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 "ℒ" 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	$\chi^2=4.69$, $P=0.030$	Short allele	1997
3.	Maestrini E et al.	Caucasian	TDT	90 families (82 multiplex and 8 singleton)	$\chi^2=0.11$, $P=0.80$	Long allele	1999
4.	Persico AM, et al.	①Italian, ②Caucasian-American	TDT	①54 trios ②44 trios	①$\chi^2=0.51$, $P=0.48$ ②$\chi^2=0.02$, $P=0.89$ ①+②$\chi^2=0.40$, $P=0.53$	Short allele Long allele Long allele	2000
5.	Yirmiya N et al.	?? (Israel)	HRR design TDT	34 families	Likelihood ratio=5.99, $P=0.014$ $\chi^2=5.44$, $P<0.025$	Long allele	2001
6.	Tordjman S et al.	Caucasian (French child hospital)	TDT	71 trios	$\chi^2=4.00$, $P=0.046$	Long allele	2001
7.	Persico AM et al.	Italian, Caucasian-American	HHRR	①155 trios ②57 unaffected sibs	①$\chi^2=1.47$, $P=0.23$ ②$\chi^2=0.45$, $P=0.50$ ①vs. ②$\chi^2=0.81$, $P=0.37$	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	①$\chi^2=1.73$, $P<0.19$ ②$\chi^2=0.62$, $P<0.43$ Total: $\chi^2=2.03$, $P<0.15$	Short allele	2002
9.	Kim SJ et al.	Caucasian, African-American, Hispanic-American, Asian-American	TDT	①81 trios ②115 trios	①$\chi^2=2.32$, $P=0.128$ ②$\chi^2=7.31$, $P=0.007$ ②$\chi^2=7.31$, $P=0.007$	Short allele	2002
10.	Conroy J et al.	Irish	TDT	84 trios	$\chi^2=4.5252$, $P=0.0334$	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	$\chi^2=0.086$, $P=0.77$	Long allele	2005
13.	Our results	Japanese	TDT	104 trios	$\chi^2=1.92$, $P=0.17$	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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Rapid communication

No association of *FOXP2* and *PTPRZ1* on 7q31 with autism from the Japanese population

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Abstract

Autism is a child-onset pervasive developmental disorder, with a significant role of genetic factors in its development. Genome-wide linkage studies have suggested a 7q region as a susceptibility locus for autism. We investigated several single nucleotide polymorphisms (SNPs) of *Forkhead Box P2* (*FOXP2*) and *Protein-Tyrosine Phosphatase, Receptor-type, Zeta-1* (*PTPRZ1*) at the 7q region in Japanese patients with autism and healthy controls. No significant difference was observed, after correction for the multiple testing, in allele, genotype or haplotype frequencies of the SNPs of *FOXP2* or *PTPRZ1* between patients and controls. No evidence was thus obtained for a major role of *FOXP2* or *PTPRZ1* in the development of autism.

Keywords: Autism; Chromosome 7q; FOXP2; PTPRZ1; Genetic association

Autism is a neurodevelopmental disorder characterized by impairment in reciprocal social interaction and communication (or language), restricted and stereotyped pattern of interest and activities, occurring within the first 3 years of life. A disrupted growth of the brain, with unknown mechanism, is suggested on the background of autism. Twin and family studies have indicated a robust role of genetic factors in the development of autism (Folstein and Rosen-Sheidley, 2001). A number of genome-wide linkage studies have been conducted in Caucasian families with autism and its spectrum disorders. Although the results of the linkage studies were controversial, several studies have provided evidence for a chromosomal 7q region (7q21-31.3) as a susceptibility locus (or loci) of autism (reviewed by Folstein and Rosen-Sheidley, 2001). Candidate genes of autism on this chromosomal region may include *Forkhead Box P2* (*FOXP2*) and *Protein-Tyrosine Phosphatase, Receptor-type, Zeta-1* (*PTPRZ1*), which are both located on 7q31.

FOXP2 encodes a forkhead family protein, which plays diverse and important roles in embryogenesis (Kaufmann and Knochel, 1996). The gene contains 17 exons spanning over 274.9 kb. *FOXP2* is expressed in confined regions of the brain during embryogenesis, in contrast to its ubiquitous expression during adulthood, suggesting its role in the prenatal brain development (Lai et al., 2001). Interestingly, mutations of *FOXP2* may be responsible for speech and language disorder in a unique three-generation pedigree and an independent individual (Lai et al., 2001). Affected members of the family had disturbances in usage of grammar as well as phonation of the language. Voxel-based morphometry (VBM) of the brain showed reduction of the

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gray matter in some of the brain region in the affected members (Vargha-Khadem et al., 2005). Thus far, a Chinese group reported a weak support for association between the *FOXP2* gene and autism (Gong et al., 2004), while other studies observed no association (Gauthier et al., 2003; Newbury et al., 2002; Wassink et al., 2002).

PTPRZ1 is also highly expressed in the brain during embryogenesis (Levy et al., 1993). The gene contains 30 exons spanning over 188.6 kb. PTPRZ1 is a large receptor-type protein of tyrosine phosphatase. Phosphorylation of tyrosine-residues plays a key role in the signaling of cell growth and differentiation. PTPRZ1 plays a role in recovery and survival of oligodendrocytes in demyelinating disease (Harroch et al., 2002). Glial cells including oligodendrocytes may be involved in neurodevelopmental disorders including autism (Dong and Greenough, 2004). Thus far, Bonora et al. (2004) observed no association between *PTPRZ1* and autism.

