significantly associated with TD after Bonferroni correction. An attempt was made to replicate the association of 63 SNPs, which were allelic p-values <0.002 and located within 10 kb from known genes with the TaqMan genotyping assay (Table 1). A potential association was found for four SNPs (allelic $p<0.05$) (Table 1). However, no significant association was found after correction for multiple testing of 63 SNPs in the replication sample only. Among these four SNPs, an association between *GABRG3* SNP and TD has already been reported (Inada *et al*, 2008). The lowest allelic p-value for the association was found for rs2445142 ($p=2\times10^{-5}$) when the initial genome-wide sample and replication sample were combined. The SNP is located in the *HSPG2* gene.

Next, we tested associations of 24 tag SNPs including rs2445142 in the *HSPG2* gene with TD and found a nominal significant association for five SNPs in addition to rs2445142 (Table 2). Other than rs2445142, we found a significant association of rs2124368 located in intron 43 of the *HSPG2* gene with TD even after applying Bonferroni's correction for multiple testing (uncorrected allelic $p=0.0003$, corrected $p=0.007$). The SNP rs2124368 was not in LD with rs2445142, which was located in intron 1 ($D'=0.13$, $r^2=0.01$).

Subsequently, we genotyped the SNPs of rs2501255 (intron 1), rs2501257 (intron 1), rs897474 (intron 3), rs2254357 (exon 6), rs2254358 (exon 6), and rs2497632 (intron 9), because of the expected complete LD between these SNPs and rs2445142 based on the HapMap database. We confirmed that they were in complete LD with rs2445142 ($r^2=1.00$). These SNPs were located in introns 1–9 of the *HSPG2* gene. Age, sex, and age of onset were not associated with these SNPs. Acute extrapyramidal symptoms were associated with rs2445142 and the six SNPs in complete LD with rs2445142 (allelic $p=0.00002$). Resequencing all exons of the *HSPG2* gene in patients with TD did not reveal novel SNPs. Finally, we genotyped missense SNPs of rs3736360, rs2229493, s2291827, rs2228349, rs2229491, rs2229490, rs2229489, rs2229475, rs897471, rs2229481, and rs989994, which were listed in dbSNP and did not find significant associations of these SNPs with TD (data not shown). Thus, we tested a total of 103 SNPs, including 41 SNPs in the *HSPG2* gene, in our total subjects of 86 TD and 136 non-TD patients.

Association Between *Hspg2* Expression Levels in the Postmortem Prefrontal Cortex and Rs2445142

The transcription level in the postmortem prefrontal cortex, as measured by TaqMan real-time polymerase chain reaction, was not significantly different by diagnosis, age, sex, postmortem intervals, or pH of brain samples. A significant genotype effect on *HSPG2* gene expression levels was observed in 20 Australian subjects ($F(2,17)=4.9$, $p=0.02$) and replicated in 54 Japanese subjects ($F(2,51)=3.5$, $p=0.04$). The association was significant in the combined subjects ($F(2,71)=7.6$, $p=0.001$). Tukey's *post hoc* tests showed that *HSPG2* expression levels were significantly higher in the subjects with the GG genotype than in those with the CC genotype (Figure 1). Unfortunately, information about TD in the brains we analyzed was not available.

Table 2 Allelic p-Values of Tag SNPs in the *HSPG2* Gene for Association with TD

	Location	Allele frequency*			
		Allele	TD group	Non-TD group	Allelic p
rs3736360	exon 96 (N4331S)	A/G	0.19	0.20	0.8715
rs3767137	intron 77	A/G	0.23	0.19	0.2759
rs10917053	intron 71	A/G	1.00	0.99	0.3308
rs7355045	intron 64	G/A	0.84	0.81	0.4235
rs2290501	intron 60	C/A	0.22	0.21	0.7134
rs1563370	intron 52	A/G	0.35	0.27	0.0687
rs2229475	exon 47 (I1967V)	G/A	0.01	0.01	0.9477
rs2305562	intron 43	A/G	0.61	0.49	0.0117
rs4654991	intron 42	G/A	0.39	0.36	0.5605
rs2124368	intron 42	G/A	0.77	0.60	0.0003
rs897472	intron 36	C/A	0.09	0.05	0.1098
rs897471	exon 36 (V1503A)	A/G	0.88	0.87	0.7005
rs2229478	exon 8 (L248L)	A/G	0.53	0.42	0.0273
rs3767141	intron 6	G/A	0.66	0.60	0.1811
rs2445142	intron 1	G/C	0.58	0.38	0.00002
rs878949	intron 1	A/G	0.22	0.20	0.5867
rs1545593	intron 1	C/A	0.41	0.30	0.0122
rs1002480	intron 1	G/C	0.41	0.32	0.0368
rs6698486	intron 1	G/A	0.46	0.38	0.0754
rs10799719	intron 1	G/A	0.80	0.75	0.1789
rs9426785	intron 1	A/G	0.57	0.55	0.7389
rs4654773	intron 1	A/G	0.45	0.45	0.9165
rs11587857	intron 1	G/A	0.50	0.46	0.3465
rs4233280	5′ flanking	A/G	0.07	0.03	0.0588

