virtually all cells of the body. Accumulating evidence suggest that TGF-β1 has emerged as a crucial regulator of nervous system physiology, although this cytokine has been widely considered an injury-related cytokine (Gomes et al., 2005). First, TGF-β1 and its receptor are expressed in the developing nervous system, suggesting the role of TGF-β1 in brain development (Böttner et al., 2000; Gomes et al., 2005). Second, the knock-out mice for TGF-β1 gene show severe impairment in cortical development with widespread increased neuronal cell death and microgliosis (Brionne et al., 2003). Third, in adult neural stem and progenitor cell cultures and after intracerebroventricular infusion, TGF-β1 induced a long-lasting inhibition of neural stem and progenitor cell proliferation and a reduction in neurogenesis, suggesting the potential implications for neurogenesis in a variety of TGF-β1 associated CNS diseases and pathologic conditions (Wachs et al., 2006). However, no studies demonstrating on the role of TGF-β1 in autism have been reported.

Considering the key role of TGF-β1 in brain development (Gomes et al., 2005), it is of great interest to study the role of TGF-β1 in the pathophysiology of autism. The purpose of the present study is to examine whether serum levels of TGF-β1 in autistic patients are altered as compared to age-matched normal controls. Furthermore, we also examined relationships between serum TGF-β1 levels and clinical variables in autistic patients.

2. Methods

2.1. Subjects

Nineteen male autistic subjects (23.4±2.6 years (mean±S.D.), 18–28 years (range)) and twenty-one age-matched male healthy control subjects (22.7±2.3 years (mean±S.D.), 18–26 years (range)) were included in this study (Table 1). All participants for both groups were Japanese. The autistic subjects were recruited through advocacy groups in Nagoya and Hamamatsu cities, which are located in the middle of the mainland of Japan. For diagnosis of autism, the recruited individuals were initially assessed according to the Diagnostic and Statistical Manual of Mental Disorders, Forth Edition (DSM-IV) (American Psychiatric Association, 1994), followed by assessment using the Autism Diagnostic Interview-Revised (ADI-R) (Lord et al., 1994) by clinicians. Participants were excluded from the study, if they had a diagnosis of fragile X syndrome, epileptic seizures, obsessive–compulsive disorder, affective disorders, or any additional psychiatric or neurological diagnoses. All the autistic subjects were drug naive or free of psychoactive medications for at least 6 months: the majority of autistic participants have never previously received psychoactive medications, and the minority participants had been given sedatives more than 6 months before this study. Healthy controls were recruited from Hamamatsu City by advertisement. All control group participants underwent a comprehensive assessment of medical history to eliminate individuals with any neurological or other medical disorders. The Structured Clinical Interview for the DSM-IV (SCID) was also conducted to scrutinize any personal or familial history of past or present mental illness. None of the comparison subjects initially recruited was found to fulfill these exclusion procedures. This study received approval from the ethics committee of the Hamamatsu University School of Medicine. After the participants were given a complete description of the study, written informed consent was obtained from all subjects before they entered the study.

Table 1
Clinical characteristics of subjects

Characteristic	Control (n=21)	Autism (n=19)
Age, year	23.4±2.61 (18–26)	22.7±2.26 (18–28)
Gestational age at birth, week		38.63±1.54 (37–41)
ADI-R		
Domain A score		22.05±4.82 (14–29)
Domain BV score		15.69±4.82 (6–21)
Domain C score		5.16±1.74 (3–10)
Domain D score		2.94±1.06 (1–5)
Y-BOCS		10.76±5.08 (2–26)
Obsession		6.24±3.09 (1–14)
Compulsion		4.65±3.50 (0–14)
AQ—aggression		51.53±11.98 (33–69)
Theory of mind—Faux Pas Test		22.37±9.21 (3–34)
IQ		
Full scale IQ		97.65±20.65 (59–140)
Verbal IQ		96.00±20.11 (53–131)
Performance IQ		100.6±19.05 (40–137)

Values are expressed as mean±S.D. (range).
ADI-R: Autism Diagnostic Interview-Revised, Y-BOCS: Yale-Brown Obsessive–Compulsive Scale, AQ: Aggression Questionnaire, IQ: Intellectual Quotient.

