

ている「聴こえとことばの教室」に通い始めた。2年生に進級した後、学習障害の疑いにて来院した。来院時には、構音障害は消失していた。児は文字を読むときに指を折ったり、五十音表をつぶやいたりしながら文字を探す傾向が見られた。

### 〔神経心理学的検査所見〕

WISC-Rでは、VIQ103、PIQ86と正常であったが、視覚課題で得点が低下していた。立方体透視図の模写も困難で、視覚的および視空間的課題の成績が低下していた。また、RCPM検査、Benton視覚記銘検査（直後再生）とも低下しており、視覚、視空間的な認知障害が認められた。

### 〔言語病理学的所見〕

7歳5カ月時のSLTAでは、小学校2年生の平均正答率と比べて音読と理解の項目、漢字の項目でそれぞれ5%以内の有意な差で正答率が低かった。音読、書き取りの検査では仮名1文字（清音44音）の音読、書き取りは65%程度が可能であったが、時間がかかった。

小学校2年生までの国語の教科書から選択した仮名单語の音読は5割の正答で、書き取りでは6割の正答を得た。また、小学校1年生で習う漢字単語の音読と書き取りの正答は2割以下であった。漢字書き取りの誤反応パターンを見ると7割が無反応で、その他は形態的誤りや意味的誤りが見られた。

以上の特徴から、本例を発達的読み書き障害と診断した。

### 〔局所脳血流低下部位〕

後頭葉内側面や側頭葉下部、前頭葉での左右差は4 ml/以内であったが、左角回を含む下頭頂小葉において右側に比べて12ml/100g/min低下していた。

### 〔考 察〕

本例は小学校2年生になっても仮名の読み書き

で文字と音の1対1の対応関係が完成されていない。このため音と文字の関係を直接要求すると誤りやすく、すぐに正答できないが、五十音表の配列にしたがって仮名の探索を行うと正確で比較的早く可能となった。すなわち、「あ」列の1行を横方向に探索し、次にその音を手がかりに各行を縦に探索することで、音と文字の対応を引き出す傾向が観察された。

五十音表は、視空間的に構成されていると同時に、きわめて自動性の高い音のつながりを持っている。たとえば「あかさたな」という音のつながりや、「あいうえお」「かきくけこ」などの各行の自動性の強い音は、五十音表の文字配列と強く結びついている。本例では、音と文字の直接的な変換過程と並列に五十音表という迂回路を形成することで、仮名の読み書きがより正確で安定したものと変化していったと思われる。本例はWISC-R、K-ABC、立方体の模写、RCPM検査の結果から視覚-視空間認知能力に障害があるため、この「音の配列」を手がかりとして、五十音表の文字配列を引き出している可能性が考えられた。

本例の漢字仮名双方に見られる読み書き障害の臨床経過は、左角回病変の失読失書に似た症状を呈していたと考えられる。その意味で本症例が左角回の血液低下を示していたことは読字困難児の機能的局在病変を初めて示したという点で重要である。

## そのほかの学習障害

### 1. 算数障害

本障害については現在、はっきりとした概念は確立されておらず、明確な定義はなされていない。一部は読字困難に起因する者もあるが、純粋な計算障害例も存在するようである。現象としての計算障害や文章題の理解困難などが、本人のいかなる能力障害と関連しているか、神経心理検査を含めて詳細に検討するべきと思われる。

### 2. 言語性意味理解障害

非言語的な意味理解力が保たれるものの、言語

表7 精神遅滞の診断基準 (DSM-IV)

- |   |
|---|
| <p>A. 明らかに平均以下の知的機能：個別実施による知能検査で、およそ70またはそれ以下のIQ（幼児においては、明らかに平均以下の知的機能であるという臨床的判断による）。</p> <p>B. 同時に、現在の適応機能（すなわち、その文化圏でその年齢に対して期待される基準に適合する有能さ）の欠陥または不全が、以下のうち2つ以上の領域で存在：<br/>意思伝達，自己管理，家庭生活，社会的／対人的技能，地域社会資源の利用，自律性，発揮される学習能力，仕事，余韻，健康，安全。</p> <p>C. 発症は18歳未満である。</p> |
|---|

的な意味理解能力の低下が見られる特異的な発達障害児である。多くは言語発達の遅れで気づかれ、心理検査上、WISC-IVの動作性IQがほぼ正常域にあり、言語性IQとの乖離が見られること、復唱と音読、とくに仮名の音読は正確にできるのに内容の理解が悪いこと、ITPAでは聴覚・言語-音声性項目が視覚・非言語-運動性項目よりも得点が低いという所見を示す。

