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Clinical Efficacy of Fluvoxamine and Functional Polymorphism in a Serotonin Transporter Gene on Childhood Autism

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We studied the correlation between response to fluvoxamine and serotonin transporter gene promoter region polymorphism (5-HTTLPR). Eighteen children with autistic disorder completed a 12-week double-blind, placebo-controlled, randomized crossover study of fluvoxamine. Behavioral assessments were obtained before and at 12 weeks of treatment. 5-HTTLPR (long (l) or short(s)), was analyzed by the PCR method. Ten out of 18 patients responded to fluvoxamine treatment; allele type analysis revealed that clinical global effectiveness was noted significantly more in the l allele than in the s allele. However, with respect to language use, a significant effectiveness was noted in the s allele. 5-HTTLPR may influence the individual responses to fluvoxamine administration.

KEY WORDS: Autistic disorder; selective serotonin transporter inhibiter; serotonin transporter gene; 5-HTTLPR.

Clinical characteristics of autistic disorders include fundamental disturbances in social interaction, communication impairments and a markedly restricted repertoire of activities and interests.

The pathogenesis of autistic disorder is not yet fully understood, but abnormalities in the serotonin (5-HT) system, one of the neurotransmitters, were identified in some groups of autistic patients (Chugani *et al*., 1999; Cook & Leventhal, 1996; McDougle *et al*., 1996). Schain and Freedmann (1961) first reported hyperserotonemia in autistic patients, followed by several similar studies reporting that approximately one-fourth to one-third of autistic patients exhibited hyperserotonemia (Anderson *et al*., 1987; Cook, 1990). Chugani et al. (1999) found that autistic children produced far less 5-HT in the brain than normal children. Chugani *et al*. (1997) revealed the low 5-HT synthesis in the left hemisphere in five of seven autistic boys with normal 5-HT synthesis in the right hemisphere using a 5-HT precursor (alpha-C11-methyltriptophan) by positron emission tomography.

The clinical effectiveness of a selective inhibitor of the 5-HT transporter (5-HTT), which reduces reuptake of 5-HT, to treat autistic disorder was reported (Gordon, State, Nelson, Hamburger, & Rapoport 1993; Mehlinger, Scheftner, & Poznanski, 1990). McDougle, Price, & Goodman, (1990) also reported the clinical effectiveness of fluvoxamine in treating repetitive thoughts and behavior, aggression, and social relatedness in the case of one adult. This was confirmed in 30 cases in a double-blind placebo-controlled study (McDougle *et al*., 1996). DeLong, Teague, & Kamran, (1998) reported the clinical

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effectiveness of fluoxetine in treating idiopathic autism in young children aged 2 to 7 years in an open-label treatment trial. Twenty-two of 37 children exhibited a good treatment response, particularly in terms of behavioral, language, cognitive, affective, and social improvements.

It has lately drawn considerable attention that individual responses to the drug used may differ with genetic polymorphism. Human 5-HTT is encoded by a single gene on chromosome 17q 11. 2–12. There are two reported functional polymorphisms, i.e., l and s variations with a 44-bp length difference in the promoter locus of the 5-HTT gene. (5-HTTLPR) (Heils *et al*., 1996). These genetic polymorphisms in the promoter regulate the expression of 5-HTT in transfection assays and lymphoblastoid cell lines (Lesch *et al*., 1996). Clinical studies (Semerald *et al*., 1998 and Kim *et al*., 1999) also showed the involvement of genetic polymorphism in 5-HTTLPR in the differential response to fluvoxamine in patients with depression.

With the above as a background, evaluated the clinical effectiveness of fluvoxamine in Japanese children with autistic disorder in a crossover double–blind, placebo-controlled study. In addition, we evaluated the effectiveness by focusing on the correlation between clinical responses and the genetic polymorphism of the 5-HTT gene in our young patients.

