Table 1
Results of linkage disequilibrium (D'/r^2 value) between the SNPs and two- and three-SNP-based haplotype analyses.

SNPs		D'/r^2 [a]		Haplotypic global *P*-value	
				2 windows	3 windows
rs3825251		0.91/0.82	0.93/0.86	0.85	0.84
rs3918347	0.96/0.89		0.95/0.88	0.87	
rs4964770	0.94/0.87	0.98/0.97			

[a] For D'/r^2 value, right upper diagonal: schizophrenia, left lower diagonal: controls.

In our previous stage 1 study, the 3′ regions of *DAO* showed a strong LD, including three SNPs (rs3825251–rs3918347–rs4964770) that showed an association with Japanese schizophrenia. In particular, haplotype GGC may be a protective haplotype in schizophrenia (Ohnuma et al., 2009). But in our current stage 2 study that had adequate statistical power, our results did not show any significant association between schizophrenia and single SNPs or two- and three-SNP-based haplotypes. Thus, the results from our previous study should be considered a type I error (false positive), because they did not have adequate statistical power to produce reliable results. Previously, two representative meta-analyses showed an association between rs4623951 in the 5′ region and schizophrenia; however, none of the studies that only used Japanese subjects showed positive findings for rs4623951, whereas the studies with only Japanese subjects showed marginal positive findings in the 3′ region (Ohnuma et al., 2009; Yamada et al., 2005). In addition, Japanese subjects were not included in all studies that reported positive associations between schizophrenia and SNPs situated in the 5′ region of *DAO* (Chumakov et al., 2002; Corvin et al., 2007; Schumacher et al., 2004; Wood et al., 2007). Taken together, these results suggest that there may be some ethnic differences in *DAO*, and that a schizophrenia susceptibility locus in Japanese patients may have a nominal effect and may be situated in the 3′ region. Indeed, the minor allelic frequencies of the three investigated SNPs, especially rs3825251, were different among different ethnic populations, and the LD pattern in the 3′ region was also different between Japanese and Caucasian individuals (http://www.hapmap.org/index.html.ja). In addition, symptomatic differences and schizophrenia subtypes were not considered in the current study, and thus the heterogeneity of this disease may also lead to inconsistent results in genetic studies of schizophrenia.

Thus, further genetic case-control studies of *DAO* with careful consideration of schizophrenia heterogeneities (i.e., differences in ethnicity, clinical subtypes, and symptoms) and investigation of gene–gene interactions among D-serine-related genes may be needed to determine a possible association with schizophrenia. Thus, we currently conclude that *DAO* does not have an apparent degree of association with Japanese schizophrenia.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.schres.2010.01.028.

References

Allen, N.C., Bagade, S., McQueen, M.B., Ioannidis, J.P., Kavvoura, F.K., Khoury, M.J., et al., 2008. Systematic meta-analyses and field synopsis of genetic association studies in schizophrenia: the SzGene database. Nat. Genet. 40, 827–834.
Chumakov, I., Blumenfeld, M., Guerassimenko, O., Cavarec, L., Palicio, M., Abderrahim, H., et al., 2002. Genetic and physiological data implicating the new human gene G72 and the gene for D-amino acid oxidase in schizophrenia. Proc Natl Acad Sci U S A 99, 13675–13680.
Corvin, A., McGhee, K.A., Murphy, K., Donohoe, G., Nangle, J.M., Schwaiger, S., et al., 2007. Evidence for association and epistasis at the DAOA/G30 and D-amino acid oxidase loci in an Irish schizophrenia sample. Am J Med Genet B Neuropsychiatr Genet 144, 949–953.
Fallin, M.D., Lasseter, V.K., Avramopoulos, D., Nicodemus, K.K., Wolyniec, P.S., McGrath, J.A., et al., 2005. Bipolar I disorder and schizophrenia: a 440-single-nucleotide polymorphism screen of 64 candidate genes among Ashkenazi Jewish case-parent trios. Am. J. Hum. Genet. 77, 918–936.
Jonsson, E.G., Saetre, P., Vares, M., Andreou, D., Larsson, K., Timm, S., et al., 2009. DTNBP1, NRG1, DAOA, DAO and GRM3 polymorphisms and schizophrenia: an association study. Neuropsychobiology 59, 142–150.
Liu, Y.L., Fann, C.S., Liu, C.M., Chang, C.C., Wu, J.Y., Hung, S.I., et al., 2006. No association of G72 and D-amino acid oxidase genes with schizophrenia. Schizophr. Res. 87, 15–20.
Ohnuma, T., Shibata, N., Maeshima, H., Baba, H., Hatano, T., Hanzawa, R., Arai, H., 2009. Association analysis of glycine- and serine-related genes in a Japanese population of patients with schizophrenia. Prog. Neuropsychopharmacol. Biol. Psychiatry 33, 511–518.
Schumacher, J., Jamra, R.A., Freudenberg, J., Becker, T., Ohlraun, S., Otte, A.C., et al., 2004. Examination of G72 and D-amino-acid oxidase as genetic risk factors for schizophrenia and bipolar affective disorder. Mol Psychiatry 9, 203–207.
Shi, J., Gershon, E.S., Liu, C., 2008. Genetic associations with schizophrenia: meta-analyses of 12 candidate genes. Schizophr. Res. 104, 96–107.
Shinkai, T., De Luca, V., Hwang, R., Muller, D.J., Lanktree, M., Zai, G., et al., 2007. Association analyses of the DAOA/G30 and D-amino-acid oxidase genes in schizophrenia: further evidence for a role in schizophrenia. Neuromolecular Med 9, 169–177.
Tsai, G., Yang, P., Chung, L.C., Lange, N., Coyle, J.T., 1998. D-serine added to antipsychotics for the treatment of schizophrenia. Biol. Psychiatry 44, 1081–1089.
Verrall, L., Burnet, P.W., Betts, J.F., Harrison, P.J., 2009. The neurobiology of D-amino acid oxidase and its involvement in schizophrenia. Mol Psychiatry 29, 1–16.
Vilella, E., Costas, J., Sanjuan, J., Guitart, M., De Diego, Y., Carracedo, A., et al., 2008. Association of schizophrenia with DTNBP1 but not with DAO, DAOA, NRG1 and RGS4 nor their genetic interaction. J Psychiatr Res 42, 278–288.
Wood, L.S., Pickering, E.H., Dechairo, B.M., 2007. Significant support for DAO as a schizophrenia susceptibility locus: examination of five genes putatively associated with schizophrenia. Biol. Psychiatry 61, 1195–1199.
Yamada, K., Ohnishi, T., Hashimoto, K., Ohba, H., Iwayama-Shigeno, Y., Toyoshima, M., et al., 2005. Identification of multiple serine racemase (SRR) mRNA isoforms and genetic analyses of SRR and DAO in schizophrenia and D-serine levels. Biol. Psychiatry 57, 1493–1503.

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Association analysis of *SIGMAR1* with major depressive disorder and SSRI response

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SUMMARY

Background: Several investigations have suggested the possible involvement of sigma1 non-opioid intracellular receptor 1 (sigma1 receptor) in the pathophysiology of major depressive disorder (MDD). Sigma1 receptors are also one of the major pharmacological therapeutic targets of selective serotonin reuptake inhibitors (SSRIs). To evaluate the association of sigma1 receptor gene (*SIGMAR1*) and MDD and SSRIs therapeutic response in MDD, we conducted a case–control study of Japanese samples (466 MDD patients, 516 controls and 208 MDD patients treated by fluvoxamine or sertraline).

Method: We defined a clinical response as a decrease of more than 50% in baseline the Structured Interview Guide for Hamilton Rating Scale for Depression (SIGH-D) within 8 weeks, and clinical remission as an SIGH-D score of less than 7 at 8 weeks. Therefore, we selected rs1800866 in *SIGMAR1* for the following association analysis.

Results: In the logistic regression analysis, we detected an association of the phenotypes (MDD or controls) with rs1800866 genotype. However, we did not detect an association between rs1800866 and SSRI therapeutic response in Japanese MDD. In addition, remission with SSRI was not associated with rs1800866. Also, we did not detect a novel polymorphism in *SIGMAR1* when we performed a mutation search using MDD treated by SSRIs samples.

Conclusion: Our results suggest that rs1800866 in *SIGMAR1* may play a role in the pathophysiology of MDD in the Japanese population. Also, *SIGMAR1* does not play a role in the therapeutic response to SSRI in Japanese MDD patients. However, because our sample was small, a replication study using another population and larger sample will be required for conclusive results.

1. Introduction

Several investigations have suggested the possible involvement of sigma1 non-opioid intracellular receptor 1 (sigma1 receptor) in the pathophysiology of major depressive disorder (MDD) and the pharmacological therapeutic response in MDD (Bermack and Debonnel, 2005; Hashimoto and Ishiwata, 2006; Hayashi and Su, 2008; Ishikawa et al., 2007; Kulkarni and Dhir, 2009).

Preclinical studies suggest that fluvoxamine and sertraline, which are selective serotonin reuptake inhibitors (SSRIs), have effects on sigma1 receptors (Ishikawa et al., 2007; Narita et al., 1996). The sigma1 receptor is more abundant in the dentate gyrus of the hippocampal formation, facial nucleus and various thalamic and hypothalamic nuclei (Bouchard and Quirion, 1997). Sigma1 receptors regulate dopamine release in various areas of the brain (Gonzalez-Alvear et al., 1995), and regulate dopaminergic, N-methyl-D-aspartate (NMDA) and glutamatergic neurotransmission in limbic areas, including the nucleus accumbens and the ventral tegmental area (Ault and Werling, 1999; Gronier and Debonnel, 1999; Weatherspoon et al., 1996; Yamazaki et al., 2002). These mechanisms are known to be involved in the pathophysiology of MDD (Miller et al., 2009; Nestler and Carlezon, 2006). Neurodevelopmental and neurodegenerative models have been suggested to be involved in other aspects of the pathophysiology of

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MDD (Ansorge et al., 2007). The hippocampal volume in MDD patients is significantly smaller than in control subjects (Kronmuller et al., 2009). Wang and Duncan reported that both neural survival and growth pathways are activated and the caspase-3-dependent apoptosis pathway is inactivated when sigma1 receptors are functionally active (Wang and Duncan, 2006). Nishimura and colleagues reported that sigma1 receptor agonist is involved in the mechanisms causing the drugs' potentiation of nerve growth factor (NGF)-induced neurite outgrowth in PC12 cells (Nishimura et al., 2008). Moreover, sigma1 receptors were shown to interact with inositol 1,4,5-triphosphate (IP3) receptors (Nishimura et al., 2008). Sigma1 receptor knockout mice also showed a depressive-like phenotype (Sabino et al., 2009). On the other hand, sigma1 receptors were reported to play an important role in the pathogenesis of anxiety and response to stress (Kamei et al., 1998; Lambert et al., 1998; Maurice et al., 1999), and depression (Akunne et al., 1997; Bermack and Debonnel, 2001).

From this evidence, we thought that the sigma1 receptor gene (*SIGMAR1*: OMIM *601978, 2 exons in this genomic region spanning 3.850 kb, and located on 9p13) was a good candidate gene for the pathophysiology of MDD. Several genome-wide association studies reported no association between *SIGMAR1* and MDD (Muglia et al., 2008; Sullivan et al., 2009) or clinical response to treatment with antidepressants in MDD (Garriock et al., 2010; Laje et al., 2009). However, we found no genetic association study that used Japanese samples. To evaluate the association of *SIGMAR1* and MDD and SSRIs therapeutic response in MDD, we conducted a case–control study of Japanese samples (466 MDD patients, 516 controls and 208 MDD patients treated by SSRIs).