その神経心理学的特徴は音韻処理と意味処理過程の機能的乖離であり、優位半球である左側頭葉の局所脳血流低下が知られている。言語学の領域で最近、特異的言語機能障害 (SLI) として報告されている状態像と重なる概念であり、のちに述べる広汎性発達障害児に見られることもある。抽象語理解力検査を行い、評価する必要がある。

## 鑑別診断

### 1. 知的障害 (精神遅滞)

知的障害 (精神遅滞, Mental retardation ; MR) とは全般的知的機能が同年齢の平均よりも明らかに低く、適応機能の明らかな制限がある状態を言う (表7)。精神遅滞は発達期に生じ長期にわたって見られる。発達期とは通常、出生前の胎生期から概ね18歳までを言う。DSM-IVの診断基準を表7に示す。精神遅滞は状態を示す疾患名で、中枢神経系の機能に影響を与えるさまざまな病態で見られる疾患群であると考えられる。

全般的知的機能は、WISC-IVなど標準化された個別施行による1つ以上の知能検査によって得られた知能指数 (IQ) によって評価される。「明らかに平均より低い」とは、IQが70以下という定義が一般的に用いられる。しかしIQ低下とと

もに適応能力に明らかな制限がなければ精神遅滞とは診断されない。適応機能とは、日常生活でその人に期待される要求に、いかに効率よく適切に対応し自立しているかを表す機能である。

### 2. 注意欠陥多動性障害 (Attention Deficit/Hyperactivity Disorder ; ADHD)

ADHDは多動と不注意があり、衝動的な行動のため社会的な不利益を被っている状態を言う。ADHDは学習障害とくに読字困難を合併する場合がある。注意力の問題や多動によって学習が損なわれてしまう、いわゆる二次障害による学業不振の場合にはADHD + LD合併例なのかADHD単独なのかを見極める必要がある。診断その他については、他稿のADHDの項を参考にされたい。

### 3. 自閉症スペクトラム、とくに高機能自閉症

自閉症の中で、WISC-IVなどのIQ検査で70以上である、すなわち知的機能が決して低くない例を高機能自閉症と言うことがある。自閉症では、認知機能の偏りを示す場合が多く、学習障害との類似性あるいは鑑別が必要となる。幼児期に視線の合いにくさがあり、社会的な関係を築くことが困難な行動特徴があるものの、それらが目立たなくなると同時に学習障害様の症状を示す例もときにあるため、一例ごと経過を長く観察する必要があると思われる。自閉症の診断・対応については他稿に詳しいのでそちらを参照されたい。

### 学習障害の対応について

学習障害は小学校に入学してから初めて明らか

になることが多い。通常、本人の努力だけでは読み書きの障害は改善しない。教師や親からは、「さぼっている」「話ができるのに読めないのは、できないふりではないか」と誤解されることもある。安易な叱咤激励によって、本人の意欲がなくなり、自信をなくしていることもしばしば見られる。したがって、本人の自信をつけさせながら、指導するということがもっとも大事である。

学習障害の診断と評価に関しては、認知機能をさまざまな側面から解析して、機能低下が見られるところと機能が十分働いているところを明確にするのがよい。すなわち、より良好に保たれている機能を活用するというアプローチが重要となってくる。先に述べた漢字と仮名の読み書き障害児に対しては、五十音表を介したルートを活用したひらがなの書字が有効であったと述べられている。これは、視空間的に構成され音の配列とも強く結びついている五十音表が迂回路として活用できた一例であろう。

発達性読み書き障害に対する書字訓練法として確立されたものはいまだないが、視覚や聴覚などモダリティ別情報処理過程に焦点をあてた訓練が最近、報告されている。一方、平仮名や漢字の書字障害について、コンピュータを用いた支援法も出版されている。学習障害児一人ひとりの病態は異なっているので、同じ指導法（ないしソフトウェア）がまったく同じ効果を生むとは思えない。しかし、個人の特徴に基づいた使用法や指導法を行えば、本ソフトも有用なものと思われる。

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# Non-Skewed X-Inactivation May Cause Mental Retardation in a Female Carrier of X-Linked $\alpha$ -Thalassemia/Mental Retardation Syndrome (ATR-X): X-Inactivation Study of Nine Female Carriers of ATR-X

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X-linked  $\alpha$ -thalassemia/mental retardation syndrome (ATR-X) is a syndromic form of X-linked mental retardation. We investigated the X-inactivation status of nine female ATR-X carriers by methylation-specific PCR of the *HUMARA* gene. Six carriers demonstrated a skewed X-inactivation pattern (>90:10) and one showed a non-skewed pattern (72:28), while two were uninformative because of homozygosity for the CAG repeat polymorphic alleles in the *HUMARA*. Only the carrier mother who showed non-skewed X-inactivation had moderate mental retardation. These findings suggest that mutations in *ATR-X* may cause mental retardation in females, if the X chromosome carrying mutated *ATR-X* is not properly inactivated. © 2005 Wiley-Liss, Inc.

