Table 1
Power analysis.

		Screening sample		Confirmation sample		Joint analysis	
		$P=0.05$ [a]	$P=0.001$ [a]	$P=0.05$ [a]	$P=0.001$ [a]	$P=0.05$ [a]	$P=0.001$ [a]
GRR [b]	0.05 [c]	1.51	1.78	1.50	1.76	1.30	1.56
	0.30 [c]	1.25	1.36	1.24	1.35	1.18	1.26
Number of SNPs		16		1		1	
Sample size	Cases	831		793		1624	
	Controls	850		771		1621	
Total		1681		1564		3245	

[a] *P*-value.
[b] Genotype relative risk in order to achieve 80% of power at given minor allele frequency (prevalence = 0.01).
[c] Minor allele frequencies.

(KREMEN1), which predicted disease status correctly 55.76% of the time (Table 4).

4. Discussion

We conducted this study on two genes related to the wnt signaling pathway. The study is relatively large (>3000 case-control subjects), and a pathway-based approach was used for candidate gene selection. Results of this study provide genetic evidence for the involvement of KREMEN1 locus in schizophrenia. The associated SNP is located in the promoter region of KREMEN1. This association was also observed in haplotype-wise analysis. The observed association at *KREMEN1* locus was stronger in the single marker analysis (compared with haplotype analysis), which might be an indication that rs713526 is a better proxy of genetic association then the associated haplotype. Results from the screening sample could not be replicated in the confirmation sample (probably due to relatively low power), but combined analysis yielded evidence for a genetic association.

Table 2
Results (Screening sample).

Gene	dbSNP	Minor allele	Screening sample							
			Single marker (allelewise)					Multimarker (sliding window) [a]		
			Cases [b]	Controls [b]	P [c]	L95 [d]	U95 [d]	2 markers	3 markers	4 markers
DKK1	rs1896368	T	0.407	0.397	0.586	0.903	1.198			
								0.863		
	rs1896367	T	0.288	0.289	0.954	0.855	1.159		0.943	
								0.874		0.620
	rs1528877	G	0.288	0.293	0.744	0.837	1.135		0.414	
								0.254		0.626
	rs1569198	G	0.199	0.219	0.164	0.748	1.051		0.435	
								0.383		
	rs2288335	A	0.116	0.115	0.875	0.820	1.263			
KREMEN1	rs134603	T	0.307	0.315	0.646	0.831	1.122			
								0.117		
	rs134656	C	0.332	0.342	0.541	0.825	1.106		0.764	
								0.585		0.824
	rs5752866	A	0.475	0.453	0.220	0.949	1.253		0.673	
								0.585		0.785
	rs134672	T	0.176	0.180	0.750	0.810	1.164		0.681	
								0.925		0.730
	rs8135301	T	0.108	0.112	0.731	0.771	1.200		0.858	
								0.768		0.787
	rs134683	A	0.234	0.245	0.452	0.799	1.105		0.678	
								0.603		0.575
	rs5762996	A	0.467	0.451	0.362	0.928	1.226		0.857	
								0.575		0.764
	rs132277	T	0.309	0.328	.254 0	.791	1.064		0.596	
								0.371		0.211
	rs5763001	A	0.130	0.119	0.315	0.903	1.374		0.219	
								0.380		0.236
	rs2301446	A	0.107	0.098	0.429	0.872	1.379		0.070	
								0.026		
	rs713526	A	0.152	0.186	0.009	0.650	0.941			

[a] Log likelihood ratio test *P* value.
[b] Minor allele frequency.
[c] Fisher's exact test.
[d] 95% confidence intervals (odds ratio).

Table 3
Results (confirmation sample).

Gene	dbSNP	Minor allele	Confirmation sample					Joint analysis					
			Single marker (allelewise)					Single marker (allelewise)					
			Cases[a]	Controls[a]	P[b]	L95[c]	U95[c]	Cases[a]	Controls	P[d]	L95[c]	U95[c]	P_Bd[e]
KREMEN1	rs713526	A	0.155	0.172	0.193	0.726	1.067	0.155	0.177	0.018	0.745	0.9727	0.642

[a] Minor allele frequency.
[b] Fisher's exact test.
[c] 95% confidence intervals (odds ratio).
[d] Cochran–Mantel–Haenszel test.
[e] Breslow–Day test.

Table 4
SNP–SNP interaction.

Model[a]				TA[b]	CVC[c]	P value[d]
rs1896368 (DKK1)	rs2288335 (DKK1)	rs5752866 (KREMEN1)		0.5576	10	<0.001
rs1528877 (DKK1)	rs1896368 (DKK1)	rs8135301 (KREMEN1)	rs5752866 (KREMEN1)	0.5542	10	<0.01
rs1528877 (DKK1)	rs1896368 (DKK1)			0.5484	7	<0.01

[a] Best model for particular number of SNPs.
[b] Testing accuracy.
[c] Cross validation consistency (out of 10).
[d] Based on 1000 permutations.

After examining the main effects of each SNP, we analyzed potential interactions between these SNPs. It was of interest to identify interactions that predict the risk for schizophrenia, even in the absence of main effects. The tSNPs kept in the optimal MDR model were not associated with schizophrenia in single-locus association analysis, which is consistent with epistasis, as the effect of one locus may be too weak to be detected when the effect of another locus is not accounted for.

Some limitations are inherent in this type of study. Our study is not comprehensive, as we did not conduct a study regarding all wnt signaling pathway genes in the linkage regions for schizophrenia. Another potential concern was population admixture, a known confounding factor for association. The Japanese population has a small genetic diversity (Haga et al., 2002). However, even in such a genetically homogeneous population, a small amount of stratification may produce a spurious genetic association signal (Yamaguchi-Kabata et al., 2008).

Our study demonstrated that a representative tSNP of KREMEN1 might modulate the risk of schizophrenia in the Japanese population. However, these results were obtained solely through the genetic analysis, and pathophysiological explanations need to be determined. We conclude that these results are suggestive of an association with schizophrenia at this locus, but that our findings cannot be considered conclusive without replication.

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Contributors

BA, IK, YI and YN performed laboratory assays and the data-analysis. BA drafted the manuscript. HU, MS, TI, RH, MT, NI and NO participated in the design of the study, and coordinated sample collection. All authors contributed to and have approved the final manuscript.

Conflict of interest

The authors have no financial conflicts to declare.

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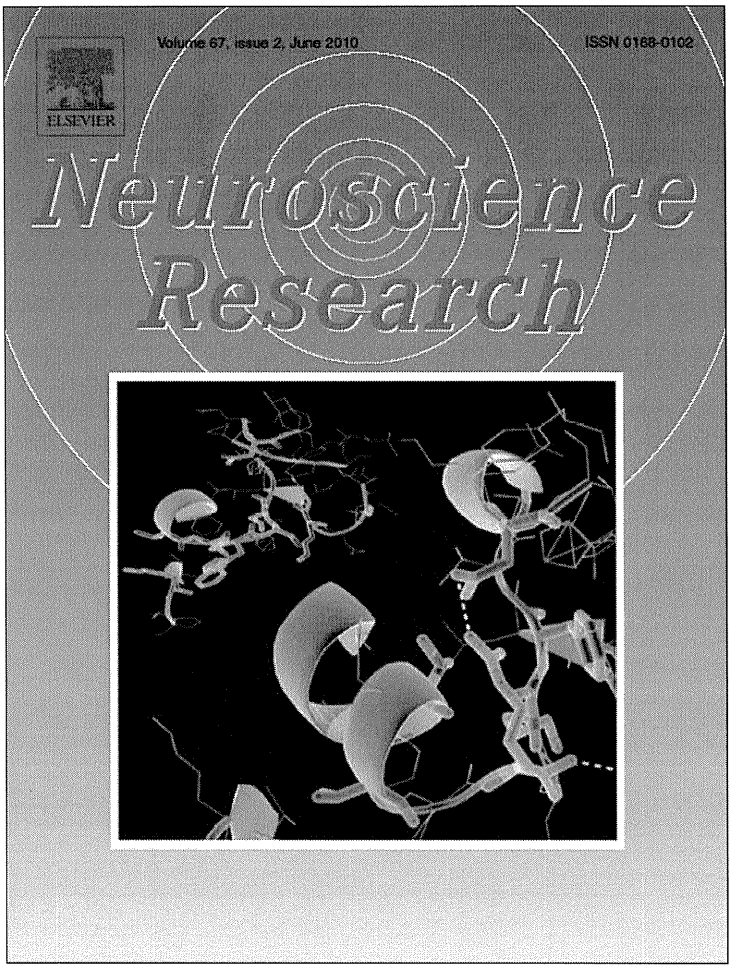

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Two genetic variants of *CD38* in subjects with autism spectrum disorder and controls

Toshio Munesue[a,b,c,e,j,k,1], Shigeru Yokoyama[a,f,j,1], Kazuhiko Nakamura[l,1], Ayyappan Anitha[l,1], Kazuo Yamada[m,1], Kenshi Hayashi[d,f,g], Tomoya Asaka[n], Hong-Xiang Liu[a,f], Duo Jin[a,f], Keita Koizumi[a,b,f,k], Mohammad Saharul Islam[a,f,j], Jian-Jun Huang[a,f], Wen-Jie Ma[a,f,j], Uh-Hyun Kim[o], Sun-Jun Kim[p], Keunwan Park[q], Dongsup Kim[q], Mitsuru Kikuchi[c,e,r], Yasuki Ono[e], Hideo Nakatani[e], Shiro Suda[l], Taishi Miyachi[l], Hirokazu Hirai[a,s], Alla Salmina[t], Yu A. Pichugina[f,u], Andrei A. Soumarokov[u], Nori Takei[k,l,v], Norio Mori[k,l,v], Masatsugu Tsujii[k,w], Toshiro Sugiyama[a,x], Kunimasa Yagi[g], Masakazu Yamagishi[g], Tsukasa Sasaki[y,z], Hidenori Yamasue[j,z], Nobumasa Kato[j,z,A], Ryota Hashimoto[k,B], Masako Taniike[k,B], Yutaka Hayashi[h], Junichiro Hamada[h], Shioto Suzuki[i], Akishi Ooi[i], Mami Noda[C], Yuko Kamiyama[C], Mizuho A. Kido[D], Olga Lopatina[a,f,j,t], Minako Hashii[f,j], Sarwat Amina[a,f], Fabio Malavasi[E], Eric J. Huang[F], Jiasheng Zhang[F], Nobuaki Shimizu[G], Takeo Yoshikawa[m], Akihiro Matsushima[n], Yoshio Minabe[a,b,e,k,r], Haruhiro Higashida[a,b,f,j,k,*]

