hyperactive. He did not respond when his name was called out. When he turned 2-year old, he was diagnosed as having autism.

After the fourth birthday, he began having severe tantrums when he saw the very slightly disorganized tableware on the dinner table.

While in special classes at the elementary school (6-year old), his hyperactivity remained unabated. He threw in a fit of temper when he was not allowed to do familiar routines or when his schedule was upset. With the administration of haloperidol, his condition turned better. As a fifth grader, he had an epileptic seizure in autumn. Although EEG showed nothing abnormal, treatment with valproic acid commenced.

In January, of the sixth year at elementary school, Tourette syndrome (TS) appeared abruptly. The major symptoms he exhibited included facial grimacing, hiccupping, and rapid jerking of the body concurrent with the utterance of bizarre sounds. These well-defined forms of tics had disappeared when he moved up to the second year of junior high school. Several months later, however, self-injurious behavior emerged. There were sudden outbursts during which he banged his head against the table violently.

In October, he took to hanging his clothes on the hanger and taking them off again and again. He also repeatedly said "itadaki-masu," a short prayer of thanks before a meal in Japan. These repetitive actions lasted from 5 min to more than 10 min. Toward the end of the year, increased slowness affecting movements became conspicuous. In May, of the third year at junior high school, he again started loosing his temper easily. Daily doses of pimozide were increased to 4 mg and these temper tantrums diminished as a result.

After he entered senior high school for mentally handicapped children, he persisted to the characteristic pattern of behavior in everyday life without relapse of temper tantrums.

He finished senior high school and found a job at 18. It seemed that he somehow managed to do his own part, although it was said that he had poor ability of concentration. He rarely lost his temper. It seemed that all things were going well with him at home and work.

Repetitive actions increased in October, of the year, when he turned 19-year old. In January of the next year, he became slow moving. In April, he began exhibiting such symptoms as the repetition of bizarre behaviors, and freezing in postures during activity such as making tea. The manifestations of these abnormalities of behavior and posture lasted several minutes. In May, he became unusually concerned with keeping the tableware on the dinner table in perfect order or arranged exactly. Repetition of such words and phrases as "I'm home!", "Good night," and "itadaki-masu" increased in frequency. Also, repetition of a word or phrase just spoken by another person increased remarkably.

In April of the following year, he became fussier and manifested motoric immobility more frequently regardless of mood, which varied greatly from

day to day. In September, he maintained a rigid posture while in standing position for several hours a day. Prompted to move, he wouldn't budge an inch. When forced to move, he returned to where he stood and continued to stand as stiff as a stature. This symptomatology caused disruption of his occupational functioning.

When the patient was additionally dosed with bromazepam (BZP) 4 mg, these symptoms tended to abate, but were not ameliorated completely. At the end of the third year, he resigned from his job and moved to a sheltered workshop in April. Through 1 year, after he became 21, motoric immobility occurred every day. Once it occurred, it lasted for 10 min to several hours.

When he was 22, the frequency of the psychomotor disturbance decreased to three times a week. Three years later, when he was 25 , his condition improved considerably. The maintenance of a rigid posture lasted only several minutes. A few months later he moved to a residential welfare institution. As immobility had almost disappeared, the dosage of BZP 5 mg was tapered off.

Soon after that, he had an epileptic seizure after an absence of over 5 years. Once the seizure returned, it occurred once or twice a month. With an increase in the dosage of an antiepileptic drug, the occurrence rate was on the decline. Relapse of the seizure did not change the residual catatonic symptoms. Epileptic seizures occurred infrequently for the 2 years that followed and have not occurred for more than 1 year now. There are no MRI abnormalities. ECG examinations revealed only a slight degree of paroxysmal abnormalities.

Case 7: 27-year-old male; IQ 19, Ohta Stage III-1

This is the case in which catatonic symptoms appeared twice and disappeared quickly.

No abnormalities were observed during the prenatal and perinatal periods.

At age 1, he started walking by himself. It was not long before he exhibited signs of hyperkinesia, mutism, and apathy. From infancy, he had a strong inclination to adhere to a pattern of behavior in everyday life. Even now, he has been occupied in doing a ritualistic custom at mealtime and has a mania for collecting plastic models of monsters.

In July, when he was a first grade junior high school student, he began to act rudely or take a defiant attitude. When he got angry, he slapped or pinched his opponent's hand.

At the age of 15 months, he started walking up and down or extending his hands compulsively. It was around that time that motoric immobility emerged. He rejected any approach when he was prompted to move. However, it disappeared in a year or so.

When he was 16-year old, he visited us, as he could not shake off the self-injurious behaviors. With the use of an antipsychotic agent, self-injurious and aggressive behaviors were reduced notably.

His condition remained in remission till he reached 21 years and 6 months of age, when eye-rolling suddenly occurred. This symptom responded to treatment with anticholinergic medication.

Two months later, he began to show bizarre behavior. He stood motionless with one leg raised. He also began to threaten to scratch his family members with his nails. After his dose of antipsychotic medication was increased, these abnormalities gradually faded away.

At present, he is not aggressive and freezing has disappeared. He still follows his characteristic pattern of behavior in every day life. He entered the 2005 Special Olympics World Winter Games and won three Gold medals.

V. Results

Average age at onset and frequency in ASDs: The average age at onset of catatonia was 19 years (SD 6, age range: 15–23). Out of 69 cases, who were 20 years or over and visited outpatient clinic of "乙" center, 8 (11.6%) had current symptoms of catatonia or had a past history of catatonia. As for the remaining three cases, who had been followed-up till 3 years ago; the whereabouts of two were unknown, whereas the third one was under treatment in another hospital (See Table I).

A. Symptoms and Severity of Catatonia

In DSM-IV-TR (APA, 2000), five symptoms characterize catatonia or catatonic disorder due to a general medical condition—that is, the maintenance of imposed postures (catalepsy), including waxy flexibility, or the absence of movements

TABLE II
Distribution of DSM-IV Criteria for Catatonia in 13 Catatonic Episodes in Patients with ASD

Case	1	2		3	4	5	6	7	8	9	10	11	12
Times of catatonic phase	1	1	2	1	1	1	1	1	2	1	1	1	1
Motoric immobility	1	1	1	1	1	1	1	1	1	1	1	1	1
Excessive motor activity	0	1	1	0	0	1	0	0	0	0	0	0	1
Extreme negativism or mutism	0	1	1	0	1	1	1	1	1	1	1	1	1
Peculiarities of voluntary movement	1	1	1	1	1	1	1	1	1	1	1	1	1
Echolalia or echopraxia	0	1	1	1	1	0	1	0	0	0	0	0	0
Number of positive items	2	5	5	3	4	4	4	3	3	3	3	3	4

1: Present.
0: Not present.

(akinesia) with the manifestation of stupor; hyperactivity; extreme negativism (evidently nonmotivated resistance or the maintenance of a stiff posture against attempts to be moved), mutism; stereotypy, significant mannerism or the strangeness of volitional movements demonstrated by a significant grimace; and echolalia or echopraxia. Should there be two or more such symptoms, it can be argued that catatonia coexists. After "*M*" had selected probable catatonic cases on the basis of the worst state, "*K*" independently evaluated them, and confirmed the cases to have catatonia by the existence of more than two of the five symptoms. Four cases were considered severe, six cases moderate, and one mild (See Table II).