Abbreviations: SNP, single nucleotide polymorphism; TD, tardive dyskinesia
*The frequency of the first allele.

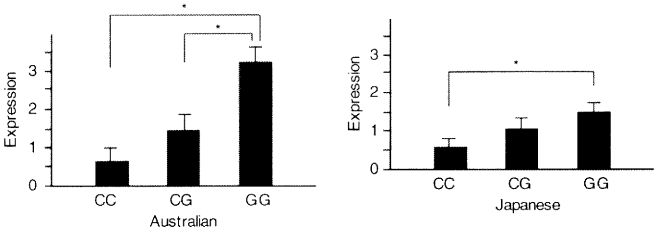

Figure 1 *HSPG2* expression levels in the postmortem prefrontal region by genotype. The vertical scores show the average (SEM) of relative expression levels in each of the three genotype groups, compared with the mean gene expression in the total samples. *Indicates $p<0.05$ by Tukey's *post hoc* tests.

Hspg2 Gene Expression in the Mouse Brains by HDL Treatment

Hspg2 expression levels were evaluated in the mouse brain after treatment with the antipsychotic drug, HDL. The expression of *Hspg2* levels did not alter after a 4-week treatment of HDL except for the striatum where *Hspg2* was expressed significantly higher than after the saline

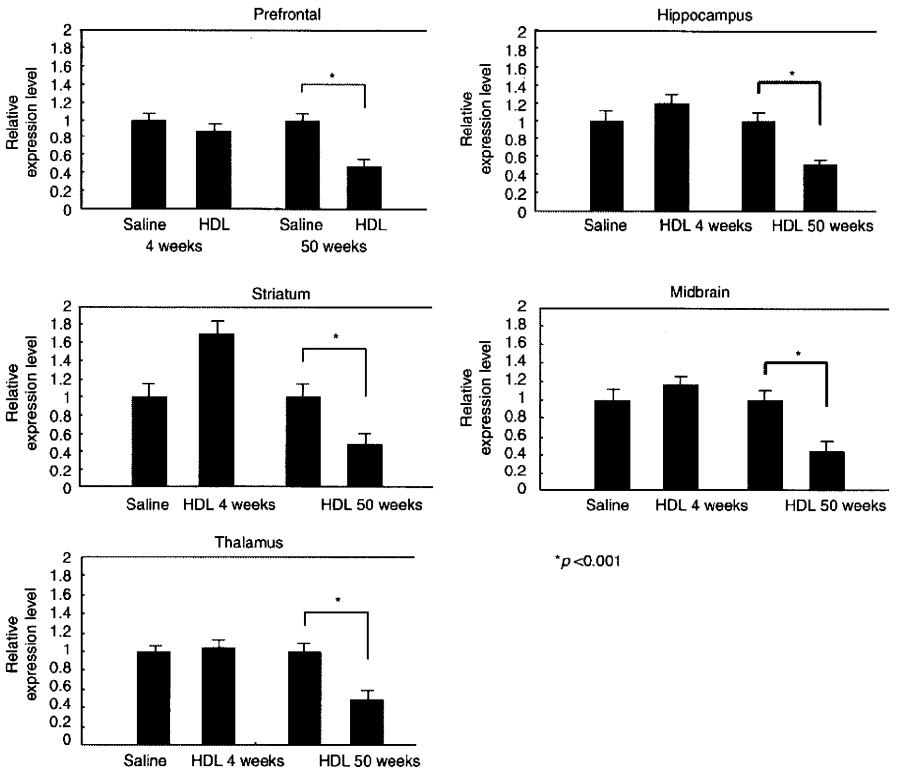

Figure 2 Effects of haloperidol (HDL) on *HSPG2* gene expression in the mouse brains. Relative expression levels of *Hspg2* from the prefrontal cortex, midbrain, hippocampus, thalamus, and striatum in the mouse brains after treatment with HDL for 4 weeks ($n = 10$) and HDL for 50 weeks ($n = 10$) were compared with the saline groups for 4 weeks ($n = 10$) and 10 weeks ($n = 10$) by Student's *t*-test.