[a] *Kanazawa University 21st Century Center of Excellence (COE) Program on Innovative Brain Science on Development, Learning and Memory, Kanazawa 920-8640, Japan*
[b] *Osaka-Hamamatsu-Kanazawa Universities Joint Research Centers, Kanazawa University Center for Child Mental Development, Kanazawa 920-8640, Japan*
[c] *Department of Child Psychiatry, Kanazawa University Hospital, Kanazawa 920-8641, Japan*
[d] *Department of Clinical Laboratory, Kanazawa University Hospital, Kanazawa 920-8641, Japan*
[e] *Department of Psychiatry and Neurobiology, Kanazawa University Graduate School of Medicine, Kanazawa 920-8640, Japan*
[f] *Department of Biophysical Genetics, Kanazawa University Graduate School of Medicine, Kanazawa 920-8640, Japan*
[g] *Department of Internal Medicine, Kanazawa University Graduate School of Medicine, Kanazawa 920-8640, Japan*
[h] *Department of Neurosurgery, Kanazawa University Graduate School of Medicine, Kanazawa 920-8640, Japan*
[i] *Department of Molecular and Cellular Pathology, Kanazawa University Graduate School of Medicine, Kanazawa 920-8640, Japan*
[j] *Core Research for Evolutional Science and Technology, Tokyo 102-0075, Japan*
[k] *United Graduate School of Child Development, Osaka-Kanazawa-Hamamatsu Universities, Osaka 565-0871, Japan*
[l] *Department of Psychiatry and Neurology, Hamamatsu University School of Medicine, Hamamatsu 431-3192, Japan*
[m] *Laboratory for Molecular Psychiatry, RIKEN Brain Science Institute, Saitama 351-0198, Japan*
[n] *Nanao National Hospital, Nanao 920-8531, Japan*
[o] *Department of Biochemistry, Chonbuk National University Medical School, Jeonju, Republic of Korea*
[p] *Department of Pediatrics, Chonbuk National University Medical School, Jeonju, Republic of Korea*
[q] *Bio and Brain Engineering, Korea Advanced Institute of Science and Technology, Republic of Korea*
[r] *Hokuriku Innovation Cluster for Health Science, Kanazawa 920-8640, Japan*
[s] *Department of Neurophysiology, Gunma University Graduate School of Medicine, Gunma 371-8511, Japan*
[t] *Department of Biochemistry and Medical Chemistry, Krasnoyarsk State Medical University, Krasnoyarsk 660022, Russia*
[u] *Department of Psychiatry, Krasnoyarsk State Medical University Krasnoyarsk 660022, Russia*
[v] *Osaka-Hamamatsu-Kanazawa Universities Joint Research Centers, Hamamatsu University Center for Child Mental Development, Medical, Hamamatsu 431-3197, Japan*
[w] *Faculty of Sociology, Chukyo University, Toyota, Aichi 470-0393, Japan*
[x] *Aichi Children's Health and Medical Center, Aichi 474-8710, Japan*
[y] *Office for Mental Health Support, Division for Counseling and Support, University of Tokyo, Tokyo 113-0033, Japan*
[z] *Department of Neuropsychiatry, Graduate School of Medicine, University of Tokyo, Tokyo 113-8655, Japan*
[A] *Department of Psychiatry, Showa University School of Medicine, Tokyo 157-8577, Japan*
[B] *Osaka-Hamamatsu-Kanazawa Universities Joint Research Centers, Molecular Research Center for Children's Mental Development, Osaka University, Osaka 565-0871, Japan*
[C] *Laboratory of Pathophysiology, Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka 812-8582, Japan*
[D] *Department of Oral Anatomy and Cell Biology, Graduate School of Dental Science, Kyushu University, Fukuoka 812-8582, Japan*
[E] *Laboratory of Immunogenetics, Department of Genetics, Biology and Biochemistry and CeRMS, University of Torino Medical School, Torino 10126, Italy*
[F] *Department of Pathology, University of California San Francisco and Pathology Service, Veterans Affair Medical Center, San Francisco, CA 94121, USA*
[G] *Institute of Nature and Environmental Technology, Kanazawa University, Kanazawa 920-1192, Japan*

* Corresponding author at: Department of Biophysical Genetics, Kanazawa University Graduate School of Medicine, Kanazawa 920-8640, Japan.
E-mail address: haruhiro@med.kanazawa-u.ac.jp (H. Higashida).
[1] These authors contributed equally to the work.

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ABSTRACT

The neurobiological basis of autism spectrum disorder (ASD) remains poorly understood. Given the role of CD38 in social recognition through oxytocin (OT) release, we hypothesized that CD38 may play a role in the etiology of ASD. Here, we first examined the immunohistochemical expression of CD38 in the hypothalamus of post-mortem brains of non-ASD subjects and found that CD38 was colocalized with OT in secretory neurons. In studies of the association between *CD38* and autism, we analyzed 10 single nucleotide polymorphisms (SNPs) and mutations of *CD38* by re-sequencing DNAs mainly from a case–control study in Japan, and Caucasian cases mainly recruited to the Autism Genetic Resource Exchange (AGRE). The SNPs of *CD38*, rs6449197 ($p < 0.040$) and rs3796863 ($p < 0.005$) showed significant associations with a subset of ASD (IQ > 70; designated as high-functioning autism (HFA)) in the U.S. 104 AGRE family trios, but not with Japanese 188 HFA subjects. A mutation that caused tryptophan to replace arginine at amino acid residue 140 (R140W; (rs1800561, 4693C>T)) was found in 0.6–4.6% of the Japanese population and was associated with ASD in the smaller case–control study. The SNP was clustered in pedigrees in which the fathers and brothers of T-allele-carrier probands had ASD or ASD traits. In this cohort OT plasma levels were lower in subjects with the T allele than in those without. One proband with the T allele who was taking nasal OT spray showed relief of symptoms. The two variant *CD38* poloymorphysms tested may be of interest with regard of the pathophysiology of ASD.

1. Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disease manifesting in childhood but extending through to adulthood. The disorder is more common than previously supposed, with a worldwide frequency of >0.6% (Honda et al., 2005; Baird et al., 2006; Williams et al., 2006). The region with the maximum reported rate (3% of births) is the Nagoya/Hamamatsu region in Japan (Sumi et al., 2006). ASD can be sporadic or familial and is far more common in males than in females (Zhao et al., 2007). Because ASD is etiologically heterogeneous and forms a continuum, it is likely to involve many genes (Sutcliffe, 2008; Levitt and Campbell, 2009). *De novo* mutations and copy number variations (CNVs) are reported in a small fraction of ASD cases (Sebat et al., 2007; Glessner et al., 2009), but common variants also underlie the disease (Wang et al., 2009), and a unified mechanism for both forms of genetic inheritance has been proposed (Zhao et al., 2007).

Oxytocin (OT) is secreted into the brain by hypothalamic neuronal dendrites and plays important roles in social recognition and memory (Insel and Fernald, 2004; Takayanagi et al., 2005; Donaldson and Young, 2008; Neumann, 2008). This hormone mediates behavioral effects, such as pair bonding, mate guarding, and parental care in rodents (Ferguson et al., 2000; Ludwig and Leng, 2006; Campbell, 2008) and may be involved in romantic love, trust, and fear in humans (Kosfeld et al., 2005; Zeki, 2007; Domes et al., 2007; Ditzen et al., 2009). Recently, evidence has accumulating to suggest that the polymorphisms of multiple OT-related genes are associated with ASD (Wu et al., 2005; Jacob et al., 2007; Ebstein et al., 2009; Gregory et al., 2009; Wermter et al., 2010).

Peripheral or nasal administration of OT facilitates social recognition and trust in healthy humans (Guastella et al., 2008a,b, 2010; Ditzen et al., 2009) and increases eye contact and recognition in autistic subjects (Hollander et al., 2007; Yamasue et al., 2009). Those observations are based mostly on a small number of administrations, and the effects of long-term OT treatment on human social behavior in ASD patients is unknown.

Human CD38 is a type II transmembrane antigen (Malavasi et al., 2008). The *CD38* gene consists of 8 exons on 4p15 (Nakagawara et al., 1995) and spans a genomic stretch of 70.51 kb. The mRNA contains 1227 bases, and single nucleotide polymorphisms (SNPs) have been reported (Nata et al., 1997; Yagui et al., 1998; Ebstein et al., 2009; see Fig. 2A). CD38 has been studied extensively because it is a reliable negative prognostic marker for chronic lymphocytic leukemia (Deaglio et al., 2008). CD38 is expressed in the brain (Lee, 2001; Higashida et al., 2007) and can catalyze the formation of cyclic ADP-ribose (cADPR) from NAD^+ (Lee, 2001; Guse, 2005; Malavasi et al., 2008). cADPR mobilizes Ca^{2+} from intracellular Ca^{2+} stores, thus acting as a second messenger (Lee, 2001). Little was known about the CD38-dependent cADPR/Ca^{2+} signaling pathway in the brain until recent studies in our laboratory. They showed that CD38 regulates OT secretion in the mouse hypothalamus and posterior pituitary, which is critical for mouse social behavior (Jin et al., 2007; Liu et al., 2008). The precise role of CD38 in the human hypothalamus, however, has not been clarified.

As OT seems to be an important factor for the understanding of ASD, we examined the relationships among human *CD38* polymorphisms and mutations in Japanese, Korean, and Caucasian subjects. We identified two functional polymorphisms in subgroups: ASD and high-functioning autism (HFA) based on IQ (>70 classified as HFA). We measured the carrier's serum OT levels and examined each subject for ASD. Here, we also discuss the possibility of treating ASD patients who have a SNP that lowers OT levels by intranasal administration of OT.

2. Materials and methods

2.1. CD38 expression and immunohistochemistry

We measured CD38 mRNA levels by the semi-quantitative or real-time quantitative RT-PCR method (Jin et al., 2007) using commercially available total RNAs from various regions of the human brain. Control human brain tissues were obtained from archival blocks in the Departments of Pathology at the University of California San Francisco and Kanazawa University Graduate School of Medicine. The use of this tissue followed the institutional guidelines established by the Committee on Human Research (CHR) in both universities. The immunofluorescent stainings for human CD38 and OT were performed according to the procedures described previously (Zhang et al., 2007). Briefly, sections of the hypothalamus were treated with antigen retrieval protocol (0.01 M citrate acid buffer pH 6.0, plus heating for 121 °C for 5 min). The sections were then incubated with primary antibody against human CD38 (1:50, sc-7325, Santa Cruz Biotechnology, Santa Cruz, CA, USA) and OT (1:500, AB911, Chemicon, Billerica, MA, USA) overnight at room temperature, followed by washing in Tris-buffered saline (TBS), and incubation with secondary antibodies that were conjugated with Alexa 488 or Alexa 568 (1:300, A11001 and A11011; Invitrogen) for 1 h at room temperature. Images for the co-localization of CD38 and OT in the paraventricular nucleus of the hypothalamus were

Table 1
Data sets for the 4 case–control study cohorts.