**KEY WORDS:** *ATR-X*; *XLMR*; mental retardation; X inactivation; female carrier

## INTRODUCTION

X-linked  $\alpha$ -thalassemia/mental retardation syndrome (ATR-X, MIM #301040) is a syndromic form of X-linked mental retardation, and characterized by severe mental retardation (MR) in males, dysmorphic facies, hemoglobin H (HbH) inclusions, genital abnormalities, skeletal abnormalities, and characteristic posture and/or behavior [Gibbons and Wada, 2004]. More than 160 patients from 90 families were diagnosed as ATR-X [Wada et al., 2000; Gibbons and Wada, 2004]. ATR-X is caused by a mutation in the *ATR-X* gene (*ATR-X*). *ATR-X* is also involved in other syndromes, including Juberg-Marsidi syndrome, X-linked MR with spastic paraplegia, Carpenter-Waziri syndrome, Holmes-Gang syndrome and Smith-Fineman-Myers syndrome, Chudley-Lowry syndrome, and non-specific MR in males [Abidi et al., 2004; Gibbons and Wada, 2004]. A few reports demonstrated that most female carriers of an *ATR-X* gene mutation had a skewed X-inactivation (XI) pattern and no mental retardation (MR) [Gibbons et al., 1992].

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However, no MR female with an *ATR-X* mutation has been described.

Here we describe the result of X-inactivation study in nine female carriers of *ATR-X* mutations. One of them had MR and showed non-skewed XI. To our knowledge, this is the first report suggesting that *ATR-X* causes MR in females.

## MATERIALS AND METHODS

### Subjects Studied

We investigated 17 female relatives of ATR-X probands, including 11 mothers, 5 sisters, and an aunt, from 11 Japanese families in which the disease of the probands were diagnosed molecular genetically. Of the 11 patients, 1 was familial case (two patients from Family 2), and 10 were sporadic cases. We obtained informed consent from all these families, and the study protocol was approved by IRBs of Shinshu University School of Medicine and Hokkaido University Graduate School of Medicine. None of the female relatives had HbH inclusions in their peripheral blood cells, and none but one (mother of Family 8) had mental retardation. The mother who had moderate MR never presented with evidence of any other ATR-X manifestations, such as dysmorphic facies or HbH inclusions. She was 56 years old, but her communication ability is low and her age of social life was estimated as 9 years and 8 months. She cannot write a letter or spend money adequately.

### Mutation Detection

Genomic DNA was purified from peripheral blood leukocytes by standard methods. PCR-based direct sequencing was performed in the 17 female relatives to know whether they had same mutations as had the ATR-X probands. Once *ATR-X* mutations were observed in the females, the mutations were confirmed by PCR-RFLP as described previously [Wada et al., 2000].

### X-Inactivation Study

An XI pattern was analyzed at the human androgen receptor (*HUMARA*) locus using a methylation-specific PCR (M-PCR) method [Kubota et al., 1999]. Briefly, genomic DNA (1  $\mu$ g) was modified with sodium bisulfite using an EZ DNA methylation Kit™ (ZYMO RESEARCH, Orange, CA). The modified DNA was PCR-amplified using two pairs of methylation-specific PCR primers, AR-M that amplifies the methylated (inactive X) sequence, and AR-U that amplifies the unmethylated (active X) sequence. Two alleles inherited from different parents were differentiated by heterozygosity for the CAG repeat polymorphic alleles in the *HUMARA* gene. PCR products were separated on an 8% acrylamide gel and analyzed on ALF Express DNA automated sequencer (Pharmacia, Uppsala, Sweden).