treatment. Significantly lower expression of *Hspg2* was observed in all brain regions after a 50-week treatment with HDL than after a 50-week treatment with saline (Figure 2) ($F(1, 18) = 42.9$, $p < 0.0001$ at the prefrontal cortex; $F(1, 18) = 20.1$, $p = 0.0003$ at the hippocampus; $F(1, 18) = 15.9$, $p = 0.0009$ at the striatum; $F(1, 17 = 19.3$, $p = 0.0004$ at the midbrain; $F(1, 18) = 16.5$, $p = 0.0007$ at the thalamus).

Analysis of VCMs Induced by Haloperidol–Reserpine in *Hspg2* Knockout Mice

As we could not induce VCMs by administration of HDL only to mice, VCMs induced by long-term treatment with HDL and reserpine in female *Hspg2* hetero-knockout mice and female wild-type gene litters were measured to evaluate the relationship between expression levels of *Hspg2* and TD (Figure 3a). *Hspg2*-null knockout mice were embryonic lethal. The relative expression levels of *Hspg2* in *Hspg2* hetero-knockout mouse brains were almost half of that in the wild littermates (data not shown). Body weight, locomotor activities, and performance in the rotarod test before and after 48 days of administration of HDL and reserpine were not significantly different between *Hspg2* hetero-knockout and wild litters (data not shown). There was a significant effect of genotype ($F(1, 545) = 36.8$, $p < 0.0001$), post-treatment time ($F(4, 495) = 6.15$, $p < 0.0001$), and treatment ($F(3, 543) = 5.7$, $p = 0.0008$) for the number of VCMs for 5 min. *Post hoc* analysis showed that the number of VCMs were significantly lower in hetero-knockout mice than in wild-type mice after the last injection of HDL and reserpine after 48 or 49 consecutive days of administration of HDL and reserpine, and subsequent injection of physostigmine on the 50th day, or saline on the 53rd day (Figure 3b). The response of VCMs to physostigmine was subsequently evaluated (Figure 3c). There was a significant effect of genotype ($F(1, 128) = 36.9$, $p < 0.0001$), but not post-treatment time ($F(4, 125) = 1.03$, $p = 0.39$) for individual differences in the number of VCMs between pre-injection and post-treatment time. As for saline treatment, there was no significant effect of genotype ($F(1, 118) = 0.13$, $p = 0.72$) and post-treatment time ($F(4, 115) = 0.31$, $p = 0.87$). The numbers of VCMs were significantly reduced by injection of physostigmine compared with those before the injection at 24 h after HDL and reserpine injection in the wild-type mice but the differences in the numbers of VCMs before and after injection of physostigmine were not significant in hetero-knockout mice. The number of VCMs did not significantly alter after injection of saline in hetero-knockout mice and wild-type mice.

DISCUSSION

From a genome-wide association analysis, this study identified the role of *HSPG2* in neuroleptic-induced TD. The association was not significant in the initial screening and second confirmation after correction for multiple testing. However, screening with the tag SNPs for *HSPG2*,