B. Preceding Conditions

Before the manifestation of a typical catatonic symptom in which movements come to a halt in a strange posture, eight cases had prodromal symptoms, typically a gradually emerging sluggishness with compulsive behaviors lasting for more than 1 year. In the other three cases, onset of catatonia was abrupt with no preceding prodromal phase.

C. Psychiatric Complication, Family History, and Medication

Three cases were diagnosed with TS. At present, one of them (Case 11) still has it (see Table I).

The obsessive-compulsive symptoms in Case 9 seemed to increase in periods of greater family turmoil and conflict with his schizophrenic sibling. Catatonia may have been precipitated by increased stress.

Case 10 had a family history of mood disorder. Complications with epilepsy were observed in three cases, and in one of them, epileptic seizures recurred twice or so a month after the alleviation of catatonia (Case 4). In Case 10, the onset of epilepsy came for the first time at the age of 20 after the alleviation of catatonia. Only three cases were on antipsychotics before onset of catatonia. Of the remaining 8 cases, Case 7 took antipsychotics when he had catatonia for the second time. For Case 7, above all, a discreet differentiation was required between catatonia and antipsychotic-induced parkinsonism (see the case presentation).

D. Courses

Catatonic symptoms showed considerable fluctuations during the span of a day, in all cases. Those changes could be observed even at the worst time. The alleviation of symptoms did not signify full improvements in the attitude of refusal

or spontaneity. A review of the long-term course showed that catatonic symptoms came out twice in two cases. Of them, Case 2 was complicated with TS. With the exclusion of Case 6—whose whereabouts are unknown—and the inclusion of two cases with two catatonic episodes, the average duration was 27 months (SD 31.8, duration range 4–108 months). Out of nine cases interviewed on clinic visits or by telephone, five cases no longer had catatonia at the time of the current examination, whereas one case remained moderate and three cases were mild. When comparing the three cases with sudden onset to the eight cases with gradual onset, it was found that the rate of remission within 1 year was higher in the sudden onset cases (100%) than in the gradual onset cases (25%) with no statistical significance.

VI. Discussion

Wing and Shah (2000) operationally defined catatonia in individuals with ASDs. In their definition, four features were taken up—that is, (1) increased slowness affecting movements and verbal response, (2) difficulty in initiating and completing action, (3) increased reliance on physical or verbal prompting by others, and (4) increased passivity and apparent lack of motivation. As often-associated symptoms, they referred to (5) reversal of day and night, (6) parkinsonian features (tremor, eye-rolling, dystonia, odd stiff posture, freezing in postures, etc.), (7) excitement and agitation, and (8) an increase in repetitive and ritualistic behavior.

Unlike the criteria set forth by Wing and Shah, we adopted suspension in an odd posture as the core of the diagnostic criteria for catatonia in this study.

However, it was confirmed that our diagnostic criteria are fully compatible with those of DSM-IV-TR (APA, 2000), and their validity was ascertained.

Wing and Shah (2000) reported that the age-of-onset of catatonia ranged from 10 to 30 years of age, with a peak at 15–19 and the prevalence of catatonia was 6% in outpatients with ASDs. In our cases, the prevalence in ASDs was rather higher than that. But the age of onset roughly came within the ranges set by them. As the "こころ" center is the tertiary facility for developmental disorders and a large portion of the patients visiting the center has various behavioral problems regardless of level of intelligence, the prevalence in our study would be higher than that in the previous study. Among ASD patients with remarkable social impairment, the prevalence of catatonia might be higher than that expected. It is said that catatonia can occur as intrinsic symptoms of ASDs or comorbid psychiatric condition of ASDs or aversive side effects related to antipsychotics (Chaplin, 2000; Dhossche, 1998; Leary and Hill, 1996; Realmuto and August, 1991).

First, we examined relationship between catatonia and ASDs in terms of comorbidity.

It is known that catatonia comes out regardless of levels of intelligence (Howlin, 2000). In this study, most of the subjects are individuals who had severe or moderate mental retardation. Therefore, it is difficult to diagnose complications with mood disorders and schizophrenia. On the other hand, TS is relatively easy to diagnose (Baron-Cohen *et al.*, 1999; Kano *et al.*, 1988) and was found in three subjects in this study.

They are the first cases of ASDs reported to have both catatonia and TS. It seems to be worthy to examine relationship between catatonia and TS, which is closely associated with ASDs.

Second, we examined the relationship between catatonia and ASDs from the viewpoint of course of catatonia.

We found that some cases developed catatonia with preceding gradual slowness and other cases had sudden onset of catatonic symptom, in accord with the findings of Wing and Shah (2000).

It may also be pointed out that there existed cases in which catatonia repeatedly aggravated over short spans of time.

In addition, it should be emphasized catatonia continued for more than 2 years on average, and there were cases with no significant change for nearly 9 years.

VII. Suggestions on Treatment

First of all, it should be emphasized that it is inappropriate to force ASD patients with catatonia to act on their own initiative.

And it should be considered that, for any clinical case, the severity of catatonia changes in a day. It is effective to approach catatonic ASD patients during minutes or hours when severity of catatonia diminishes within a day. The severity of catatonia often fluctuates throughout the day. It is most effective to approach catatonic ASD patients when catatonic symptoms are at their lowest point during the day.

Catatonic ASD patients assume a negative attitude toward approaches from other persons when the disease is at its worst, and it may well be argued that they offer strong resistance to treatment as suggested by Wing (1996). It seems to be impossible to approach such patients with oral instructions. However, the patients may be able to take an action, albeit at a slow pace, when their bodies are touched and moved toward the place to which he presumably wanted to move. As regards pharmacotherapy, it can be said from our experience that the use of both benzodiazepine and antipsychotics will be effective in the long run.

VIII. Limitation of this Study

There is the need to examine if the diagnostic criteria for catatonia set forth here are in harmony with those worked out by Wing and Shah. It is also necessary to prepare diagnostic criteria for the screening of catatonia and to systematically review the medical records of outpatients who are suspected of having catatonia.

It is convenient to screen patients for catatonia in ASDs at age 20, because, at that time, a comprehensive review and diagnostic assessment is done in order to file applications for pensions payable to physically or mentally handicapped in Japan. On the basis of outpatient services, there is the need to study patients at younger ages. As many ASD patients with severe or moderate mental retardation were taken up in this study, it was difficult to come to grips with mood disorders and schizophrenia. Though two catatonic ASD patients in this study carry a family history of mood disorders or schizophrenia, it cannot be hastily concluded which complications are closely related to catatonia. It is necessary to investigate relationship between catatonia and other complications in ASD cases without mental retardation.

IX. Conclusions

Our diagnostic criteria of catatonia are fully compatible with those of DSM-IV-TR (APA, 2000), and their validity was ascertained.

Catatonia in ASDs seems to be a chronic condition in most cases. However, there were also a few cases in which catatonia repeatedly aggravated over short spans of time. Catatonia in ASDs may be considered an epiphenomenon of ASD or a manifestation of comorbidity in adolescence or early adulthood.

Further studies in patients with ASDs are needed to compare different diagnostic criteria for catatonia and to examine the biological correlates of catatonia in ASDs.