Here we studied these two genes in 170 unrelated Japanese patients with autism (147 males and 23 females, mean age = 20.8 years with the range of 3–41 years) and 214 unrelated healthy volunteers (145 males and 69 females, mean age = 34.6 years with the range of 21–65 years). All patients met the DSM-IV criteria for autistic disorder. The patients were recruited from the outpatient clinics of the departments of psychiatry, Tokyo University Hospital and Tokai University Hospital, and seven daycare facilities for subjects with developmental disorders. All the hospital and facilities were located around Tokyo. Apparent physical anomalies were not observed in the subjects. Controls were mainly recruited from the hospital and facility staff. All controls resided in the same area (Kanto District or around Tokyo) as the patients. All the patients and controls were ethnically Japanese, with no parents or grandparents of ethnicity other than Japanese.

Confirmation of the diagnosis was conducted as follows. Semi-structured behavior-observation of the patients and interview of them and their parents were conducted, for most of the cases, by two experienced child psychiatrists independently. When one of the parents was not available, mothers were interviewed in most of the cases. At the interview of the parent(s), the Child Behavior Questionnaire Revised (Izutsu et al., 2001) was used to assist the evaluation of the autism-specific behaviors and symptoms. Diagnosis was made according to the DSM-IV criteria. After the initial observation and interview, the patients were followed up to examine the behaviors and symptoms for several months (for at least 6 months in most of the cases) and those who were not considered to meet the DSM-IV criteria during the follow-up were excluded from the sample. The present study was approved by the Ethical Committees of the University of Tokyo and Tokai University. Informed consent was obtained from all subjects and healthy controls. Peripheral blood was obtained and genomic DNA was extracted using the standard phenol–chloroform method.

Single nucleotide polymorphisms (SNPs) of the genes were analyzed, using ABI prism 7900HT sequence detective system (Applied Biosystems Foster City, CA, USA). Five SNPs and seven SNPs of the *FOXP2* and *PTPRZ1* genes, respectively, were selected from the list of Assays-on-Demand™ Products for ABI PRISM 7900HT for this association study. SNPs with putative high minor allele frequencies in Japanese, according to the database for the Applied Biosystems Assays-on-Demand™ SNP Genotyping Assays, were predominantly selected for the study. The db SNP IDs of the SNPs are shown in Tables 1 and 2. Primers and probes of the ABI Assays-on-Demand™ kit were used for the genotyping.

Statistical analyses including chi-square tests and others were performed using the SAS/Genetics 9.1 software (SAS Institute Inc. Cary, North Carolina, USA). D′ and r2 of the linkage disequilibrium between SNPs and frequencies of haplotypes consisting of SNPs, which were at high linkage disequilibrium, were estimated. Exact p-values based on the likelihood ratio test with 10,000 permutations were calculated for comparison of haplotype frequencies between patients and controls.

Allele frequencies of the SNPs of *FOXP2* and *PTPRZ1* are summarized in Tables 1 and 2. The frequency of the minor allele of the SNPs was higher than 30%, except for the SNP 4 of *FOXP2* and the SNPs 4 and 6 of *PTPRZ1*. For the most of the SNPs, the allele frequencies were almost same between the patients and controls. No significant difference was observed in the allele frequencies of any of the SNPs

Table 1
Allele frequencies of five SNPs of the FOXP2 gene in autism patients and controls

Locus	db SNP ID	Allele A/B[a]	Patients		Controls		Chi-square	p-Value	Odds ratio	95% Confidence intervals		Chromosome position
			Allele B		Allele B							
			N	%	N	%				Lower	Upper	
SNP 1	rs2106900	[C/T]	102	30	124	30	0.043	0.837	0.97	0.71	1.32	113145303
SNP 2	rs2061183	[G/C]	84	25	105	25	0.007	0.933	0.99	0.71	1.37	113280812
SNP 3	rs1456029	[A/G]	143	42	203	48	2.354	0.125	1.25	0.94	1.67	113313550
SNP 4	rs1005958	[A/G]	27	8	31	7	0.140	0.709	0.90	0.53	1.54	113325658
SNP 5	rs1058335	[C/T]	114	34	156	37	0.860	0.354	1.15	0.85	1.56	113356466

The SNPs 1–5 are located at introns 1, 27, 14 and the 3′ region of the gene, respectively. Chromosome position on the SNPs is according to TCAG consortium.
[a] Alleles "B" are minor alleles.

Table 2
Allele frequencies of seven SNPs of the *PTPRZ1* gene in autism patients and controls

Locus	db SNP ID	Allele A/B[a]	Patients		Controls		Chi-square	*p*-Value	Odds ratio	95% Confidence intervals		Chromosome position
			Allele B		Allele B							
			N	%	*N*	%				Lower	Upper	
SNP 1	rs740960	[G/T]	113	34	143	34	0.014	0.904	1.02	0.75	1.38	120551052
SNP 2	rs1206504	[A/G]	102	30	144	34	1.312	0.252	1.20	0.88	1.63	120592038
SNP 3	rs1196490	[G/A]	112	33	153	36	0.721	0.396	0.88	0.65	1.19	120612421
SNP 4	rs1196509	[C/T]	20	6	26	6	0.012	0.914	1.03	0.57	1.89	120640281
SNP 5	rs1196475	[C/T]	119	35	142	33	0.362	0.547	0.91	0.67	1.23	120673905
SNP 6	rs1147489	[A/G]	62	18	67	16	0.864	0.353	0.84	0.57	1.22	120703821
SNP 7	rs1206381	[A/C]	111	33	128	31	0.584	0.445	0.89	0.65	1.21	120723515

The SNPs 1–7 are located at introns 1 (SNPs 1 and 2), 2, 4, 11, 19 and 27, respectively. Chromosome position on the SNPs is according to TCAG consortium.
[a] Alleles "B" are minor alleles.

between the groups. No significant difference was found in genotype frequencies between the groups, either (not shown in the table). When analyzed by sex, a difference was observed in allele frequencies of the SNP 3 (rs1456029) of *FOXP2* at intron 7 between male patients and controls (chi-square = 5.06, d.f. = 1, $p = 0.024$, uncorrected). The difference was not statistically significant after correction for the multiple testing. No other significant difference was observed. Hardy-Weinberg disequilibrium ($p < 0.05$) was significant for rs740960 marker in the patients (PTPRZ1, $p = 0.00570$), not for other 11 markers. The disequilibrium for rs740960 was not statistically significant after the correction for the 24 tests (cases and controls of the 12 SNPs).