METHODS

Subjects

The twenty patients enrolled in this study were evaluated at the Department of Pediatric Neurology at Hamamatsu City Medical Center for Developmental Medicine and affiliated hospitals of Hamamatsu University School of Medicine, and were diagnosed as having autism by pediatric neurologists and clinical psychologists based on DSM-IV (Diagnostic and Statistical Manual of Mental Disorders (4th ed.), Criteria, American Psychiatric Association 1994). In 19 of the 20 cases, written informed parental consent was obtained, and final approval was given by the ethical committee of this center to participate in the protocol using fluvoxamine. They were all Japanese individuals: 15 males and 4 females with ages ranging from 3 to 8 years and 5 months (mean age: 5 years and 4 months). After full neurological evaluation and laboratory tests including hematological check, chromosomal analysis and neuroradiological assessment, patients with evident underlying diseases, such as chromosomal aberration, congenital rubella syndrome and apparent neurological deficits, were not included in this study.

Study design and medication

Subjects had not taken any psychotropic drugs for at least 4 weeks before the trial. After parents gave their written informed consent, the patients were randomly allocated according to a computer-generated list to 12 weeks of double-blind two-way crossover treatment with fluvoxamine or the placebo in identical appearing powders. To ensure compliance, medication was administered by parents. Fluvoxamine or placebo administration (Fig.1) was started at a dose of 1 mg/kg body weight/day for 2 weeks, then increased to 2 mg/kg body weight/day for 4 weeks, 3 mg/kg body weight/day for 6 weeks and then decreased to 1.5 mg/kg body weight/day for 2 weeks (first stage). After a 2-week washout period, fluvoxamine and the placebo were switched for the crossover trial (second stage). Since the half-life of fluvoxamine ranges 19–22 hours (Sproule, Naranjo, Brenmer, & Hassan, 1997), we set a two-week washout period before switching the medication for the crossover trials. The prescribing pediatric neurologist, the clinical psychologist who performed the behavioral ratings, the patients, and all family and other members of the patients' treatment teams were unaware of the drug assignment (blind).

Ten patients were randomly assigned to receive fluvoxamine and 9 to receive the placebo in the first stage and then switched in the second stage.

Assessments

Each patient was evaluated in detail with respect to behavioral symptoms by clinical psychologists before and after initiating the administration of fluvoxamine or the placebo. The new scales used were the Behavioral Assessment Scale (BAS) (Sugiyama, Sugie, Igarashi, Ito, & Fukuda, 1998), designed originally by the Division of Clinical Psychology at Hamamatsu City Medical Center for Developmental Medicine. BAS, presented in Table I, is designed to assess seven categories, namely, facial expression and eye movement, emotion and mood, interest and will, activities, attention, personal relationships and language. Each category has several items, totaling 20 items. Patients are scored based on

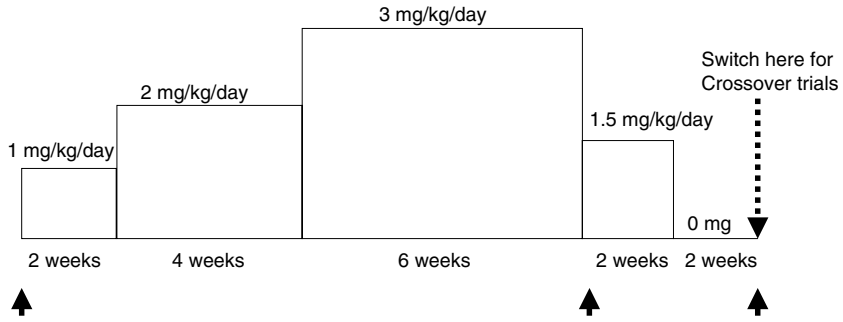

Fig. 1. Arrows indicate the time when behavioral symptoms, and blood 5-HT levels were determined, and hematological tests were conducted.

a four-grade scale from 1, indicating the least symptomatic, to 4, the most symptomatic. We examined the validity of BAS by comparing it with the Childhood Autism Rating Scale (CARS) (Schopler, Reichler, Devellis, & Dafy, 1980); we assessed the behavior of 108 children with autistic disorder using BAS and CARS. Specifically, 108 children with autistic disorder were assessed using both BAS and CARS, and statistical analyses were performed by the Pearson's correlation coefficiency using the total scores obtained by pediatric neurologists and psychologists. Because of the high correlation coefficiency evaluated to be 0.802 ($p < 0.0001$), we decided to adopt BAS as one of the tools for assessing autistic children.