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Short communication

Serotonin transporter gene promoter polymorphism and autism: A family-based genetic association study in Japanese population

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Abstract

Autism is now widely accepted as a biological disorder which, by and large, starts before birth. It has been shown that serotonin (5-HT) is associated with several psychological processes and hyperserotoninemia is observed in some autistic patients. The results of previous reports about family-based association studies between the serotonin transporter (5-HTT) gene promoter polymorphism and autism are controversial. In this study, an analysis using the transmission/disequilibrium test (TDT) between the 5-HTT gene promoter polymorphism and autism in 104 trios, all ethnically Japanese, showed no significant linkage disequilibrium ($P=0.17$). Recently, it has been reported that some haplotypes at the serotonin transporter locus may be associated with the pathogenesis of autism. Therefore, further investigations by haplotype analyses are necessary to confirm the implications of genetic variants of the serotonin transporter in the etiology of autism.

Keywords: Autism; Serotonin transporter (5-HTT); Serotonin transporter gene promoter polymorphism; Transmission/disequilibrium test; Ethnic differences

Autism is a neuro-developmental disorder characterized by abnormalities in social, communicative and behavioral functioning. Although etiological studies have indicated that autism is a disorder with strong genetic susceptibility [1], the genes responsible for this complex disorder have not been defined yet.

Serotonin (5-hydroxytryptamine, 5-HT) is associated with several psychological processes, including mood, anxiety, obsessive-compulsive symptoms and social interaction. The role of the serotonergic system in neuroplastic events has been explored. The 5-HT blood level is known to be elevated by roughly 30% in autistic patients [2]. It was also reported that selective serotonin reuptake inhibitors (SSRIs) were effective for some symptoms of autism, such as repetitive behavior, aggression and impediment of language usage [3]. These facts suggest that the serotonin transporter (5-HTT) gene can be involved in the etiology of autism.

The 5-HTT gene contains in its promoter region a deletion/insertion polymorphism, a short allele and a long allele (14- and 16-repeat alleles), profoundly affecting expression levels [4]. Previous genetic association studies between the 5-HTT gene promoter polymorphism and autism failed to show consistent results (Table 1) [5–16]. The inconsistencies among the studies may be due to the influence of racial differences or the heterogeneity of the pathogenesis. In this study, we performed a family-based association study between the 5-HTT gene promoter

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Table 1
Results of family-based association studies between 5-HTT gene promoter polymorphism and autism

		Ethnics	Study design	Number of samples	5-HTT gene promoter polymorphism	Preferential transmission	Publication
1.	Klauck SM et al.	Caucasian (one family; Asian)	TDT	A: 52 trios fulfilling stringent criteria for autism B: 65 trios including patients showing no language delay in first 3 years of life	A (P=0.248) A and B (P=0.032)	Long allele	1997
2.	Cook EH et al.	Caucasian, African-American, Hispanic-American, Asian-American	TDT	86 trios	χ^2=4.69, P=0.030	Short allele	1997
3.	Maestrini E et al.	Caucasian	TDT	90 families (82 multiplex and 8 singleton)	χ^2=0.11, P=0.80	Long allele	1999
4.	Persico AM, et al.	①Italian, ②Caucasian-American	TDT	①54 trios ②44 trios	①χ^2=0.51, P=0.48 ②χ^2=0.02, P=0.89 ①+②χ^2=0.40, P=0.53	Short allele Long allele Long allele	2000
5.	Yirmiya N et al.	?? (Israel)	HRR design TDT	34 families	Likelihood ratio=5.99, P=0.014 χ^2=5.44, P<0.025	Long allele	2001
6.	Tordjman S et al.	Caucasian (French child hospital)	TDT	71 trios	χ^2=4.00, P=0.046	Long allele	2001
7.	Persico AM et al.	Italian, Caucasian-American	HHRR	①155 trios ②57 unaffected sibs	①χ^2=1.47, P=0.23 ②χ^2=0.45, P=0.50 ①vs. ②χ^2=0.81, P=0.37	Long allele	2002
8.	Betancur C et al.	Caucasian (Austria, Belgium, France, Italy, Norway, Sweden and United states)	TDT	①43 trios ②53 sib-pair families	①χ^2=1.73, P<0.19 ②χ^2=0.62, P<0.43 Total: χ^2=2.03, P<0.15	Short allele	2002
9.	Kim SJ et al.	Caucasian, African-American, Hispanic-American, Asian-American	TDT	①81 trios ②115 trios	①χ^2=2.32, P=0.128 ②χ^2=7.31, P=0.007 ②χ^2=7.31, P=0.007	Short allele	2002
10.	Conroy J et al.	Irish	TDT	84 trios	χ^2=4.5252, P=0.0334	Short allele	2004
11.	McCauley JL et al.	New England, Autism Genetic Resource Exchange (AGRE)	PDT	137 multiplex families	P=0.01	Short allele	2004
12.	Mulder EJ et al.	Dutch	TDT	125 trios	χ^2=0.086, P=0.77	Long allele	2005
13.	Our results	Japanese	TDT	104 trios	χ^2=1.92, P=0.17	Short allele	2005

TDT, transmission/disequilibrium test; HRR, haplotype relative risk; HHRR, haplotype-based haplotype relative risk; PDT, pedigree disequilibrium test.

Table 2
Distribution of genotypes and alleles of the 5-HTT gene promoter polymorphism in autistic patients and their parents

	Genotype distribution (%)			Allele distribution (%)	
	Short/Short	Long/Short	Long/Long	Short	Long
Autistic patients ($n=104$)	60 (57.7)	34 (32.7)	10 (9.6)	154 (74.0)	54 (26.0)
Mothers ($n=104$)	62 (59.6)	36 (34.6)	6 (5.8)	160 (76.9)	48 (23.1)
Fathers ($n=104$)	61 (58.7)	39 (37.5)	4 (3.8)	161 (77.4)	47 (22.6)

polymorphism and autism using the transmission/disequilibrium test (TDT) in the Japanese population for the first time.

One hundred and four trios (12 female and 92 male autistic probands; mean ages of probands, mothers and fathers are 17.4 ± 10.5 SD, 45.9 ± 10.7 SD and 48.2 ± 11.6 SD, respectively), all ethnically Japanese, were examined. The subjects assessed in this study were recruited from the outpatient department of Tokai University Hospital and two institutions devoted to autism which are located close together in the Kanto district, Japan. Two experienced child psychiatrists, who are two of the authors (K Yamazaki and S Koishi), independently conducted a semi-structured behavioral observation and an interview with all patients and their parents, and made final diagnoses according to the ICD-10 DCR (World Health Organization, 1993) and DSM-IV (American Psychiatric Association, 1994). Only cases, which fulfilled the ICD-10 criteria for childhood autism and DSM-IV criteria for autistic disorder were selected by both child psychiatrists. After that, the observations by the child psychiatrists continued and we excluded cases, which were found not to fulfill both criteria within six months of their participation in this study. In order to exclude cases secondary to other genetic syndromes or neurological diseases, the subjects were included only after a thorough clinical evaluation and medical examination comprising a full exploration of medical and family history, physical and neurological examinations such as brain imaging, EEG, urinalysis, standard karyotyping and fragile-X testing according to molecular genetic testing for the trinucleotide repeat expansion in the FMR-1 gene [17]. The study was approved by the ethical committee of Tokai University and other collaborating organizations. Informed consent forms were completed by the patients and/or their parents.