Strength of linkage disequilibrium, denoted as D′, between pairs of the SNPs of *FOXP2* and *PTPRZ1* is summarized in Table 3(a) and (b), respectively. The SNP 1 and 2 of *FOXP2* were at high LD and the SNPs 4–7 of *PTPRZ1* appear to form a LD block, both in controls and patients. Haplotype frequencies of the *FOXP2* SNPs 1–2 and the *PTPRZ1* SNPs 4–7 were estimated and compared between patients and controls using permutation test. No significant difference was observed.

Table 3
The strength of LD (denoted as D′) between pairs of SNPs of (a) *FOXP2* and (b) *PTPRZ1*in autism patients (the lower diagonal) and controls (the upper diagonal)

SNP	1	2	3	4	5
(a) *FOXP2*					
1		0.94	0.37	0.35	0.14
2	0.92		0.40	0.47	0.82
3	0.53	0.43		0.21	0.22
4	0.59	0.29	0.88		0.74
5	0.15	0.54	0.09	1.00	

SNP	1	2	3	4	5	6	7
(b) *PTPRZ1*							
1		0.40	0.91	0.24	0.53	0.50	0.56
2	0.62		0.44	1.00	0.21	0.34	0.24
3	0.90	0.67		0.26	0.59	0.55	0.61
4	0.15	0.09	0.28		1.00	1.00	1.00
5	0.67	0.52	0.85	1.00		1.00	0.99
6	0.66	0.52	0.85	1.00	1.00		1.00
7	0.67	0.56	0.85	1.00	1.00	1.00	

The 95% confidence intervals of odds ratios were within 0.53 and 1.67 in 11 of the 12 SNPs of the two genes. The only exception was rs1196509, where minor allele frequency was extremely low (6%) compared with the others. Thus, our results might have adequate statistical power to contradict the effects of the genes with odds ratios of approximately 1.8 or more. However, the present sample size is not adequate to detect smaller effect of the genes.

Genetic association between these genes and autism has been explored in a limited number of studies. *PTPRZ1* was previously investigated in a study, with other six genes on the 7q area. While the study provided suggestive evidence for the role of other genes, including *LAMB1* and *NRCAM*, no support was observed for the role of *PTPRZ1* in autism, (Bonora et al., 2004). This result on *PTPRZ1* may be consistent with the present study. Regarding *FOXP2*, a Chinese group found an association of one SNP (rs1456031 at intron 9 of *FOXP2*) out of three with autism using transmission disequilibrium test (TDT) (Gong et al., 2004). The level of the significance did not reach statistical significance after correction for the multiple testing of the three SNPs. We attempted to investigate this polymorphism. However, another SNP (rs6966051) was within 2bp of the rs145631, which made the precise genotyping unable at present. Other studies that investigated the role of *FOXP2* provided no evidence for the linkage or association between the gene and autism, which may be consistent with the present result (Gauthier et al., 2003; Newbury et al., 2002; Wassink et al., 2002). Studies thus far to our knowledge, including the present one, may therefore yield no or few support for the genetic association between *PTPRZ1* or *FOXP2* with autism.

A major limitation of the present study may be the limited sample size. Although the present result may contradict the effect of the genes with odds ratio of 1.8 or more, smaller effects might not be detected in the present sample. Another concern may be population stratification of the sample,

which could affect studies in case-control design. This might not however significantly affect the present study in homogeneous Japanese population. No subjects in this study had parents or grandparents of ethnicity other than Japanese. Also acknowledged might be that the controls in this study were not age-matched to the patients. But this may not be likely to significantly affect the result, considering the homogeneity of the population and no major effect of environmental factors in autism (Folstein and Rosen-Sheidley, 2001).

In conclusion, no evidence was provided for the association of *FOXP2* and *PTPRZ1* with autism. However, a weak tendency for the association was observed in male subjects for one SNP of *FOXP2*. The present sample size may not have adequate power to detect very small effects of the genes. Further investigations with larger samples are recommended to detect more subtle effects of these genes on autism.

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REVIEW ARTICLE

A 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,

whereas uninfected control rats showed slightly decreased ambulation when compared in a less aversive, dimly lit open field. Moreover, compared to controls, neonatally BDV-infected rats exhibited attenuated habituation of the acoustic startle at PND 23 and decreased startle responsiveness at PND 30. Prepulse inhibition of the acoustic startle reflex remained unaltered in BDV-infected rats (Pletnikov *et al.* 2001).

Another behavioral task requiring the integrity of the limbic system, particularly the hippocampus, is contextual fear conditioning. Freezing behavior and defecation response can be used in rats for assessing the amount of contextual fear conditioning. BDV-infected rats demonstrated attenuated conditional freezing in the context previously paired with either sudden loud noise or foot shock compared to control rats (Pletnikov *et al.* 1999a).

Both the hippocampus and cerebellum play a major role in the acquisition phase of the spatial navigation task and lesioning of these areas impairs acquisition of a hidden platform location in the Morris water maze (MWM). In the MWM, neonatally BDV-infected rats exhibited a performance deficit. At PND 72, BDV-infected rats had difficulties in learning the location of the platform over a series of swim trials (Rubin *et al.* 1999).