2. Materials and methods

2.1. Subjects

The subjects in the association analysis were 466 MDD patients (218 males and 248 females; mean age ± standard deviation 48.7 ± 16.0 years) and 516 healthy controls (266 males and 250 females; 39.4 ± 17.1 years). The patients were diagnosed according to DSM-IV criteria with the consensus of at least two experienced psychiatrists on the basis of unstructured interviews and a review of medical records. 265 of the 466 MDD patients, who were treated with fluvoxamine (136 patients), sertraline (72 patients) or paroxetine (57 patients), were diagnosed according to DSM-IV criteria with the consensus of at least two experienced psychiatrists on the basis of a review of medical records and assessments with the Structured Interview Guide for Hamilton Rating Scale for Depression (SIGH-D) (Williams, 1988). Our subjects were hospital in-patients or outpatients, unrelated to each other and ethnically Japanese; cases were collected between January 2000 and December 2009. Almost patients were treated in the Department of Psychiatry, Fujita Health University Hospital, and University of Occupational and Environmental Health, Japan. The healthy controls were psychiatrically screened based on unstructured interviews. None had severe medical complications such as liver cirrhosis, renal failure, heart failure or other Axis-I disorders according to DSM-IV. No structured methods were used to assess psychiatric symptoms in the controls, which included hospital staff and medical students. The healthy controls were also unrelated to each other and ethnically Japanese, enrolled between January 2000 and December 2009. The study was described to subjects and written informed consent was obtained from each. This study was approved by the Ethics Committees at Fujita Health University, Nagoya University Graduate School of Medicine and the University of Occupational and Environmental Health.

2.2. Data collection

Fluvoxamine and sertraline affect on sigma1 receptors (Ishikawa et al., 2007; Narita et al., 1996). Therefore, we performed a pharmacogenic study using 208 MDD patients treated with fluvoxamine or sertraline. The 208 MDD patients in this study had scores of 12 or higher on the 17 items of the SIGH-D (Peveler and Kendrick, 2005). We defined a clinical response as a decrease of more than 50% in baseline SIGH-D within 8 weeks, and clinical remission as a SIGH-D score of less than 7 at 8 weeks. Fluvoxamine and sertraline were increased gradually to a maximum of 150 mg and 100 mg, respectively, depending on the patient's condition. Patients with insomnia and severe anxiety were prescribed benzodiazepine drugs, but no other psychotropic drugs were permitted during the study. Detailed information on data collection was described in a previous paper (Saito et al., 2006). The clinical characteristics of patients in this study, classified according to these definitions, can be seen in Table 1.

2.3. SNP selection and LD evaluation

We first consulted the HapMap database (release#24/phase II, Nov 2008, www.hapmap.org, population: Japanese Tokyo, minor allele frequencies (MAFs) of more than 0.05) and included one SNP (rs10814130) covering *SIGMAR1*. However, we thought that information of only one SNP on *SIGMAR1* from the HapMap database would be insufficient to reflect the detailed linkage disequilibrium (LD) background in the Japanese population. Rs1799729 and rs1800866 were reported to possibly be biologically functional SNPs (Prasad et al., 1998; Seth et al., 1997). Uchida and colleagues reported that LD from rs1800866 and rs1799729 was very tight ($r^2 = 1.00$) (Fig. 1) (Uchida et al., 2003). Therefore, we selected rs1800866 for LD evaluation. In order to reflect the LD background in the Japanese population, we genotyped rs1800866, which was reported to have a substitution from glutamine (CAG) to proline (CCG) that may perturb appropriate regulation of transportation from the endoplasmic reticulum to the plasma membrane of sigma1 receptors (Seth et al., 1997), and rs10814130 using our 40 of our own control samples, and made a more detailed LD evaluation of *SIGMAR1*. We determined the LD between rs1800866 and rs10814130 using HAPLOVIEW software (Barrett et al., 2005). Because LD from rs1800866 to rs10814130 was very tight in our control samples (r^2 more than 0.923) (Fig. 1), we selected rs1800866 for the following association analysis.

2.4. SNP genotyping

We used TaqMan assays (ABI: Applied Biosystems, Inc., Foster City, CA,) for rs1800866. One allelic probe was labeled with FAM dye and the other with fluorescent VIC dye. The plates were heated for 2 min at 50 °C and 95 °C for 10 min,

Table 1
Clinical characteristics of the patients in both definition groups.

	N			Patients permitted with SSRIs, *n*[c]		Age, (mean ± SD)	Baseline SIGH-D, (avg ± SD)	SSRIs dose at eight weeks[d]	Number of previous episode, (avg ± SD)	Patients permitted with anxiolytics/ hypnoticse, *n* (%)
	Total	Male	Female	FLV	STL					
Overall	208	100	108	136	72	47.8 ± 16.8	20.1 ± 5.48	119 ± 39.7	1.89 ± 0.818	69 (32.4)
Clinical response group[a]										
Responders	117	52	65	70	47	47.5 ± 15.9	21.1 ± 5.46	118 ± 37.9	1.83 ± 0.781	45 (21.1)
Nonresponders	91	48	43	66	25	48.4 ± 18.0	18.8 ± 5.11	122 ± 42.2	2.02 ± 0.871	24 (11.3)
P-value	0.249			0.0862		0.694	**0.00140**	0.462	0.179	0.103
Clinical remission group[b]										
Remitters	86	40	46	54	32	46.9 ± 15.8	19.7 ± 4.60	113 ± 39.6	1.94 ± 0.716	30 (14.1)
Nonremitters	122	60	62	82	40	48.6 ± 17.5	20.5 ± 5.98	124 ± 39.2	2.04 ± 0.878	39 (18.3)
P-value	0.814			0.397		0.499	0.291	**0.0462**	0.112	0.878

[a] Clinical response was defined as a 50% or greater decrease in the baseline SIGH-D score.
[b] Clinical remission was defined as a final SIGH-D score of less than 7.
[c] FLV: Fluvoxamine and STL: Sertraline.
[d] Imipramine-equivalent.

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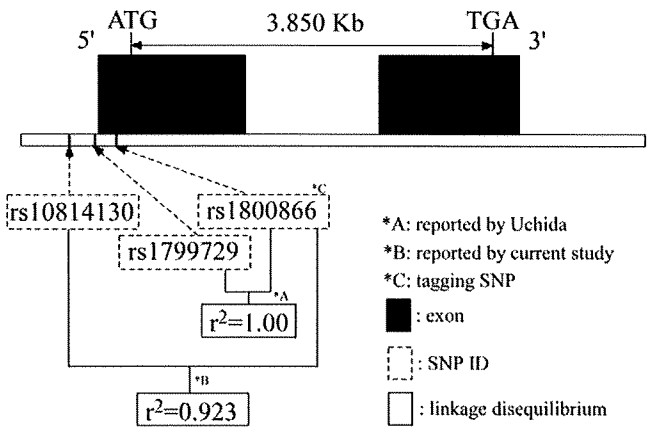

Fig. 1. LD evaluation in *SIGMAR1*. ATG is the start codon and TAG is the stop codon. Vertical bars represent exons. SNPs in *SIGMAR1*, which were already known, are represented by black boxes. The r^2 value is represented by broken line boxes. *A: reported by Uchida. *B: reported by current study. *C: tagging SNP.

followed by 45 cycles of 95 °C for 15 s and 58 °C for 1 min. Please refer to ABI for the primer sequence. We also used direct-sequence for rs10814130. Detailed information is available on request.

2.5. Mutation screening

We detected a significant association between *SIGMAR1* and MDD. In addition, therapeutic response and remission with fluvoxamine or sertraline was also marginally associated with rs1800866. Therefore, we performed mutation screening with *SIGMAR1* (promoter region, all exons including branch site) in 32 MDD treated by SSRIs patients (16 males and 16 females) using the primer extension method. We randomly selected 16 MDD patients of each sex after dividing by sex. The PCR method, including primer sequence and PCR condition, was adopted with reference to the study of Uchida and colleagues (Uchida et al., 2003). More detailed information about the primer extension method were published elsewhere (Suzuki et al., 2003).

2.6. Statistical analysis

Genotype deviation from the Hardy-Weinberg equilibrium (HWE) was evaluated by chi-square test (SAS/Genetics, release 8.2, SAS Japan Inc, Tokyo, Japan). The distribution of patient characteristics between MDD group and healthy control group was analyzed using a t test or a chi-square test. We found a significant difference in age among these groups ($P_{age} < 0.0001$), however, there was no difference in gender distribution ($P_{gender} = 0.339$). Although we investigated the difference in rs1800866' MAF by gender in the MDD cases, we did not detect a significant difference between MAF in males (0.257) and females (0.262). However, the number of male MDD patients was larger than the number of male healthy controls. We therefore performed a logistic regression analysis to compare the phenotype among rs1800866 genotypes to adjust possible confounding. The phenotype (MDD or controls) was the dependent variable, and gender, age at the time of recruitment and rs1800866 genotype were set as the independent variables. In the pharmacogenic study, individual *t* test and chi-square tests were used to compare means and categorical proportions (responders or nonresponders and remitters or nonremitters), respectively. Among the clinical characteristics of the patients in this study, a significant difference with total SIGH-D score was detected at the baseline in relation to SSRI therapeutic response (*P*-value = 0.00140) (Table 1). We also found another significant difference in SSRI dose at eight weeks between remitters and nonremitters (*P*-value = 0.0462) (Table 1). Therefore, we performed an analysis of the possible correlations between response or remission, SSRI treatment, and several clinical factors, using logistic regression. In these analyses, response classification was set as the dependent variable, and gender, age at the time of recruitment, SSRIs dose at eight weeks (imipramine-equivalent), SIGH-D total score at the baseline, and rs1800866 genotype were set as the independent variables. Polymorphisms were categorized into three genotypes, homozygous wild-type, heterozygous, and homozygous variant. Homozygosity for the more common allele was treated as the reference category. Tests for linear trend of odds ratio (additive model) were calculated using an ordered categorical variable by assigning scores to the genotypes: 0 (no variant allele), 1 (carrying one variant allele), and 2 (carrying two variant alleles). The statistical package JMP for Windows was used for logistic regression analysis (JMP 5.0. 1J, SAS Japan Inc, Tokyo, Japan).

Power calculation was performed using a Genetic Power Calculator (Purcell et al., 2003). We set each item in each value in the Genetic Power Calculator as follows: Prevalence: 0.2 in MDD and MDD treated with SSRI, respectively, User-defined: 0.05. The significance level for statistical tests was 0.05.

Table 2
Rs1800866 in *SIGMAR1* and major depressive disorder in the logistic regression analysis.

rs1800866	MDD, *n* (%)	Controls, *n* (%)	*P*-value[a]	OR[b]	95% CI[c]
AA	254 (54.5)	236 (45.7)			
AC	182 (39.1)	233 (45.2)	0.743	1.09	0.661–1.77
CC	30 (0.0644)	47 (9.11)	**0.0202**	0.563	0.346–0.913

Reference genotype is AA. Adjustment for age and gender.
[a] Bold numbers represent significant *P*-value.
[b] Odds ratio.
[c] 95% confidence interval.

3. Results

LD between rs1800866 and rs10814130 was very tight in our control samples (r^2 more than 0.9) (Fig. 1). Therefore, we selected rs1800866 for the association analysis.

Genotype frequencies of rs1800866 were in HWE. We performed a logistic regression analysis to compare the phenotype among rs1800866 genotypes to adjust possible confounding. In the logistic regression analysis, we detected an association of the phenotypes (MDD or controls) with rs1800866 genotype (Table 2).