Genomic DNA was isolated from peripheral blood leukocytes using standard procedures, and genotyping for 5-HTT gene promoter polymorphism was carried out as previously described [4]. Linkage/association analyses were performed applying the transmission/disequilibrium test (TDT), whereby preferential allelic transmission from heterozygous parents to affected offspring is tested by applying the $(b-c)^2/(b+c)$ statistics and the χ^2 ('McNemar test') [18].

Table 2 shows the distribution of the genotypes and alleles of 5-HTT gene promoter polymorphism. There was no evidence of deviation from the Hardy-Weinberg equilibrium in this polymorphism ($\chi^2=0.47$, $P=0.49$) when the entire sample was examined. These results of genotypic and allelic distribution are in accordance with the previous report that there was a difference in the frequencies for the genotypes and alleles between Japanese and Caucasians [19]. No preferential transmission of either short or long alleles using the TDT was detected in the present study ($\chi^2=1.92$, df$=1$, $P=0.17$) (Table 3).

This is the first report of a family-based genetic association study of autism using the TDT in the Japanese autistic population. There are few studies about the association between the 5-HTT promoter polymorphism and autism using the family-based method with more than 100 trios consisting of ethnically homogeneous subjects and, moreover, previous results have been chaotic to date (Table 1). In the present study, all subjects were recruited in a limited, small area in Japan. The Japanese population is considered to consist of a single ethnicity. The frequencies of the alleles and the genotypes of the 5-HTT gene promoter polymorphism in the Japanese population were quite different from those in other populations [19], so it was interesting to make this study. Our results do not support a linkage/association between the 5-HTT gene promoter polymorphism and autism. Recent reports suggested that some haplotypes at this locus may be associated with the early development of the brain in autism [13,14]. It was also reported that polymorphisms in the 5-HTT gene may modify the severity of behavioral problems in social and communication domains [10] or behavioral phenotypes such as the rigid compulsive behavior in autism [16]. Therefore, further investigations of the relationship between haplotypes and behavioral features are necessary to confirm the implications for genetic variants of the serotonin transporter in the etiology of autism or its phenotypic variability.

Finally, the results of the present study showing no association between the 5-HTT gene promoter polymorphism and autism need to be viewed cautiously, especially since the number of families examined was small. Further

Table 3
Transmission/disequilibrium test (TDT) of 5-HTT gene promoter alleles and autism

Transmitted	Not transmitted	
	Short	Long
Short	123	31
Long	44	10

TDT $\chi^2=1.92$, df$=1$, $P=0.17$.

investigations with an increased number of subjects in ethnically homogeneous groups are necessary.

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Combined analysis of association between personality traits and three functional polymorphisms in the tyrosine hydroxylase, monoamine oxidase A, and catechol-*O*-methyltransferase genes

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Abstract

Several molecular genetic studies have been conducted with regard to the association between catecholamine-related genes and personality traits. However, the results of replication studies did not always coincide. One of the possible reasons may be that the effect exerted by the individual gene is small. In the present study, we investigated the association between personality traits and systematic combination of functional polymorphisms in three genes that regulate the metabolism of catecholamines, namely, tyrosine hydroxylase (TH), monoamine oxidase A (MAOA), and catechol-*O*-methyltransferase (COMT). The $(TCAT)_n$ repeat in the TH gene, the promoter variable number tandem repeat (VNTR) in the MAOA gene, and Val158Met in the COMT gene were genotyped in 256 healthy Japanese volunteers. Personality traits were evaluated using the NEO Personality Inventory-Revised (NEO PI-R). As a result, the score for Neuroticism increased, and those for Extraversion and Conscientiousness decreased according to the degree of functional polymorphic change, i.e., the lower synthesis/higher catalysis of catecholamines. A statistically significant difference was observed in the change of Extraversion ($p = 0.04$, after Bonferroni correction). These results may provide evidence for the association between metabolic change of catecholamines and personality traits, which may be due to the additive effect of the three genes.

Keywords: Tyrosine hydroxylase (TH); Monoamine oxidase A (MAOA); Catechol-*O*-methyltransferase (COMT); NEO Personality Inventory-Revised (NEO PI-R); Personality trait; Association study

1. Introduction

Catecholamines, including dopamine and norepinephrine, are neurotransmitters that affect various mental functions and behavior. Pharmacological research on schizophrenia has proposed the dopamine hypothesis, which states that schizophrenia results from excessive dopaminergic activity (Meltzer and Stahl, 1976). Personality traits have also been considered to depend on the secretion and metabolism of neurotransmitters such as dopamine and norepinephrine (Cloninger, 1987). Based on this concept, several molecular genetic studies on personality have been conducted with respect to dopamine-related genes. Particularly, the association between the dopamine D4 receptor gene (DRD4) and novelty seeking has been studied intensively ever since the first study conducted by Ebstein et al. (1996). However, the results of replication studies did not always coincide, and several reasons were considered for this inconsistency (Van Gestel and Van Broeckhoven, 2003; Savitz and Ramesar, 2004). One of the reasons may be investigating association of individual receptor gene, which is believed to exert a minor effect. Therefore, it appears reasonable to assess the effect of a systematic combination of genes that regulate the metabolism of catecholamines. Tyrosine hydroxylase (TH), monoamine oxidase (MAO), and catechol-*O*-methyltransferase

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(COMT) are genes of the major metabolic enzymes of catecholamines.

TH is the rate-limiting enzyme involved in the synthesis of catecholamines; it converts tyrosine to dihydroxyphenylalanine (DOPA). Among several polymorphisms known within this gene, a $(TCAT)_n$ repeat polymorphism defining five main alleles, namely, T6, T7, T8, T9, and T10 (the numbering denotes the number of repeats), is located in the first intron (Polymeropoulos et al., 1991). This polymorphism has been suggested to have a relationship with catecholamine turnover rates. Among the five alleles, T9 has been suggested to be related to the low excitability of noradrenergic nerves (Wei et al., 1997; Sharma et al., 1999), although this was not replicated by another study (Zhang et al., 2004). To our knowledge, only one study has investigated the association of TH with personality traits. Persson et al. (2000) described a tendency for high scores for Neuroticism in carriers of T8 allele, and high scores for Conscientiousness and low scores for Openness in women with T6/T10 genotype. They did not provide any evidence for the association of the T9 allele with personality traits.

In contrast, MAO and COMT act as catalytic enzymes for monoamines. MAO oxidatively deaminates monoamines to their corresponding aldehydes. MAO type A (MAOA), an isozyme of MAO, preferentially deaminates norepinephrine and serotonin. The gene encoding MAOA is mapped to the chromosomal region Xp11 (Chen et al., 1992). A 30-bp variable number tandem repeat (VNTR) that affects MAOA transcription has been identified in the promoter region of this gene (Sabol et al., 1998). The polymorphism mainly consists of 3, 3.5, 4, and 5 repeats. Transcription efficiency was shown to be two- to three-fold higher for longer alleles (3.5, 4, and 5) than for shorter alleles (3 and a rare 2 repeat allele) (Deckert et al., 1999). To our knowledge, four studies have been conducted with respect to personality traits. Males with longer alleles were observed to have lower scores for aggressiveness and impulsivity (Manuck et al., 2000) or higher scores for Neuroticism (Eley et al., 2003). Samochowiec et al. (2004) described that males with shorter alleles had lower scores for Openness. In contrast, Garpenstrand et al. (2002) did not observe any significant association between the MAOA genotype and personality traits.