2.2. Psychological measures

ADI-R is a semi-especially-formulated structured psychiatric interview with a parent, especially a mother, which is administered to the parent. It is used to confirm diagnosis and also to evaluate the core symptoms of autism. ADI-R is based on three separate scores. ADI-R domain score A quantifies impairment in social interaction (range of score: 0–32), domain score BV quantifies impairment in communication (range of score: 0–26), and domain score C quantifies restricted, repetitive, and stereotyped patterns of behavior and interests (range of score: 0–16). Higher scores on each indicate worse performance. ADI-R domain D corresponds to age of onset criterion for autistic disorder. If the score is 1 or higher, the subject is quite likely to have the age of onset earlier than or equal to 3 years old. All of the subjects with autism have age of onset no later than 3 years old since none had ADI-R domain D score lower than 1 (Table 1).

Obsessional/repetitive behavior was rated using the Yale-Brown Obsessive–Compulsive Scale (Y-BOCS) (Goodman

et al., 1989a,b); additional aggression symptoms were also assessed using the Aggression Questionnaire (AQ) (Buss and Perry, 1992). We used a Faux Pas Test to evaluate the function of "Theory of Mind" (*mentalizing*) (Baron-Cohen et al., 1999; Stone et al., 2003). The performance of individuals with autism on the *Faux Pas Test* is an experimental demonstration of their theory-of-mind deficit at a higher level. There were 40 points possible for *Faux Pas*—related questions about 10 stories (range: 0–40, 1 point for each question).

2.3. Procedures

The serum samples of autistic patients and normal comparison subjects were collected during 11:00–noon, and stored at −80 °C until assay. The serum levels of TGF-β1 were measured using TGF-β1 ELISA Kit (R&D Systems, Inc., Minneapolis, MN), which involved a sandwich enzyme-linked immunosorbent assay (ELISA) using anti-TGF-β1 monoclonal antibody and one enzyme-linked polyclonal antibody for TGF-β1. The assay was performed according to the supplier's recommendation. The calibrator consisted of recombinant human TGF-β1. All samples were measured in duplicates and respective mean value was calculated.

2.4. Statistical analysis

The data were presented as the mean±standard deviation (S.D.). The data were analyzed using a Mann–Whitney *U*-test. Evaluation of relationships between levels of TGF-β1 and clinical variables among patients with autism was determined by Pearson correlations or Spearman correlations. A *p*-value of less than 0.05 was considered to be statistically significant.

3. Results

The serum levels (7.34±5.21 ng/mL (mean±S.D.)) of TGF-β1 in the patients with autism were significantly ($z=-5.106$, $p<0.001$) lower than those (14.48±1.64 ng/mL (mean±S.D.)) of normal controls (Fig. 1).

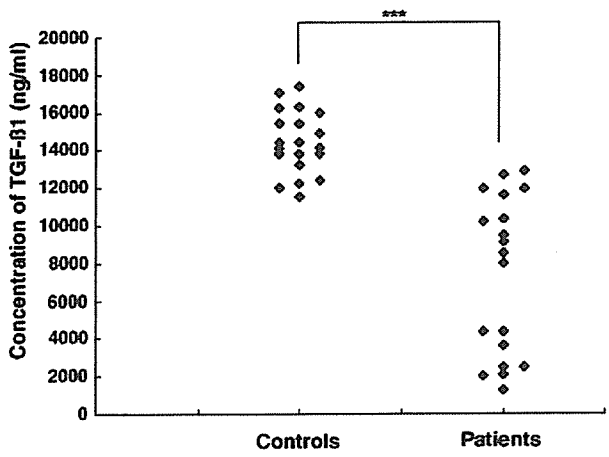

Fig. 1. The serum levels of TGF-β1 in autistic patients and normal controls. The serum levels of TGF-β1 in autistic patients ($n=19$) were significantly lower than those of normal controls ($n=21$). ***$p<0.001$ (Mann–Whitney *U*-test).