Table I. Behavior Assessment Scale (BAS)*

Facial expression and eye movement
(1) Little natural change in expression (still expression)
(2) Nonspontaneous expression (stiff or loose expression)
(3) Avoidance of looking at people's faces (lack of eye contact)
(4) Flighty eye movements
Emotion and mood
(5) Poor emotional expression (lack of natural change in feeling)
(6) Inadequate emotional change (presenting emotions unsuitable for the situation)
(7) Emotional instability (moodiness)
Interest and will
(8) Narrow range of interest in things, activities and topics (obsession)
(9) Poor interest in or concern for stimuli or topics (indifference)
(10) Subjects of interest or concern easily changes (easily distracted)
Activities
(11) Hyperactive (inconstant behavior)
(12) Hypoactive (reticent behavior)
Attention
(13) Unable to appropriately shift focus of attention (selectivity)
(14) Poor concentration (sever degree, deep degree)
(15) Unsustainable attention (sustainability)
Personal relationships (ability to appropriately interest with adults or children)
(16) Inattentive toward other persons or social interaction
(17) Abnormal attitude towards others (overly familiar or rejective)
(18) Unwillingness to interact or have contact with others
Language
(19) Delayed speech or peculiar or inappropriate speech
(20) Indifferent or unresponsive to or noncomprehensive of oral instruction

* Patients are scored on a four-grade scale: 1, rare; 2, occasional; 3, sometimes; 4, frequent.

We calculated the efficacy points by subtraction of the score for the pretreatment from that for the post-treatment, and then evaluated the total efficacy points for the 20 items of BAS for each patient as well as the efficacy points for each item individually.

Finally, all of the patients were also rated based on the Clinical Global Impression Scale (CGI, National Institute of Mental Health, 1985) by a pediatric neurologist. The CGI score was 7 for a very-much-improved condition, and 1 for a very-much-worse condition. Patients with CGI scores indicating a very-much-improved or much-improved condition were classified as being excellent responders, and those who showed any improvements were classified as responders.

In order to evaluate the side effects of fluvoxamine, all of the patients received treatment every 2 weeks as outpatient, and hematological and blood 5-HT levels were determined before and after administration of fluvoxamine or placebo. The hematological tests included the determination of complete blood cell counts and blood chemistries. Moreover, blood 5-HT levels carried out by high-performance liquid chromatography (HPLC) (Anderson *et al.*, 1985) before and after treatments. The blood 5-HT levels in seven normal age-matched subjects served as control.

Molecular genetic analysis

Genomic DNA was extracted from peripheral blood leucocytes using a G NOME DNA extraction

kit (Bio 101 Inc., La Jolla, CA). Analysis of genetic polymorphism in 5-HTTLPR was performed as described by Cook *et al.* (1997). The PCR products were electrophoresed on 2% agarose gel and stained with ethidium bromide. Based on the amplified size of the PCR products, three genotypes (l/l, l/s and s/s) were identified.

Statistical analyses

Comparison of clinical effectiveness between patients of two genotypes or allele variations were analyzed based on scores obtained at the end of the treatment using the CGI scale, which was analyzed by the Mann–Whitney *U*-test. The ratings of behavioral symptoms of the patients administered the drug or the placebo were analyzed by the Wilcoxon signed-rank test based on the efficacy points. We compared the blood 5-HT level between controls and patients. When the *F*-value was considered significant at $p < 0.05$, Welch's *t*-test was applied for statistical evaluation; otherwise, the unpaired Student's *t*-test was used to compare differences between the two genotypes with respect to the blood 5-HT level. The paired Student's *t*-test was applied to compare the blood 5-HT level between pre- and post-treatment in all patients, patients with the two genotype groups and allele variations. Statistical data are presented as means ($\pm$SD) and estimated as significant at $p < 0.05$ (2-tailed). The statistical software employed was Stat View Version 5.0 (SAS Institute, Inc.).