Among the clinical characteristics of the patients in this study, a significant difference with total SIGH-D score was detected at the baseline in relation to SSRI therapeutic response (*P*-value = 0.00140) (Table 1). We also found another significant difference in SSRI dose at eight weeks between remitters and nonremitters (*P*-value = 0.0462) (Table 1). In addition to SSRI treatment in this cohort, one patient each was prescribed alprazolam, loflazepate, and bromazepam. Three patients each were prescribed estazoram, etizolam and quazepam. Sixteen patients each were prescribed triazolam and flunitrazepam. Twenty-five patients each were prescribed zolpidem. In the logistic regression analysis, we detected the significant correlation between SIGH-D total score at the baseline and SSRIs the therapeutic response (*P*-value: 0.00346). Also, SSRIs dose at eight weeks also did not correlate with remission (*P*-value: 0.486). However, response or remission with SSRI treatment, were not associated with rs1800866 (Table 3).

Although we performed mutation screening in *SIGMAR1* using MDD treated by SSRIs samples, we detected three SNPs (rs10814130, rs1800866 and rs1799729), which are already known (Fig. 1). LD among rs10814130, rs1800866 and rs1799729 was very tight (r^2 more than 0.9) (Fig. 1). LD from rs1799729 and rs1800866 in our controls was complete linkage disequilibrium, the same as in Uchida's study (Uchida et al., 2003). We did not find a novel SNP in *SIGMAR1*. Also, other previously reported SNPs were not detected in the Japanese MDD patients.

Table 3
Logistic regression analysis of rs1800866 in *SIGMAR1* in both definition groups.

rs1800866	Responder, *n* (%)	Nonresponder, *n* (%)	*P*-value[a]	OR[b]	95% CI[c]
AA	54 (46.2)	54 (59.3)			
AC	56 (47.9)	34 (37.4)	0.899	0.928	0.273–2.85
CC	7 (5.98)	3 (3.30)	0.273	2.88	0.476–23.9
rs1800866	Remitter, *n* (%)	Nonremitter, *n* (%)	*P*-value[a]	OR[b]	95% CI[c]
AA	39 (45.3)	69 (56.6)			
AC	40 (46.5)	50 (41.0)	0.484	0.667	0.199–2.00
CC	7 (8.14)	3 (2.46)	0.0769	5.49	0.915–45.4

Reference genotype is AA. Adjustment for age, gender, SIGH-D score at baseline, SSRI dose at 8 weeks and number of episode.
[a] Bold numbers represent significant *P*-value.
[b] Odds ratio.
[c] 95% confidence interval.

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In the power analysis, we obtained more than 80% power for the detection of association when we set the genotype relative risk at 1.35 and 2.28 in MDD and SSRI response in MDD, respectively, for rs1800866 in *SIGMAR1* under a multiplicative model of inheritance.

4. Discussion

We first performed a gene-based association analysis between *SIGMAR1* and MDD in the Japanese population. We performed an analysis of the possible correlations between phenotype and rs1800866 genotype and several clinical factors using logistic regression. In the logistic regression analysis, we detected an association of the phenotypes (MDD or controls) with rs1800866 genotype. Our results suggest that *SIGMAR1* plays a role in the pathophysiology of MDD in the Japanese population. On the other hand, the significant difference in baseline SIGH-D scores between responders and nonresponders might have affected the results; the baseline SIGH-D scores could predict clinical response in the logistic regression analysis. We did not find an association between rs1800866 genotype and SSRI therapeutic response in the logistic regression analysis. Remission with fluvoxamine or sertraline was marginally associated with rs1800866 in the case-control study. We also found another significant difference in SSRI dose at eight weeks between remitters and nonremitters. Therefore, we also performed an analysis of the possible correlations between remission with SSRI treatment and several clinical factors using logistic regression. Rs1800866 genotype was not predictive of the therapeutic remission. Therefore, we suggest that rs1800866 in the *SIGMAR1* genotype was not a predictor of SSRI treatment remission in Japanese MDD.

There was a significantly lower percentage of the C allele among MDD patients (MAF: 26.0%) than among controls (MAF: 31.7%). On the other hand, there was the trend toward higher percentage of the C allele among patients (MAF in responders and remitters: 29.9% and 31.5%, respectively) than among nonresponders and nonremitters (MAF in nonresponders and nonremitters: 22.0% and 23.0%, respectively). Rs1800866 (also known as A61C) was reported to be a possible functional SNP in exon 1, leading to an amino acid change from glutamine (CAG) to proline (CCG) at position 2 of the receptor protein (Seth et al., 1997). It is considered that this SNP may be a biologically functional SNP (Seth et al., 1997). LD from rs1800866 and rs1799729 in our MDD samples was completed as in Uchida's study using a Japanese sample (Uchida et al., 2003).

Miyatake and colleagues reported functional polymorphisms (T-485A) in the promoter region of *SIGMAR1* (Miyatake et al., 2004). However, MAF of this SNP is less that 5% according to studies of Miyatake and colleagues (Miyatake et al., 2004) and Satoh and colleagues (Satoh et al., 2004). Therefore, we designed the study based on the common disease-common variants hypothesis (CD–CV hypothesis) (Chakravarti, 1999), and did not perform an analysis on this SNP. Also, although we performed mutation screening in *SIGMAR1*, we did not detect this SNP. We considered that this result might have been caused by a small sample size.

Recently, Ishikawa and colleagues reported that rs1799729 and T-485A in *SIGMAR1* were not associated with fluvoxamine binding potential (Ishikawa et al., 2007). These authors suggested that that rs1799729 and T-485A in *SIGMAR1* may not contribute to differences in sigma1 receptor in the human brain (Ishikawa et al., 2007). Moreover, they suggested that it will be necessary to conduct a replication study using larger samples, since T-485A, which is located in the promoter region and has a biologically functional effect, has very low MAF (less than 5% in the Japanese population) (Ishikawa et al., 2007).

Ishiguro and colleagues' haplotype analysis showed that SNPs in *SIGMAR1* (rs1799729 and rs1800866) were associated with schizophrenia (Ishiguro et al., 1998). These SNPs were also reported to be associated with Japanese alcoholism (Miyatake et al., 2004) and schizophrenia (Ishiguro et al., 1998). In addition, rs1800866 was reported to be associated with Alzheimer disease/Alzheimer type dementia (Uchida et al., 2005). On the other hand, rs1799729 and rs1800866 in *SIGMAR1* were not associated with Japanese methamphetamine use disorder (Inada et al., 2004). Ohmori and colleagues reported that rs1799729 was not associated with Japanese schizophrenia (Ohmori et al., 2000), and Satoh and colleagues reported that SNPs in *SIGMAR1* (examined SNP: rs1799729, rs1800866 and T-485A) were not associated with Japanese schizophrenia (Satoh et al., 2004). In addition, Uchida and colleagues reported no association between rs1800866 and Japanese schizophrenia in a case-control study and a meta-analysis (Uchida et al., 2003).

A few points of caution should be mentioned with respect to our results. Firstly, the association between *SIGMAR1* and MDD patients may be due to biased samples, such as small sample sizes or unmatched age. We therefore performed a logistic regression analysis to compare the phenotypes among rs1800866 genotypes, using several clinical factors as other independent variables to adjust possible confounding. In the power analysis, we obtained more than 80% power for the detection of association when we set the genotype relative risk at 1.35 and 2.28 in MDD and SSRI response in MDD, respectively, for rs1800866 in *SIGMAR1* under a multiplicative model of inheritance. Because our samples were small, statistical errors are possible in the results of these statistical association analyses. On average, the controls were much younger than the patients. This means that a number of young controls may go on to develop one of these disorders, most likely MDD, since the incidence of major depression is as high as 5% or more. Our subjects did not undergo structured interviews, and it is reported that MDD patients who are not diagnosed by structured interview may develop bipolar disorder in the future (Bowden, 2001; Kishi et al., 2008, 2009a–d). However, in this study patients were carefully diagnosed according to DSM-IV criteria with consensus of at least two experienced psychiatrists on the basis of a review of medical records. In addition, when we found a patient who had been misdiagnosed, we promptly excluded the misdiagnosed case to maintain the precision of our sample (Kishi et al., 2008, 2009a–d). Also, Yamaguchi-Kabata and colleagues reported that different proportions of individuals from different regions of Japan in case and control groups can lead to statistical error (Yamaguchi-Kabata et al., 2008). Therefore, the positive association with MDD could be due to type I error, possibly because of population stratification. Secondly, we performed a mutation scan of *SIGMAR1*. Thirdly, the analysis of epigenetic controls and other genetic variations such as CNV in *SIGMAR1* were not performed in our study. Fourthly, sixty-nine of the 208 fluvoxamine and sertraline treatment patients were prescribed anxiolytics/hypnotic. Since benzodiazepines are also effective antidepressant molecules, our results for these 69 MDD patients may have been influenced by the anxiolytics/hypnotic. Because we consider it to be difficult to evaluate the association of extremely rare variants from the viewpoint of statistical power, a replication study using a larger sample will be required for conclusive results.

Our results suggest that *SIGMAR1* plays a role in MDD in the Japanese population. Also, *SIGMAR1* does not play a role in the therapeutic response to SSRI in Japanese MDD patients. However, because our samples are small, it will be important to replicate and confirm these findings in other independent studies using larger samples.

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References

Akunne, H.C., Whetzel, S.Z., Wiley, J.N., Corbin, A.E., Ninteman, F.W., Tecle, H., Pei, Y., Pugsley, T.A., Heffner, T.G., 1997. The pharmacology of the novel and selective sigma ligand, PD 144418. Neuropharmacology 36, 51–62.

Ansorge, M.S., Hen, R., Gingrich, J.A., 2007. Neurodevelopmental origins of depressive disorders. Curr. Opin. Pharmacol. 7, 8–17.

Ault, D.T., Werling, L.L., 1999. Phencyclidine and dizocilpine modulate dopamine release from rat nucleus accumbens via sigma receptors. Eur. J. Pharmacol. 386, 145–153.

Barrett, J.C., Fry, B., Maller, J., Daly, M.J., 2005. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 21, 263–265.

Bermack, J.E., Debonnel, G., 2001. Modulation of serotonergic neurotransmission by short- and long-term treatments with sigma ligands. Br. J. Pharmacol. 134, 691–699.

Bermack, J.E., Debonnel, G., 2005. The role of sigma receptors in depression. J. Pharmacol. Sci. 97, 317–336.

Bouchard, P., Quirion, R., 1997. [^{3}H]1,3-di(2-tolyl)guanidine and [3H](+)pentazocine binding sites in the rat brain: autoradiographic visualization of the putative sigma1 and sigma2 receptor subtypes. Neuroscience 76, 467–477.

Bowden, C.L., 2001. Strategies to reduce misdiagnosis of bipolar depression. Psychiatr. Serv. 52, 51–55.

Chakravarti, A., 1999. Population genetics–making sense out of sequence. Nat. Genet. 21, 56–60.

Garriock, H.A., Kraft, J.B., Shyn, S.I., Peters, E.J., Yokoyama, J.S., Jenkins, G.D., Reinalda, M.S., Slager, S.L., McGrath, P.J., Hamilton, S.P., 2010. A genomewide association study of citalopram response in major depressive disorder. Biol. Psychiatry 67, 133–138.

Gonzalez-Alvear, G.M., Thompson-Montgomery, D., Deben, S.E., Werling, L.L., 1995. Functional and binding properties of sigma receptors in rat cerebellum. J. Neurochem. 65, 2509–2516.