We then examined the correlations between serum TGF-β1 levels and clinical variables among patients with autism. There was a trend toward a positive correlation ($r=0.400$, $p=0.089$) between serum TGF-β1 levels and gestational age at birth in patients. However, there were no marked or significant correlations between serum TGF-β1 levels and other clinical variables, including ADI-R scores, Y-BOCS, aggression, Theory of Mind, and IQ.

4. Discussion

The finding of the present study is that serum levels of TGF-β1 in the patients with autism are significantly lower than those of age-matched normal healthy controls. To the best of our knowledge, this is the first report demonstrating the decreased serum levels of TGF-β1 in autistic patients. It remains unclear whether serum TGF-1β levels reflect the levels of TGF-1β in the brain since it is shown that TGF-β1 does not cross the intact blood–brain barrier (BBB) whereas TGF-β1 can cross the disrupted BBB (Kastin et al., 2003). Therefore, it may be of great interest to study whether cerebrospinal fluid (CSF) levels of TGF-1β are altered in autistic patients. Given the key role of TGF-β1 in brain development (Gomes et al., 2005), our findings lead us to the hypothesis that decreased levels of TGF-β1 may be implicated in the pathophysiology of autism although the result does not necessarily indicate either causation or its direction. Furthermore, it is also of interest to measure serum levels of TGF-β1 in children with and without autism in order to determine the role of TGF-β1 as a serological marker for children who will go on to develop autistic disorder.

A recent immunohistochemical study and ELISA assay using postmortem brain samples showed that a number of cytokines, and growth factors including TGF-β1 were altered in the middle frontal gyrus, anterior cingulated gyrus, and cerebellum in the patients with autism, although there was no difference in CSF levels of TGF-β1 between two groups (Vargas et al., 2005). These findings suggest that innate neuroimmune reactions play a pathogenic role in an undefined proportion of autistic patients (Vargas et al., 2005). Taken together, our findings suggest that TGF-β1 may play a role in the pathophysiology of autism but further work is necessary to study its precise role and how specifically TGF-β1 is linked to core autism. Given the sample size of the groups, the present results may not have the statistical power. Therefore, further studies using a large sample will be necessary.

Accumulating evidence suggests that brain-derived neurotrophic factor (BDNF) plays a role in the pathophysiology of psychiatric disorders, including schizophrenia, mood disorders, and eating disorders (Hashimoto et al., 2004, 2005a,b). It has been demonstrated that serum levels of BDNF were significantly correlated with serum levels of TGF-β1, suggesting that BDNF and TGF-β1 could be anatomically and functionally related in the human blood (Lommatzsch et al., 2005). Interestingly, we recently reported that serum levels of BDNF in patients with autism were significantly lower than those of

age-matched normal controls, suggesting that reduced BDNF levels may be implicated in the pathophysiology of autism (Hashimoto et al., 2007). In addition, we found a positive correlation (r=0.738, p<0.001) between serum BDNF levels and serum TGF-β1 levels in the same sample of patients, suggesting the possible relationship of BDNF and TGF-β1 in patients with autism. It has been reported that TGF-β1 enhances expression of BDNF and its receptor TrkB in neurons from rat cerebral cortex (Sometani et al., 2001), suggesting that BDNF may require TGF-β1 as a cofactor to exert its neurotrophic activities. Taken together, it is likely that decreased levels of TGF-β1 and BDNF may be implicated in the pathophysiology of autism although the precise mechanism(s) underlying the functional role of BDNF and TGF-β1 in the autism are currently unclear.

5. Conclusion

Our findings suggest that reduced levels of TGF-β1 might be implicated in the pathophysiology of autism. Further studies on the potential mechanisms and physiological implications of reduced TGF-β1 levels in autism will be necessary in the future.