RESULTS

Genetic polymorphism in patients with autistic disorders

One case was excluded because of noncompliance to the drug administration. Eighteen patients completed the 26-week study and were thus included in the efficacy analysis.

Among our patients, one had genotype l/l, seven had genotype l/s and ten had genotype s/s. The l-allele frequency of 5-HTT LPR was estimated to be 0.25. The patient with genotype l/l was a 5-year-old female, those with l/s consisted of 6 males and 1 female with a mean age of 6 years and 2 months, and those with s/s consisted of 9 males and 1 female with a mean age of 5 years and 10 months.

Clinical global impressions (Table II)

From a clinical point of view, five of the 18 (28%) cases were classified as excellent responders. In the case of those who exhibited minimal improvement, as estimated by CGI, fluvoxamine treatment was clinically effective in 10 of the 18 (56%) cases. Moreover, correlational analyses between genotype or allele variation of 5-HTTLPR and the CGI scores revealed that fluvoxamine tended to be more effective in patients with genotype l/l + l/s than in those with genotype s/s, and was significantly more effective in the l allele variant than the s allele variant. Undesirable symptoms, such as hyperactivity in three patients and nausea during the initial period of treatment in four patients, were observed.

Clinical effectiveness assessed using BAS and genotype (Table III)

A comparison between drug and placebo effects with respect to individual items of BAS is shown in Table III. Statistically significant drug effects were noted for the flighty eye movements (item 4) and the delayed speech or peculiar or inappropriate speech (item 19). Regarding the genotype, no significant drug effects were demonstrated in the patients with genotype l/l + l/s, however a significant improvement with respect to the delayed speech or peculiar or inappropriate speech (item 19) in patients with genotype s/s was observed.

Clinical effectiveness assessed using BAS and allele variation

At the initial assessment performed before treatment, there was no significant difference between the l

Table II. Clinical Global Impression Scale

	CGI scores	Total No.	Number of genotype l/l + l/s	Number of genotype s/s
Very much improved	7	1	1	0
Much improved	6	4	3	1
Minimally improved	5	5	2	3
No change	4	8	2	6
Minimally worse	3	0	0	0
Much worse	2	0	0	0
Very much worse	1	0	0	0
Total No.		18	8	10

No.: Number of patients.
l/l + l/s v s/s $p = 0.065$. (Mann–Whitney *U* test).
l v s $p = 0.0471$* (Mann–Whitney *U* test).

Table III. Fluvoxamine effect on behavior of patients with autistic disorder

BAS item number	Total	Genotype		Allele	
		l/l + l/s	s/s	l	s
1	0.2879	0.3173	0.4795	0.3173	0.1511
2	0.1316	0.1025	0.4795	0.0588	0.1171
3	0.8256	0.1573	0.4142	0.1573	0.8892
4	0.0470*	0.1573	0.1797	0.1573	0.0209*
5	0.3147	0.0588	0.9999	0.0339*	0.4786
6	0.4000	0.7055	0.3173	0.7055	0.1851
7	0.5208	0.7389	0.1067	0.7389	0.0824
8	0.5417	0.7921	0.2059	0.4705	0.0903
9	0.7432	0.2878	0.4290	0.1463	0.6314
10	0.6555	0.9999	0.5887	0.7825	0.4634
11	0.4575	0.3173	0.4530	0.3173	0.4699
12	0.2604	0.5637	–	0.3173	0.1936
13	0.8138	0.3657	0.8918	0.1898	0.9415
14	0.8087	0.9999	0.9999	0.9999	0.9638
15	0.4413	0.3173	0.9999	0.1597	0.8601
16	0.4863	0.1573	0.9999	0.1573	0.5271
17	0.8601	0.2568	0.4142	0.1290	0.4170
18	0.6819	0.5637	0.7055	0.5637	0.4669
19	0.0400*	0.9999	0.0256*	0.6547	0.0013*
20	0.3872	0.3173	0.6547	0.3173	0.2850

BAS: Behavioral Assessment Scale.
Assessment of individual items of BAS between drug and placebo (Wilcoxon signed-rank test).
*$p < 0.05$.

and s alleles except for the high scores observed in emotional instability (item 7) in the s allele compared to the l allele. Significant improvement with respect to poor emotional expression (item 5) was observed in the l allele. On the other hand, significant improvements with respect to flighty eye movements and delayed speech or peculiar or inappropriate speech (item 4 and 19) were noted in the s allele.