Neonatal BDV infection induced abnormal social interaction and communication in Lewis rats when tested as old as 30–35 days of age. Studies were conducted using the resident/intruder paradigm. A resident rat was isolated for one week in order to increase social motivation. An unfamiliar rat (intruder) was placed in the resident's cage. This scenario is conducive to social interactions between the rats, often resulting in play behavior (measured as number of pins, similar to a pin observed in a wrestling match) (Pletnikov *et al.* 1999b). As the result, control rat pairs exhibited significantly more pins than the pairs where either one or both rats were BDV-infected rats. Similarly, play soliciting behaviors (e.g. pounce, crawl over/under and darting) were reduced in BDV-infected rats. Nonsocial exploratory activity (e.g. ambulation and rearing) was similar in BDV-infected and non-infected residents. Duration of non-play social investigation (e.g. sniffing, approach, and follow) was higher in BDV-infected rats as compared to non-infected controls.

However, there is little serological evidence that suggests BDV infects humans (Chalmers *et al.* 2005), and its role in psychiatric disorders remains controversial.

Chemical teratogenic model of autism

Thalidomide exposure: rats

Thalidomide (THAL) was used worldwide at the end of the 1950s and beginning of the 1960s for the treatment of anxiety and insomnia. Lenz carried out analysis of hypoplastic malformations of the limbs and reported a correlation between the intake of THAL during pregnancy and the observed birth defects (Lenz *et al.* 1962). In addition to limb defects, THAL may give rise to a wide spectrum of malformations of various organ systems. Anomalies noted are heart defects, laryngeal and tracheal abnormalities, anotia, microtia, and hearing impairment, choanal atresia, microphthalmia, cloboma, intestinal atresia, aplastic or hypoplastic gallbladder, renal anomalies, criptorchism, vaginal and anal atresia, as well as dysfunction of cranial nerves, notably the 6th and 7th nerve (Miller & Stromland 1999).

Recent epidemiological studies have revealed that THAL exposure during the first trimester in humans causes higher incidence of autism in the offspring. Exposure between the 20th and 24th day of gestation led to an incidence of autism of 5 out of 15 cases (Stromland *et al.* 1994; Miller *et al.* 2005). This critical period for exposure corresponds to the time of early development of the Central Nervous System (CNS), when the neural tube begins to form. On the basis of somite numbers in early embryos of rats and humans, E9-E11 in rats is considered to be from early somite stage corresponding to approximately E20-E24 in human embryos (Rice & Barone 2000). Models exposed to THAL showed a reduction of cell numbers in the cranial nerve motor nuclei, reductions in Purkinje cell number and cerebellar volume, and retarded migration of 5-HT neuron (Rodier *et al.* 1997; Narita *et al.* 2002). Narita *et al.* (Narita *et al.* 2002) reported that a significant increase of hippocampal serotonin concentration was observed in the group exposed to THAL on E9. E9 THAL exposure resulted in an increase of hippocampal serotonin and frontal cortex dopamine, as well as hyperserotonemia. These observations all parallel the reported human autistic pathologic findings (Rodier *et al.* 1997).

Although neurobehavioral investigations have been scarce, Vorhees *et al.* (Vorhees *et al.* 2001) have reported that male THAL exposed rat pups show significant increases in errors and latency in the multiple-T Cincinnati water maze. They also indicated that THAL exposure induced increased preweaning mortality and male specific, late onset reduction in growth in rat pups (Vorhees *et al.* 2001).

Valproic acid exposed rat model

While THAL may have a teratogenic effect in rodents that differs from that in primates (Schumacher *et al.* 1972), valproic acid (VPA) has a similar effect in rodents and humans (Kemper & Bauman 1993).The effect of VPA are observed if the rat brainstem is exposed to VPA *in utero* and the somatic effects are similar to those of THAL (Kemper & Bauman 1993). Offspring of female rats injected with VPA at the time of neural tube closure show brain abnormalities resembling those found in autistic patients (Christianson *et al.* 1994). There are several brainstem abnormalities found so far in rats exposed to VPA *in utero*; (i) diminished number of motor neurons in the oculomotor, trigeminal, abducens, and hypoglossus nuclei of cranial nerves; (ii) shortening of the region caudal to the facial nucleus and lengthening of the region rostral to the facial nucleus; (iii) smaller cerebellum with reduction of a number of Purkinje cells both in the hemispheres and vermis; and (iv) reduced cerebellar nucleus interpositus (Rodier *et al.* 1997; Ingram *et al.* 2000a)

Schneider *et al.* (Schneider *et al.* 2001) have suggested that rats exposed to VPA during gestation may resemble the abnormalities seen in autism both neurophysiologically and behaviorally. They have demonstrated that VPA exposed rat offspring exhibit (i) lower sensitivity to pain and higher sensitivity to non-painful stimuli; (ii) diminished acoustic prepulse inhibition; (iii) locomotor and repetitive/stereotypic-like hyperactivity combined with lower levels of exploratory activity; and (iv) decreased number of social behaviors and increased latency to social behaviors.

Neonatal amygdala lesioned rat

Results from neuroanatomical studies indicate that medial temporal lobe structures, especially amygdala, may be implicated in the pathogenesis of autism (Bachevailier 1996; Baron-Cohen *et al.* 2000). Some authors have noted similarities between autism and the Kluver–Bucy syndrome, a syndrome caused by bilateral lesions to the anterior temporal lobes in monkeys (Baron-Cohen *et al.* 2000). Monkeys with the Kluver–Bucy syndrome display features often seen in autistic subjects such as absence of social chattering, lack of facial expression and absence of emotional reactions. Other such similarities include repetitive abnormal movement patterns, increased aggression, and the tendency to examine objects by mouth or smell. Several post-mortem studies in autistic subjects

have demonstrated amygdale abnormalities with small neuronal size and increased cell-packing density (Bauman & Kemper 1985; Kemper & Bauman 1993; Bailey *et al.* 1998).

Experimental lesion studies in non-human primates provide further evidence for medial temporal lobe involvement in autism. Bilateral lesions to the medial temporal lobe in infant rhesus monkeys have resulted in long-term deficits in social behavior, an effect that is absent in monkeys receiving similar lesions in adulthood (Bachevailier 1996). Monkeys subjected to bilateral removal of the amygdala, hippocampus, and adjacent cortical areas were uninterested in and avoided social contacts. Those monkeys also developed autistic-like characteristics, such as unexpressive faces, very little eye contact, locomotor stereotypies, and self-directed activity (Prather *et al.* 2001; Bauman *et al.* 2004).