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Decreased Serum Levels of Epidermal Growth Factor in Adult Subjects with High-Functioning Autism

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Background: *The neurobiological basis for autism remains poorly understood. Given the role of growth factors in brain development, we hypothesized that epidermal growth factor (EGF) may play a role in the pathophysiology of autism. In this study, we examined whether serum levels of EGF are altered in adult subjects with high-functioning autism.*

Methods: *We measured serum levels of EGF in the 17 male subjects with high-functioning autism and 18 age-matched healthy male subjects.*

Results: *The serum levels of EGF in the subjects with high-functioning autism (72.4 ± 102.8 pg/mL [mean ± SD]) were significantly lower (Mann-Whitney* $U = 22.0$, $p < .001$*) than those of normal control subjects (322.3 ± 122.0 pg/mL [mean ± SD]). However, there were no correlations between serum EGF levels and clinical variables in the subjects with autism.*

Conclusions: *This study suggests that decreased levels of EGF might be implicated in the pathophysiology of high-functioning autism.*

Key Words: Autism, developmental disorders, ELISA, epidermal growth factor, growth factors, human blood

Autism is a neurodevelopmental disorder resulting in pervasive abnormalities in social interaction and communication, repetitive behaviors, and restricted interests. However, the precise mechanisms underlying the pathophysiology of this disorder remains to be determined (Volkmar and Pauls 2003; Rubenstein and Merzenich 2003; Belmonte et al 2004; Polleux and Lauder 2004; Levitt et al 2004; Baron-Cohen and Belmonte 2005; Cohly and Panja 2005).

Epidermal growth factor (EGF) is a member of the EGF family of growth factors, and this growth factor binds to the epidermal growth factor receptor (EGFR) with high affinity. Epidermal growth factor is detected in the majority of neurons and in maturing astrocytes in the developing and adult brain of humans and different species of animals (Ferrer et al 1996; Xian and Zhou 1999). Recently, it has been demonstrated that levels of EGF are significantly decreased in the postmortem brains of schizophrenia (also a neurodevelopmental disorder) and that serum EGF levels in both the medicated and drug-free patients with schizophrenia are also markedly reduced as compared with normal control subjects (Futamura et al 2002). However, a recent study using a large sample revealed no changes of serum EGF levels in drug-naive patients or medicated patients with schizophrenia (Hashimoto et al 2005).

At present, no studies demonstrating alterations in the EGF in autism have been reported. Considering the role of EGF in brain development, it is of interest to study the role of EGF in the pathophysiology of autism. The purpose of the present study is to examine whether serum levels of EGF in adult subjects with high-functioning autism are altered as compared with age-matched normal control subjects. Furthermore, we also examined any relationship between serum levels of EGF and clinical variables in autistic patients.

Methods and Materials

This study received approval from the ethics committee of the Hamamatsu University School of Medicine. After the participants were given a complete description of the study, written informed consent was obtained from all subjects before they entered the study.

Seventeen male autistic subjects (23.1 ± 2.52 years [mean ± SD], 19–28 years [range]) and 18 age-matched male healthy control subjects (23.0 ± 2.03 years [mean ± SD], 20–26 years [range]) were included in this study (Table 1). All participants for both groups were Japanese. The autistic subjects were recruited through advocacy groups in Nagoya and Hamamatsu, which are cities located in the middle of the mainland of Japan. The diagnosis of autism was made on the basis of the Autism Diagnostic Interview-Revised (ADI-R) (Lord et al 1994), Japanese version. One of the authors (K.J.T.), having established reliability of diagnosing autism with the authors, conducted the interview for all subjects. Then, based on the results, a DSM-IV (American Psychiatric Association 1994) diagnosis of autistic disorder was made for all subjects. We also conducted the Wechsler Adult Intelligence Scale-Revised (WAIS-R) to exclude subjects with a full-scale intelligence quotient (IQ) of less than 70, resulting in a group of 17 subjects with high-functioning autism. Participants were excluded from the study if they had a diagnosis of fragile X syndrome, epileptic seizures, obsessive-compulsive disorder, affective disorders, or any additional psychiatric or neurological diagnoses. All the autistic subjects were drug naive or had been free of psychoactive medications for at least 6 months: the majority of autistic participants ($n = 12$) had never previously received psychoactive medications and a minority ($n = 5$) of participants had been given sedatives more than 6 months before this study. Healthy control subjects were recruited from Hamamatsu City by advertisement. All control group participants underwent a comprehensive assessment of their medical history to eliminate individuals with any neurological or other medical disorders. The Structured Clinical Interview for DSM-IV (SCID) was also conducted to scrutinize any personal or family history of