TABLE I. Summary of Mutation Carrier Status and XI Patterns in Female Relatives

Family no.	Mutation in patients	Relationship to patient	Mutation carrier	XI ratio	XI pattern
1	Y1847C	Mother	Yes	>99:1	Skewed
2	V1552F <sup>a</sup>	Mother	Yes	>99:1	Skewed
3	P190L	Mother	No	—	Uninformative
		Sister	No	67:33	Random
4	R246C	Mother	Yes	—	Uninformative
5	R246C	Mother	Yes	>99:1	Skewed
		Sister	Yes	92:8	Skewed
6	V194I	Mother	No	73:27	Random
		Sister	No	—	Uninformative
		Sister	No	62:38	Random
7	L1645S	Mother	Yes	>99:1	Skewed
8	V194A	Mother	Yes	72:28	Non-Skewed
		Sister	No	70:30	Random
9	S576stop	Mother	No	70:30	Random
10	Y266C	Mother	Yes	—	Uninformative
11	R246C	Mother	Yes	>99:1	Skewed
		Aunt	No	—	Uninformative

<sup>a</sup>Familial case.

Peak images of separated PCR products were measured by Allele-links software (Pharmacia), and the ratio of XI between the two products was calculated. The XI ratios were confirmed by conventional PCR using methylation-sensitive restriction enzymes as described previously [Allen et al., 1992]. We defined a ratio of more than 90:10 as "skewed," and 90:10 or less as "random." In carrier females in whom a skewed pattern was expected, lack of the pattern was described as "non-skewed."

## RESULTS

Eight (72.7%) out of the 11 mothers examined and one (Family 5) of five sisters from four families were carriers and heterozygous for an *ATR-X* mutation (Table I). Altogether, we identified nine female carriers. Results of the XI analysis are shown in Table I and Figure 1. Two carrier and three non-carrier females were uninformative because of homozygosity for the CAG repeat polymorphic alleles in the *HUMARA* gene. Among seven informative carriers, six mothers (Families 1, 2, 5, 7, and 11) and one sister (Family 5) showed a skewed XI pattern and the remaining one mother (Family 8) a non-skewed pattern (72:28). Although six such carriers had normal intelligence, the last mother had moderate MR. The ATR-X child of this mother had a V194A mutation, who showed typical manifestations of ATR-X but no HbH inclusions. All five informative non-carrier females had random XI patterns.

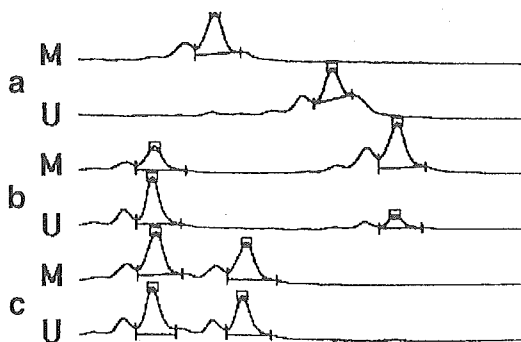


Fig. 1. Results of methylation-specific PCR assay. Peak images of mother of Family 7 (a), and mother (b) and her daughter (c) of Family 8, showing skewed (a), non-skewed (b), and random (c) XI patterns, respectively. M: AR-M amplification, U: AR-U amplification.

## DISCUSSION

Reproductive fitness of ATR-X patients is presumed to be zero. Our observation that 8 (72.7%) of the 11 mothers were carriers of an *ATR-X* mutation is consistent with an estimate that one third of all copies of a mutated gene are lost in each generation, and one third of all persons with severe X-linked disorders are predicted to carry a new mutation [Nussbaum et al., 2001]. However, 7 (70%) of 10 mothers of sporadic cases were carriers (Table I), the figure being lower than the previously estimated prevalence of 85% [Bachoo and Gibbons, 1999]. We could not exclude in the remaining three mothers the possibility of germline mosaicism that was previously reported in two ATR-X families [Bachoo and Gibbons, 1999].

Female carriers of X-linked disorder are usually asymptomatic, and the same seems true for female ATR-X carriers [Gibbons et al., 1992; Lossi et al., 1999; Yntema et al., 2002]. One reason for this phenomenon is skewed XI, where the mutated allele is selectively inactivated through the following two alternative mechanisms: a bias in the initial choice of X chromosome to inactivate; and cell selection following the initial, random XI. It was reported that an *XIST* mutation could have caused skewed XI and suggested that *XIST* may affect the primary choice of X chromosome [Newall et al., 2001]. Alternatively, the cell selection secondary to the random XI may result in leaving cells with the preferentially active wild-type allele, because such cells may have some advantage for their proliferation or growth. A recent study revealed that female carriers of X-linked MR likely showed skewed XI [Plenge et al., 2002], suggesting that a selection mechanism may regulate XI in these carriers.