COMT catalyzes the transfer of a methyl group to catecholamines. A common single nucleotide polymorphism (SNP) (G/A), at the first position of codon 158 of the gene, results in a functional valine (high activity) to methionine (low activity) transition associated with a three- to four-fold difference among homozygous subjects (Lotta et al., 1995). COMT has been assessed as a candidate gene for anxiety and depressive disorders. The Met allele was significantly more common in patients with obsessive–compulsive disorder (OCD); the effect appeared to be recessive because it was seen only in homozygous genotypes (Karayiorgou et al., 1997). A higher frequency of social drinking was observed in individuals with the Met/Met genotype (Kauhanen et al., 2000). In addition, studies on personality also showed that the Met/Met genotype is associated with aggressive personality traits (Rujescu et al., 2003), Neuroticism (Eley et al., 2003), or harm avoidance (Enoch et al., 2003).

In the present study, we investigated the association between three functional polymorphisms in TH, MAOA, and COMT genes and personality traits that were evaluated using the NEO Personality Inventory-Revised (NEO PI-R). The effect of systematic combination of the three genes was assessed, in addition to the analysis of the effect of the individual genes. To our knowledge, the combined effect of these genes has not been studied, except for the study of Eley et al. (2003), in which interaction between MAOA and COMT was explored with respect to Neuroticism.

2. Subjects and methods

The subjects included 256 unrelated healthy volunteers (64 males and 192 females; age, 37.4 ± 11.9 years (mean ± S.D.)) recruited from the staff of several mental and general hospitals around Tokyo. The subjects filled out the NEO PI-R, a self-report inventory based on the five-factor model of personality (Costa and McCrae, 1992). The validity and reliability of the NEO PI-R have been confirmed in a variety of populations and cultures (Costa and McCrae, 1992). The research protocol was approved by the ethics committee of the University of Tokyo. Written informed consent was obtained from all the subjects.

Genome-DNA was extracted from leukocytes by using a standard method. The $(TCAT)_n$ repeat polymorphism in the TH gene, the promoter VNTR polymorphism in the MAOA gene, and the Val158Met polymorphism in the COMT gene were genotyped. The polymorphisms in the MAOA and TH genes were analyzed using the Fragment Analysis module of the CEQ 8000 Genetic Analysis System (Beckman Coulter, Inc., USA). The COMT gene polymorphism was analyzed using restriction fragment length polymorphism (RFLP), as described by Hallikainen et al. (2000).

Statistical analysis was performed as follows; initially, the NEO PI-R scores were compared on the basis of genotypes of each polymorphism. With respect to the TH gene, carriers of the T9 allele were compared to others because the allele has been suggested to be related to a decrease in the synthesis of catecholamines (Wei et al., 1997; Sharma et al., 1999). It was also attempted to replicate the significant associations observed in the previous study (Persson et al., 2000). With respect to the MAOA gene, females and males were analyzed separately. The alleles were grouped as the short alleles (s) and the long alleles (l) based on the previous knowledge (Deckert et al., 1999). Analyses were performed by using one-way analysis of variance (ANOVA) (or Student's *t*-test) and analysis of covariance (ANCOVA) adjusting sex and/or age when these effects were significant.

In addition to the study of the individual genes, the effects of the combination of the three genes on the NEO-PI-R scores were investigated as follows. First, the genotypes in each polymorphism were classified into two groups according to their effects on the synthesis or catalysis of catecholamines; genotypes with the T9 allele versus others for the TH gene, genotypes with the long allele versus others for the MAOA gene, and genotypes with the Val allele versus the other (the Met/Met genotype) for the COMT gene. Genotypes with the T9 allele of the TH gene may have an effect to decrease the synthesis of catecholamines. Genotypes with the long allele of the MAOA gene and genotypes with the Val allele of the COMT gene may have an increasing effect on the catalysis of catecholamines.

Then, the analysis was performed in two methods. One method employed analysis of epistasis by using multivariate three-way ANOVA. The significance of interaction between the genotypes was examined after testing the statistical independence among genotype frequencies of each gene. NEO PI-R scores were entered as the dependent variable; the genotypes, as the independent variables; and sex and age, as covariates. *p*-Values were adjusted by Bonferroni correction for multiple testing.

Another method was as follows. We allocated points to the subjects according to the number of the synthesis-decreasing or catalysis-increasing genotypes, i.e., genotypes with the T9 allele versus others for the TH gene,

genotypes with the long allele versus others for the MAOA gene, and genotypes with the Val allele versus the other (the Met/Met genotype) for the COMT gene. Thus, subjects who had the most synthesis-decreasing/catalysis-increasing combination of the genotypes were scored "three" and those with the least synthesis-decreasing/catalysis-increasing combination was scored "zero". The rests were scored "one" or "two" and therefore subjects were classified into four groups. The NEO PI-R scores were compared among those groups. Subjects with the score "three" were compared with the others (with the scores "zero" to "two"), in addition to the comparison among the four groups. ANOVA, *t*-test or ANCOVA adjusting sex and/or age was used based on the significance of covariates. The statistical package SPSS for Windows (SPSS Inc., USA, 1999) was used for all analyses.

3. Results

Genotypic distributions of the three polymorphisms in the TH, MAOA, and COMT genes are shown in Tables 1 and 2. The distributions of all three polymorphisms follow the Hardy–Weinberg equilibrium.

Table 2 summarizes the scores for NEO PI-R factors according to the genotypes of each polymorphism. There was a significant difference in the score for Extraversion between the subjects with and without T9 allele of the polymorphism in the TH gene ($t = 3.35$, $p = 0.001$, t-test, uncorrected). No other significant difference in the scores for the NEO PI-R factors was observed during the comparison of each polymorphism. The association between Neuroticism and T8 allele or Openness and T6/T10 genotype observed in the previous study (Persson et al., 2000) was not confirmed (data not shown).

In the epistatic analysis, we initially confirmed no deviation of independence among genotype frequencies of each gene. Multivariate three-way ANOVA showed no significant interaction of TH × MAOA × COMT, TH × MAOA, TH × COMT, and MAOA × COMT on the NEO PI-R scores (Hotelling's Trace and p-value were 0.008 and 0.88, 0.004 and 0.97, 0.011 and 0.77, and 0.031 and 0.24, respectively). Subsequent univariate three-way ANOVA showed no significant interaction among the three genes either. Significant main effect of TH was observed when Extraversion was the dependent variable ($F = 5.89$, $p = 0.016$, corrected); and that of COMT, when Agreeableness ($F = 4.56$, $p = 0.034$, corrected). No other main effect was observed on each NEO PI-R score during subsequent analyses.