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Table 1. Clinical Characteristics of Normal Control Subjects and Subjects with High-Functioning Autism

Group (*n*)	Control Subjects (18)	High-Functioning Autism (17)
Age (Year)	23.0 ± 2.03 (20–26)	23.1 ± 2.52 (19–28)
EGF (pg/mL)	322.3 ± 122.0 (120.7–539.8)	72.4 ± 102.8 (.10–320.2)[b]
ADI-R		
Domain A Score		22.5 ± 4.8 (14–29)
Domain BV Score		16.5 ± 3.8 (9–22)
Domain C Score		5.3 ± 1.8 (3–10)
Domain D Score		3.7 ± 1.1 (1–5)
Y-BOCS (Total Score)		11.2 ± 5.6 (2–26)
Hamilton Depression Scale Score		2.4 ± 3.7 (0–15)
Hamilton Anxiety Scale Score		4.1 ± 3.3 (0–11)
AQ Total Score		50.6 ± 12.7 (34–69)
Faux Pas Test-Theory of Mind		23.4 ± 8.8 (3–34)
WAIS-R (Full-Scale IQ)		98.9 ± 18.9 (71–140)
Gestational Age (Week)[a]		38.8 ± 1.7 (34–41)
Birth Weight (g)[a]		3382 ± 502 (2376–4148)
Head Circumference at Birth (cm)[a]		34.0 ± 2.3 (29.2–37.6)

Values are expressed as mean ± SD (range).

EGF, epidermal growth factor; ADI-R, Autism Diagnostic Interview-Revised; Y-BOCS, Yale-Brown Obsessive Compulsive Scale; AQ, Aggression Questionnaire; WAIS-R, Wechsler Adult Intelligence Scale-Revised.

[a]One subject had no available information.

[b]$p < .001$ as compared with control (Mann-Whitney *U* test).

past or present mental illness. None of the comparison subjects initially recruited was found to fulfill these exclusion procedures.

Serum samples of autistic patients and normal comparison subjects were collected during 11:00 AM to noon and stored at −80°C until assay. Serum levels of EGF were measured using EGF ELISA Kit (R&D Systems, Inc., Minneapolis, Minnesota). The data were presented as the mean ± SD. The data were analyzed using the Mann-Whitney *U* test. Among patients with autism, relationships between serum EGF levels and clinical variables were determined by Pearson or Spearman correlations. A p value of less than .05 was considered to be statistically significant.

Results

The serum levels of EGF in the subjects with high-functioning autism (72.4 ± 102.8 pg/mL [mean ± SD]) were significantly lower (Mann-Whitney $U = 22$, $p < .001$) than those of normal control subjects (322.3 ± 122.0 pg/mL [mean ± SD]) (Table 1 and Figure 1). We then examined the correlations between serum EGF levels and clinical variables among subjects with autism. There were no marked or significant correlations between serum EGF levels and clinical variables, including ADI-R scores, Yale-Brown Obsessive-Compulsive Scale (Y-BOCS) scores, Hamilton Depression Scale scores, Hamilton Anxiety Scale scores, aggression, Faux Pas Test-Theory of Mind scores, WAIS-R scores, gestational age, birth weight, and head circumference at birth (Table 1).

Discussion

The finding of the present study is that serum levels of EGF in the adult subjects with high-functioning autism were significantly lower than those of age-matched normal healthy control subjects. To the best of our knowledge, this is the first report demonstrating the decreased serum levels of EGF in autism. It has been reported that ^{125}I-labeled epidermal growth factor (^{125}I-EGF) crosses the blood-brain barrier (BBB) rapidly and that the fast rate of influx is significantly decreased by co-administration of nonradiolabeled EGF, suggesting that a rapid, saturable, and unidirectional transport system on the BBB enables EGF to enter the brain (Pan and Kastin 1999). Therefore, it is likely that decreased levels of EGF may occur in the brain of autistic patients.