Blood 5-HT level (Table IV)

Blood 5-HT levels are presented in Table IV. The basal blood 5-HT level was significantly higher in the children with autistic disorder than in the control children. A marked decease in the blood 5-HT level was observed after the fluvoxamine treatment but no change was observed after the placebo treatment. No significant correlation between CGI and blood serotonin level before fluvoxamine treatment was observed (correlation coefficient of 0.194, $p = 0.4317$) (Fig.2a) There was also no significant correlation between CGI and the ratio of blood serotonin level reduction (level after treatment/level before fluvoxamine treatment) (Correlation coefficient of −0.073, $p = 0.7711$) (Fig. 2b).

Table IV. Blood serotonin level

		Number	Before treatment	After treatment
Control		7	172.1 ± 45.0*	
Patient		18	261.1 ± 113.9	48.9 ± 28.9**
Patient	Genotype			
	l/l + l/s	8	263.4 ± 131.6	47.9 ± 29.9**
	s/s	10	259.3 ± 105.0	50.2 ± 30.8**
	Allele			
	l	9	271.1 ± 125.3	48.3 ± 28.0**
	s	27	257.7 ± 110.0	49.4 ± 29.9**

ng/ml.
Mean ± SD.
*Patient and control; $p < 0.01$ (Welch's *t*-test).
**Before and after treatment of patients; $p < 0.0001$ (paired student's *t*-test).
Comparison of two genotypes (l/l + l/s vs s/s) or allele; Ns (unpaired student's *t*-test).

There was no difference in the blood 5-HT level before and after fluvoxamine treatment between the genotypes or allele variations.

DISCUSSION

There are many studies on the effectiveness of SSRIs for treating autistic disorder (DeLong *et al.*, 1998; Gordon *et al.*, 1993; McDougle *et al.*, 1990; 1996; Mehlinger *et al.*, 1990). This study is the first to describe a therapeutic response to fluvoxamine administered to children with autistic disorder using a double-blind placebo-controlled study design.

Several studies on the clinical effectiveness of SSRIs and 5-HTTLPR, that is, fluvoxamine for patients with depression (Smeraldi *et al.*, 1998), fluvoxamine or paroxetine for patients with major depression (Kim *et al.*, 1999) and paroxetine for patients with late-life depression (Pollock *et al.*, 2000) have been reported. Our study provides the first clinical evidence that allelic variation in 5-HTTLPR may affect the response of young patients with autistic disorder to fluvoxamine.

Although the study sample was small, based on the therapeutic efficacy of fluvoxamine administered to Japanese children with autism, considerable clinical global improvement was recognized in five cases (27.8%); when we included those showing slight improvement the number increases to 10 out of 18 cases (55.6%). Several studies have been conducted using SSRIs for patients with autism. McDougle *et al.*, (1996) demonstrated that 8 (53%) out of 15 adult patients with autism responded favorably to