Almost all previous reports stated that female ATR-X carriers showed skewed XI, suggesting that the mutated *ATR-X*-bearing X chromosomes were preferentially inactivated [Gibbons et al., 1992; Lossi et al., 1999; Yntema et al., 2002]. Only a mentally normal female ATR-X carrier who had HbH inclusions and non-skewed XI inactivation pattern has been reported [Gibbons et al., 1992]. In this context, the mentally retarded mother of Family 8 whose XI pattern was non-skewed is unusual and merits comment. A few explanations are possible for how female ATR-X carriers escape skewed XI. Some mutations would be less harmful for cell growth and/or survival than other mutations. But this is unlikely in our case, because the patient with V194A also leads to typical phenotype. Secondly, some mutations could make X chromosome to escape skewed inactivation via their unknown effect on silencing of other gene(s) on X chromosomes. The ADD domain

in ATRX protein found also in the de novo DNA methyltransferase 3b (*DNMT3B*) gene is implicated in immunodeficiency-centromeric instability-facial anomalies (ICF) syndrome (MIM #242860) [Xie et al., 1999], and mutations in *DNMT3B* lead to disruption of silencing genes on the inactive X chromosome [Hansen et al., 2000]. Thus, ATRX protein may play a role in the establishment and maintenance of DNA methylation, although it has no intrinsic methyltransferase activity [Gibbons et al., 2000]. We could not test the hypothesis that her mutation caused the aberrant XI because we have no other female carriers with the same mutation. Thirdly, female carriers could have some genetic factors that control randomness or nonrandomness of XI, e.g., an *XIST* mutation causing a strong bias towards the inactive X chromosome.

Although it is difficult to analyze the XI ratio in the brain, we speculate that her MR without HbH inclusions may be due to cell type-specific XI, i.e., her mutated allele (V194A) is not completely inactivated, leading to either the expression of mutant ATRX protein or decreased expression of normal protein in her neuronal cells, although the mutated allele may be favourably inactivated in the erythroid cell. It remains to be investigated whether cell type-specific XI is seen in her mesodermal (blood cells), endodermal (buccal cells), and ectodermal (hair root) tissues. A recent study has demonstrated differential XI patterns between neuronal and non-neuronal tissues in Rett syndrome, an X-linked MR disorder [Gibson et al., 2004]. It is likely that in female *ATRX* mutation carriers, neurons in which the wild-type allele is inactivated have a significant selective disadvantage during development, because the brain tissue is one of the most severely affected organs in the disease. Mouse models of ATR-X will provide an important resource for investigation of the XI status in the brain, as did a model for Rett syndrome [Young and Zoghbi, 2004].

If skewed XI favoring the wild-type allele expression prevents manifestations in ATR-X female carriers, interruption of skewed XI in the brain may cause partial disease phenotype. Therefore, our data suggest that *ATRX* mutations can cause MR in females, unless the mutated allele is properly inactivated in the brain. It remains to be investigated whether other female carriers with MR show non-skewed X-inactivation.

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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 inhibitor; 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-methyltryptophan) 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 hematolog-

ical 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, Brenner, & 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



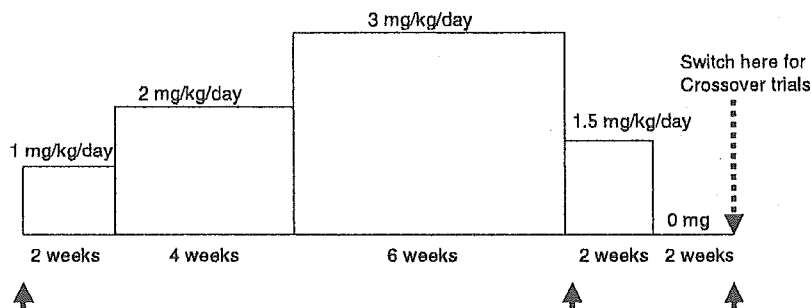


Fig. 1. Arrows indicate the time when behavioral symptoms, and blood 5-HT levels were determined, and hematological tests were conducted.