Neonatal ibotenic acid lesion of the amygdala in the rat has also been proposed as an animal model of autism. Excitotoxic lesions of the amygdala at PND 7, but not PND 21 in rat, produce multiple behavioral abnormalities persisting into adulthood, indicating neurodevelopmental deficits of structures connected to the amygdala (Daenen *et al.* 2002a). Lesioning the amygdala on PND 7 resulted in an adult animal with stereotypic-like increased ambulatory behaviors and decreased investigatory behaviors. Moreover, those animals exhibited increased locomotor reactivity to challenge with a low dose of apomorphine, reminiscent of supersensitivity of postsynaptic dopamine systems in the nucleus accumbens (Wolterink *et al.* 2001; Daenen *et al.* 2002a).

Other lesioned animals

There have been several reports suggesting that neonatally ventral hippocampus (VH) lesioned rats show many aspects of abnormalities in behavior and cellular formation reminiscent of schizophrenia. When tested as juveniles (PND 35), rats with the neonatal VH lesions are less social than controls (Sams-Dodd *et al.* 1997), but otherwise behave normally in motor tests involving exposure to stress and dopamine agonists. In adolescence and adulthood (PND 56 and older), lesioned animals display markedly changed behaviors such as motor hyperresponsiveness to stress and stimulants, and enhanced stereotypies. They also show deficits in PPI and latent inhibition, impaired social behaviors and working memory problems (Lipska & Weinberger 1993; Lipska & Weinberger 1994; Lipska *et al.* 1995).

However, other reports found that rats lesioned in the VH on PND 7 or PND 21, showed no differences in social behavior related or unrelated to social play behavior early in life or in adulthood (Wolterink *et al.* 2001; Daenen *et al.* 2002b). In monkeys, emotional behavior was not disturbed with damage in the hippocampal area only (Bauman *et al.* 2004). Wood *et al.* (Wood *et al.* 1997) suggested that the pattern of impairments associated with the excitotoxic VH lesion varies depending on the age at which lesioning occurs. Consequently, VH lesioned rats are still considered to be controversial as a model of autism.

Early prefrontocortical damage in humans has been shown to impair cooperative and reciprocal behavior, social interactions, and social cognition (Eslinger *et al.* 2004). It is suggested that dysfunctions and morphological abnormalities of the prefrontal cortex (PFC) are implicated in the pathophysiology of autism (Baron-Cohen *et al.* 1999). Neonatal PFC lesions have also been proposed as an adequate model to investigate early developmental aberrations (Schneider & Koch 2004). The total amount of self-grooming and social behaviors was reduced in PFC lesioned animals compared to controls. Neonatal PFC lesions reduced pinning in juvenile rats and lesioned rats showed an increase in the total number of so called 'partial rotations'. Partial rotation is an adult-like pattern of defense, so investigators suggested that neonatal lesions of PFC lead to a behavioral shift of social play in juvenile rats to an adult-like pattern of defense (Schneider & Koch 2004).

There is growing evidence that the cerebellum is implicated in autism. Recently, many studies have demonstrated that the cerebellum is involved not only in the regulation of motor skills, but also in more complex integrated functions, such as classical conditioning, learning of motor skills, spatial learning, habituation of exploratory behavior and the acoustic startle response (McCormick & Thompson 1984; Leaton & Supple 1986; Leaton & Supple 1991; Dahhaoui *et al.* 1992a,b; Molinari *et al.* 1997). The cerebellum is further implicated in motivations and emotional behavior as well (Heath *et al.* 1980; Caston *et al.* 1998). Adult rats with midline lesions of the cerebellum performed at PND 10 exhibited the hyperactivity in the open field test as well as overt disinhibition tendencies in the anxiety and social discrimination tests (Bobee *et al.* 2000). These results indicate the involvement of the cerebellar vermis in the pathology of autism, considering a number of autistic subjects have a hypoplasia of cerebellar vermal lobules.

Genetic model

Recently, overwhelming evidence of genetic underpinnings of autism has generated much research. As this field is rapidly developing, many candidate loci for autism have been published in recent years. Spontaneous mutants or transgenic animal models can greatly help to delineate the role of these candidate genes.

The nonapeptide oxytocin (OT) is synthesized in the hypothalamus and released into the blood stream via axon terminals in the posterior pituitary or neurophypophysis. OT receptors are concentrated in several brain regions involved in social behavior in the mouse, including the olfactory bulbs, piriform cortex, amygdala and lateral septum. OT facilitates the formation of the mother-infant bond in sheep and stimulates nurturing behaviors in rodent females toward pups. In male rats, chronic OT treatment doubles the time spent in social contact. OT knockout mice (OTKO) fail to remember recently encountered individuals despite apparently normal olfactory and general cognitive abilities (Young 2001; Winslow & Insel 2002). Central injections of OT prior to the first encounter, but not after, completely rescue this very specific deficit and infusions of an OT antagonist inhibit social recognition in normal wild-type (WT) mice (Ferguson *et al.* 2000). Both WT and OTKO mice showed a similar neuronal activation in the initial encounter, as evidenced by the comparable c-Fos immunoreactivity in olfactory bulbs, piriform cortex, cortical amygdala, and the lateral septum. However, WT mice, but not OTKO mice, exhibited an induction of c-Fos in the medial amygdala, whereas OTKO, but not WT mice, showed dramatic increases in c-Fos in the somatosensory cortex and the hippocampus (Ferguson *et al.* 2000). These findings have an interesting parallel with recent neuroimaging studies in autistic human patients, suggesting that people with autism utilize alternative cortical areas to process social cues, areas that are typically activated by non-social cues in normal subjects (Schultz *et al.* 2000).