Data set[a]	Cohort 1 Subjects, age (range), male/female	Cohort 2 Subjects, age (range), male/female	Cohort 3 Subjects	Cohort 4 Subjects, male/female
Cases (Probands)	29, 22.8 ± 7.6 (12–44), 23/6	301, 11.9 ± 6.7 (3–64), 263/38	16	263, 263/0
Controls	315, 34.1 ± 4.3 (8–75), 171/144	417, 28.6 ± 14.4 (5–65), 229/188	150	–
Trio families	3	334		252
Family member	25, 53.0 ± 4.5 (21–84), 15/10	297, 39.2 ± 15.5 (3–93), 143/154	–	–
HFA subjects	–	188		104
Usage/analysis	Intronic SNP	–	–	Intronic SNP
	Exonic mutation	R140W	R140W	R140W
	Family-based association	HFA association		HFS association
	OT/AVP measurement			

[a] Cohort 1 was from the Kanazawa area, Japan; Cohort 2, the Nagoya/Hamamatsu, Tokyo, and Osaka areas, Japan; Cohort 3, Jeonju, Korea; Cohort 4, study of 252 trio samples from the Autism Genetic Resource Exchange comprising 252 U.S., 7 Russian, and 4 Italian ASD patients. Age ± SEM years (year range).

captured using a Leica confocal microscope (TCS SP, Bannockburn, IL, USA) and imported into the Photoshop software.

2.2. Participants

The participants consisted of cohorts organized by the Osaka-Hamamatsu-Kanazawa University Joint Research Centers for Child Mental Development, the Kanazawa University COE Program, the Core Research for Evolutional Science and Technology Program in Japan, which includes DNA samples collected at the University of Tokyo (Table 1). Three hundred and fifty-seven ASD subjects were recruited from outpatient psychiatry or pediatric clinics of each university hospital. All subjects fullfilled the DSM-IV criteria for autistic disorder. The diagnoses were made by two experienced child psychiatrists through interviews and clinical record reviews, and the subjects had no apparent physical anomalies. We also recruited patients' parents, grandparents, siblings, and other relatives from 322 families. The controls consisted of unrelated healthy Japanese volunteers—315 from the first cohort and 417 from the second. We recruited adult controls mainly from among hospital and facility staff and medical schools, and age-matched children as controls. In the Japanese cohorts, all subjects resided in Kanazawa, Hamamatsu/Nagoya, Tokyo, or Osaka, Japan, and all patients and controls were Japanese with no non-Japanese parents or grandparents. Two experienced child psychiatrists independently confirmed the diagnosis of ASD for most patients by semi-structured behavior observation and interviews with the subjects and their parents. At the interview with the parents, which was helpful in the evaluation of autism-specific behaviors and symptoms, the examiner used one of the following instruments: the Asperger Syndrome Diagnostic Interview (Gillberg et al., 2001), Autism Diagnostic Interview-Revised (ADI-R; Lord et al., 1994), Pervasive Developmental Disorders Autism Society Japan Rating Scale (2006), or Diagnostic Interview for Social and Communication Disorders (Wing et al., 2002).

In addition, for a third case–control study, we recruited 16 male ASD patients and 150 non-ASD male controls from Jeonju University Hospital in Korea. For a fourth study, we recruited 252 families from the Autism Genetic Resource Exchange (http://www.agre.org; AGRE cohort; Geschwind et al., 2001). Additional selection criteria required that (i) there be no possible non-idiopathic autism flags and (ii) all the trios be Whites, with the exclusion of Hispanic and Latino races (Anitha et al., 2008). Seven Russian male patients from Krasnoyarsk State Medical University Hospital, and 4 lymphoblastoid cells from Italian male ASD patients from Trino University Medical Hospital were also included in the study. These subjects met the DSM-IV or ADI-R criteria for autistic disorder.

For the HFA group, the U.S. autistic offspring of 104 trios (patient plus two parents) among the 252 AGRE trios, who had IQ > 70, were considered. In the second cohort, we selected 188 trio families as Japanese HFA cases (Table 1).

Using the Autism-Spectrum Quotient (AQ) (Baron-Cohen et al., 2006; Munesue et al., 2008), we evaluated members of families in which older subjects performed self-evaluation by recalling how they behaved in their 20 s. Subjects of autism traits in 3 kindreds was defined by AQ scores, above the average (>27) but less than the higher level (<32), during interviews by two psychiatrists.

This study was approved by the ethics committees of Kanazawa University, Hamamatsu Medical University, University of Tokyo, Osaka University, RIKEN, and the other participating institutes.

2.3. Marker selection

The genomic structure of *CD38* is based on the University of California, Santa Cruz, March 2006 draft assembly of the human genome (http://www.genome.ucsc.edu). We selected SNPs using information from the International HapMap Project (http://www.hapmap.org) and the National Center for Biotechnology Information (NCBI dbSNP: http://www.ncbi.nlm.nih.gov/SNP). Initially, all the SNPs with MAF > 0.1 were selected. Tags, which

Table 2
Oligonucleotides. Designed for amplification of coding sequences including 60–100 bp of flanking intronic sequences. Primers were followings.

Exon	Up	Down
1	5′-AGGGAAACAGAGAAAAGGCAAGTGU-3′	5′-GGCCAGCTGCTCCTGAAAG-3′
2	5′-GGCATATAATAGATGCTTCC-3′	5′-TGGACCTATGAATTGTTACC-3′
3	5′-GACATGCTAAATTGATCTCAG-3′	5′-CAGCAGAAGTCACTCTGTTC-3′
4	5′-TCCACTATGACTGAACAGCC-3′	5′-AGCACTGACTGAGTAACG-3′
5	5′-CTTAACCAGCTATTGCTAAG-3′	5′-ACTGTGATATTTGCAACAGG-3′
6	5′-TCTGCCTGCTGGTTGTTGAG-3′	5′-TCCTGAGTCAATTTGTTCCC-3′
7	5′-CCCAACAGCCTCTTAACTTT-3′	5′-ATCACCAGAGGTTGCCAT-3′
8	5′-AGCGAATTGGACGACAGATG-3′	5′-CATTGACCTTATTGTGGAGG-3′

Usually, we used the following temperatures: 15 min at 95 °C, 35 cycles of 30 s at 95 °C, 30 s at annealing temperature at 52 °C, 5 min at 72 °C for extension followed by a final extension of 10 min at 72 °C. SNPs of these samples were examined by the methods described.

Table 3
Sequence information for SNPs1-10 and R140W.

SNP	Sequence	Strand	ON GENE	UCSC No
SNP01 (rs3796878)	TTGCTCTGTTCCCAGGTTGGTCCTCC[A/G]CATACCTCCCACTGCAGGATCCTGG	–	T/C	15,394,757
SNP02 (rs3796875)	GTTTTTCAAGAGTCCTAAGACAAAGA[A/G]GAAAGGAAGAAGCAGAGAAGCCATG	–	T/C	15,396,312
SNP03 (rs6449197)	CAGGTTGAGGAAATTTTATTTCTAAT[C/T]TGCTCAGTGTTTTTTTCATCACAAG	+	C/T	15,424,020
SNP04 (rs11574927)	AAAATTGTGTACCCCAATTCAGTAGT[A/G]AAACTACTACCGGGAACATCGGGAA	+	A/G	15,449,341
SNP05 (rs10805347)	ATTAACATTTCAGAATTTTATGATCT[A/G]ATATTATGGTTCAAGCACTTGAAAC	+	A/G	15,449,937
SNP06 (rs3796863)	GGGAGGGGAGCTATCCATGCCACCTG[A/C]TGGTCAAAAAAACAGCAGGAGCAGC	–	T/G	15,459,084
SNP07 (rs1130169)	TGTACCCTTCCT ACAGATAGTCAAAC[C/T]ATAAACTTCATGGTCATGGGTCATG	+	C/T	15,459,783
SNP08 (rs13137313)	AAATAAACCATATGTGTTGAACAAAG[A/G]ATTAATAAATTAATTTGAGACTCAA	+	A/G	15,461.066
SNP09 (rs3733593)	ATCTTGAACAAAATCGCCTAACCTTTC[C/T]GAACTTCAACTTCCTTGCCACTCCT	+	C/T	15,461,202
SNP10 (rs3733593)	CTGCCTCCGAATTCCATAGTTTCCAC[C/T]GCCTTGCGCTACTTGCATTCTCTGATT	–	G/A	15,463,823
R140W (rs1800561)	TGGCCCATCAGTTCACACAGGTCCAG[C/T]GGGACATGTTCACCCTGGAGGACAC	+	C/T	15,435,656

could capture the common allelic variants with $r^2 > 0.8$ by pairwise tagging, were picked from this set using Haploview v4.0 (http://www.broad.mit.edu/mpg/haploview).

2.4. Genetic analysis

We isolated genomic DNA from venous blood samples using the standard phenol/chloroform method (Easy-DNA kit, Invitrogen, Carlsbad, CA). We amplified *CD38* exons (Tables 2 and 3) and flanking introns (Table 3) by PCR (Taq PCR Core Kit, Qiagen, Hilden, Germany). We used Assay-on-Demand SNP genotyping products (Applied Biosystems, Foster City, CA) to score SNPs based on the TaqMan assay method described previously (Anitha et al., 2008). An ABI 7900 Sequence Detection System (SDS) was used to determine genotypes and analyses were performed with SDS v2.0 software (Applied Biosystems). Fig. 2A shows the SNPs and mutations analyzed in this study and their locations.

2.5. Enzyme immunoassay for OT and vasopressin

Blood samples for measuring OT and vasopressin concentrations were collected in two hospitals in the Kanazawa area between 10:00 and 12:00 or 15:00 and18:00 o'clock from subjects who had been asked to fast for the previous 2 h. Qualified lab technicians drew 10 ml of blood from an arm vein into heparinized tubes in less than 15 min. The samples were centrifuged at 0 °C at 2600 × *g* for 15 min and the plasma was separated off, divided into 2 tubes, and stored at −80 °C. We performed the peptide assay for OT and vasopressin (AVP) as described previously (Jin et al., 2007; Liu et al., 2008).