Gronier, B., Debonnel, G., 1999. Involvement of sigma receptors in the modulation of the glutamatergic/NMDA neurotransmission in the dopaminergic systems. Eur. J. Pharmacol. 368, 183–196.

Hashimoto, K., Ishiwata, K., 2006. Sigma receptor ligands: possible application as therapeutic drugs and as radiopharmaceuticals. Curr. Pharm. Des. 12, 3857–3876.

Hayashi, T., Su, T.P., 2008. An update on the development of drugs for neuropsychiatric disorders: focusing on the sigma 1 receptor ligand. Expert. Opin. Ther. Targets 12, 45–58.

Inada, T., Iijima, Y., Uchida, N., Maeda, T., Iwashita, S., Ozaki, N., Harano, M., Komiyama, T., Yamada, M., Sekine, Y., Iyo, M., Sora, I., Ujikec, H., 2004. No association found between the type 1 sigma receptor gene polymorphisms and methamphetamine abuse in the Japanese population: a collaborative study by the Japanese genetics initiative for drug abuse. Ann. N.Y. Acad. Sci. 1025, 27–33.

Ishiguro, H., Ohtsuki, T., Toru, M., Itokawa, M., Aoki, J., Shibuya, H., Kurumaji, A., Okubo, Y., Iwawaki, A., Ota, K., Shimizu, H., Hamaguchi, H., Arinami, T., 1998. Association between polymorphisms in the type 1 sigma receptor gene and schizophrenia. Neurosci. Lett. 257, 45–48.

Ishikawa, M., Ishiwata, K., Ishii, K., Kimura, Y., Sakata, M., Naganawa, M., Oda, K., Miyatake, R., Fujisaki, M., Shimizu, E., Shirayama, Y., Iyo, M., Hashimoto, K., 2007. High occupancy of sigma-1 receptors in the human brain after single oral administration of fluvoxamine: a positron emission tomography study using [11C]SA4503. Biol. Psychiatry 62, 878–883.

Kamei, H., Kameyama, T., Nabeshima, T., 1998. Effects of sigma receptor ligands on conditioned fear stress. Methods Find. Exp. Clin. Pharmacol. 20, 613–618.

Kishi, T., Kitajima, T., Ikeda, M., Yamanouchi, Y., Kinoshita, Y., Kawashima, K., Okochi, T., Okumura, T., Tsunoka, T., Inada, T., Ozaki, N., Iwata, N., 2009a. Association study of clock gene (CLOCK) and schizophrenia and mood disorders in the Japanese population. Eur. Arch. Psychiatry Clin. Neurosci. 259, 293–297.

Kishi, T., Kitajima, T., Ikeda, M., Yamanouchi, Y., Kinoshita, Y., Kawashima, K., Okochi, T., Okumura, T., Tsunoka, T., Ozaki, N., Iwata, N., 2009b. CLOCK may predict the response to fluvoxamine treatment in Japanese major depressive disorder patients. Neuromolecular Med. 11, 53–57.

Kishi, T., Kitajima, T., Ikeda, M., Yamanouchi, Y., Kinoshita, Y., Kawashima, K., Okochi, T., Ozaki, N., Iwata, N., 2008. Association analysis of nuclear receptor Rev-erb alpha gene (NR1D1) with mood disorders in the Japanese population. Neurosci. Res. 62, 211–215.

Kishi, T., Kitajima, T., Tsunoka, T., Ikeda, M., Yamanouchi, Y., Kinoshita, Y., Kawashima, K., Okochi, T., Okumura, T., Inada, T., Ozaki, N., Iwata, N., 2009c. Genetic association analysis of serotonin 2A receptor gene (HTR2A) with bipolar disorder and major depressive disorder in the Japanese population. Neurosci. Res. 64, 231–234.

Kishi, T., Kitajima, T., Tsunoka, T., Okumura, T., Ikeda, M., Okochi, T., Kinoshita, Y., Kawashima, K., Yamanouchi, Y., Ozaki, N., Iwata, N., 2009d. Possible association of prokineticin 2 receptor gene (PROKR2) with mood disorders in the Japanese population. Neuromolecular Med. 11, 114–122.

Kronmuller, K.T., Schroder, J., Kohler, S., Gotz, B., Victor, D., Unger, J., Giesel, F., Magnotta, V., Mundt, C., Essig, M., Pantel, J., 2009. Hippocampal volume in first episode and recurrent depression. Psychiatry Res. 174, 62–66.

Kulkarni, S.K., Dhir, A., 2009. Sigma-1 receptors in major depression and anxiety. Expert Rev. Neurother. 9, 1021–1034.

Laje, G., Allen, A.S., Akula, N., Manji, H., John Rush, A., McMahon, F.J., 2009. Genome-wide association study of suicidal ideation emerging during citalopram treatment of depressed outpatients. Pharmacogenet. Genomics 19, 666–674.

Lambert, M.T., Trutia, C., Petty, F., 1998. Extrapyramidal adverse effects associated with sertraline. Prog. Neuropsychopharmacol. Biol. Psychiatry 22, 741–748.

Maurice, T., Phan, V.L., Urani, A., Kamei, H., Noda, Y., Nabeshima, T., 1999. Neuroactive neurosteroids as endogenous effectors for the sigma1 (sigma1) receptor: pharmacological evidence and therapeutic opportunities. Jpn. J. Pharmacol. 81, 125–155.

Miller, A.H., Maletic, V., Raison, C.L., 2009. Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. Biol. Psychiatry 65, 732–741.

Miyatake, R., Furukawa, A., Matsushita, S., Higuchi, S., Suwaki, H., 2004. Functional polymorphisms in the sigma1 receptor gene associated with alcoholism. Biol. Psychiatry 55, 85–90.

Muglia, P., Tozzi, F., Galwey, N.W., Francks, C., Upmanyu, R., Kong, X.Q., Antoniades, A., Domenici, E., Perry, J., Rothen, S., Vandeleur, C.L., Mooser, V., Waeber, G., Vollenweider, P., Preisig, M., Lucae, S., Muller-Myhsok, B., Holsboer, F., Middleton, L.T., Roses, A.D., 2008. Genome-wide association study of recurrent major depressive disorder in two European case–control cohorts. Mol. Psychiatry.

Narita, N., Hashimoto, K., Tomitaka, S., Minabe, Y., 1996. Interactions of selective serotonin reuptake inhibitors with subtypes of sigma receptors in rat brain. Eur. J. Pharmacol. 307, 117–119.

Nestler, E.J., Carlezon Jr., W.A., 2006. The mesolimbic dopamine reward circuit in depression. Biol. Psychiatry 59, 1151–1159.

Nishimura, T., Ishima, T., Iyo, M., Hashimoto, K., 2008. Potentiation of nerve growth factor-induced neurite outgrowth by fluvoxamine: role of sigma-1 receptors, IP3 receptors and cellular signaling pathways. PLoS One 3, e2558.

Ohmori, O., Shinkai, T., Suzuki, T., Okano, C., Kojima, H., Terao, T., Nakamura, J., 2000. Polymorphisms of the sigma(1) receptor gene in schizophrenia: an association study. Am. J. Med. Genet. 96, 118–122.

Peveler, R., Kendrick, T., 2005. Selective serotonin reuptake inhibitors: THREAD trial may show way forward. BMJ 330, 420–421.

Prasad, P.D., Li, H.W., Fei, Y.J., Ganapathy, M.E., Fujita, T., Plumley, L.H., Yang-Feng, T.L., Leibach, F.H., Ganapathy, V., 1998. Exon–intron structure, analysis of promoter region, and chromosomal localization of the human type 1 sigma receptor gene. J. Neurochem. 70, 443–451.

Purcell, S., Cherny, S.S., Sham, P.C., 2003. Genetic Power calculator: design of linkage and association genetic mapping studies of complex traits. Bioinformatics 19, 149–150.

Sabino, V., Cottone, P., Parylak, S.L., Steardo, L., Zorrilla, E.P., 2009. Sigma-1 receptor knockout mice display a depressive-like phenotype. Behav. Brain Res. 198, 472–476.

Saito, S., Takahashi, N., Ishihara, R., Ikeda, M., Suzuki, T., Kitajima, T., Yamanouchi, Y., Iwata, N., Yamada, M., Yoshida, K., Inada, T., Ozaki, N., 2006. Association study between vesicle-associated membrane protein 2 gene polymorphisms and fluvoxamine response in Japanese major depressive patients. Neuropsychobiology 54, 226–230.

Satoh, F., Miyatake, R., Furukawa, A., Suwaki, H., 2004. Lack of association between sigma receptor gene variants and schizophrenia. Psychiatry Clin. Neurosci. 58, 359–363.

Seth, P., Leibach, F.H., Ganapathy, V., 1997. Cloning and structural analysis of the cDNA and the gene encoding the murine type 1 sigma receptor. Biochem. Biophys. Res. Commun. 241, 535–540.

Sullivan, P.F., de Geus, E.J., Willemsen, G., James, M.R., Smit, J.H., Zandbelt, T., Arolt, V., Baune, B.T., Blackwood, D., Cichon, S., Coventry, W.L., Domschke, K., Farmer, A., Fava, M., Gordon, S.D., He, Q., Heath, A.C., Heutink, P., Holsboer, F., Hoogendijk, W.J., Hottenga, J.J., Hu, Y., Kohli, M., Lin, D., Lucae, S., Macintyre, D.J., Maier, W., McGhee, K.A., McGuffin, P., Montgomery, G.W., Muir, W.J., Nolen, W.A., Nothen, M.M., Perlis, R.H., Pirlo, K., Posthuma, D., Rietschel, M., Rizzu, P., Schosser, A., Smit, A.B., Smoller, J.W., Tzeng, J.Y., van Dyck, R., Verhage, M., Zitman, F.G., Martin, N.G., Wray, N.R., Boomsma, D.I., Penninx, B.W., 2009. Genome-wide association for major depressive disorder: a possible role for the presynaptic protein piccolo. Mol. Psychiatry 14, 359–375.

Suzuki, T., Iwata, N., Kitamura, Y., Kitajima, T., Yamanouchi, Y., Ikeda, M., Nishiyama, T., Kamatani, N., Ozaki, N., 2003. Association of a haplotype in the serotonin 5-HT4 receptor gene (HTR4) with Japanese schizophrenia. Am. J. Med. Genet. B Neuropsychiatr. Genet. 121B, 7–13.

Uchida, N., Ujike, H., Nakata, K., Takaki, M., Nomura, A., Katsu, T., Tanaka, Y., Imamura, T., Sakai, A., Kuroda, S., 2003. No association between the sigma receptor type 1 gene and schizophrenia: results of analysis and meta-analysis of case–control studies. BMC Psychiatry 3, 13.

Uchida, N., Ujike, H., Tanaka, Y., Sakai, A., Yamamoto, M., Fujisawa, Y., Kanzaki, A., Kuroda, S., 2005. A variant of the sigma receptor type-1 gene is a protective factor for Alzheimer disease. Am. J. Geriatr. Psychiatry 13, 1062–1066.

Wang, L., Duncan, G., 2006. Silencing of sigma-1 receptor induces cell death in human lens cells. Exp. Cell Res. 312, 1439–1446.

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Weatherspoon, J.K., Gonzalez-Alvear, G.M., Frank, A.R., Werling, L.L., 1996. Regulation of [^{3}H] dopamine release from mesolimbic and mesocortical areas of guinea pig brain by sigma receptors. Schizophr. Res. 21, 51–62.

Williams, J.B., 1988. A structured interview guide for the Hamilton Depression Rating Scale. Arch. Gen. Psychiatry 45, 742–747.