Recent genetic reports implicate a number of genes in the causation of autism and the Reelin gene (*RELN*) is one such gene (Fatemi *et al.* 2001). Persico *et al.* (Persico *et al.* 2001; Zhang *et al.* 2002) reported that individuals inheriting alleles of the Reelin gene that contain 11 CGG repeats in the 5′-UTR of the *RELN* mRNA have an increased risk of autism. Another group has reported that autistic patients and their first-degree relatives show significantly reduced plasma levels of full-length Reelin and its low molecular weight isoforms (Fatemi *et al.* 2001). The reeler mutation is a spontaneous recessive mutation in mice that leads, in the homozy-

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gous state, to the absence of Reelin and to severe disorganization of cortical, hippocampal, and cerebellar development. In comparison to WT mice, heterozygous reeler mice (rl/+) displaying Reelin levels reduced by 50% do not show gross developmental abnormalities of the CNS, but do show a progressive loss of Purkinje cells in the cerebellum during the first postnatal weeks (Tueting *et al.* 1999). The loss of Purkinje cells is seen only in male rl/+, and not in female rl/+mice (Hadj-Sahraoui *et al.* 1996).

Another interesting example of genetically altered mouse models presenting autistic-like features is mice deficient for Dishevelled-1 (Dvl1) proteins. Dvl1 is one of three mouse homologs of the Drosophilla segment polarity gene Dishevelled. Mice deficient in Dvl1 were reported to exhibit abnormal social interaction as well as deficits in sensorimotor gating, as measured by impaired prepulse inhibition (PPI) (Lijam *et al.* 1997). These mice have been noted as a potent model for autism or schizophrenia, but the deficits in social memory task and PPI were not replicated in Dvl1-null mice in a later study (Long *et al.* 2004).

NEONATAL HYPOTHYROIDISM RATS

Thyroid hormone is essential for brain development and maintenance of basal metabolic rates. The manipulation of thyroid hormone in laboratory animals typically increases activity levels and decreases performance during motivated learning tasks. It is well-known that hypothyroidism during the critical period of brain development induces irreversible dysfunction of the central nervous system. The timing of thyroid hormone manipulation plays a critical role in the degree to which developmental sequelae are expressed. The anatomical bases of behavioral and intellectual deficits may result from global reductions in brain size, premature termination of neuronal proliferation, non-migrated granule cells in the cerebellar cortex and caudate nucleus, decreased synaptic junctions in cerebellar cortex and malformed dendrites on Purkinje cells (Lewis *et al.* 1976). Humans with primary or secondary congenital hypothyroidism demonstrate deficits in academic skills as children, and as adults, decreased performance on neuropsychological tests and prolongation of latencies for visual- and auditory-evoked potentials (Murphy & Nagy 1976; Osterweil *et al.* 1992).

Lactating rats receiving 0.02% propylthiouracil (PTU) in their drinking water transfer the goitrogenic effect to the offspring through their milk. This treatment induces a temporary mild hypothyroid condition of the pups (Van Middlesworth & Norris 1980). We conducted experiments to investigate the effects of temporary neonatal PTU-induced hypothyroidism on the behavior of rats. Rat pups were treated with 0.02% PTU in drinking water to dams from day 0–19 post partum (Kato *et al.* 1982). The serum T4 level was depressed below the limit of detection at 2 weeks of age, but recovered to the normal level at 4 weeks of age (Akaike *et al.* 1991). The open field test was conducted at 3, 6, and 9 weeks of age. At 3 weeks of age, the number of ambulations did not differ between PTU rats and controls. At 6 and 9 weeks of age, the number of ambulations of the PTU rats was significantly greater than that of the control rats. Kato *et al.* reported extensive hyperactivity (Akaike *et al.* 1991; Akaike & Kato 1997) and attenuated habituation in the open field test in PTU rats after maturation, as shown in Figure 1 (Kato *et al.* 1992).

Spatial learning ability was further investigated in the PTU rats. Biel water maze tests at the age of 6 weeks showed an increase in errors with prolonged swimming time in the PTU rats. The radial arm maze test was performed to evaluate spatial maze learning. The test started at 13 weeks and revealed that the PTU animals required more trials until they showed the first well-performed trial. The PTU rats showed more active moving from arm to arm compared to controls. However, while the number of total choices of PTU rats was increased the number of correct choices was smaller than the control values (Akaike *et al.* 1991; Akaike & Kato 1997). The performance of PTU rats was further assessed by the modified T-maze test and then the mirror image of the first trial (Fig. 2a). The performance of PTU rats was superior to that of the controls in the initial maze test, but it was clearly inferior to that of the controls in terms of a higher error frequency and a longer running time upon reversal of the route to the mirror image of the original (Fig. 2b). This was interpreted as inability to adapt to changes in the environment and a reference for the highly repetitive and routine response pattern initially acquired.

As stated earlier, the most apparent and consistent neuropathology in autistic patients lies in the cerebellum. In this regard, it might be of significance that PTU rats have retarded granular cell migration in the external granular layer (Sadamatu & Watanabe 2005). Furthermore, PTU rats exhibited a marked susceptibility to audiogenic seizures, starting from the age 7 weeks and persisting into adulthood (Yasuda *et al.* 2000). 9

These results suggest that mild hypothyroidism around the critical period causes permanent impairment of brain function, as manifested by hyperactivity, lack of habituation, spatial learning impairment and auditory hypersensitivity. It is thus expected that PTU-treated rats may serve a useful model for autism.