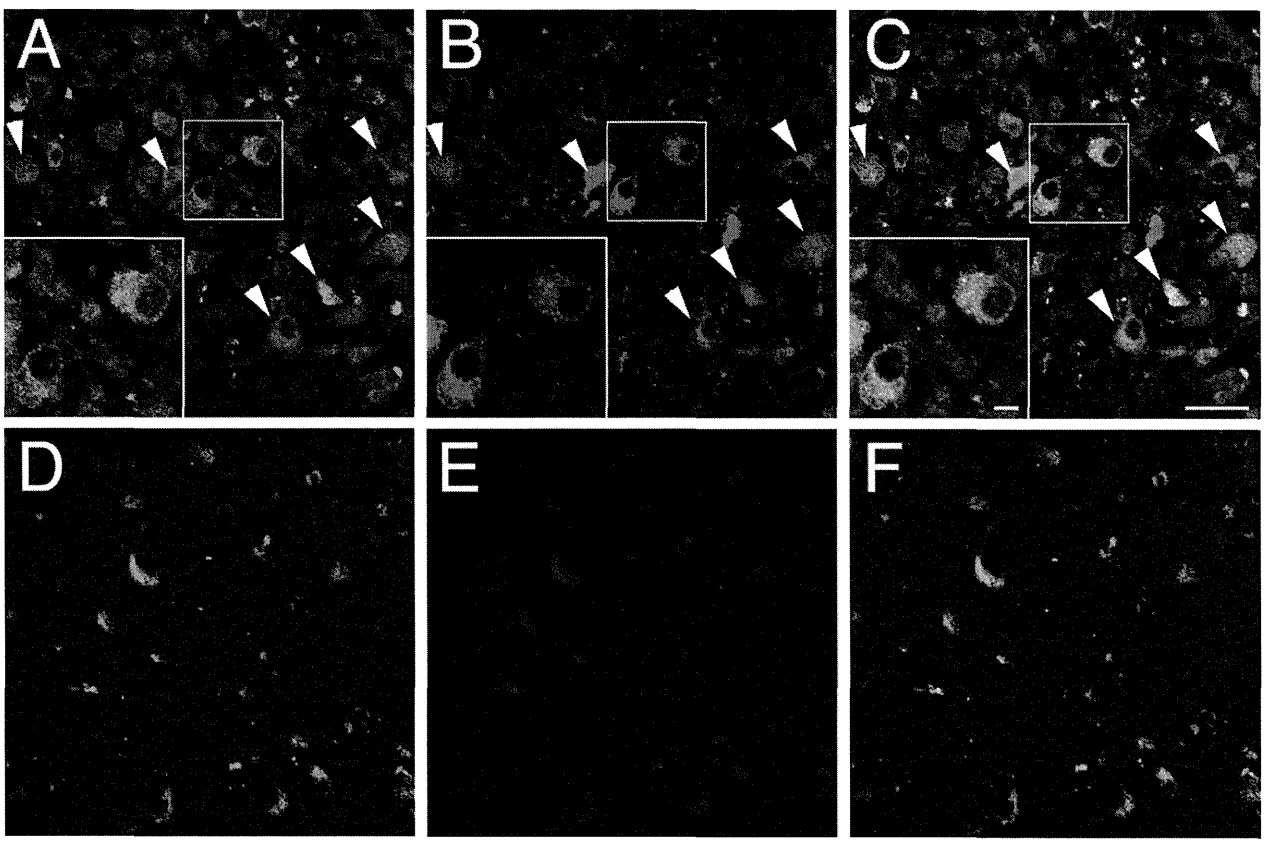

Fig. 1. Immunohistochemical analysis of CD38 (A, D) and oxytocin (B, E) in the human brain. Cell montages of panels were taken from the paraventricular nucleus (PVN) in the hypothalamus (A–C) and insular cortex (D–F) of autopsy subjects from the USA. Arrowheads indicate extensive colabeling with OT. The insets in panels are enlarged images of neurons showing coexpression of CD38 and OT. Scale bars: 40 μm in C and 8 μm in insert.

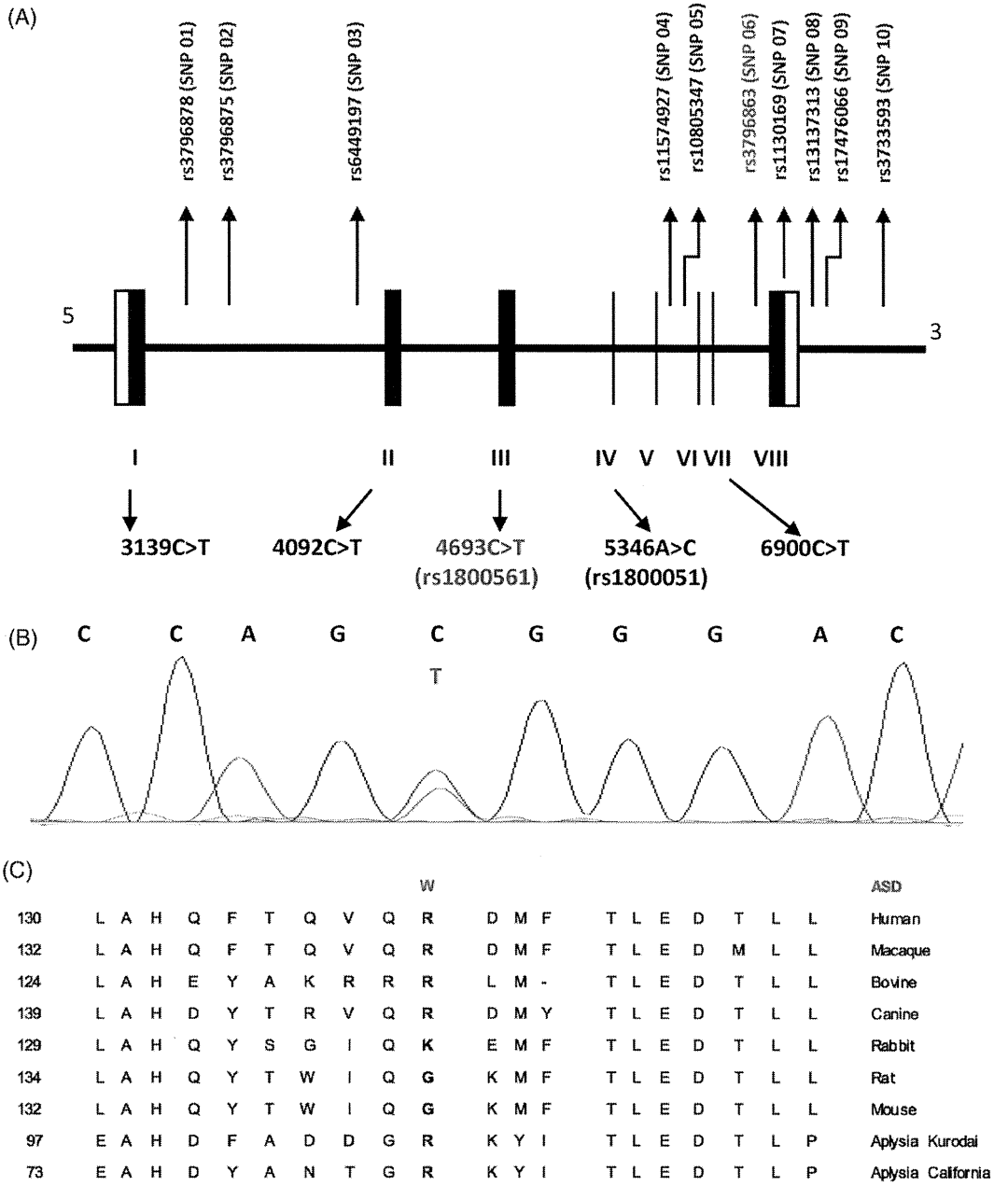

Fig. 2. Genome structure, SNPs, and mutations of *CD38*. (A) Genomic structure of *CD38* and locations of SNPs in introns (upper) and exons (lower). Exons are indicated by boxes, with translated regions in closed boxes and untranslated regions in open boxes. Numbering of the nucleotides starts at the A of ATG and refers to GenBank Accession number D84284. (B) The sequence trace was derived from a DNA sample of a 4693C/T heterozygote. (C) Amino acid sequence of CD38 showing conservation of R at the 140th amino acid among different species, except for rodents and rabbits. Sequences were obtained through the accession numbers NM001775, AY555148, NM175798, AF117714, AF272974, NM013127, NM007646, D30048, and M85206/M37644 for the indicated species.

2.6. Statistics

We analyzed the data using one- or two-way ANOVA, as appropriate. The criterion for significance in all cases was $p < 0.05$. PedCheck program v1.1 (http://watson.hgen.pitt.edu) was used to identify and eliminate all Mendelian inheritance inconsistencies in the trio genotype data. Markers were tested for association by family-based association test (FBAT), using FBAT v2.0.3 (http://www.biostat.harvard.edu/~fbat/).

3. Results

The highest level of *CD38* mRNA expression was detected in the human hypothalamus, and we detected substantial expression in the frontal cortex, amygdala, and cerebellum (data not shown). CD38 immunoreactivity was detected in the hypothalamus of the two Japanese brains (data not shown). In brains of samples from USA (Fig. 1), double immunohistochemical staining revealed high levels of CD38 immunoreactivity in many cells in the paraventricular nucleus of the hypothalamus and showed extensive colabeling with OT (Yamashita et al., 2002), while much lower CD38 expression levels and little or no detectable OT were observed in the insular cortex, which served as a control. These results suggested that CD38 may have an important role in OT release in the human hypothalamus, as in the mice (Jin et al., 2007; Liu et al., 2008). Based on this new information about the human brain, we set out to examine the human *CD38* gene.

Table 4
FBAT analysis of *CD38* SNPs in AGRE trios.

Marker	Allele	252 trios				HFA trios			
		Families[a]	Frequency	*Z*-Score	*p*-Value	Families[a]	Frequency	*Z*-Score	*p*-Value[b]
rs3796878	A	119	0.163	0.253	0.801	49	0.161	0.640	0.522
	G	119	0.837	−0.253		49	0.839	−0.640	
rs3796875	A	171	0.659	−0.740	0.459	78	0.640	−0.594	0.552
	G	171	0.341	0.740		78	0.360	0.594	
rs6449197	T	79	0.103	−0.647	0.518	30	0.103	−2.058	***0.040***
	C	79	0.897	0.647		30	0.897	2.058	
rs11574927	A	111	0.830	0.947	0.344	55	0.834	1.861	0.063
	G	111	0.170	−0.947		55	0.166	−1.861	
rs10805347	A	171	0.321	−0.614	0.539	71	0.309	−0.663	0.508
	G	171	0.679	0.614		71	0.691	0.663	
rs3796863	A	160	0.277	−1.706	0.088	74	0.270	−2.800	***0.005***
	C	160	0.723	1.706		74	0.730	2.800	
rs1130169	T	195	0.503	1.004	0.315	85	0.517	0.659	0.510
	C	195	0.497	−1.004		85	0.483	−0.659	
rs13137313	A	168	0.742	0.765	0.445	72	0.726	0.426	0.670
	G	168	0.258	−0.765		72	0.274	−0.426	
rs17476066	T	167	0.714	0.697	0.486	69	0.714	0.655	0.513
	C	167	0.286	−0.697		69	0.286	−0.655	
rs3733593	T	178	0.451	0.962	0.336	74	0.428	0.887	0.375
	C	178	0.549	−0.962		74	0.572	−0.887	
p-Value after multimarker testing					0.295				0.053

[a] Informative families.
[b] Significant *p*-values are indicated in bold italic.