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 coefficient using the total scores obtained by pediatric neurologists and psychologists. Because of the high correlation coefficient 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)\*

<i>Facial expression and eye movement</i>	
(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
<i>Emotion and mood</i>	
(5)	Poor emotional expression (lack of natural change in feeling)
(6)	Inadequate emotional change (presenting emotions unsuitable for the situation)
(7)	Emotional instability (moodiness)
<i>Interest and will</i>	
(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)
<i>Activities</i>	
(11)	Hyperactive (inconstant behavior)
(12)	Hypoactive (reticent behavior)
<i>Attention</i>	
(13)	Unable to appropriately shift focus of attention (selectivity)
(14)	Poor concentration (sever degree, deep degree)
(15)	Unsustainable attention (sustainability)
<i>Personal relationships (ability to appropriately interest with adults or children)</i>	
(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
<i>Language</i>	
(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 decrease 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
	s/s	10	259.3 ± 105.0
	Allele		
	l	9	271.1 ± 125.3
	s	27	257.7 ± 110.0

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

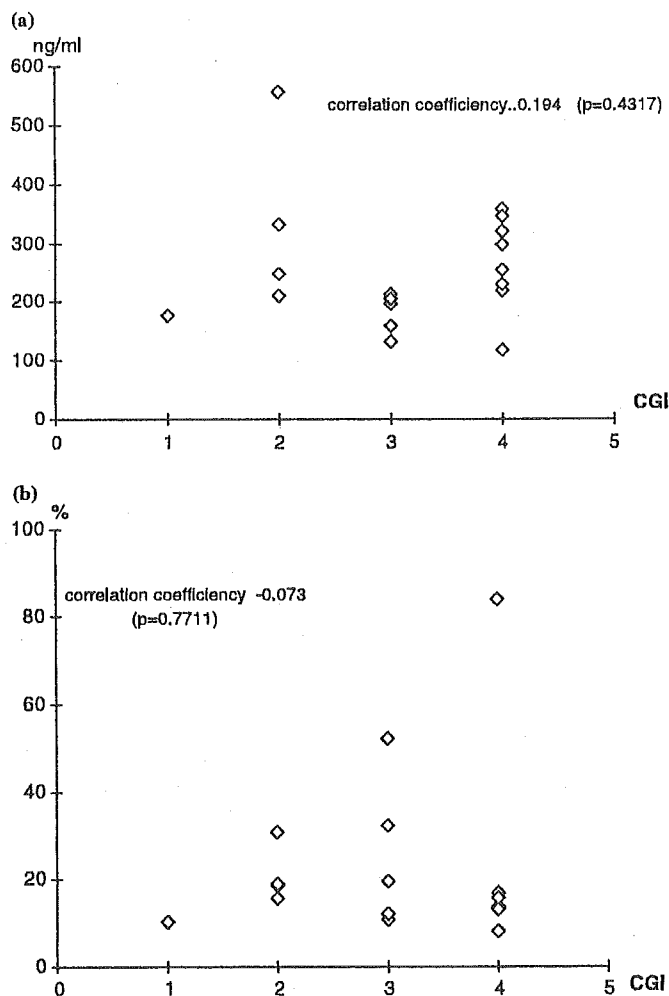


Fig. 2. (a) Relationship between CGI and blood 5-HT level. The open square indicates data for one subject. (b) Relationship between CGI and the ratio of blood serotonin level after 5-HT treatment to that before 5-HT treatment. The open square indicates data for one subject.

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 correlation 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 observed 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

exhibiting 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 allelic 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.

#### ACKNOWLEDGMENTS

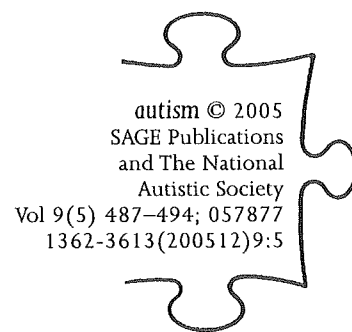
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# Neonatal factors in infants with Autistic Disorder and typically developing infants



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**ABSTRACT** The prenatal and neonatal factors of 225 children diagnosed with Autistic Disorder were compared with those of 1580 typically developing children. Each of the neonatal factors was compared between the Autistic Disorder and control groups, and between males and females. The results showed that males in the 'Autistic Disorder' group had a significantly longer gestational age and a heavier birth weight than the male controls. No significant differences in these factors were observed between females in the two groups. Both male and female children with Autistic Disorder showed a significantly higher incidence of neonatal complications than their respective controls. In the Autistic Disorder group, males had a heavier mean birth weight, and there were more post-term infants among females.