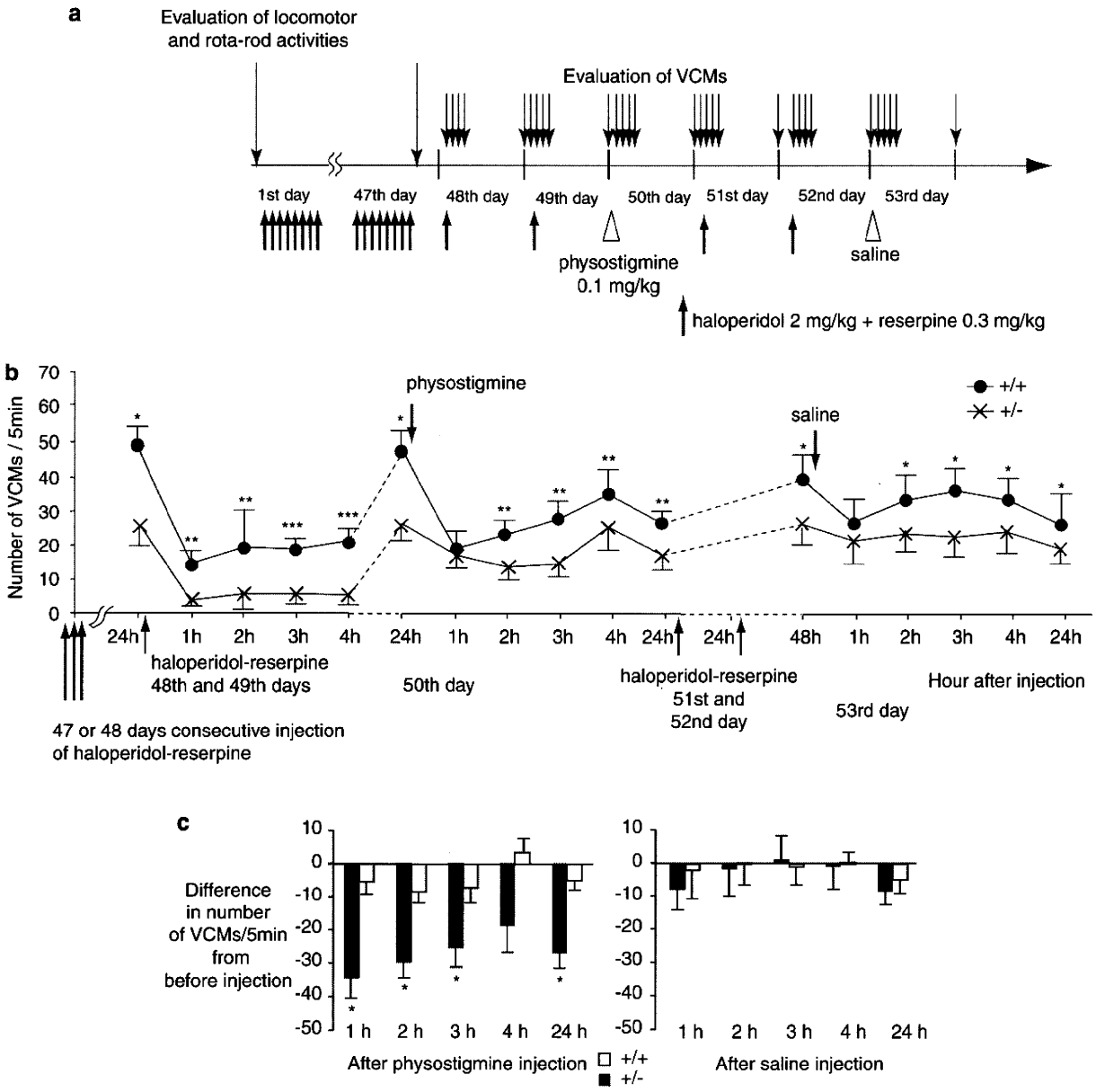

Figure 3 Analysis of vacuous chewing movements (VCMs) induced by haloperidol–reserpine in *Hspg2* knockout mice. (a) Schematic presentation of schedule of injections and measurements. (b) The average number (SEM) of VCMs for 5 min after injection. The abscissa axis shows the time after injection of HDL and reserpine, physostigmine, or saline. Significant difference between *Hspg2* hetero-knockout and wild-type mice is shown as * at $p<0.05$, ** at $p<0.01$, and *** at $p<0.001$. (c) Reduction of the number of VCMs after physostigmine or saline injection. Individual differences of the number of VCMs before injection (50th day for physostigmine or 53rd day for saline) to each time after injection grouped by the genotype are shown. Significant difference from mean = 0 is shown as * at $p<0.001$.

where the SNP (rs2445142) with the smallest *p*-value for association with TD in our genome-wide association study was located, identified one SNP (rs2124368) associated with TD even after correction for multiple testing. These two SNPs, which were found to be associated with TD, one identified by a genome-wide screening and another identified by screening with the tag SNPs, were not in LD. However, it is not obvious whether the finding for genetic association with TD of these SNPs in the *HSPG2* gene can be interpreted as significant, because of two steps of genome-wide association analyses before the step of screening of tag SNPs. Furthermore, the Human-1 BeadChip used in our initial screening is far from a complete genome coverage. This may affect the credibility of the results. Confirmation of associations in other populations is necessary.