DISCUSSION

The extremely high concordance rate of autism in monozygotic twins, as compared in dizygotic twins, clearly indicates the important role of genetic factors. The published genome screens have found convergent evidence for linkage in several genomic regions, with regions on chromosome 2, 7, 15, 16 (IMGSAC 2001a). In particular a region on chromosome 7q showed increased allele sharing in all screens (Risch *et al.* 1999; IMGSAC 2001a,b; Bartlett *et al.* 2005). *RELN* (Persico *et al.* 2001) and *HOXA1* (Ingram *et al.* 2000b), both on chromosome 7q22, are the most prospective candidate genes. In human subjects, one report showed that blood levels of Reelin were reduced (Fatemi *et al.* 2002). Although the case-control and affected sib-pair findings fail to support a role for *RELN* in susceptibility to Autism Spectrum Disorder (ASD), the more powerful family–based association study demonstrates that *RELN* alleles with larger numbers of CGG repeats may play a role in the etiology of some cases of ASD, especially in children without delayed phrase speech (Zhang *et al.* 2002; Bonora *et al.* 2003). Recent studies have reported conflicting findings of an association between a variant of the *HOXA1* gene and autism (Ingram *et al.* 2000b; Conciatori *et al.* 2004; Gallagher *et al.* 2004). Thus, so far a single gene responsible for the pathogenesis of autism has not been found and, it seems unlikely that any single gene can explain the whole picture of autism.

Genes have two broad roles, the first being the template function and the second the transcriptional function. Although the template function is largely independent of outside forces, the transcriptional function is highly regulated and responsive to environmental factors.

The question of whether or not the actual number of autistic patients has increased is also a matter of debate. Honda *et al.* (Honda *et al.* 2005) first reported that childhood autism was more frequent in Japan than previously estimated. Cumulative incidence of childhood autism up to 5 years in the birth cohort in the Yokohama increased up to 27.2 per 10 000 in 1991 in the strictest sense, whereas it was 16.2 in 1988. If indeed, the prevalence of autism is

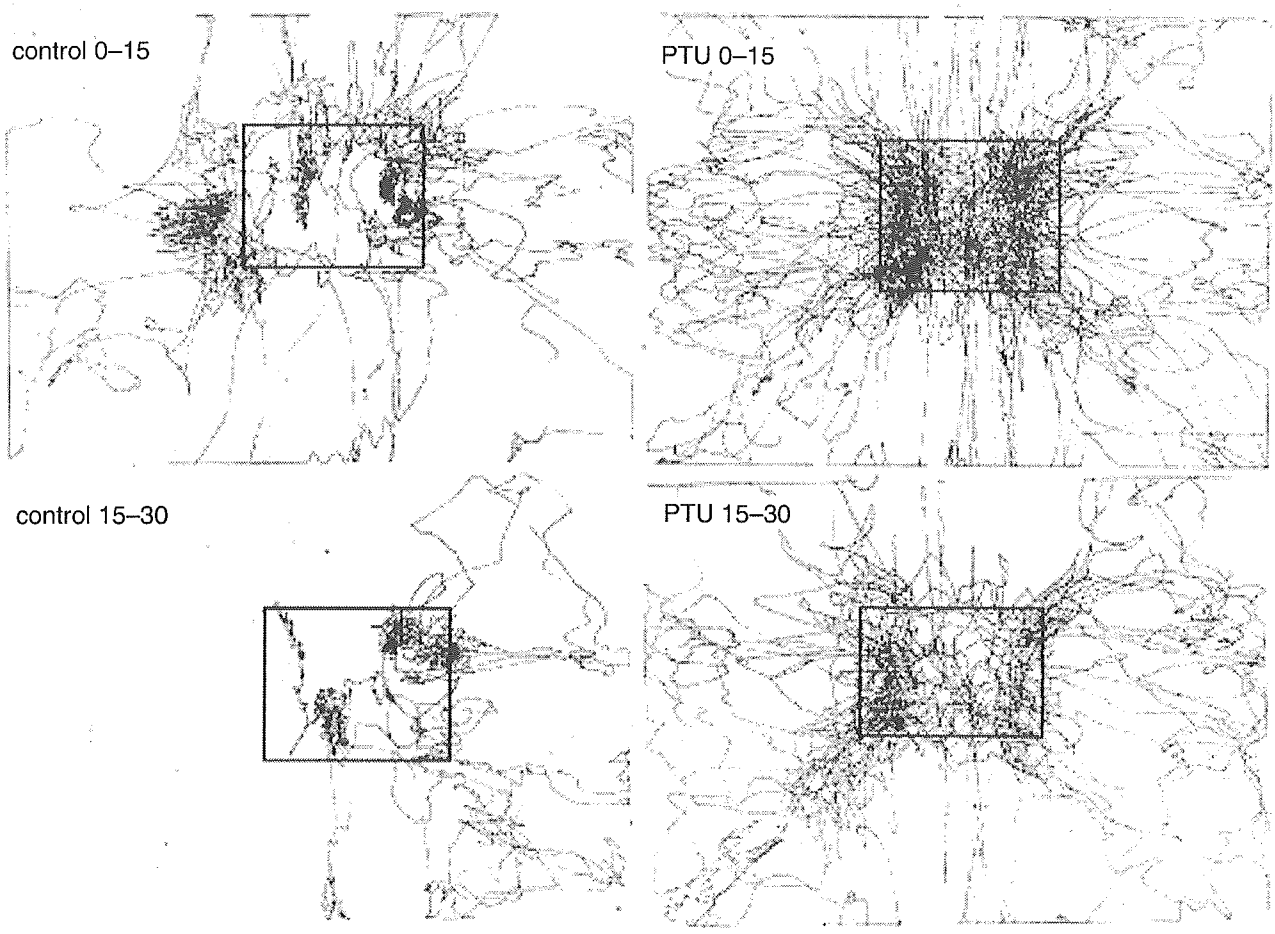

Fig. 1 Comparison of spontaneous movement in a PTU rat (right) and its littermate control (left) as detected by a multidimensional behavioral analyzer (Animex) at the age of 10 weeks. The device recorded the linear locomotion of the animal for two consecutive 15-min periods. The rectangle in the center of each figure indicates the base of the cage and traces outside the rectangle indicate rearing. (Kato *et al.* 1992)

growing recently in some urban areas like Yokohama regardless of the rate, it seems plausible that environmental factors might contribute to the incidence of autism. In view of this, autism–like syndrome(s) due to environmental factors may not necessarily be the same as classical autism with mental retardation.