3.1. Intronic SNP analysis in Japanese and U.S. subjects

An association study for 10 intronic SNPs shown in Fig. 2 was first performed in a case–control study in a Japanese population (29 ASD subjects and 315 controls, the first cohort in the Table 1). No significant association with ASD was found for these SNPs (data not shown).

Next, we analyzed U.S. ASD DNA samples (cohort 4). FBAT was performed for the whole set of 252 trios in the AGRE samples. Again, none of the SNPs showed significant associations, except rs3796863 (SNP06) with a tendency toward association ($p = 0.088$; Table 4). Therefore, we further analyzed this SNP for the U.S. HFA subgroup of 104 trios in our AGRE samples (cohort 4). In the FBAT of HFA trios, rs6449197 ($p = 0.040$) and rs3796863 ($p = 0.005$) showed significant associations; a tendency for association ($p = 0.053$) was found after multimarker testing (Table 4).

Unlike the U.S. cases, no association was detected in 188 Japanese HFA trio cases selected from cohort 2 ($p = 0.228$).

One-way ANOVA showed a significant variation in the distribution of ADI-R_C scores (restricted, repetitive, and stereotyped patterns of behavior) between the C/C, C/A, and A/A genotypes of SNP06 of *CD38*, in 252 trios ($p = 0.013$) and HFA trios ($p = 0.0067$) (Fig. 3). Following *post hoc* pairwise comparison with the Bonferroni method, the variations in the distribution of ADI-R_C between the C/C and C/A groups were found to be significant at the 0.05- and 0.01-levels, in the 252 and HFA trios, respectively.

Linkage disequilibrium (LD) analysis identified three haplotype blocks across the *CD38* gene in 104 trios in AGRE samples, with the first block comprising SNPs01-05, the second block comprising SNPs06 and 07, and the third block comprising SNPs08 and 09 (Fig. 4). The results of haplotype transmission disequilibrium test (TDT) for the HFA trios are shown in Table 5. The associations of haplotypes in the three haploblocks were examined based on the LD structure of *CD38*. The first haploblock including SNPs01-05, showed a weak tendency for association ($p = 0.055$; Table 5). The haplotypes GGCAG ($p = 0.022$) and GGTAG ($p = 0.034$) of this block showed significant associations; however, this was not significant by permutation (permutation $p = 0.157$ for GGCAG and permutation $p = 0.271$ for GGTAG). The GGCAG haplotype, with the C allele of SNP03, showed overtransmission (62.61%). Overtransmission (51.58%) of the C allele of SNP03 was also observed in single SNP TDT.

Table 5
Haplotype associations of SNPs belonging to the three LD blocks of *CD38* in HFA trios.

Block	Haplotype[a]	Frequency	*T* (%)	Individual *p*-value	Permutation[b] *p*-value	Block *p*-value
Block 1 (SNPs 1-5)	GACAA	0.304	49.41	0.914	1	
	GGCAG	0.248	62.61	***0.022***	0.157	
	GACGG	0.163	39.68	0.102	0.565	0.055
	AACAG	0.153	54.39	0.508	0.998	
	GGTAG	0.099	31.25	***0.034***	0.271	
	GACAG	0.02	62.65	0.456	0.994	
Block 2 (SNPs 6-7)	CC	0.476	48.69	0.781	1	
	AT	0.287	37.05	***0.015***	0.12	***0.001***
	CT	0.23	69.18	***0.0007***	***0.005***	
Block 3 (SNPs 8-9)	AT	0.441	54.53	0.351	0.979	
	AC	0.286	46.7	0.543	0.999	0.638
	GT	0.274	47.73	0.67	1	

T (%): Transmitted/(transmitted + untransmitted). Significant values ($p < 0.05$) are indicated in bold italic
[a] All possible combinations of haplotypes with frequency > 0.01.
[b] 10,000 permutations.

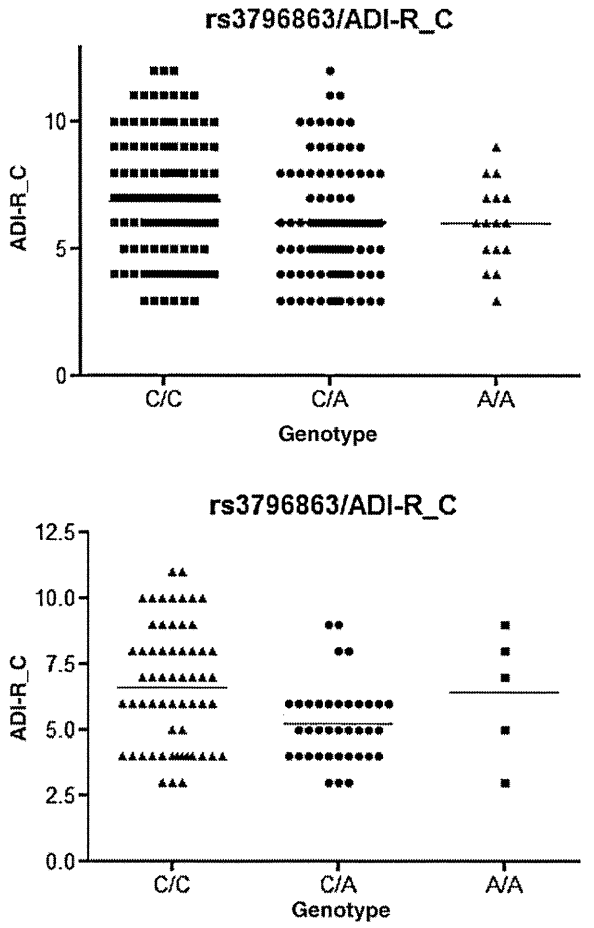

Fig. 3. Comparison of the distribution of ADI-R_C scores of autistic individuals across the C/C, C/A, and A/A genotypes of rs3796863 in 252 trios (A) and HFA trios (B). Significant variation was observed in the distribution of ADI-R_C scores between the three groups, in the 252 trios and HFA trios. The variation in the distribution of ADI-R_C between the C/C and C/A groups was significant at the 0.05- and 0.01-levels in the 252 and HFA trios, following *post hoc* pairwise comparison with Bonferroni's method. One-way analysis of variance (ANOVA), followed by *post hoc* pairwise comparison with Bonferroni's test, was used to examine the variability in the distribution of ADI-R phenotypic data (ADI-R_A, ADI-R_BV, ADI-R_C, ADI-R_D) across the homozygous and heterozygous genotypes of SNPs that showed significant associations in single SNP TDT.

The 2nd haploblock including SNPs06 and 07, showed a strong association ($p = 0.001$) in the HFA subgroup (Fig. 4 and Table 5). In this block, the haplotypes AT ($p = 0.015$) and CT ($p = 0.0007$) showed significant associations; the association shown by CT remained significant (permutation $p = 0.005$), while that of AT was not significant (permutation $p = 0.12$), by permutation. There was an overtransmission (69.18%) of the CT haplotype, with the C allele of SNP06. The C allele of SNP06 also showed overtransmission (54.52%) in single SNP TDT.

The haplotypes of the 3rd haploblock, which included SNPs 08 and 09, did not show any association with HFA. None of the haplotypes in the 252 trios showed significant association.

3.2. Mutation analysis

Next, we performed mutation and/or exonic SNP analysis in *CD38* (Fig. 2A) in cohort 1. The C3139T polymorphism in exon 1 led to an arginine-to-cysteine substitution at codon 47, the C4693T (rs1800561) polymorphism in exon 3 that led to an arginine-to-tryptophan substitution at codon 140 (R140W), and the C6900T polymorphism in exon 7 led to a serine-to-leucine substitution at codon 264. Two others mutations [C4092T (SNP14) and A5346C (rs1800051)] were synonymous.

A weak association was detected for rs1800561 (R140W) in adult ASD patients (average age = 22.8 ± 7.6 years old; allele frequency = 0.052) compared with controls (average age 34.1 ± 4.3 years old; allele frequency = 0.006) ($p < 0.05$) in cohort 1. Therefore, we focused only on the rs1800561 (R140W) polymorphism in case–control cohort 2 (Table 1) from 3 Japanese sites and genotyped in the same platform. Of 301 Japanese ASD subjects with the average age of 11.9 ± 6.7 years old, 13 male (but no female) patients were heterozygous for R140W (allelic frequency, 0.022). In 417 unscreened control subjects without ASD, 10 males and 7 females were heterozygous for the mutation and one female was homozygous (allelic frequency, 0.023). We failed to replicate the association in the larger sample set (cohort 2) ($\chi^2 = 1.20$, $p < 0.3$). Furthermore, although we detected the SNP in 5 of the 150 Korean controls, it was not detected it among the 16 Korean patients (cohort 3 in Table 1) or the 263 Caucasian patients (cohort 4 in Table 1).

3.3. rs1800561 (R140W) SNP in families

In the course of our studies of the rs1800561 SNP (R140W), we identified three families (cohort 1) in which ASD appeared to relatively segregate as a dominant trait (Fig. 5). In these families, fathers, brothers, and other relatives of 3 probands (two autistic (2-II-1 and 3-III-2) and one Asperger (1-III-1)) showed clinically identified ASD or exhibited ASD traits. AQ scores for the brothers of two probands (1-III-2 and 3-III-1) fulfilled the criteria (score >28) for ASD (Asperger disorder).

Twenty-eight family members were available (Fig. 5, not all are shown), and the R140W heterozygous SNP was found in 18 subjects whose ages ranged from 22 to 86 years old. Out of them, 8 carriers were clinically diagnosed as ASD or with ASD traits (44%). In these pedigrees, no ASD subjects were found without this mutant SNP.

Next, we examined whether mRNA from the mutant allele is expressed in the patients. We prepared blood RNA samples from one subject with the C/C genotype and 3 subjects with C/T genotype. cDNA with 4693C has the *Msp*A1I restriction site. The RT-PCR products from homozygous 4693C/C and heterozygous C/T subjects were digested by *Msp*A1I. RT-PCR products from the C/C subject gave two (digested) bands, while those from the C/T subjects gave 3 bands with an additional undigested one (data not shown). Furthermore, sequencing of RT-PCR products of the C/T samples confirmed the existence of the SNP. These results show that the mutant (W140) allele was transcribed and expressed in the 3 probands.