Yamaguchi-Kabata, Y., Nakazono, K., Takahashi, A., Saito, S., Hosono, N., Kubo, M., Nakamura, Y., Kamatani, N., 2008. Japanese population structure, based on SNP genotypes from 7003 individuals compared to other ethnic groups: effects on population-based association studies. Am. J. Hum. Genet. 83, 445–456.

Yamazaki, Y., Ishioka, M., Matsubayashi, H., Amano, T., Sasa, M., 2002. Inhibition by sigma receptor ligand, MS-377, of N-methyl-D-aspartate-induced currents in dopamine neurons of the rat ventral tegmental area. Psychopharmacology (Berl.) 161, 64–69.

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Genetic association study of KREMEN1 and DKK1 and schizophrenia in a Japanese population

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ABSTRACT

The aim of the current study was to examine the association of KREMEN1 and DKK1, two wnt pathway-related genes with schizophrenia in Japanese subjects. We genotyped 16 common genetic variants within the aforementioned genes and examined their associations with schizophrenia. Results demonstrated that a common variant in the promoter region of KREMEN1 might modulate the risk of schizophrenia in the Japanese. However, further replication will be needed for conclusive interpretation of the effect of this locus on the pathogenesis of schizophrenia.

1. Introduction

Irregularities consistent with abnormal brain development, including faulty neuronal migration, altered spatial neuronal arrangement, and absence of significant gliosis, have been reported in schizophrenia. Together with behavioral, neuromotor, and other functional abnormalities that occur in childhood and predict schizophrenia, such as low IQ, poor motor skills, and poor development of language and social skills, these morphological findings indicate a developmental origin for schizophrenia (Crow 1995, Benes et al., 1986).

Recent advances in the understanding of genes that regulate brain development offer insights into the mechanisms of developmental brain changes. One important issue is the identification of signaling pathways that coordinate changes in gene expression with dynamic changes in cell adhesion and migration, events that are important for the complex cellular architecture of the human brain. Although several growth factors affect both gene expression and cell migration, recent focus has been on the wnt signaling pathway (Clevers, 2006). wnts are powerful regulators of cell proliferation and differentiation, and their signaling pathway involves proteins that directly participate in both gene transcription and cell adhesion

Abbreviations: SNP, single nucleotide polymorphism;tSNP, tagging SNP; HWE, Hardy–Weinberg equilibrium;MDR, multifactor dimensionality reduction method;TA, testing accuracy;CVC, cross-validation consistency.

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(currently > 150 genes (Li et al., 2006). Because schizophrenia involves developmental brain changes and abnormal neuronal and synaptic organization, alterations in the transduction of wnt signaling pathway may represent such a mechanism (Clevers, 2006; Emamian et al., 2004). The genetic associations of several wnt pathway genes and schizophrenia have been reported (Proitsi et al., 2008). As coordinate changes in gene expression are important for normal brain development, the present study focused on negative modulators of the wnt signaling pathway, KREMEN1 and DKK1.

wnt/beta-catenin signaling is inhibited by the secreted protein Dickkopf1, a member of a multigene family, which induces head formation in amphibian embryos (Niehrs, 2006). Dickkopf1 inhibits wnt signaling by triggering LRP5/6 internalization through formation of a ternary complex with Kremen receptors (Mao et al., 2002). DKK1 encodes a protein that is a member of the dickkopf family. This gene maps on chromosome 10 region q11.2, a region with suggestive linkage evidence for schizophrenia (Faraone et al., 1998). *KREMEN1* encodes a high-affinity dickkopf homolog 1, a transmembrane receptor that functionally cooperates with dickkopf1 to block wnt/beta-catenin signaling. KREMEN1 maps to chromosome 22q12.1, relatively close to the region with weak, but suggestive linkage evidence for schizophrenia (Pulver et al., 1994).

We genotyped 16 common genetic variants within the aforementioned genes and examined their associations with schizophrenia. Additionally, we examined interactions between single nucleotide polymorphisms (SNPs) within KREMEN1 and DKK1.

2. Materials and methods

Subjects comprised 1624 patients with schizophrenia (mean age, 46.5 ± 14.5 years) and 1621 volunteers with no personal or family history of psychiatric illness (mean age, 45.1 ± 14.0 years). Subjects were divided into a screening sample ($n = 1681$) and a confirmation sample ($n = 1564$). A general characterization and psychiatric assessment of subjects is available elsewhere (Ikeda et al., 2008). The screening and confirmation sample were collected independently at each university hospital. All subjects provided written informed consent. This study was approved by the ethics committee at Nagoya University.

DNA was extracted from peripheral blood. Genotyping was performed using a fluorescence-based allelic discrimination assay (Taqman, Applied Biosystems, Foster City, CA, USA). Power was calculated according to the methods of Skol et al (Skol et al., 2006).

SNP tagging criteria were based on minor allele frequency (>5%) and correlation coefficient between loci (>0.8) as reported in the HAPMAP database (rel #24, Japanese population). Based on these criteria, 16 tagging SNPs (tSNP) were selected for genotyping. To exclude low-quality DNA sample or genotyping probes, data sets were filtered on the basis of tSNP genotype call rates (95% completeness) or deviation from the Hardy-Weinberg equilibrium (HWE) ($P = 0.05$) in the control sample. Subjects whose percentage of missing genotypes was >10% or who had evidence of possible DNA contamination were excluded from subsequent analyses.

All allele-wise association analyses (screening or confirmation sample) were carried out by calculating the P values for each tSNP. Significance was determined at the 0.05 level using Fisher's exact test. All P values were two-sided. Log likelihood ratio tests for assessing haplotype-wise associations between schizophrenia and combination of tagging SNPs was performed using UNPHASED software v3.04 (Dudbridge, 2008). The rare haplotype frequency threshold was set at 5%.

To reduce the total number of tests, clearly unassociated markers were removed in the first stage involving the screening sample of the present study. Next, conditional on the findings of the first stage, which used a less stringent nominal level, was tested in the second stage involving the confirmation sample using the augmented data and data from the first stage. In this joint sample analysis, P values were generated by Cochran–Mantel–Haenszel stratified analysis, and the Breslow–Day test was performed for evaluation of heterogeneous associations as implemented in PLINK v1.06 (Purcell et al., 2007).

To determine high- and low-risk genotype combinations, the multifactor dimensionality reduction method (MDR) was used (Moore et al., 2006). This procedure was developed to detect higher-order interactions between polymorphisms to predict a dichotomous trait variation, even when marginal effects are small. A 10-fold cross-validation test was conducted. Accordingly, a model was constructed based on 9/10 of the data (training data) and then evaluated using the remaining 1/10 of the data (testing data). The fitness of the MDR model was assessed by estimating the testing accuracy (TA). Models that were true positive would have an estimated TA of >0.5. The cross-validation consistency (CVC) was a measure of how many times among 10 divisions of the data the MDR found the same best model. We used the Tuned ReliefF algorithm (Moore and White, 2007) to removed noisy SNPs from the pool of possible candidates. Statistical significance was evaluated using a 1000-fold permutation test to compare observed testing accuracies with those expected under the null hypothesis. Permutation testing corrects for multiple testing by repeating the entire analysis on 1,000 data sets that are consistent with the null hypothesis. Models were significant at the 0.01 level.

3. Results

The results of the power analysis are presented in Table 1. We genotyped 16 tSNPs using the screening sample All tSNPs were in HWE, and the SNP-wise call rate was >95%. The association signal was detected in allele-wise analysis at rs713526 as well as in the haplotype-wise analysis (Table 2). To deal with multiple testing issues and to keep the statistical power uncompromised, we performed additional genotyping of rs713526 using the confirmation sample. Although the direction of the association was the same, no statistically significant results were obtained (Table 3). To maximize statistical power, we performed joint association analysis combining the data from the screening and confirmation sample and obtained a positive association signal.

We performed an exploratory SNP-SNP interaction analysis. For a specific number of SNPs we reported a single best model that maximized the TA. This is the model that is most likely to generalize to independent data sets. We also reported the CVC, which represents the number of times in a particular cross validated run that a given SNP combination was selected as the best model. The strongest evidence for SNP interaction was between rs1896368 (DKK1), rs2288335 (DKK1), and rs5752866

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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).

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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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References

Benes, F.M., Davidson, J., Bird, E.D., 1986. Quantitative cytoarchitectural studies of the cerebral cortex of schizophrenics. Arch. Gen. Psychiatry 43 (1), 31–35.
Clevers, H., 2006. Wnt/beta-catenin signaling in development and disease. Cell 127 (3), 469–480.
Crow, T.J., 1995. A Darwinian approach to the origins of psychosis. Br. J. Psychiatry 167 (1), 12–25.
Dudbridge, F., 2008. Likelihood-based association analysis for nuclear families and unrelated subjects with missing genotype data. Hum. Hered. 66 (2), 87–98.
Emamian, E.S., Hall, D., Birnbaum, M.J., Karayiorgou, M., Gogos, J.A., 2004. Convergent evidence for impaired AKT1-GSK3beta signaling in schizophrenia. Nat. Genet. 36 (2), 131–137.
Faraone, S.V., Matise, T., Svrakic, D., Pepple, J., Malaspina, D., Suarez, B., Hampe, C., Zambuto, C.T., Schmitt, K., Meyer, J., Markel, P., Lee, H., Harkavy Friedman, J., Kaufmann, C., Cloninger, C.R., Tsuang, M.T., 1998. Genome scan of European-American schizophrenia pedigrees: results of the NIMH Genetics Initiative and Millennium Consortium. Am. J. Med. Genet. 81 (4), 290–295.
Haga, H., Yamada, R., Ohnishi, Y., Nakamura, Y., Tanaka, T., 2002. Gene-based SNP discovery as part of the Japanese Millennium Genome Project: identification of 190,562 genetic variations in the human genome. Single-nucleotide polymorphism. J. Hum. Genet. 47 (11), 605–610.
Ikeda, M., Takahashi, N., Saito, S., Aleksic, B., Watanabe, Y., Nunokawa, A., Yamanouchi, Y., Kitajima, T., Kinoshita, Y., Kishi, T., Kawashima, K., Hashimoto, R., Ujike, H., Inada, T., Someya, T., Takeda, M., Ozaki, N., Iwata, N., 2008. Failure to replicate the association between NRG1 and schizophrenia using Japanese large sample. Schizophr. Res. 101 (1–3), 1–8.
Li, F., Chong, Z.Z., Maiese, K., 2006. Winding through the WNT pathway during cellular development and demise. Histol. Histopathol. 21 (1), 103–124.

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Mao, B., Wu, W., Davidson, G., Marhold, J., Li, M., Mechler, B.M., Delius, H., Hoppe, D., Stannek, P., Walter, C., Glinka, A., Niehrs, C., 2002. Kremen proteins are Dickkopf receptors that regulate Wnt/beta-catenin signalling. Nature 417 (6889), 664–667.

Moore, J., White, B., 2007. Tuning relieff for genome-wide genetic analysis. Lect. Notes Comput. Sci. 4447 (1), 166–175.

Moore, J.H., Gilbert, J.C., Tsai, C.T., Chiang, F.T., Holden, T., Barney, N., White, B. C., 2006. A flexible computational framework for detecting, characterizing, and interpreting statistical patterns of epistasis in genetic studies of human disease susceptibility. J. Theor. Biol. 241 (2), 252–261.

Niehrs, C., 2006. Function and biological roles of the Dickkopf family of Wnt modulators. Oncogene 25 (57), 7469–7481.