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. However, is any single brain region able to explain such a broad spectrum dysfunction of ASD? Baron-Cohen *et al.* (Baron-Cohen *et al.* 2000) proposed the amygdala as an area responsible for the impairment of social behavior in autism, but recent data on the effects of amygdala lesions in macaque monkeys did not support their hypothesis (Amaral *et al.* 2003). Alternatively, a different hypothesis as to the brain region responsible for ASD may be derived from recent neuroimaging studies with human patients with the disorder. When viewing images of faces, autistic subjects, compared with unaffected subjects, exhibit a decreased activation of both the amygdala and cortical 'face' areas, and interestingly, also show an increase in other cortical regions typically activated while viewing non-social objects (Critchley *et al.* 2000). Autistic patients may have genetic and/or environmental impairments in some specific brain areas, which, in turn, activate a different set of brain structures during social recognition (Ferguson *et al.* 2001).

Most of the neuroanatomical features highlighted by recent studies of autistic subjects indicate the aberration of very early fetal development, such as shortening and elongation of the brainstem, increased cell packing in the cerebral cortex and preceding enlargement of brain volume. This may imply the significance of a critical period when some genetic and/or environmental factors work in the fetus. The period determines the extent of organs involved, and each organ has its own period for maturation. Some environmental factors such as VPA or THAL disrupt specific points of cell proliferation and differentiation, and some factors such as thyroxine affect the maturation of some sets of organs in the CNS.

Neonatal mild hypothyroidism may provide a useful model for autism. The importance of thyroid hormone in brain development has been extensively documented. Recent studies further demonstrate that relatively subtle changes in circulating levels of thyroid hormone in pregnant women can affect the neurological outcome of their children (Morreale De Escobar *et al.* 2004; Pop & Vulsma 2005). One candidate that affects thyroid function is endocrine disruptors. We currently focus on bisphenol-A, one of the endocrine disruptors known to alter thyroid function (Moriyama *et al.* 2002).

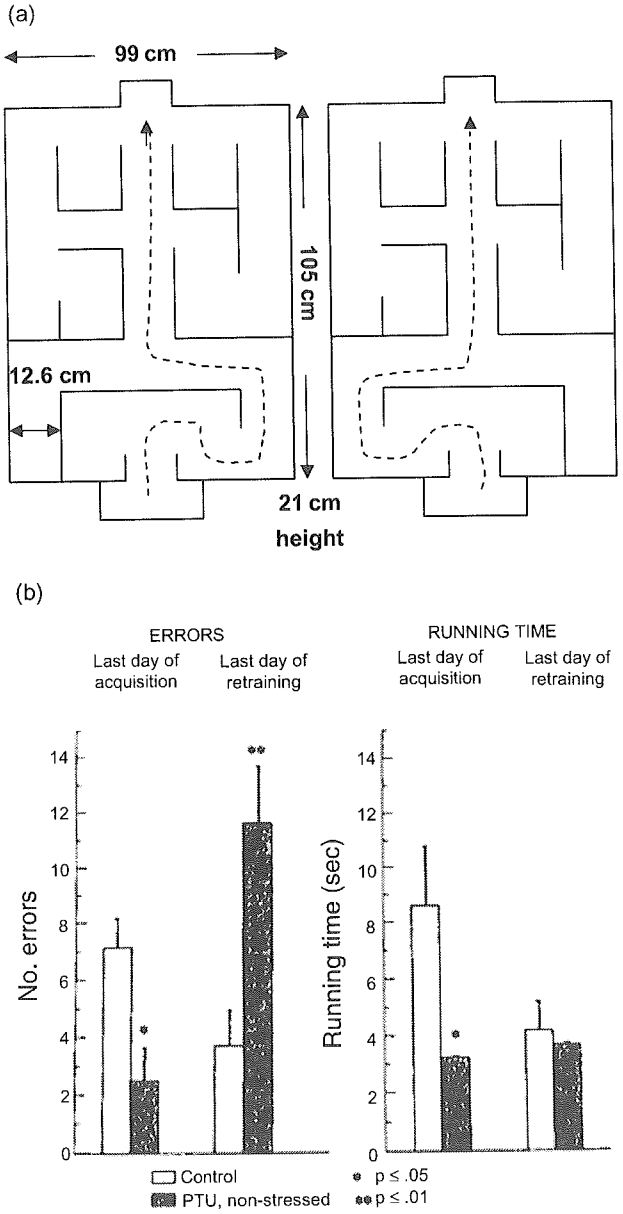

Fig. 2 (a) Diagram of T-maze for study of learning ability in rats. Rats were placed on a 23 h food deprivation schedule and trained to run the maze for food reinforcement once daily in 10 trial sessions. Route 'a' was employed for the learning phase of the study and route 'b' (the mirror image of 'a') for the relearning phase. (b) Maze-learning ability of PTU versus control rats. Average number of errors and running time were evaluated on the last day of task acquisition in the 'a' maze and the last day of retraining in the 'b' maze. Both error frequency and total running time were reduced in the PTU rats on the last day of task acquisition. In contrast, error frequency was significantly increased in the PTU rats on the last day of retraining. (Akaike *et al.* 1991; Akaike & Kato 1997)

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Our preliminary data indicates that the administration of bisphenol-A at the environmental dose during the early postnatal period induces hyperactivity and learning impairment in male, but not female, rats after maturation (unpublished data).

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Our understanding of the neuropathology of autism has advanced substantially over the past 20 years, but there are still so many questions that remain unsolved. Each of these models mentioned above seems to capture at least one of the pieces of the autism puzzle. It is hoped further studies will elucidate the whole picture of the neuropathology of autism.

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