3.4. Plasma OT and vasopressin levels

Plasma OT levels of ASD probands with the W140 allele (79.3 ± 14.9 pg/ml; $n = 3$) were significantly lower than those of ASD subjects without the W140 allele in cohort 1 (147.7 ± 15.0 pg/ml; $p < 0.01$, $n = 26$; Fig. 6). The OT levels in ASD patients with the W140 allele were significantly lower than those in control subjects with the R140 allele (198.2 ± 24.7 pg/ml; $n = 100$; $p < 0.01$). Only one control subject with the W140 allele was available for plasma OT measurement, and the value was 174.7 pg/ml. The OT levels in ASD patients with the R140 allele were almost equivalent to those in control subjects with the R140 allele, suggesting that allele (C/T) status may be a unique determinant for the plasma OT level among multiple confounding factors. In contrast, plasma AVP levels were slightly higher in the probands with the W140 allele than in ASD patients with the R140 allele (38.9 ± 3.8 pg/ml, $n = 3$, *vs.* 26.9 ± 5.0 pg/ml, $n = 26$; $p < 0.05$) (Fig. 6).

Finally, one proband (3-III-2) aged 23 years, diagnosed with autism at the age of 3 years and 9 months, began nasal OT adminis-

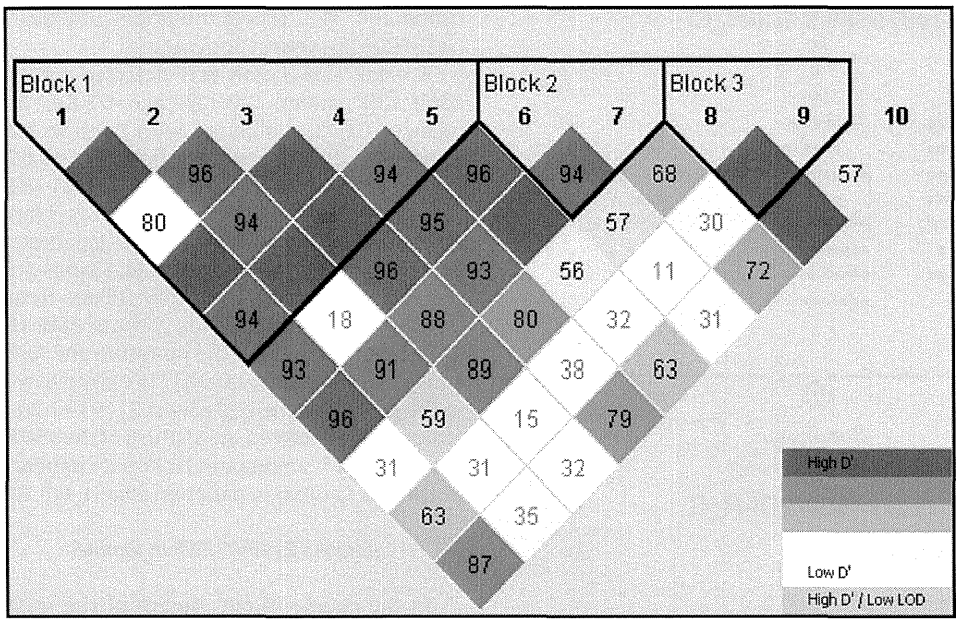

Fig. 4. LD structure of *CD38* based on *D'* values calculated from HFA trios. A linkage disequilibrium (LD) plot was constructed using the *D'* (linkage disequilibrium coefficient; Ranade, 2001) pairwise LD values between markers, estimated using the Haploview software. Based on the LD structure of the gene, haplotype associations were examined; all the haplotypes with frequency >0.01 were included for the association test.

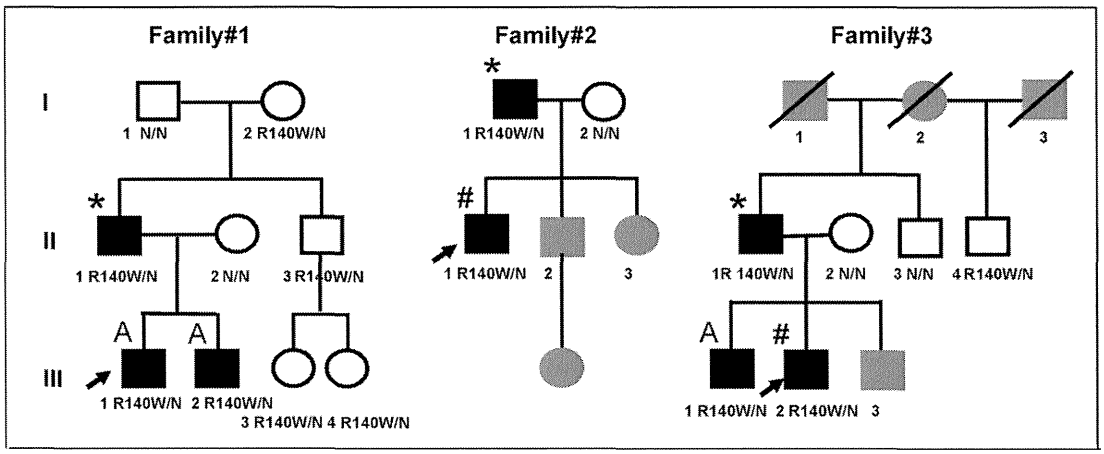

Fig. 5. Pedigrees of the 3 ASD probands carrying the W140 allele. Squares and circles represent male and female family members, respectively. Black squares represent those with ASD or ASD trait. A slash mark through symbols indicates the subject is deceased. The allele status is indicated under the symbols: N/N, two normal alleles; R140W/N, one mutant and one normal allele. Gray symbols indicate undetermined (no DNA available for analysis). The subjects are identified by Arabic numbers, and the generation by Roman numerals. The arrow indicates the proband. (*) Autism trait; (#) Autism; (A) Asperger disorder.

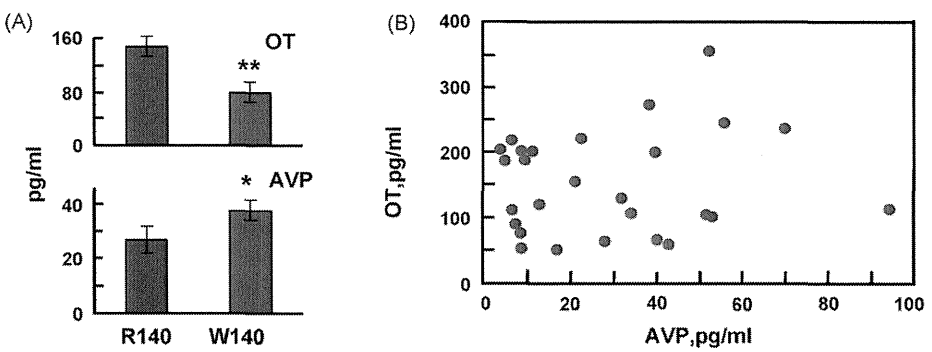

Fig. 6. Plasma oxytocin and vasopressin levels in ASD subjects. Bar graph (A) and Scatchard plot (B) of plasma concentrations of OT and AVP levels in 29 ASD patients in cohort 1 with (red) or without (green) the W140 allele. Mean ± S.E.M. *$p < 0.05$; **$p < 0.01$ (one-way ANOVA).

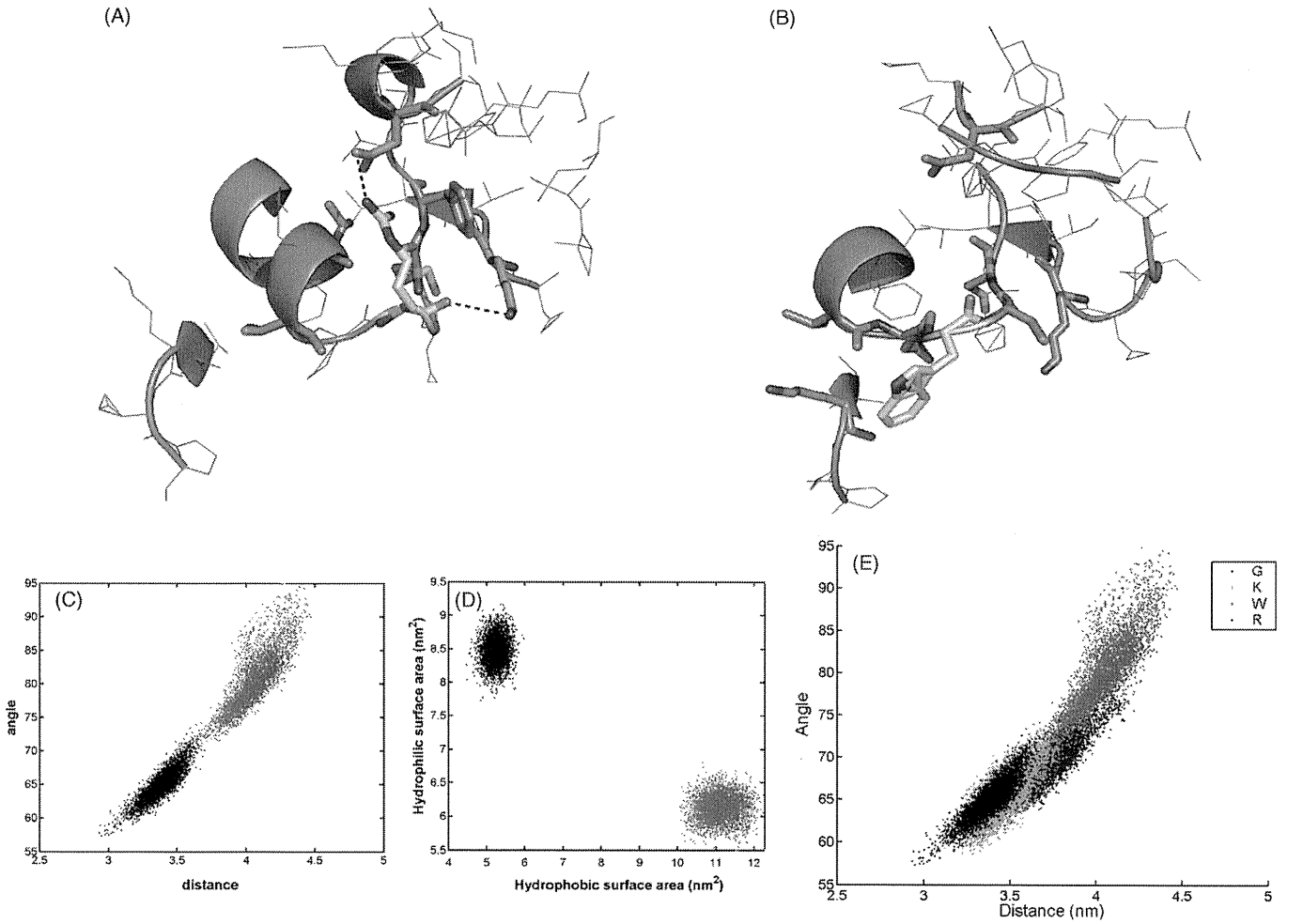

Fig. 7. Molecular structure of CD38. The interactions between R140 (A) or W140 (B) and nearby residues in CD38 protein are shown. Hydrogen bonds are shown as dashed lines. The protein residues are colored as follows: C, blue except the 140 residue (yellow); N, blue; O, red. R140 and W140 are packed into the helix, and the packing conformation seems stable. The W140 protein has an outward conformation and fewer interacting residues than the wild-type protein. (C) Distribution of distance and angle between domains. The distance between Q171 and S213 and the angle between Q171, G113 (hinge), and S213 are plotted based on domain analysis. The R140 structures are shown as black dots and the W140 as red dots. Each point representing a conformation is from MD simulation (5–9 ns). The mutant structure has a more the open conformation than the wild-type and a slightly larger degree of variation. (D) Solvent-accessible surface areas. The active site properties are significantly different between the mutant and wild-type. (E) Distribution of distance and angle between domains during 5–9 ns MD simulation as in (C): R140, black; W140, red; K140, green; and G140, blue. K140 shows almost the same distribution as the wild-type. G140 shows a slightly different distribution, but less than W140.

tration twice a day at home with parental assistance in June 2008. The immediate effect of OT was obvious after the first trial, in which he was no longer boisterous after awaking early in the morning. This quieting on awaking has been maintained for more than 12 months. He showed improvements in eye contact behavior with smiling and answering to yes/no questions in his daily life. There were no significant adverse effects.