In the comparison based on the total score for polymorphism, the scores for Neuroticism, Extraversion, and Conscientiousness changed consecutively, according to the total score for polymorphism (Table 3). The four group comparison revealed a significant difference in the Extraversion score ($F = 3.75$, d.f. = 3, $p = 0.012$, ANOVA, uncorrected). No significant difference was observed in the other scores for NEO PI-R factors in this comparison. The two group comparison revealed significant differences in the Extraversion scores (99.4 ± 13.8 versus 93.9 ± 15.9 (mean ± S.D.) between subjects with zero to two points and those with three points, respectively; $t = 2.68$, $p = 0.008$, t-test, uncorrected). Conscientiousness score was also different between subjects with zero to two points and those with three points (102.0 ± 16.1 versus 97.0 ± 15.6 (mean ± S.D.), respectively; $F = 4.17$, d.f. = 1, $p = 0.042$, ANCOVA, age included as a covariate,

Table 1
Genotypic distributions of the polymorphisms in the TH and MAOA genes

(a) $(TCAT)_n$ repeat polymorphism in the TH gene

Genotypes	T6/T6	T6/T7	T6/T8	T6/T9	T6/T10	T7/T7	T7/T8	T7/T9	T7/T10	T8/T8	T8/T9	T8/T10	T9/T9	T9/T10
N (%) ($n = 247$)	18 (7.3)	23 (9.3)	2 (0.8)	51 (20.6)	7 (2.8)	15 (6.1)	8 (3.2)	56 (22.8)	10 (4.0)	2 (0.8)	12 (4.9)	1 (0.4)	32 (13.0)	10 (4.0)

(b) Promoter VNTR polymorphism in the MAOA gene

(i) Females ($n = 189$)

Genotypes	2/3	2/4	3/3	3/4	4/4
N (%)	1 (0.5)	1 (0.5)	82 (43.5)	77 (40.7)	28 (14.8)

(ii) Males ($n = 61$)

Genotypes	2	3	4
N (%)	1 (1.6)	42 (68.9)	18 (29.5)

Table 2
The scores for five factors of NEO PI-R according to the genotypes of each polymorphism

Genotype	N (%)	Neuroticism	Extraversion	Openness	Agreeableness	Conscientiousness
$(TCAT)_n$ repeat polymorphism in the TH gene						
With T9 allele	161 (65.2)	99.7 ± 19.4	95.4 ± 14.3*	110.0 ± 13.4	112.3 ± 14.0	99.5 ± 15.7
Without T9 allele	86 (34.8)	99.2 ± 19.9	101.8 ± 14.4	110.4 ± 15.1	114.9 ± 11.2	103.0 ± 18.4
Promoter VNTR polymorphism in the MAOA gene						
Females						
s/s	83 (43.9)	99.7 ± 18.0	99.4 ± 14.6	111.3 ± 14.2	115.1 ± 12.9	103.6 ± 17.3
s/l	78 (41.3)	103.0 ± 19.4	95.9 ± 12.6	109.3 ± 13.9	115.4 ± 13.5	99.5 ± 13.2
l/l	28 (14.8)	101.3 ± 23.6	98.7 ± 16.4	108.0 ± 13.8	114.7 ± 12.7	97.9 ± 18.5
Males						
s	43 (70.5)	90.7 ± 17.2	99.0 ± 13.4	110.0 ± 13.7	109.9 ± 11.6	102.0 ± 14.9
l	18 (29.5)	98.6 ± 25.2	97.0 ± 20.0	111.3 ± 9.7	108.0 ± 10.9	95.3 ± 21.8
Val158Met polymorphism in the COMT gene						
Val/Val	115 (46.4)	100.9 ± 19.3	97.5 ± 15.4	109.6 ± 13.7	113.9 ± 13.2	100.5 ± 14.6
Val/Met	105 (42.3)	97.5 ± 19.8	97.9 ± 14.0	110.7 ± 15.0	114.2 ± 12.3	100.6 ± 18.0
Met/Met	28 (11.3)	99.5 ± 18.7	97.6 ± 14.4	110.9 ± 12.2	108.2 ± 12.1	104.7 ± 19.4

Scores presented as mean ± standard deviation; s, short allele; l, long allele.
* $p < 0.005$; compared between the subjects with and without T9 allele (t-test, uncorrected).

Table 3
The scores for five factors of NEO PI-R according to the total score for polymorphism*

Score for polymorphism	N	Neuroticism	Extraversion#	Openness	Agreeableness	Conscientiousness$
0	5	96.4 ± 14.0	110.2 ± 16.9	112.8 ± 12.9	114.0 ± 16.5	106.6 ± 25.7
1	45	98.0 ± 18.8	101.0 ± 15.5	109.5 ± 13.9	112.1 ± 11.3	102.3 ± 16.9
2	116	98.6 ± 18.3	98.4 ± 12.8	111.1 ± 14.5	113.6 ± 11.9	101.6 ± 15.4
3	71	102.3 ± 21.3	93.9 ± 15.9	108.7 ± 12.9	113.1 ± 15.0	97.0 ± 15.6

* Carriers of the T9 allele in the TH gene, carriers of the long allele in the MAOA gene, and carriers of the Val allele in the COMT gene get are allotted one point as a score for polymorphism. Subjects with other genotypes are awarded zero point.
# $p < 0.05$, comparison among four groups (ANOVA), uncorrected; and $p < 0.01$, comparison between subjects with scores of 0–2 and those with score of 3 (t-test), uncorrected.
$ $p < 0.05$, comparison between subjects with sores of 0–2 and those with score of three (ANCOVA), uncorrected. ANCOVA was employed because age had a significant effect as a covariate on Conscientiousness.

uncorrected). No significant difference was observed in the other scores for the NEO PI-R factors in this comparison.

4. Discussion

In the present study, we investigated the association of personality traits with three functional polymorphisms in the genes of the metabolic enzymes of catecholamines, namely, TH, MAOA, and COMT. In addition to the association with each polymorphism, the effect of the systematic combination of the three genes was also assessed. As a result, association was observed between the TH gene and Extraversion ($p = 0.001$). In epistatic analysis of the three genes using three-way ANOVA, no significant interaction among the three genes on personality traits was observed. However, in the comparison based on a score for polymorphism, the scores for Neuroticism increased consecutively according to the scores for polymorphism. In contrast, the scores for Extraversion and Conscientiousness decreased consecutively, according to the scores for polymorphism. Significant differences were observed in the changes in Extraversion ($p = 0.012$ in the four group comparison and $p = 0.008$ in the two group comparison) and Conscientiousness ($p = 0.042$ in the two group comparison). These results may provide evidence for the association between the lower synthesis/higher catalysis of catecholamines and personality traits such as Extraversion and Conscientiousness. The association may be due to the additive effect of the genes of metabolic enzyme.