**KEYWORDS**  
autistic disorder;  
neonatal complications;  
pervasive developmental disorders

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## Introduction

Autistic Disorder is characterized in DSM-IV by impaired social interaction, abnormal communication skills and a limited range of activities and interests. The critical mechanisms underlying autism spectrum disorders (ASD) have not yet been clarified but causation is thought to be related to several overlapping factors. Currently genetic factors are considered to be the primary cause (Folstein and Rosen-Heidley, 2001). However, reports indicate that the frequencies of pre-, peri-, and neonatal complications are



also high in children with ASD (Hoshino et al., 1980; Juul-Dam et al., 2001). If environmental factors influence the onset of these disorders, then early infancy, particularly the perinatal period, may be a critical period. Neonatal factors are therefore considered to be important variables on which children with ASD should be assessed. In this study, the neonatal factors of children with a diagnosis of Autistic Disorder were compared with those of typically developing children. Sex differences were also analyzed since the incidence of Autistic Disorder in males is between three and six times the rate in females.

### **Subjects and methods**

The Autistic Disorder group comprised 225 subjects (184 males and 41 females, born between May 1980 and November 1999) who visited Hamamatsu City Medical Center for Developmental Medicine and were diagnosed as having Autistic Disorder by pediatric neurologists and clinical psychologists. Diagnosis was based on the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition (DSM-IV criteria). All the children were Japanese. Patients with other forms of pervasive developmental disorders not otherwise specified were not included in the study. Patients with evident chromosomal aberrations (four patients), delay in motor development (five patients), and underlying diseases (four patients) were also excluded. The level of cognitive development varied: 30 individuals had normal cognitive ability, 57 showed slight retardation, 97 had moderate retardation, and 38 had severe retardation. The control group comprised 1580 children aged 3 years or older (784 males and 796 females, born between December 1981 and December 1999) who were assessed as having normal cognitive development in routine examination by pediatric neurologists. Parents' age at birth, gestational age in weeks, birth weight and neonatal factors (e.g. hyperbilirubinemia, a history of phototherapy, asphyxia and respiratory distress) were examined, based on interviews with parents and maternity health records completed by pediatricians or obstetricians. The degree of hyperbilirubinemia was estimated based on maternity records. The above neonatal factors were first compared between the Autistic Disorder and control groups. Then, pre- and neonatal factors were compared between males and females in the two groups (herein after referred to as autism and control groups).

For statistical assessment, differences in gestational age and birth weight, an unpaired t-test was used and results are presented as mean  $\pm$  standard deviation. For statistical assessment of the other factors, a  $\chi^2$  test was used with appropriate corrections for small sample size. The level of significance was set at 5 percent.

## Results

The mean age of the mothers was  $29.5 \pm 4.1$  years and that of the fathers was  $32.6 \pm 4.7$  years. These ages were comparable with those of mothers in the general population in Japan. Other data are summarized in Tables 1 and 2 (see also Figure 1).

### Comparison between the autism and control groups

The mean gestational age of the autism group was significantly older than that of the controls. The mean birth weight of the autism group was also significantly higher (see Table 1). Frequent neonatal complications included hyperbilirubinemia, history of phototherapy, premature birth (less than 37 weeks), asphyxia, post-term birth of 42 weeks or longer, fetal distress, and complications of respiratory distress (see Table 2). Significant group differences were observed in the proportion of the autism group with hyperbilirubinemia ( $p < 0.05$ ), a history of phototherapy ( $p < 0.0005$ ), asphyxia ( $p < 0.0001$ ), and post-term birth ( $p < 0.05$ ). There were no significant group differences in the proportions showing fetal distress, or those born prematurely. The proportions exhibiting some neonatal complications were 30.6 percent in the autism group and 14.8 percent in the control groups ( $p < 0.00001$ ).

### Comparison between males and females in the control group

The mean gestational age of the male subjects was significantly lower than that of females ( $p < 0.0001$ ) and the mean birth weight was significantly heavier ( $p < 0.005$ ). The proportion of male subjects with some neonatal complications ( $p < 0.01$ ), hyperbilirubinemia and/or premature birth was significantly higher than in females. The proportion of female subjects born post-term was significantly higher than in males ( $p < 0.05$ ). The percentage of male subjects with a history of phototherapy was somewhat higher than in females ( $p = 0.08$ ). No significant difference was observed between the percentages of males and females who had asphyxia.