Proitsi, P., Li, T., Hamilton, G., Di Forti, M., Collier, D., Killick, R., Chen, R., Sham, P., Murray, R., Powell, J., Lovestone, S., 2008. Positional pathway screen of wnt signaling genes in schizophrenia: association with DKK4. Biol. Psychiatry 63 (1), 13–16.

Pulver, A.E., Karayiorgou, M., Wolyniec, P.S., Lasseter, V.K., Kasch, L., Nestadt, G., Antonarakis, S., Housman, D., Kazazian, H.H., Meyers, D., Ott, J., Lamacz, M., Liang, K.Y., Hanfelt, J., Ullrich, G., DeMarchi, N., Ramu, E., McHugh, P.R., Adler, L., Thomas, M., Carpenter, W.T., Manschreck, T., Gordon, C.T., Kimberland, M., Babb, R., Puck, J., Childs, B., 1994. Sequential strategy to identify a susceptibility gene for schizophrenia: report of potential linkage on chromosome 22q12-q13.1: Part 1. Am. J. Med. Genet. 54 (1), 36–43.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A., Bender, D., Maller, J., Sklar, P., de Bakker, P.I., Daly, M.J., Sham, P.C., 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am. J. Hum. Genet. 81 (3), 559–575.

Skol, A.D., Scott, L.J., Abecasis, G.R., Boehnke, M., 2006. Joint analysis is more efficient than replication-based analysis for two-stage genome-wide association studies. Nat. Genet. 38 (2), 209–213.

Yamaguchi-Kabata, Y., Nakazono, K., Takahashi, A., Saito, S., Hosono, N., Kubo, M., Nakamura, Y., Kamatani, N., 2008. Japanese population structure, based on SNP genotypes from 7003 individuals compared to other ethnic groups: effects on population-based association studies. Am. J. Hum. Genet. 83 (4), 445–456.

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ORIGINAL ARTICLE

Association study of *ubiquitin-specific peptidase 46* (*USP46*) with bipolar disorder and schizophrenia in a Japanese population

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Recently, *ubiquitin-specific peptidase 46* (*Usp46*) has been identified as a quantitative trait gene responsible for immobility in the tail suspension test and forced swimming test in mice. Mice with 3-bp deletion in *Usp46* exhibited loss of 'behavioral despair' under inescapable stresses in addition to abnormalities in circadian behavioral rhythms and the GABAergic system. Considering the face and construct validity as an animal model for bipolar disorder, we explored an association of *USP46* and bipolar disorder in a Japanese population. We also examined an association of *USP46* and schizophrenia. We found nominal evidence for an association of rs12646800 and schizophrenia. This association was not significant after correction for multiple testing. No significant association was detected for bipolar disorder. In conclusion, our data argue against the presence of any strong genetic susceptibility factors for bipolar disorder or schizophrenia in the region *USP46*.
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Keywords: association study; bipolar disorder; schizophrenia; ubiquitin–proteasome system; *USP46*

INTRODUCTION

Biological studies have shown that the ubiquitin–proteasome system, which is highly conserved from yeast to man as the principal means of targeting cytosolic proteins for degradation, has an important role in neuronal function, such as synaptic formation, transmission and plasticity.[1–3] Genetic studies also have implicated the ubiquitin–proteasome system in a range of neuropsychiatric diseases, such as Parkinson's disease,[4] autism spectrum disorders,[5,6] mental retardation,[7–9] bipolar disorder[10,11] and schizophrenia.[11,12]

More recently, quantitative trait locus studies in mice have revealed that *ubiquitin-specific peptidase 46* (*Usp46*) is responsible for negligible immobility in the tail suspension test and forced swimming test, the experimental paradigms for assessing antidepressant activity and depression-like behavior.[13] Usp46 is one of approximately a hundred deubiquitinating enzymes. Protein deubiquitination by deubiquitinating enzymes can either antagonize or facilitate substrate presentation to the proteasome.[2] Deubiquitinating enzymes have also been associated with neurogenetic disorders, including Parkinson's disease, spinocerebellar ataxia. In the aforementioned study,[13] mice with 3-bp deletion in the exon region of *Usp46* exhibit loss of 'behavioral despair' under short-term, inescapable stresses of being suspended by their tail (tail suspension test) or being forced to swim in a water-filled cylinder (forced swimming test). 'Behavioral despair' was a characteristic immobile posture adopted by animals under stresses. Abnormalities in circadian behavioral rhythms and the GABAergic system, both of which are observed in bipolar disorder,[14] were also reported in the mice.[13] Furthermore, the *USP46* locus (4q12) corresponds to the linkage regions for bipolar disorder.[15] Considering the face and construct validity as an animal model for bipolar disorder and the findings of the linkage study, we explored an association of *USP46* with bipolar disorder in a Japanese population.

In addition, recent results from a genome-wide association study[16] and a population-based epidemiological study[17] provided evidence that schizophrenia and bipolar disorder share some common genetic causes. Therefore, we also examined an association between *USP46* and schizophrenia. It should be noted that abnormalities in circadian

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rhythms and the GABAergic system[18] have been reported in schizophrenia as well.

MATERIALS AND METHODS

Subjects

For the bipolar disorder study, 867 cases (mean age, 50.7 ± 14.2 years) and 895 age- and gender-matched controls (49.9 ± 13.5 years) were used. This sample panel was the same as used in the Collaborative Study of Mood Disorder consortium study.[19] Seven laboratories (National Institute of Neuroscience, two laboratories of RIKEN Brain Science Institute, Kohnodai Hospital, Teikyo University, Okayama University and Fujita Health University) provided case and control samples. The proportion of cases with each disorder was 67.5, 31.9 and 0.6% for bipolar I disorder, bipolar II disorder and schizoaffective disorder, respectively. For the schizophrenia study, 715 cases (47.5 ± 14.0 years) and age- and gender-matched 711 controls (46.7 ± 13.1 years) were used. Controls used in the bipolar disorder or schizophrenia studies were independent. All subjects were of Japanese descent. Consensus diagnosis of bipolar disorder or schizophrenia was made according to the criteria of the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, by at least two experienced psychiatrists, on the basis of unstructured interviews, available medical records, and information from hospital staff and relatives. Controls were psychiatrically screened using an unstructured interview to exclude subjects with brain disorders or psychotic disorders. This study was approved by the Ethics Committees of all participating institutes. All participants provided written informed consent.

Tagging SNP selection, SNP genotyping and quality control

To test for genetic association, the gene-based approach was implemented. This method implies inclusion of both gene and gene-adjacent regions in the association study.[20] Therefore, the screened region was extended 10 kb upstream of the annotated transcription start site and downstream at the end of the last *USP46* exon. Consulting the HapMap database (release #24, population: Japanese in Tokyo), tagging single-nucleotide polymorphisms (SNPs) were selected to capture common SNPs (minor allele frequency >5%) in the predefined *USP46* locus. Given the linkage disequilibrium structure, seven tagging SNPs were selected, capturing all 30 common SNPs in the *USP46* locus at correlation coefficient (r^2)=1.

Genomic DNA was extracted from leukocytes by using the standard method. SNP genotyping was performed using the TaqMan system (Applied Biosystems, Foster City, CA, USA). PCR was performed using ABI 7900HT Fast Real-time PCR system and fluorescent signals were analyzed using SDS v2.2.1 software (Applied Biosystems).

For quality control, first, deviation from the Hardy–Weinberg equilibrium was checked in controls. Second, we excluded samples with call rates <100% from analyses.

Statistical analyses

The χ^2-test was used to compare the allele or genotype frequencies between cases and controls. Deviation from the Hardy–Weinberg equilibrium was also tested by the χ^2-test. Haplotype frequencies were estimated in a two- and three-marker sliding window manner by expectation maximization algorithm. Log likelihood ratio tests were performed for global *P*-values with COCAPHASE program in the UNPHASED v2.403 program (http://www.mrc-bsu.cam.ac.uk/personal/frank/software/unphased/).[21] All *P*-values reported were two-tailed. Statistical significance was defined as $P<0.05$. Power calculation was conducted with CaTS software (http://www.sph.umich.edu/csg/abecasis/CaTS/download.html).

RESULTS

All tagging SNPs were in Hardy–Weinberg equilibrium in controls. After excluding samples with call rates <100%, 845 cases and 869 controls in the bipolar disorder study, and 699 cases and 701 controls in the schizophrenia study remained for subsequent analyses. Assuming a multiplicative genetic model and a disease prevalence of 1%, power calculations showed that our sample had sufficient power (>80%) to detect gene-wide significant associations with genotype-relative risk values of 1.21–1.58 (minor allele frequency, 0.036–0.496) and 1.23–1.58 (minor allele frequency, 0.044–0.486) in bipolar disorder and schizophrenia, respectively. The linkage disequilibrium structures around *USP46* locus in 1570 control samples (869 controls in the bipolar disorder study+701 controls in the schizophrenia study) are shown in Table 1 and are highly similar to those of the JPT HapMap samples, ensuring that our genotyping were conducted correctly. The results of analyses are shown in Tables 2 and 3. We found nominal evidence for an association of rs12646800 with schizophrenia (allelic P=0.04, genotypic P=0.01). However, this association was not significant after Bonferroni correction. In bipolar disorder, no significant association was detected in allele-/genotype-/haplotype-wise analyses.

DISCUSSION

Although we could not detect evidence of a strong association of the *USP46* locus with bipolar disorder or schizophrenia in a Japanese population, these results could be interpreted in several ways. First, the results could indicate that there is no relevance of the *USP46* locus to these psychiatric disorders. Second, although this study was based on the common disease–common variant model, the genetic architecture of psychiatric disorders might be closer to the multiple rare variant model, making detection of causal variants difficult. Concerning this, on the basis of the epidemiological data and evolution theory, Uher[22] recently argues that severe mental illnesses, including schizophrenia that confer strong reproductive disadvantage, are likely to have a large and pleiotropic contribution from rare variants of recent origin. Third, it is possible that we overestimated the effect size of disease-related variants; that is, this study might be underpowered to detect variants

Table 1 Linkage disequilibrium analysis of *USP46*

SNP	*rs346005*	*rs10034164*	*rs2244291*	*rs12646800*	*rs6554557*	*rs17675844*	*rs10517263*
rs346005		1.00	1.00	1.00	1.00	0.99	1.00
rs10034164	0.14		1.00	1.00	0.99	1.00	1.00
rs2244291	0.43	0.06		1.00	0.98	0.99	1.00
rs12646800	0.04	0.01	0.02		1.00	1.00	1.00
rs6554557	0.14	0.97	0.06	0.01		1.00	1.00
rs17675844	0.10	0.02	0.23	0.00	0.02		1.00
rs10517263	0.09	0.62	0.04	0.00	0.61	0.01	

Abbreviations: SNP, single-nucleotide polymorphism; *USP46*, *ubiquitin-specific peptidase 46.*
Values shown above the diagonal are D′ and values shown below are r^2. Data of 1570 controls (control in bipolar disorder analysis, *N*=869; controls in schizophrenia analysis, *N*=701) were used for the calculation.