The results of previous molecular genetic studies did not always coincide (Van Gestel and Van Broeckhoven, 2003; Savits and Ramesar, 2004). One of the possible reasons may be that the effect exerted by individual genes is small. Therefore, it is worthwhile to assess the combined effect of the genes of the metabolic enzyme. To date, Eley et al. (2003) have analyzed the interaction between MAOA and COMT with respect to Neuroticism; however, no combined effect was observed. In the present study, we did not observe significant association of personality traits with each gene, with the exception of Extraversion that showed significant association with the TH gene ($p = 0.005$ after Bonferroni correction). We did not observe significant effect of interactions (or epistasis) between the enzyme genes either. However, in the comparison based on

a score for polymorphism, we clearly observed consecutive association between personality traits and metabolic function of catecholamines. The association of Extraversion was statistically significant after Bonferroni correction ($p = 0.04$ in the two group comparison). When considering the role of catecholamines, it seems reasonable that the scores for Neuroticism increases and those for Extraversion and Conscientiousness decrease in accordance with the lower synthesis/higher catalysis of catecholamines. High Neuroticism, low Extraversion, and low Conscientiousness were observed to be associated with anxiety and depressive disorders, such as social phobia, agoraphobia, panic disorder, OCD, generalized anxiety disorder, and major depressive disorder (Bienvenu et al., 2004). The association between catecholamines and personality traits, observed in the present study, might suggest a biological basis for predisposition to anxiety and depressive disorders.

It must be acknowledged however that no significant evidence was obtained concerning Neuroticism, which is directly indicative of a tendency toward developing anxiety and depressive disorders. The association of Conscientiousness became statistically insignificant after Bonferroni correction ($p = 0.21$ in the two group comparison). In addition, there is a possibility that the statistical significance of the association of Extraversion in combined analysis ($p = 0.04$ in the two group comparison, corrected) may be a reflection of the significant association of the TH gene ($p = 0.005$, corrected), although the combined effect of the three genes on the score distribution was more prominent than the sole effect of the TH gene. Therefore, each of the present results should be interpreted with utmost caution. The problem of multiple testing is also need to be taken into consideration, although Bonfferoni correction was performed for five factors of the NEO PI-R. In order to confirm the present results, the study may be needed to replicate using a lager sample size. In addition, it may be necessary to consider the limitations of the methodology of combined analysis used in the present study. One of the limitations is that we allocated the same "one point" to the genotype of the all three genes, while the size of the effect of the polymorphism on the metabolism of catecholamines could be different. Especially, the effect of the TH polymorphism might be more complicated than that of MAOA or COMT (Zhang et al., 2004). The comparison of the four groups according to the score ("zero" to "three") has this weakness. To compensate the limitation, we compared between subjects with the score "three" and the other subjects, and observed a support for the association between the combination of the three polymorphisms and the personality traits.

In the analysis of each gene, we found the association between the T9 allele of the TH gene and personality traits. This was not observed in Persson et al. (2000), which to our knowledge, is the only previous study investigating the association of TH with personality traits. In addition, the associations observed in the previous study (Persson et al., 2000) were not confirmed in the present study. These contradictions might be due to ethnic differences: Japanese in our study and Caucasians in Persson et al. (2000). The sample size was relatively small ($n = 205$) in the previous study; therefore, a type II error might exist. Further investigation may be preferred, with respect to the association of the TH gene.

In conclusion, the present study indicates that carrying combination of genes that increases synthesis (and decreases catalysis) central nervous system (CNS) catecholamines may significantly affect personality traits such as Extraversion and Conscientiousness.

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REVIEW ARTICLE

Review of animal models for autism: implication of thyroid hormone

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ABSTRACT **Autism is a behaviorally defined disorder associated with characteristic impairments in social interactions and communication, as well as restricted and repetitive behaviors and interest. Its prevalence was once thought to be 2/10 000, but recently several large autism prevalence reviews revealed that the rate of occurrence was roughly 30/10 000. While it has been considered a developmental disorder, little is certain about its etiology. Neuroanatomical studies at the histological level in the brains of autistic patients provide many arguments in the etiology of autism. Results from postmortem and imaging studies have implicated many major structures of the brain including the limbic system, cerebellum, corpus callosum, basal ganglia and brainstem. There is no single biological or clinical marker for autism. While several promising candidate genes have been presented, the critical loci are yet unknown. Environmental influences such as rubella virus, valproic acid, and thalidomide exposure during pregnancy are also considered important, as concordance in monozygotic twins is less than 100% and the phenotypic expression of the disorder varies widely. It is thus hypothesized that non-genetic mechanisms contribute to the onset of autistic syndrome. In light of these ambiguities, hope is held that an animal model of autism may help elucidate matters. In this article, we overview most of the currently available animal models for autism, and propose the rat with mild and transient neonatal hypothyroidism as a novel model for autism.**

Key Words: animal model, autism, hypothyroidism, thalidomide, rat

INTRODUCTION

Autism is a severe neurobiological disorder that develops in the first 3 years of life. It is characterized by impairments in social interactions and communication, as well as restricted and repetitive behaviors and interest. Its prevalence was once thought to be 2/10 000, but recently several large autism prevalence reviews revealed that the rate of occurrence is roughly 30/10 000, and its incidence is progressively increasing (Stokstad 2001; Muhle *et al.* 2004; Honda *et al.* 2005). The etiology of autism remains to be clarified. Since the first description by Kanner in 1943, autism has been attributed as the earliest manifestation of schizophrenia and then to a failure of parental nurturing. Currently its etiology is unanimously thought to derive from some developmental disorder of communication with a neurobiological basis.

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The genetic component clearly plays an important role in the pathophysiology of the disorder, as there is a concordance rate of approximately 2–6% in dizygotic twins as opposed to the 60–95% concordance rate in monozygotic twins (Ritvo *et al.* 1985; Bailey *et al.* 1995). The prevalence rate of non-twin siblings of children with autism varies from 1–6% (Hallmayer *et al.* 2002). Nevertheless, the finding of the increasing prevalence rate of autism during the past 10 years may cast some doubt on whether genetics alone can explain the whole picture. A case-control study of a group of Swedish adults with Asperger syndrome (1999) has noted that the rate of autistic children with mild mental retardation remains relatively stable, while the rate is increased in children with severe mental retardation and with normal intelligence (Gillberg & Wing 1999). Some other epidemiological studies (Ehlers & Gillberg 1993; Kadesjo *et al.* 1999) indicate that the recent increase of autistic children is mainly attributable to the increase of so-called atypical autism characterized by a lesser degree of mental retardation or normal intelligence. This higher prevalence rate of high function autism or Asperger syndrome encouraged us to accept the concept of autism spectrum disorders (ASD).

Environmental factors can cause developmental disabilities. Case reports of autism associated with environmental factors, such as rubella virus, valproic acid, and thalidomide exposure during pregnancy, lead to the hypothesis that non-genetic mechanisms may also produce an autistic syndrome (Chess 1977). Although there clearly exists a genetic component in the pathophysiology of autism, ASD appears to be a syndrome of complex genetic traits, rather than the outcome of any single mutation. Furthermore, a varied burden of environmental factors may contribute to the broad spectrum disorders of autistic syndrome with a higher prevalence rate.

Since the 1970s, researchers have known that autism is a complex genetic disorder. Thus far a number of research groups including an international consortium have tried to identify the responsible gene(s) in autism. However, although several promising candidates have been presented, the critical loci are still not known. Therefore, the animal models for autism currently available are mainly derived from empiric data such as viral infection, thalidomide exposure and valproate exposure in human subjects. The rationale for some models arises from the similarities between clinical manifestations in autism and behavioral abnormalities exhibited by treated animals. In this review, we briefly introduce currently available animal models of autism, and then present our hypothesis, the pivotal role of mild neonatal hypothyroidism, as a putative animal model for studying the underlying mechanisms of autism and/or related neurodevelopmental disorders.