4. Discussion

Here, we demonstrated that one rs3796863 (C > A) SNP of *CD38* showed significant association with U.S. but not Japanese high-fiunctioning autism (HFA) patients, with a cutoff at IQ > 70. Based on the results of SNP- and haplotype-TDT analyses, the A allele of rs3796863 of *CD38* may be considered a protective allele and the C allele as a risk allele for U.S. HFA cases. As the allele frequency is about 0.3, this variant is common. Very recently, common variants on 5p14.1 between neural cadherin 10 and 9 have been reported to associate with ASD (Wang et al., 2009). As rs3796863 is an intronic SNP, the functional importance of this SNP remains to be determined. There were significant variations in the distribution of ADI-R_C scores (restricted, repetitive, and stereotyped patterns of behavior) between the C/C, C/A, and A/A genotypes of this SNP in 252 trios and HFA trios. The second haploblock of *CD38*, which included rs3796863, showed a significant association with HFA. The association shown by the CT haplotype of second haploblock remained significant by permutation analysis. This common variant (Arking et al., 2008) may contribute to the genetic susceptibility of HFA, in addition to other susceptiblity genes for HFA (Weiss and Arking, 2009; Wermter et al., 2010).

SNP (rs1800561) of *CD38* was reported in Japanese (allele frequency, 0.035) and Han Chinese (0.01) but not in European or African control populations in the online SNP database. However, recently, it was detected in Polish Caucasians, where 3 healthy controls out of 500 yielded an allele frequency of 0.003, and 21 W140 carriers were found among 439 B-cell chronic leukocytic leukemia patients (frequency, 0.024) (Jamroziak et al., 2009). An Italian study indicated one carrier among 25 healthy controls (frequency, 0.02; cohort 4) (Mallone et al., 2001). We found 68 carriers of the T genotype among 1384 Japanese, and they included controls, ASD patients, and family members of ASD patients. We also detected this genotype in 5 of 150 Koreans non-ASD controls with diabetes (frequency, 0.017; cohort 3) (Table 1), indicating that the polymorphism is more common among Asians than Caucasians.

The biological relevance of the inheritance patterns of the R140W allele was unclear in the context of ASD. The male offspring of W140 carriers seem to have a higher risk of ASD than females. Why the effect of the W140 variant on development of ASD varies with sex is unknown, but females may have more protective factors and/or weaker risk factors related to female hormones or OT. Plasma OT levels were lower in W140 allele ASD carriers than R140 ASD carriers. However, taking into account the results that the W140 allele is not associated with ASD in general population, we can only say that the W140 allele is deemed to have a role in decreased plasma OT levels, but not in AVP levels, regardless of disease status. This scenario is expected from our prior observation in *Cd38* knockout mice (Jin et al., 2007), in which the plasma OT but not AVP level was differentially decreased.

Our finding that one proband (3-III-2 in Fig. 5) with the W140 allele had been receiving intranasal OT was unexpected. His social behavior showed some improvement after the first administration of OT, and this improvement has been maintained for more than 12 months. The observed effects are in accordance with those reported previously for OT: improved mind- or emotion-reading and social memory, increased eye contact, and positive communication (Hollander et al., 2007; Domes et al., 2007; Guastella et al., 2008a,b, 2010). However, to our knowledge, this is the first report of a long-term therapeutic effect of OT on the social deficits in ASD, suggesting that it may benefit a broader group of patients, disorders, or typical adults and individuals carrying this rare allele.

We conducted a preliminary structural analysis of CD38 and various mutant proteins by Dyndom and MD simulation to analyze domain motion (Hayward and Lee, 2002) (Fig. 7). W140-CD38 had a completely different conformation than R140-CD38 (Fig. 7A and B). The charge change at the packed site caused by the R → W substitution was likely the primary reason for the outward conformation of the mutant protein. The mutant structure is more open and has a slightly larger degree of variation (Fig. 7C and E). Fig. 7D shows the solvent-accessible surface area of the active site of the mutant protein altered by the closure motion, significantly changing its properties. Thus, W140 CD38 showed changes that may affect the substrate binding affinity and eventually enzyme activity. The amino acid substitution can cause severe perturbations of the predicted protein structure in comparison with wild-type human (R140), rabbit (K140), and mouse (G140) CD38. In CHO cells, in fact, the W140-CD38 protein possesses only one third of the ADP-ribosyl cyclase activity of R140-CD38 (Yagui et al., 1998). Moreover, social amnesia was not rescued by local re-expression of human W140-CD38 in the hypothalamus in the *Cd38* null mice (Jin et al., 2007). Taken together, these observations indicate functional abnormality of W140-CD38.

In conclusion, despite their statistical limitations, our results suggest that the rs3796863 SNP may contribute to genetic susceptibility to HFA in U.S. but not necessarily in Japanese subjects (at least to the limits of our current analysis). Our results call for functional and expression assay to assess the biological effects of the variant. Furthermore, the W140 allele could be a potential risk factor for a subset of Japanese ASD patients, *i.e.*, males with low blood OT levels. Patients in this subgroup are candidates for a clinical trial of OT treatment, although further systematic case–control investigations are required to verify its effects.

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うつ病治療の実際

最近のAugmentation療法

簗詰健次郎[*1)]，岡本長久[*2)]

1957年にRoland Kuhnが初めてうつ病患者にイミプラミンを投与してから，これまでに多くの抗うつ薬が導入されてきた。それによりうつ病治療の選択肢が広がったものの，依然として多くの患者が1種類以上の抗うつ薬を使用しても寛解に至らないことも事実である。Augmentation（増強）療法は，1960年代頃からそれらの治療抵抗性うつ病の治療において一定の位置を占めてきた。治療抵抗性うつ病の治療において，これまでに比較的強いエビデンスが得られている薬剤としては，炭酸リチウム，甲状腺ホルモン，非定型抗精神病薬が挙げられる。昨今，海外を中心に，これらの薬剤の薬理機序や抗うつ効果の研究結果が多数示されてきている。特に非定型抗精神薬の治療抵抗性うつ病増強療法における効果のエビデンスの蓄積は，欧米を中心として，近年，目覚ましいものがある。

今回は増強療法について，最近の研究結果を交えて分かり易く述べていく。

Ⅰ．はじめに

臨床の現場では，治療抵抗性うつ病患者に遭遇することは稀では無い。実際にファーストラインの抗うつ薬単剤治療の反応率は47%，寛解率は33%であり，約7割の患者は初回の抗うつ薬では寛解に至らないとの報告[1)]がある。そのような場合，次の治療戦略としてAugmentation（増強）療法を選択する臨床家は多いと考えられる。

Texas Medication Algorithm Project（TMAP）の非精神病性うつ病治療アルゴリズム2008年版（表1）においては，うつ病治療におけるAugmentation（増強）療法の位置付けは，ファーストラインの選択的セロトニン再取り込み阻害薬（SSRI）やセロトニン-ノルアドレナリン再取り込み阻害薬（SNRI）等の投与にて部分的に有効（不完全寛解）であった際の，次の治療戦略の一つとしての位置づけである。また他のうつ病治療アルゴリズム（表1）においてもほぼ同様の位置づけである。

増強療法の利点としては，これまでに効果を認めた薬剤を中止することなく継続して使用するため，症状の悪化のリスクが小さいということや，他の薬に変更する際にその置換にある程度の時間がかかるものの，増強療法では付加するだけのため，時間的な短縮が望め，患者の症状による苦痛の軽減という意味からも望ましい点，退薬症候群を起こすリスクが無いことなどが挙げられる。

短所は，薬剤の相互作用の影響が否定できないこと（リチウムによるSSRI血中濃度の上昇など），最適用量や投与継続期間などが十分に確立していないこと，

*1) Kenjiro Yanazume, 2) Nagahisa Okamoto　国立精神・神経医療研究センター病院

表1　うつ病治療における各種アルゴリズム

いずれのアルゴリズムにおいても増強療法は，1つ目の抗うつ薬にて寛解に至らなかった場合の次の治療戦略としての位置づけである。

	STAR*D	TMAP (2008)	CANMAT
Step 1	SSRI (citalopram)	SSRI，SNRI mirtazapine*または bupropion	SSRI または mirtazapine*，venlafaxine，nefazodone bupropion，moclobemide，trazodone* 増量，lithium*または T3 による増強療法
Step 1A (部分反応時)		SSRI，SNRI，T3 bupropion，buspirone，mirtazapine*による増強療法	
Step 2	sertraline*，venlafaxine，bupropion への変更 bupropion または buspirone による増強療法	SSRI，SNRI，mirtazapine bupropion への変更	SSRI，mirtazapine*，venlafaxine.nefazodone, bupropion，moclobemide，trazodone*への変更
Step 2A (partial responders)		SSRI，SNRI，T3，bupropion，buspirone，mirtazapine による増強療法	
Step 3	lithium*または T3 による増強療法 mirtazapine*または nortriptyline*への変更	a）TCA ± lithium．b）MAOI c）TCA + SSRI d）bupropione + SSRI/SNRI e）mirtazapine*+ SSRI/SNRI の併用療法 f）SSRI + AAP lamotrigine*，bupropion．mirtazapine*，D_2 agonist による増強療法	lithium*，T3，buspirone．AAP または psychostimulant による増強療法 他のクラスへの変更 (TCA/MAOI)
Step 3A (partial responders)			
Step 4	tranylcypromine への変更 mirtazapine*+ venlafaxine の併用療法	step 3 にて a）または b）：c）～ f）を選択 c）～ f）：a）または b）を選択	
Step 5		ECT	