Accumulating evidence suggests the role of immune signaling in the pathophysiology of autism (Belmonte et al 2004; Cohly and Panja 2005). It has been reported that excessive production of tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), and interleukin-1 receptor antagonist (IL-1RA) with stimulation of endotoxin lipopolysaccharide is shown in children with autism spectrum disorders (Croonenberghs et al 2002; Jyonouchi et al 2002, 2005), suggesting that increased production of proinflammatory cytokines could play a role in the pathophysiology of autism. A recent study using postmortem brain samples showed abnormality of proinflammatory and anti-inflammatory cytokines and growth factors in the brains with autism, suggesting that innate neuroimmune reactions play a pathogenic role in an underlined proportion of autistic patients (Vargas et al 2005). Taken together, it is also likely that abnormality of growth factors, including EGF, may be implicated in the mechanisms associated with neural dysfunction in autism, although further studies using a large sample will be necessary.

Recently, we reported that serum levels of brain-derived neurotrophic factor (BDNF) in adult subjects with autism were significantly lower than those of age-matched normal control subjects (Hashimoto et al, in press), inconsistent with previous reports (Nelson et al 2001; Connolly et al 2006). One possible reason for this discrepancy may be the difference in the age range of the subjects: neonatal blood in the Nelson et al (2001) study and 5.9 ± 3.9 years in the Connolly et al (2006) study versus 18 to 26 years in the (Hashimoto et al, in press) study. This may be significant, since BDNF levels in the blood of human subjects have been found to be significantly affected by age (Nelson et al 2006). In contrast, the cerebrospinal fluid (CSF) levels of nerve growth factor (NGF) in children with infantile autism were not altered, whereas CSF levels of NGF were decreased in children with Rett syndrome, suggesting that CSF levels of NGF could be used as a biochemical marker for differentiation of patients with autism from those with Rett syndrome (Riikonen and Vanhala 1999). Taken together, these