NEUROANATOMICAL AND NEUROIMAGING ASPECTS OF AUTISM

Neuroanatomical studies at the histological level in the brains of autistic patients provide many arguments in the etiology of autism.

Results from postmortem and imaging studies have implicated many major structures of the brain including the limbic system, cerebellum, corpus callosum, basal ganglia and brainstem.

Areas of the forebrain that have been found to be histologically abnormal include the hippocampus, subiculum, entorhinal cortex, amygdala, mammillary body, anterior cingulate gyrus and septum, structures which comprise a major portion of the limbic system. In comparison with controls, these areas showed reduced neuronal cell size and increased cell packing density (increased numbers of neurons per unit volume) bilaterally (Bauman & Kemper 1994). Golgi analysis of CA1 and CA4 pyramidal neurons has shown decreased complexity and extent of dendritic arbors in these cells (Raymond *et al.* 1996). In the amygdala, the most significant increase in cell packing density was noted in the most medially placed nuclei. With the exception of a single child of normal intelligence, the lateral nucleus has appeared to be uninvolved.

Outside of the limbic system, the most apparent and consistent abnormalities have been confined to the cerebellum and related inferior olive. Regardless of age, sex, or cognitive abilities, all the autistic brains reported to date have shown a significant decrease in the number of Purkinje cells (Bailey *et al.* 1998). With few exceptions, there has been an absence of glial hyperplasia (Bauman & Kemper 1996; Bailey *et al.* 1998) suggesting the cerebellar lesions are acquired early in development. A similar pattern of change in cell size has also been observed in the inferior olive of the brainstem but the number of neurons has been found to be preserved. Bailey *et al.* (Bailey *et al.* 1998) have noted neocortical malformations to be a prominent feature in their autopsy material. They found evidence of thickened cortices, areas of increased neuronal density, irregular laminar patterns, increased number of neurons in layer I, and abnormally oriented pyramidal cells.

The observation of postnatal brain enlargement is intriguing and a number of hypotheses have been posed to explain its origins. Clinically, the head circumference of the autistic child has been said to be either normal or slightly small at birth but later increases in size during early to mid-childhood (Courchesne *et al.* 2003; Lainhart 2003). More recently, imaging studies have indicated increased brain volume in autism (Aylward *et al.* 1999; Sparks *et al.* 2002; Herbert *et al.* 2003; Courchesne & Pierce 2005), most prominent between 2–4.5 years of age, and later appear to plateau during adolescence (Courchesne *et al.* 2001). Schumann *et al.* (Schumann *et al.* 2004) reported that children with autism (7.5–12.5 years of age) had larger right and left amygdala volumes as well as a right hippocampal volume larger than typically developing controls, even after controlling for total cerebral volume. Brambilla *et al.* (Brambilla *et al.* 2003) reviewed original MRI research papers published from 1966 to 2003 and concluded that increased total volumes of the brain, parieto-temporal lobe, and cerebellar hemisphere were the most replicated abnormalities in autism. Interestingly, recent papers suggest the size of amygdala, hippocampus, and corpus callosum may also be abnormal, although the results are controversial (Abell *et al.* 1999; Aylward *et al.* 1999; Sparks *et al.* 2002).

CURRENT ANIMAL MODELS FOR AUTISM

Neonatally Borna disease virus infected rat

Borna disease virus (BDV) is a negative strand, non-segmented RNA virus that is the prototypic member of Bornaviridae, a new class of virus in the Mononegavirales order, and is a human pathogen (De La Torre 1994). Host factors including the age at the time of inoculation, the genetic background and the immune status, as well as viral factors, influence the course of infection. In adult rats, BDV usually causes an immune-mediated biphasic behavioral disease. After a varied incubation period, the onset of a hyperactive phase is observed, which can lead to rapid death in some animals. Excitability and hyperactivity, together with movement and posture disorders, are consistent clinical features in both natural and experimental infections. Some animals may have stereotyped behaviors. A chronic hypoactive phase with somnolence follows in conjunction with a decrease in the inflammatory reaction and high levels of virus in the Central Nervous System (CNS). During this chronic phase, symptoms resembling those of the initial phase may reemerge in the form of recurrent episodes (Taieb *et al.* 2001). Heightened viral gene expression in limbic system structures, together with astrocytosis and neuronal structural alterations within the hippocampal formation are the main histopathologic hallmarks of BDV infection in adult rats (De La Torre 2002).

When BDV is inoculated into a newborn Lewis rat, it causes a life-long persistent infection that is characterized by the lack of any significant inflammatory cell infiltration within the central nervous system (CNS) and the absence of clinical signs of BDV (Pletnikov *et al.* 1999a).

Intracranial injection of the BDV in a newborn rat pup within the first 24–48 h after birth is the most common way of inducing neonatal BDV infection in rats (Pletnikov *et al.* 2003). Infected rats have normal body shape and proportion but are overall smaller than uninfected control pups. Injury to the cerebellum is one of the most salient morphological features of neonatal infection. BDV infection induces a prominent loss of Purkinje cells (PC), with up to 75% of PCs dropping out by seven months. A loss of PCs and their dendrites in the molecular layer has been suggested to play a major role in markedly reducing cerebellum size (Hornig *et al.* 1999).

In addition to injury of the cerebellum, neonatal BDV infection affects the postnatal maturation of the hippocampus. BDV infection of dentate gyrus neurons is associated with their continuing loss and eventual complete disappearance by 45–55 postnatal days (PNDs) and replaced by reactive glial cells (Hornig *et al.* 1999; Gonzalez-Dunia *et al.* 2000).

Neonatal BDV infection also induces cortical shrinkage. It has been shown that up to 30% of cortical neurons are lost in BDV-infected rats by PND 45. Similar to the hippocampus, diminished immunoreactivity for GAP-43 and synaptophysin is observed in the neocortex of neonatally BDV-infected rats (Gonzalez-Dunia *et al.* 2000).

Neonatally BDV-infected rats have very robust astrocytosis. Astrocytes, oligodendrocytes, ependymal cells and Schwann cells in the peripheral nervous system all express BDV markers (Bautista *et al.* 1995; Pletnikov *et al.* 2002). In the late stages of neonatal infection, BDV spreads centrifugally by anterograde axonal transport and infects most inner organs innervated by peripheral or autonomic nerves.

BDV-induced neuroanatomical damage is likely to underlie the behavioral abnormalities observed in BDV-infected rats. BDV-associated behavioral deficits are as follows; (i) selectively deficient social behaviors; (ii) changes in emotional behavior; (iii) selectively reduced cognitive abilities in spatial memory and learning/contextual fear conditioning; and (iv) spontaneous locomotor hyperactivity, hyper-reactivity and stereotypy along with mild gait ataxia (Dittrich *et al.* 1989; Hornig *et al.* 1999; Bauer *et al.* 2002). Neonatally BDV-infected rats show no evidence of gross ataxia and have normal swimming speeds despite the significant cerebellar lesions (Bautista *et al.* 1995).

Pletnikov *et al.* (Pletnikov *et al.* 1999a) showed that BDV-infected adult Lewis rats exhibited locomotor hyperactivity and elevated defecation in a highly aversive and brightly lit open field,