STAR*D：Sequenced Treatment Alternatives to Relieve Depression，TMAP：Texan Medication Algorithm Project，CANMAT：Canadian Network for Mood and Anxiety treatment，AAP：atypical antipsychotic，MAOI：monoamine oxidase inhibitor，SSRI：selective serotonin reuptake inhibitors，TCA：tricyclic antidepressants，ECT：electroconvulsive therapy，SNRI：serotonin-norepinephrine reuptake inhibitor

＊はわが国で上市済。

(An algorithm for the pharmacological treatment of depression：Acta psychiar Scand 121：180-189, 2010 を改編)

表2 増強療法における長所と短所の紹介

長所，短所共に存在し，臨床ではこれらの 特性を考慮した上で増強療法を行っていく必要がある。

長所	短所
1．部分反応があった抗うつ薬を中止する訳では無いので，症状の急激な悪化を防げる。 2．抗うつ薬の置換に要する時間が省け，治療期間の短縮につながる。 3．退薬症候群のリスクが無い。	1．作用機序が不明確である。 2．薬剤相互作用によるリスクがある。 3．投与量や投与期間におけるコンセンサスが得られていない。 4．保険適応が無い。

などである。(表2)。

また，注意しないといけない点は，わが国においてうつ病に対する増強療法は，どの薬剤においても保険適応となっていないことである。

Ⅱ．増強療法の使用薬剤

1．リチウム

これまでに多くの研究結果が示されており，わが国において増強療法にて使用される薬剤の中で最も使用頻度の高い薬剤の一つである。リチウムの作用機序は未だ不明であるが，その主要な働きはセカンドメッセンジャー機構に働きかけ，セロトニンの神経伝達効果を増強すると言われている。また最近の研究では脳内の BDNF（神経保護因子）の濃度を高めることで神経保護作用による抗うつ効果も指摘されている。

リチウム増強療法については，これまでに 30 以上のオープン試験と 10 個のプラセボ比較対照試験により，最も古くから効果の実証がなされている。

これまでに行われた3つのメタ解析[2)-4)]では，薬物治療抵抗性うつ病の 50%近くに4週間以内の効果増強が認められるとされ，特に血中濃度 0.5 mEq/L 以上に維持した場合は改善率が高い，との結果であった。ただ，最も最近行われたメタ解析[4)]では，対象の 10 個の二重盲検試験（N＝269）のうち，9個の試験の被験者は 35 人以下であり，さらに対象としたいくつかの二重盲検試験に双極性うつ病の患者が含まれていたことに留意する必要がある。

投与量については，低用量でも有効とする報告があるが，一般的には気分安定薬としての使用量の目安である 400 mg/ 日～ 1,200 mg/ 日，血中濃度にて 0.4 ～ 1.2 mEq/L の範囲が望ましいとされている。効果発現時期については 1981 年に De Montigny らがリチウムを追加することで 48 時間以内に急速な改善が見られるというケースシリーズ報告を最初に行い，同様の報告が幾つか見られたものの，その後のプラセボ比較対照試験（Browne M, et al, 1990）では否定されており，通常は効果を判定するまでに3～4週間は必要と考えられる。ただし Thase ら（1989）のオープン試験では5～6週目に反応する群も認める，という報告もある。リチウムによる増強療法が有効であった場合に，どの位の期間に渡って治療を続けることが望ましいかについては，これまでに2件の無作為化比較試験（RCT）（Bauer M, et al, 2000, Bschor T, et al, 2002）で検討されており，その結果からは，症状が寛解した後，1年間以上は内服治療を続けることが必要であることが示されている。

以上のようにリチウム増強療法の効果についての研究は多く，ある程度のエビデンスは蓄積されていることは事実であるが，これらの研究の限界点もいくつか挙げられる。

まず，これらの研究では三環系抗うつ薬（TCA）に対する増強療法が大部分で，他の抗うつ薬（SSRI など）への増強療法の研究は，RCT については SSRI に対す

TMAP (Texas Medication Algorithm Project)
SNRI (serotonin-norepinephrine reuptake inhibitor)
TCA (tricyclic antidepressants)
SSRI (selective serotonin reuptake inhibitor)
BDNF (brain derived neutrotrophic factor)

る増強療法についてのRCTが2つ[5][6]しかなく，その中の1つ[5]はプラセボとの間に効果に有意差はあったものの，N＝24と小規模で，さらに投与期間が1週間と短く，他方[6]はプラセボとの間に有意差は得られない結果となり，非TCA薬に対するエビデンスは乏しいのが現状である。

また，長期間投与についてのリチウム増強療法の効果についてのエビデンスは乏しく，プラセボ薬では無く他の種類の抗うつ薬との増強療法における抗うつ効果の比較試験のエビデンスも乏しい。さらに，心機能障害や腎機能障害，甲状腺機能障害などの副作用に注意を払うことは勿論，適宜，血中濃度のモニターが必要であるのは言うまでもないことである。

2．甲状腺ホルモン

甲状腺ホルモンにはT3とT4があり，T4はT3に代謝され脳内での活性を持つ。欧米ではT3の使用が多いが，T3は半減期が短く血中濃度の変動が大きいため，わが国では伝統的にT4の使用が一般的である。作用機序については，甲状腺ホルモンがセロトニンやノルアドレナリンの神経伝達効果を増強することに加え，潜在的に低下した甲状腺機能を是正することにより効果を発揮すると考えられている。

T3増強療法については，有効性の報告はある一方で否定的な見解もある。T3をTCAに追加した効果はリチウムとTCAの併用と同等であるという報告がJoffeら(1993)を始めとして6つのRCTと2つのメタ解析にて示され，有効性が確認されている。

ただAronsonら(1996)による292人を対象としたT3のメタ解析では23%に効果増強を認めたが，二重盲検試験に限った解析では効果は認めなかったという報告[7]もある。

またJoffeら(2006)による2週間の短期治療の報告ではリチウム，T3，リチウムとT3の併用，プラセボで治療効果に差が無いことがわかっている。

SSRIなどの非TCA薬に対するT3増強療法についての報告は，少数[8]あるのみで，効果については未知数である。

T3の投与量については，研究は少ないものの5～50μg/日の投与で約半数の患者に効果が見られるとの指摘[9]がある。また投与期間についても同様に，研究は少ないものの甲状腺機能が正常な患者ではT3の使用は2～3週間に留めるべきであり，これを超えるとT3誘発性の甲状腺機能低下症を起こすリスクがあるとの指摘[9]がある。

T4については高用量のT4追加が治療抵抗性のうつ病患者に効果を認めたとのオープン試験[10]が報告されている。

STAR*D (Sequenced Treatment Alternatives to Relieve Depression)では，2種類の治療戦略に失敗した患者がリチウム群，T3群に割り付けられ，最長14週間の治療が行われた。寛解率は，リチウム群では15.9%，T3群では24.7%であり，有効性に有意な差は認められず，副作用による中断はリチウム群23.2%，T3群9.6%とリチウム群の方が忍容性が低い結果となっている。しかし，本研究はオープンスタディであること，citalopram抵抗性の患者に限定されていること，短期間投与ということで，長期の安全性については（T3の女性の骨に対する長期的な影響についてなど）考慮されていない点を留意する必要がある。まとめると，これまでにT3のTCAに対する増強療法は効果がある程度確認されているものの，非TCA薬に対する効果は未知である，T4増強療法についての効果のエビデンスは現在のところ乏しい，というのが現状である。

3．非定型抗精神病薬

1999年，OstroffとNelsonが非定型抗精神病薬の有効性を報告して以降，STAR*D開始時にはエビデンスの乏しかった非定型抗精神病薬の治療抵抗性うつ病に対する増強療法のエビデンスが近年急速に増加した。2007年，Papakostasらは1,500人を対象としたオランザピン，リスペリドン，クエチアピンのメタ解析を行って有効性を示した[11]。その後，アリピプラゾールとクエチアピンの臨床試験のエビデンスが急速に蓄積し，米国食品医薬品局(FDA)では単極性うつ病

FDA（米国食品医薬品局）

の増強療法として2008年に最初にアリピプラゾールが認可され，2009年にクエチアピンも承認された。さらに治療抵抗性うつ病に対しSSRIであるフルオキセチンとオランザピンの合剤も承認された。2009年に行われた16個の二重盲検試験の3,480人を対象としたメタ解析[12]では非定型抗精神病薬（オランザピン，リスペリドン，クエチアピン，アリピプラゾール）のプラセボに対する反応率のオッズ比は1.69，寛解率のオッズ比は2.00であることが報告され，その有効性に非定型抗精神病薬間での有意差は無かった。このメタ解析は，今まで最もエビデンスが高いとされたリチウムやT3のメタ解析の実に10倍以上の人数を対象としており，これらの結果からは，現在治療抵抗性うつ病に関して最もエビデンスが高い増強療法は非定型抗精神病薬ということになる。ただこの研究では，有害事象による治療中断率が9.1%で，プラセボ群に対するオッズ比は3.91であったことと，維持療法時などの長期投与における有効性と安全性は未解明であることを留意する必要がある。

抗精神病薬の併用による作用機序は未だ不明な点が多いが，Zangら（2000）は前頭前野におけるノルアドレナリンやドパミンの放出増加作用に関与する可能性を指摘している。

同じ気分障害圏である躁うつ病における非定型抗精神病薬の導入は近年，目覚ましいものがあり，一定の効果を挙げていることを踏まえれば，今後，更に非定型抗精神薬のうつ病の増強療法においての役割も重要となると考えられ，注目すべき薬剤であることは間違いないであろう。

表3　治療抵抗性うつ病に対する増強療法における各種薬剤の抗うつ効果のエビデンスを表した表

現時点では非定型抗精神病薬が最もエビデンスレベルが強い薬剤となっている。この表は，それまでに行われたすべての無作為比較対照試験とSTAR*Dの結果に基づいて作られている。

Srategy	Evidence Grade
非定型抗精神病薬	A
ω-3脂肪酸	A⁻
モダフィニル リチウム T3 ブプロピオン*	B
テストステロン デシプラミン*	B⁻
ピンドロール ブスピロン* イノシトール	C
ラモトリギン* メチルフェニデート	C

A＝強い　B＝強弱混合　C＝弱い

(Managing Partial Respose Nonresponse : Switching, Augmentation, and Combination Strategies for Major Depressive Disorder. J Clin Psychiatry 2009；70；16-25を改変)

*わが国では未承認。ラモトリギンは抗てんかん薬としては承認済み。

MAO-B（モノアミン酸化酵素B）