Table 2 Allele-/genotype-/haplotype-wise analyses in bipolar disorder

		Genotype counts						Single SNP		Haplotype-wise[a]	
		Case (N=845)			Control (N=869)						
dbSNP	*M/m*	*M/M*	*M/m*	*m/m*	*M/M*	*M/m*	*m/m*	*Allele -wise*	*Genotype -wise*	*2-window*	*3-window*
rs346005	A/C	213	427	205	215	432	222	0.61	0.83		
										0.26	
rs10034164	A/G	632	197	16	674	179	16	0.22	0.39		0.30
										0.34	
rs2244291	A/G	419	363	63	413	378	78	0.25	0.45		0.34
										0.35	
rs12646800	G/A	769	72	4	807	61	1	0.10	0.19		0.42
										0.25	
rs6554557	T/G	630	199	16	669	183	17	0.30	0.46		0.30
										0.37	
rs17675844	T/G	713	128	4	716	147	6	0.25	0.50		1.00
										1.00	
rs10517263	G/C	709	130	6	739	122	8	0.62	0.66		

Abbreviations: M, major allele; m, minor allele; SNP, single-nucleotide polymorphism.
[a]Sliding window analysis, rare haplotype threshold 10%.

Table 3 Allele-/genotype-/haplotype-wise analyses in schizophrenia

		Genotype counts						Single SNP		Haplotype-wise[a]	
		Case (N=699)			Control (N=701)						
dbSNP	*M/m*	*M/M*	*M/m*	*m/m*	*M/M*	*M/m*	*m/m*	*Allele -wise*	*Genotype -wise*	*2-window*	*3-window*
rs346005	A/C	169	342	188	170	342	189	1.00	1.00		
										0.95	
rs10034164	A/G	526	160	13	533	155	13	0.76	0.94		0.93
										0.92	
rs2244291	A/G	346	278	75	336	293	72	0.75	0.74		0.84
										0.55	
rs12646800	G/A	661	36	2	640	61	0	0.04	0.01		0.82
										0.79	
rs6554557	T/G	527	157	15	533	156	12	0.67	0.83		0.89
										0.75	
rs17675844	T/G	579	115	5	571	122	8	0.41	0.62		1.00
										1.00	
rs10517263	G/C	590	101	8	590	106	5	0.93	0.67		

Abbreviations: M, major allele; m, minor allele; SNP, single-nucleotide polymorphism.
[a]Sliding window analysis, rare haplotype threshold 10%.

with small effect. For example, the range of odds ratios was 1.15–1.24 in seven markers, which were recently reported to show genome-wide significant association with schizophrenia.[23] Although the association between rs12646800 and schizophrenia was not significant after Bonferroni correction in our study, this correction may be too stringent because of the presence of linkage disequilibrium. For this reason, we checked whether there was an association of *USP46* with schizophrenia in a recent genome-wide association study. In the genome-wide association study by Need *et al*.[24], *USP46* locus included one SNP nominally associated with schizophrenia in a Caucasian population (rs2244291; allelic P=0.027). Although rs2244291 is not associated with schizophrenia in our study (allelic P=0.75, genotypic P=0.74) and rs12646800 is not polymorphic in HapMap Caucasian samples, it should be noted that rs2244291 is only ~200 bp away from rs12646800. This might point to the relevance of this region within *USP46* to risk for schizophrenia.

In addition, we searched two databases for further evidence of association of *USP46* with bipolar disorder or schizophrenia. First, we referred to the Stanley Medical Research Institute Online Genomics Database (https://www.stanleygenomics.org/) to examine the differences in USP46 expression in post-mortem brains.[25] Although we did not find a significant difference in USP46 expression between patients with schizophrenia and controls in a combined analysis of the results from 16 studies, we detected evidence of a trend for association in USP46 expression change between patients with bipolar disorder and controls in a combined analysis of the results from 18 studies (P=0.089), with USP46 expression in bipolar disorder reduced. Second, we referred to the Database of Genomic Variants[26] in search

of copy number variations with functional implication at *USP46* locus. Although we could not find copy number variations in this locus, it cannot be ruled out that unknown copy number variations located in this locus have an important role in the etiology of psychiatric disorders.

In conclusion, our data argue against the presence of any strong genetic susceptibility factors for bipolar disorder or schizophrenia in the region *USP46*. However, considering the limitations of this genetic association study and supportive evidence from various datasets, expansion of samples or resequencing strategy would be required for a more conclusive result.

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1 Hegde, A. N. & DiAntonio, A. Ubiquitin and the synapse. *Nat. Rev. Neurosci.* **3,** 854–861 (2002).
2 Tai, H. C. & Schuman, E. M. Ubiquitin, the proteasome and protein degradation in neuronal function and dysfunction. *Nat. Rev. Neurosci.* **9,** 826–838 (2008).
3 Yi, J. J. & Ehlers, M. D. Ubiquitin and protein turnover in synapse function. *Neuron* **47,** 629–632 (2005).
4 Kitada, T., Asakawa, S., Hattori, N., Matsumine, H., Yamamura, Y., Minoshima, S. *et al.* Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature* **392,** 605–608 (1998).
5 Glessner, J. T., Wang, K., Cai, G., Korvatska, O., Kim, C. E., Wood, S. *et al.* Autism genome-wide copy number variation reveals ubiquitin and neuronal genes. *Nature* **459,** 569–573 (2009).
6 Bucan, M., Abrahams, B. S., Wang, K., Glessner, J. T., Herman, E. I., Sonnenblick, L. I. *et al.* Genome-wide analyses of exonic copy number variants in a family-based study point to novel autism susceptibility genes. *PLoS Genet.* **5,** e1000536 (2009).
7 Froyen, G., Corbett, M., Vandewalle, J., Jarvela, I., Lawrence, O., Meldrum, C. *et al.* Submicroscopic duplications of the hydroxysteroid dehydrogenase HSD17B10 and the E3 ubiquitin ligase HUWE1 are associated with mental retardation. *Am. J. Hum. Genet.* **82,** 432–443 (2008).
8 Tarpey, P. S., Raymond, F. L., O'Meara, S., Edkins, S., Teague, J., Butler, A. *et al.* Mutations in CUL4B, which encodes a ubiquitin E3 ligase subunit, cause an X-linked mental retardation syndrome associated with aggressive outbursts, seizures, relative macrocephaly, central obesity, hypogonadism, pes cavus, and tremor. *Am. J. Hum. Genet.* **80,** 345–352 (2007).
9 Field, M., Tarpey, P. S., Smith, R., Edkins, S., O'Meara, S., Stevens, C. *et al.* Mutations in the BRWD3 gene cause X-linked mental retardation associated with macrocephaly. *Am. J. Hum. Genet.* **81,** 367–374 (2007).
10 Ryan, M. M., Lockstone, H. E., Huffaker, S. J., Wayland, M. T., Webster, M. J. & Bahn, S. Gene expression analysis of bipolar disorder reveals downregulation of the ubiquitin cycle and alterations in synaptic genes. *Mol. Psychiatry* **11,** 965–978 (2006).
11 Bousman, C. A., Chana, G., Glatt, S. J., Chandler, S. D., Lucero, G. R., Tatro, E. *et al.* Preliminary evidence of ubiquitin proteasome system dysregulation in schizophrenia and bipolar disorder: Convergent pathway analysis findings from two independent samples. *Am. J. Med. Genet. B Neuropsychiatr Genet.* (2009). (e-pub ahead of print).
12 Chen, X., Wang, X., Sun, C., Chen, Q., O'Neill, F. A., Walsh, D. *et al.* FBXL21 association with schizophrenia in Irish family and case-control samples. *Am. J. Med. Genet. B Neuropsychiatr Genet.* **147B,** 1231–1237 (2008).
13 Tomida, S., Mamiya, T., Sakamaki, H., Miura, M., Aosaki, T., Masuda, M. *et al.* Usp46 is a quantitative trait gene regulating mouse immobile behavior in the tail suspension and forced swimming tests. *Nat. Genet.* **41,** 688–695 (2009).
14 Brambilla, P., Perez, J., Barale, F., Schettini, G. & Soares, J. C. GABAergic dysfunction in mood disorders. *Mol. Psychiatry* **8,** 721–737, 715 (2003).
15 Lambert, D., Middle, F., Hamshere, M. L., Segurado, R., Raybould, R., Corvin, A. *et al.* Stage 2 of the Wellcome Trust UK-Irish bipolar affective disorder sibling-pair genome screen: evidence for linkage on chromosomes 6q16-q21, 4q12-q21, 9p21, 10p14-p12 and 18q22. *Mol. Psychiatry* **10,** 831–841 (2005).
16 Purcell, S. M., Wray, N. R., Stone, J. L., Visscher, P. M., O'Donovan, M. C., Sullivan, P. F. *et al.* Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. *Nature* **460,** 748–752 (2009).
17 Lichtenstein, P., Yip, B. H., Bjork, C., Pawitan, Y., Cannon, T. D., Sullivan, P. F. *et al.* Common genetic determinants of schizophrenia and bipolar disorder in Swedish families: a population-based study. *Lancet* **373,** 234–239 (2009).
18 Lewis, D. A., Hashimoto, T. & Volk, D. W. Cortical inhibitory neurons and schizophrenia. *Nat. Rev. Neurosci.* **6,** 312–324 (2005).
19 Iwayama, Y., Hattori, E., Maekawa, M., Yamada, K., Toyota, T., Ohnishi, T. *et al.* Association analyses between brain-expressed fatty-acid binding protein (FABP) genes and schizophrenia and bipolar disorder. *Am. J. Med. Genet. B Neuropsychiatr Genet.* (2009). (e-pub ahead of print).
20 Neale, B. M. & Sham, P. C. The future of association studies: gene-based analysis and replication. *Am. J. Hum. Genet.* **75,** 353–362 (2004).
21 Dudbridge, F. Pedigree disequilibrium tests for multilocus haplotypes. *Genet. Epidemiol.* **25,** 115–121 (2003).
22 Uher, R. The role of genetic variation in the causation of mental illness: an evolution-informed framework. *Mol. Psychiatry* **14,** 1072–1082 (2009).
23 Stefansson, H., Ophoff, R. A., Steinberg, S., Andreassen, O. A., Cichon, S., Rujescu, D. *et al.* Common variants conferring risk of schizophrenia. *Nature* **460,** 744–747 (2009).
24 Need, A. C., Ge, D., Weale, M. E., Maia, J., Feng, S., Heinzen, E. L. *et al.* A genome-wide investigation of SNPs and CNVs in schizophrenia. *PLoS Genet.* **5,** e1000373 (2009).
25 Higgs, B. W., Elashoff, M., Richman, S. & Barci, B. An online database for brain disease research. *BMC Genomics* **7,** 70 (2006).
26 Iafrate, A. J., Feuk, L., Rivera, M. N., Listewnik, M. L., Donahoe, P. K., Qi, Y. *et al.* Detection of large-scale variation in the human genome. *Nat. Genet.* **36,** 949–951 (2004).

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Diagnostic classification of schizophrenia by neural network analysis of blood-based gene expression signatures

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ABSTRACT

Gene expression profiling with microarray technology suggests that peripheral blood cells might be a surrogate for postmortem brain tissue in studies of schizophrenia. The development of an accessible peripheral biomarker would substantially help in the diagnosis of this disease. We used a bioinformatics approach to examine whether the gene expression signature in whole blood contains enough information to make a specific diagnosis of schizophrenia. Unpaired *t*-tests of gene expression datasets from 52 antipsychotics-free schizophrenia patients and 49 normal controls identified 792 differentially expressed probes. Functional profiling with DAVID revealed that eleven of these genes were previously reported to be associated with schizophrenia, and 73 of them were expressed in the brain tissue. We analyzed the datasets with one of the supervised classifiers, artificial neural networks (ANNs). The samples were subdivided into training and testing sets. Quality filtering and stepwise forward selection identified 14 probes as predictors of the diagnosis. ANNs were then trained with the selected probes as the input and the training set for known diagnosis as the output. The constructed model achieved 91.2% diagnostic accuracy in the training set and 87.9% accuracy in the hold-out testing set. On the other hand, hierarchical clustering, a standard but unsupervised classifier, failed to separate patients and controls. These results suggest analysis of a blood-based gene expression signature with the supervised classifier, ANNs, might be a diagnostic tool for schizophrenia.