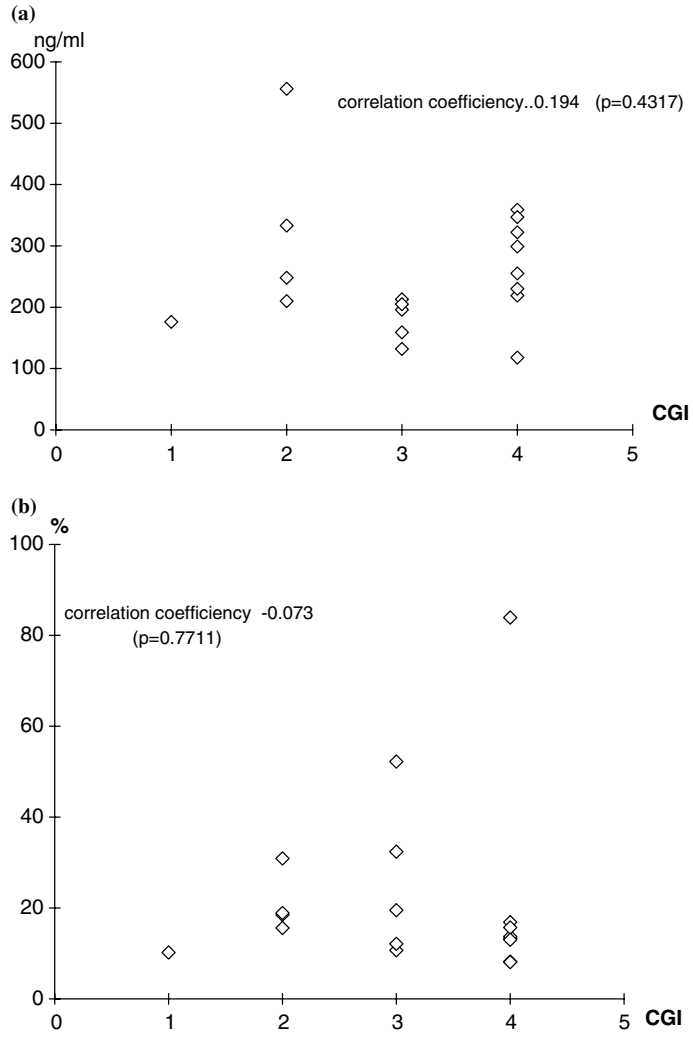

Fig. 2. (a) Relationship between CGI and blood 5-HT level. The open square indicates data for one subject. (b) Relbationship between CGI and the ratio of blood serotonin level after 5-HT treatment to that before 5-HT treatment. The open square indicates data for one subject.

fluvoxamine. Marked improvement was noted in 11 (29.7%) of 37 cases; when patients regarded as having only slight improvement were included, 22 cases (59.5%) were considered to be responders based on the case study of DeLong *et al*. (1998) using fluoxetine on young children. The observation period by DeLong *et al*. (1998) was 13–33 months, which was longer than our study period (12 weeks). Because DeLong *et al*. (1998) suggested that negative changes could occur several months after the initiation of fluoxetine treatment, our study design might only be determining the short-term effects; therefore, we should carefully monitor the patients for a longer period to determine the long-term effects.

DeLong *et al*. (1998) reported that clinical efficacy was noted in terms of behavior, language, cognition, affection and social skill in an open-label trial of fluoxetine for autistic children. They also reported that marked improvement in language ability was observed in children following the fluoxetine treatment compared with an adult or other treatment batteries for autism. Our results agree with theirs particularly in terms of the clinical efficacy of fluvoxamine with regard to the delayed speech or peculiar or inappropriate speech, although they are not as dramatic as those of DeLong *et al*. (1998). It may be effective to treat children with SSRIs because it is at this stage that they acquire language abilities.

DeLong (1999) also proposed the intriguing hypothesis that the idiopathic form of autistic disorder represents a low 5-HT state, usually localized in the left hemisphere. SSRIs specifically increase 5-HT activity in the brain by increasing 5-HT availability in synapses. It may be possible to put forth that improvement in language ability is due to improvement of the 5-HT state in the left hemisphere, as supported by our results.

The 5-HTTLPR polymorphism may play an important role in 5-HTT gene expression (Lesch *et al*., 1996 and Karley et al., 1998), therefore it is worth studying the corelation between the effects of SSRIs and genetic polymorphism of 5-HTT. Our subjects exhibited an s-allele frequency of 0.75, almost identical to the reported frequency of 0.83 (Nakamura *et al*., 1997) or 0.86 (Ishiguro *et al*., 1997) in the Japanese population; however, a frequency of 0.41 (Cook *et al*., 1997) in the Caucasian population has been reported, suggesting an apparent ethnic difference in the genetic polymorphism of 5-HTTLPR. It is reported that the frequency of genotype l/l in the Japanese population is low (Ishiguro *et al*., 1997; Nakamura *et al*., 1997), and we had only one case with the genotype l/l.