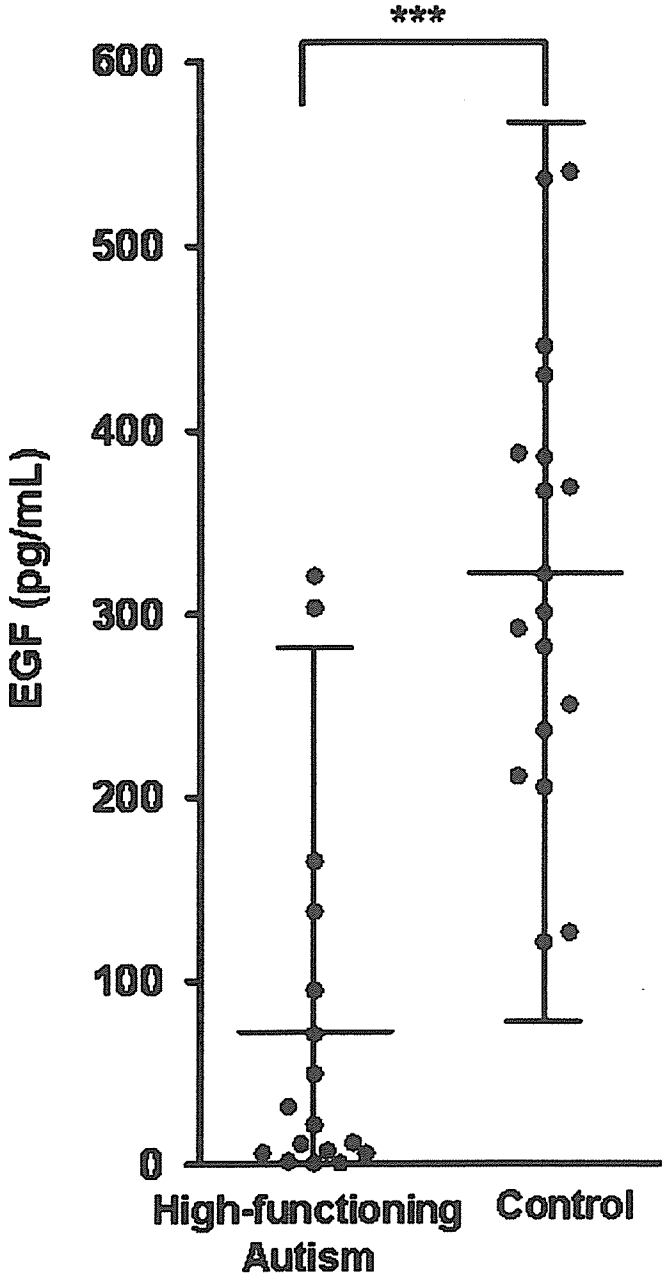

Figure 1. The serum levels of EGF in subjects with high-functioning autism and normal control subjects. The serum levels of EGF in subjects with autism (n = 17) were significantly lower than those of normal control subjects (n = 18). The bars show the mean ± 2 SD. ***$p < .001$ (Mann-Whitney U test). EGF, epidermal growth factor.

findings suggest that reduced levels of growth factors and/or neurotrophic factors, including EGF and BDNF, may play a role in the pathophysiology of autism.

Given the key role of EGF in brain development, our findings lead us to the hypothesis that decreased levels of EGF may be implicated in the pathophysiology of high-functioning autism. It is, therefore, of interest to measure serum levels of EGF in children with and without autism to determine the role of this growth factor as a serological marker for children who will go on to develop autistic disorder.

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Decreased serum levels of hepatocyte growth factor in male adults with high-functioning autism

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Abstract

Background: The mechanisms underlying the pathophysiology of autism are currently unknown. Given the role of hepatocyte growth factor (HGF) in brain development, we hypothesized that HGF plays a role in the pathophysiology of autism. In this study, we studied whether serum HGF levels are altered in subjects with high-functioning autism.
Methods: Using an enzyme-linked immunosorbent assay (ELISA), we measured serum levels of HGF in 17 male adults with high-functioning autism and age-matched 18 male healthy subjects.
Results: The serum levels (503.5±160.5 pg/mL (mean±SD)) of HGF in the subjects with high-functioning autism were significantly (Mann–Whitney $U=34.0$, $p<0.001$) lower than those (817.6±232.4 pg/mL (mean±SD)) of control subjects. However, there were no correlations between serum HGF levels and clinical variables in the patients.
Conclusions: This study suggests that reduced HGF levels may play a role in the pathophysiology of high-functioning autism.

Keywords: Autism; Developmental disorders; ELISA; Hepatocyte growth factor; Human blood

1. Introduction

Autism is a neurodevelopmental disorder characterized by severe and sustained impairment in social interaction, deviance in communication, and patterns of behavior and interest that are restricted, stereotyped, or both. However, the precise mechanisms underlying the pathophysiology of this disorder remain to be determined (Volkmar and Pauls, 2003; Levitt et al., 2004; Baron-Cohen and Belmonte, 2005).

Hepatocyte growth factor (HGF) is a polypeptide growth factor which acts by binding to the MET tyrosine kinase receptor. HGF influences the growth, motility and morphogenesis of various epithelial and endothelial cells and functions as a trophic factor for organ regeneration (Maina and Klein, 1999; Levitt et al., 2004). Accumulating evidence suggest that HGF and its receptor MET play a role in neuronal cell development (Maina and Klein, 1999; Levitt et al., 2004). First, HGF and its receptor MET are widely expressed in the developing and mature mouse brain, with expression beginning as early as embryonic day 12 (E12) and E13, respectively (Achim et al., 1997; Maina and Klein, 1999). Second, HGF promotes the migration

Abbreviations: ADI-R, Autism Diagnostic Interview-Revised; ELISA, enzyme-linked immunosorbent assay; GABA, γ-aminobutyric acid; HGF, hepatocyte growth factor; IQ, Intelligence Quotient; MCHH, Mother and Child Health Handbook; uPAR, urokinase plasminogen activator receptor; Y-BOCS, Yale–Brown Obsessive Compulsive Scale.

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