1. Introduction

Genome-wide gene expression analysis by DNA microarray and bioinformatics procedures has been conducted to elucidate common gene pathways that underpin the biological mechanisms of schizophrenia (Aston et al., 2004; Hakak et al., 2001; Hemby et al., 2002; Iwamoto et al., 2005, 2004; Mirnics et al., 2000, 2001; Sugai et al., 2004; Tkachev et al., 2003). However, the clinical use of microarray technology is not so widespread in schizophrenia research as compared with cancer research (Rhodes et al., 2004), due to the difficulty in interpreting results obtained from postmortem

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brain tissue that are complicated by agonal factors, anatomical inconsistency, and cellular heterogeneity of the cortical and subcortical regions. Postmortem brain studies use less accessible materials and therefore are limited by small sample size and repeated use of the same cohorts (Iwamoto and Kato, 2006). As a more accessible tissue, several researchers have undertaken expression profiling of peripheral blood cells (Sullivan et al., 2006; Tsuang et al., 2005; Vawter et al., 2004; Zvara et al., 2005). On a transcriptional expression level, peripheral blood cells were reported to share significant similarities with tissues from multiple brain regions (Sullivan et al., 2006). Interestingly, Tsuang et al. (2005) and Middleton et al. (2005) have shown that a set of genes extracted from gene expression signature of isolated peripheral blood cells can discriminate between schizophrenia and control groups.

These studies suggest that analysis of high dimensional data is useful to generate a biomarker of schizophrenia since it can combine data from several molecules, each of which shows small difference but is not exclusively associated with this disease (Schwarz and Bahn, 2008). In cancer research, classification by gene expression signature is widely used to predict tumor classes, drug responses, and prognosis of individual subjects (Khan et al., 2001; Lin et al., 2007; O'Neill and Song, 2003). Development of such classifier will greatly help our diagnosis of schizophrenia that is solely dependent on clinical symptoms so far. There are two approaches in classification: supervised and unsupervised methods. In contrast to unsupervised clustering, supervised classifiers learn a function from training data that consist of pairs of input objects (e.g., gene expression signatures) and desired outputs (e.g., diagnoses) (De Bruyne et al., 2007). The artificial neural network (ANN) is one of those classifiers that works very well, at identifying patterns or trends in a large amount of data with little theory.

Purpose of the present study is to examine whether microarray date obtained from whole blood cells contain enough information to classify schizophrenia. We present here that ANN model can correctly predict the diagnosis with sufficient accuracy.

2. Materials and methods

2.1. Subjects

Samples from 52 patients with schizophrenia and 49 normal controls were analyzed. Patients with schizophrenia or schizophreniform disorder were recruited from outpatients or inpatients of psychiatry unit at 6 centers across Japan. Those who were antipsychotics-free and had no comorbidity were included in the study. Control subjects were recruited from hospital staff and student volunteers who showed no evidence of present or past mental illness. All subjects were evaluated using the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I). At least two trained psychiatrists confirmed diagnosis of schizophrenia and specified its subtypes. In patients with schizophreniform disorder, the diagnosis of schizophrenia was reconfirmed 6 months after the onset of the first psychotic episode. The study protocol was approved by the ethics committee of each institution, and written informed consent was obtained from all subjects. If patients were floridly psychotic, informed consent was provided first by their parents or husband/wife, and then the consent was provided again by patients themselves after the psychotic symptoms were ameliorated.

We assessed 611 patients to recruit 52 antipsychotic-free patients without commorbidity. The patients and controls were of similar age (31.8 vs. 31.2 years; $p=0.776$) but significantly different in gender due to our sampling bias (21 males, 31 females vs. 35 males, 14 females; $p=0.0017$) (Table 1). In both patients and controls, there was no abnormal finding in standard laboratory workup including blood chemistries, complete blood count, urinalysis and electrocardiogram. Among 52 patients, 34 patients were drug-naïve, 8 patients were antipsychotics-naïve, and 10 patients were drug-free. All of the patients manifested active-phase symptoms. Current episode was the first psychotic episode for 23 of 34 drug-naïve patients. It was also the first episode for 8 neuroleptic-naïve patients, but they were taking antidepressants, benzodiazepines, or mood stabilizers for prodromal symptoms. Drug-free patients were those who stopped taking medication and relapsed due to non-adherence. They were drug-free for more than 8 weeks.

2.2. RNA isolation and microarray procedures

We extracted total RNA from whole blood because in vitro handling for cell isolation could alter gene expression (Ohmori et al., 2005). A total of 5 ml venous blood was collected in PAXgene Blood RNA Tubes (Qiagen, Valencia, CA) and frozen at □ 80 °C within 2 h after blood withdrawal. Total RNA was isolated from each of the frozen samples with PAXgene Blood RNA Kit (Qiagen) according to the manufacturer's instructions. Quantity and quality of total RNA were checked by A260/280 readings of spectrophotometry and an

Table 1
Demographic details of patients and controls.

	Control	Schizophrenia	Subtypes			
			Paranoid	Disorganized	Catatonic	Undifferentiated
N	49	52	21	9	1	21
Age	31.2±9.5	31.8±11.4	38.1±13.0	26.1±6.5	30	27.8±8.5
Sex (M/F)	35/14	21/31*	9/12	4/5	1/0	7/14
Age at onset	–	24.5±9.4	30.9±11.5	19.8±3.2	30	20.8±6.0
Medication history						
Drug-naïve	–	34	18	5	1	10
Drug-free	–	10	2	3	0	5
Neuroleptics-naïve	–	8	1	1	0	6

* $p<0.01$ compared to controls by chi-square test.

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Agilent BioAnalyzer (Agilent, Santa Clara, CA). Mean ratio of 28S/18S rRNA was 1.8 ± 0.3 and 1.9 ± 0.3 (mean ± SD) for patient and control groups, respectively.

Gene expression profiles were determined using CodeLink Human Whole Genome Bioarray (GE Healthcare Bio-Sciences, Chandler, AZ) according to the manufacturer's protocol. Briefly, cDNA was synthesized with 0.5 μg of total RNA and transcribed into biotinylated cRNA using iExpress Assay Reagent Kit (GE Healthcare Bio-Sciences, Chandler, AZ). Ten micrograms of the biotinylated cRNA was fragmented at 94 °C for 20 min and hybridized to CodeLink Human Whole Genome Bioarray, which contains probes for 54,847 transcripts. The hybridized cRNA probes to oligonucleotide arrays were stained with Cy5-streptavidin and scanned with an Agilent DNA Microarray Scanner (Agilent, Santa Clara, CA).

2.3. Data processing and normalization

The TIFF image from the microarray scanner was quantified using CodeLink Expression Analysis ver4.2 (GE Healthcare Bio-Sciences, Chandler, AZ). The mean intensity was taken for each spot and background corrected by subtracting the surrounding median local background intensity. Raw intensities were global median normalized for each bioarray. Probes were filtered to include only those present in more than two thirds of samples and those with average signal intensities between the 30th and

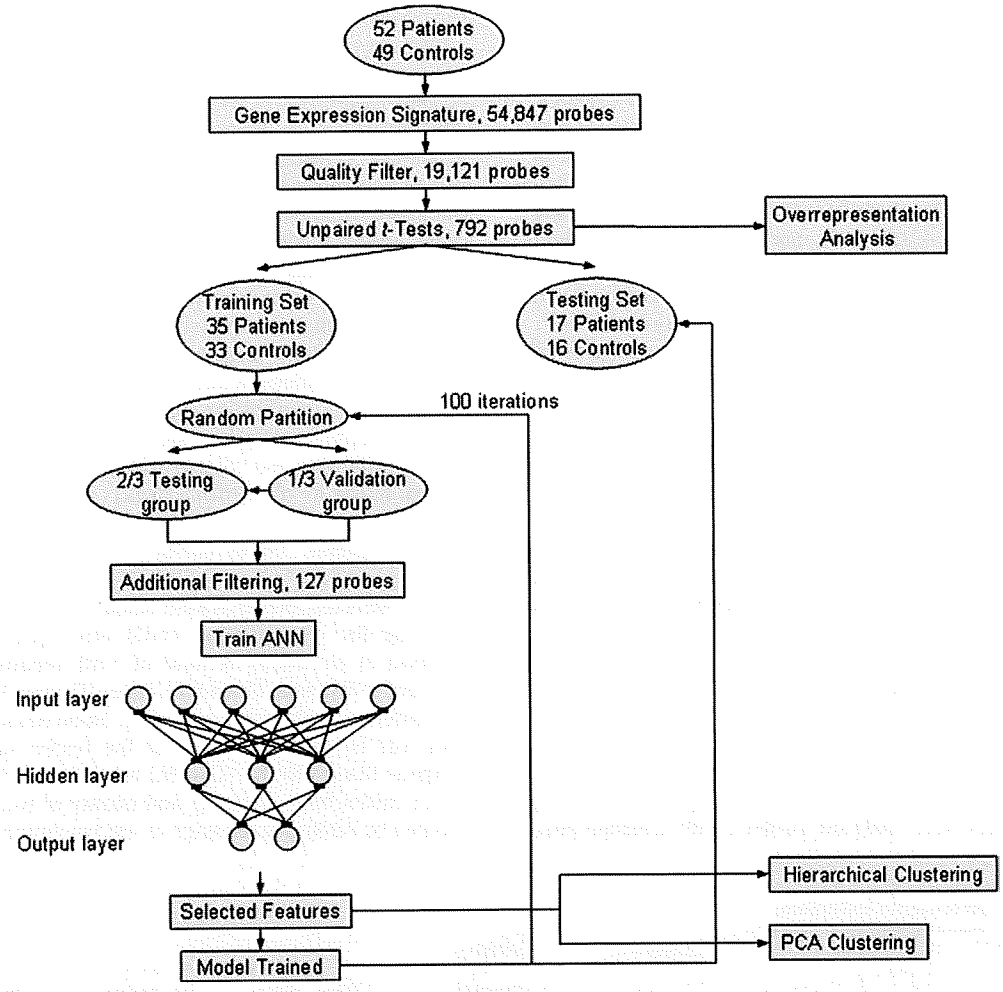

Fig. 1. Workflow for a 3-fold cross-validation artificial neural network (ANN) analysis. A gene expression signature was obtained from whole blood cDNA from 52 patients with schizophrenia and 49 normal controls using CodeLink Human Whole Genome Bioarray, containing about 55,000 probes. After a quality filter, 19,121 probes were used as a dataset for further analysis. To reduce dimensionality and skewness of the data, we selected 127 probes with moderate signal intensity and small coefficient of variation (CV) from 792 differentially expressed probes and subjected them to stepwise forward selection. Subjects were subdivided into training and testing sets to perform hold-out cross-validation. Training sets were randomly partitioned into three groups. One group was selected as a validation set, whereas the remaining two groups were used to train the network. The output produced by the first data passage through the ANN was compared with the ideal output, a known diagnosis, and an error is generated. The error was backpropagated through the ANN, and the weights of various connections between the neural units were adjusted. Then, a different validation set was selected from the same partitioning, and the remaining groups were used for training. The same step was repeated again to use each of the three groups as a validation set. Stepwise forward selection identified the best feature at each round of 3-fold cross-validation cycled with the repartitioning of the groups until an optimal feature size was determined. ANNs were then trained 100 times with selected predictors as the input, and the constructed model was used to classify testing set samples.

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