### Sex differences in the autism and control groups

Mean gestational age (weeks) was significantly greater for males in the autism group than in the control group ( $p < 0.01$ ); mean gestational ages for females did not differ significantly ( $p = 0.55$ ) between the groups. The mean birth weight of males in the autism group was significantly heavier than that of the control males ( $p < 0.005$ ), whereas the mean birth weights for females did not differ between groups ( $p = 0.86$ ). The percentages of both males ( $p < 0.0001$ ) and females ( $p < 0.001$ ) with some neonatal complications were significantly higher in the autism group than in the

**Table 1 Gestational age, birth weight, and frequency of neonatal complications in the autism and control groups**

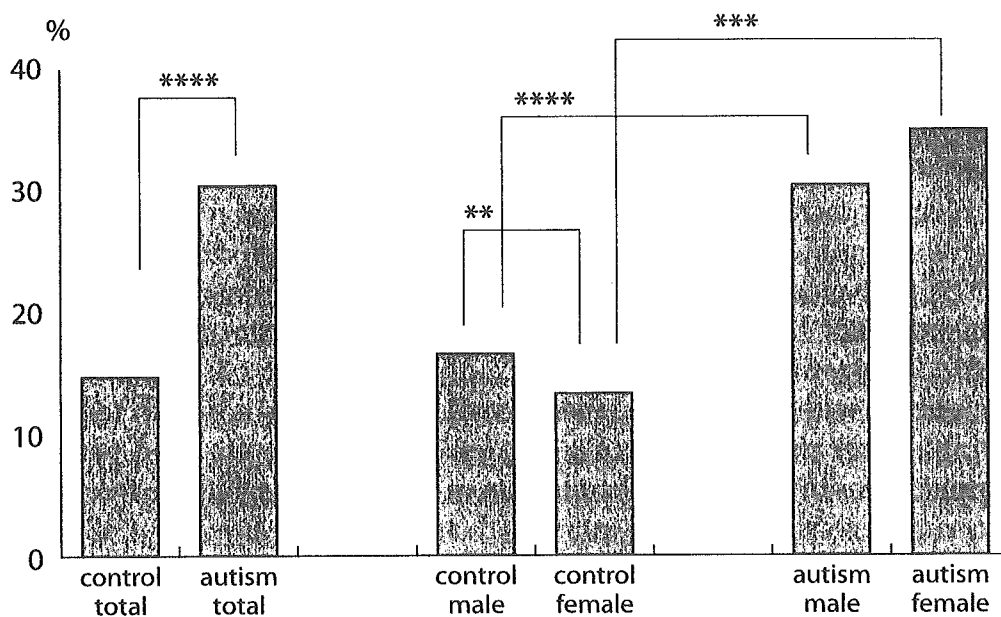
	Autism group			Control group		
	Total	Male	Female	Total	Male	Female
Number of subjects	225	184	41	1580	784	796
Gestational age (weeks)	39.11 ± 1.49	39.15 ± 1.42	38.95 ± 1.77	38.97 ± 1.45	38.82 ± 1.48	39.12 ± 1.40
Birth weight (g)	3138.07 ± 466.52	3173.9 ± 413.8	2985.92 ± 499.44	3037.6 ± 389.5	3076.1 ± 403.6	2999.7 ± 371.4
Neonatal complications:						
Present	64	51	13	215	129	105
Absent	152	121	24	1427	695	732
Unknown	16	12	4	0	0	0

**Table 2** Frequencies of neonatal complications in the autism and control groups

Complication	Autism group			Control group		
	No. (%)	Male	Female	No. (%)	Male	Female
Hyperbilirubinemia	31 (14.8)	24	7	109 (6.9)	65	44
Phototherapy	23 (11.1)	18	5	76 (4.8)	45	31
Prematurity	10 (4.7)	8	2	81 (5.1)	50	31
Asphyxia	9 (4.3)	6	3	8 (0.5)	4	4
Post-term	7 (3.3)	6	1	19 (1.9)	4	15
Fetal distress	5 (2.4)	3	2	14 (0.9)	9	5
Respiratory distress syndrome	4 (1.9)	3	1	20 (1.3)	10	10
Others <sup>a</sup>	13 (6.2)	11	2	21 (1.3)	8	13

<sup>a</sup> Others included one or two cases of vomiting, diarrhea, hypoglycemia, and hypocalcemia in the neonatal period.

controls. The percentages of males ( $p < 0.005$ ) and females ( $p < 0.001$ ) with hyperbilirubinemia was significantly higher in the autism group. The proportion of cases with asphyxia was also significantly higher in the autism group than in the controls for both sexes. No significant group

**Figure 1** Frequency of neonatal complications of autistic group and control group

Control group total v. autism group total  $\chi^2$  test  $p < 0.00001$ .

Control group male v. control group female  $\chi^2$  test  $p = 0.0072$ .

Control group male v. autism group male  $\chi^2$  test  $p < 0.00001$ .

Control group female v. autism group female  $\chi^2$  test  $p < 0.001$ .