Our results concerning allele types reveal that fluvoxamine use with autistic children is more effective in the l allele variant than the s allele variant based on CGI. These results agree with those of 6-week treatment with fluvoxamine for depression (Smeraldi *et al*., 1998). Namely, homozygotes for the long variant (l/l) and heterozygotes (l/s) of the 5-HTT promoter exhibited a better response to fluvoxamine than homozygotes for the short variant (s/s). Controversial results of 6-week treatment with fluoxetine or paroxetine for Korean patients with depression have been reported (Kim *et al*., 1999); patients exhibiting homozygous s/s in the 5-HTTLPR showed better responses than those exhibiting other genotype. Pollock *et al*., demonstrated that patients of genotype l/l were associated with a more rapid response to paroxetine treatment than those possessing one or two s alleles, and no significant differences in the number of responders were obsereved between l/l and s groups at 12 weeks. Lesch *et al*. (1996) found that the s allele reduces the transcriptional efficiency of the 5-HTT gene promoter, resulting in a decrease in the 5-HTT expression level and 5-HT uptake in human lymphoblasts. Little *et al*. (1998) showed that 5-HTT mRNA levels in human postmortem brain subjects exhibiting the l/s and s/s genotype were clearly lower than in subjects exhibitng the l/l genotype. The amount of 5-HT in the synaptic space may be different between allele variations in response to SSRIs, which may cause the different responses through the 5-HT receptors, resulting in different fluvoxamine effects between allele variations in autistic disorder. In our study, although clinical global effectiveness was noted in the l allele rather than in the s allele when each item of BAS was analyzed, drug efficacy was noted in the emotional expression in the l allele, and in the eye movement and language use in the s allele. These observations suggest that allelic variation of 5-HTTLPR may contribute to the variable responses in young patients with autistic disorder treated with fluvoxamine. It may be speculated that clinical manifestations in autistic children depend upon serotonin-related events in the brain although they are also probably influenced by other monoamines. Therefore it should be determined whether the effect of fluvoxamine administrations is related to the correction or increase in serotonin level or not.

Although the mechanism underlying the difference in clinical efficacies with respect to genetic polymorphism is as yet unknown, the effects of fluvoxamine may be attributed to the increase in 5-HTT levels within the synaptic cleft facilitating stimulation of neural transmission. It is difficult to compare the changes in 5-HT levels in the brain before and after SSRI treatment. We determined the blood 5-HT levels before and after fluvoxamine treatment. Although a significant decrease in the blood 5-HT level was observed after fluvoxamine administration, there was no significant correlation between CGI and the ratio of reduced blood 5-HT level (level after fluvoxamine treatment/levels before fluvoxamine treatment). No significant correlation between genetic polymorphisms in 5-HTTLPR for blood 5-HT levels before and after fluvoxamine treatment was also observed. Moreover, there was no significant correlation between CGI and blood 5-HT level before fluvoxamine treatment. These results suggest the difficulty in predicting the clinical efficacy of fluvoxamine administration on the basis of both blood 5-HT level before fluvoxamine treatment and the rate of reduction in blood 5-HT level. There was also no significant difference in this level between groups, in terms of genetic polymorphism in this study. It is considered that the changes in the 5-HT level in the brain after fluvoxamine administration may be so irregular that the blood 5-HT level does not always reflect the changes in the 5-HT level in the brain.

The limitations of this study are as follows. First, only one patient with genotype l/l (ethnic difference) was observed because of the small number of patients enrolled in this study. Second, we used BAS, an unconventional but reliable tool compared with CARS, in the assessment of the outcomes of our autistic patients. Third, a lower dose of fluvoxamine was used in this study than in studies in Europe and the USA., which was based on the recommended dose of 50 to 150 mg/day for Japanese adults, about half of that for their European/American counterparts. Further studies are necessary to resolve these issues.

We conclude that fluvoxamine is significantly effective in treatment of young children with autistic disorder. The drug was well tolerated without significant adverse effects, other than transient nausea and hyperactivity. Also, our study indicates that the allele variation of 5-HTTLPR in children with autistic disorder may influence their clinical response to fluvoxamine treatment. In addition, the evaluation of the clinical responses should be carried out taking into account the doses of fluvoxamine or duration of fluvoxamine treatment.

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