The SNP rs2445142 that showed the lowest association *p*-value in this study was associated with the expression levels of *HSPG2* in the human postmortem prefrontal cortex. The risk allele was associated with increased expression of *HSPG2*. The SNP rs2445142 is located in intron 1 of the *HSPG2* gene and is in complete LD with at least six SNPs located from introns 1–9. Among the SNPs associated with TD found in this study, the program TFSEARCH (http://www.cbrc.jp/research/db/TFSEARCH.html) predicts alteration of the transcription factor, LYF-1, binding affinity between the T and C alleles of rs897474 in intron 3.

Synonymous SNPs, rs2254357 (exon 6), and rs2254358 (exon 6) that were associated with TD might affect mRNA decay rates. Unfortunately, the mechanism of the association between these SNPs and HSPG2 expression levels could not be elucidated in this study.

From findings in human postmortem brain samples, we speculated that increased expression of *HSPG2* is a risk factor for TD and interpreted that decreased expression of *Hspg2* in mouse brains after chronic administration of HDL was a compensatory or adaptive response to neuroleptic drugs. We, therefore, hypothesized that decreased expression level of *HSPG2* is protective for TD. We examined our hypothesis using hetero-knockout mice and confirmed it after finding lower numbers of VCMs in hetero-knockout mice than in the wild-type littermates after chronic administration of HDL and reserpine. We carried out the experiment using only female mice; therefore, we do not have the data on the sex difference.

The mechanism behind our hypothesis that increased expression levels of *HSPG2* may induce a susceptibility to neuroleptic-induced TD is not known at present. A potential efficacy of cholinergic drugs in the treatment of TD has been reported (Caroff *et al*, 2001; Tammenmaa *et al*, 2004). AChE terminates neurotransmission at cholinergic synapses by hydrolyzing acetylcholine. At the neuromuscular junction, AChE is in the basal lamina, where AChE tetramers bind the collagen ColQ, which interacts in turn with the dystroglycan complex through perlecan (Peng *et al*, 1999). Perlecan is an essential component of the ColQ–AChE localization in neuromuscular junction (Rotundo *et al*, 2005). At central synapses, AChE tetramers bind directly to the PRiMA (Perrier *et al*, 2002). Although ColQ also anchors AChE in brain and heart in addition to skeletal muscle (Feng *et al*, 1999), the role of perlecan in acetylcholine receptor signaling in central synapses is unclear. In this study, we tested the effect of the AChE inhibitor, physostigmine, on HDL- and reserpon-induced VCMs in mice. We found significant reduction in the number of VCMs only in wild-type mice and the number of VCMs was not reduced in hetero-knockout mice. These findings indicate that perlecan may be involved in the role of AChE in TD and the genotyping and/or levels of *HSPG2* may provide useful information about the effectiveness of treatment of TD with AChE.

The other important molecule to which perlecan and TD may be related is FGF2. Perlecan promotes FGF2–FGFR1 binding (Whitelock *et al*, 1996) and HSPGs including perlecan were upregulated by responding to injury and may have a role in intracellular trafficking of FGF2 in neurons and glia in the adult rat cerebral cortex (Leadbeater *et al*, 2006). Clozapine increases FGF2 expression and, on the basis of the neuroprotective activity of FGF2, a potential use of clozapine in TD was proposed (Riva *et al*, 1999).

Perlecan is expressed at the capillary endothelial cells in the brain and perlecan at the blood–brain barrier (BBB) may have a role in maintaining the blood–brain barrier function because of acceptance of the FGF2 secreted from astrocytes (Deguchi *et al*, 2002). It is reported that neuroleptics, such as HDL and chlorpromazine, alter the blood–brain barrier function and increase brain iron levels, which affect neuroleptic-induced dopamine receptor supersensitivity (Ben-Shachar *et al*, 1993).

Although the exact mechanisms of the association between HSPG2 and TD are unclear, this study identified the role of *HSPG2* in neuroleptic-induced TD.

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DISCLOSURE

The authors declare that no financial support or compensation has been received from any individual or corporate entity over the past 3 years for research or professional service and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest.

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ORIGINAL PAPER

Translin-Associated Factor X Gene (*TSNAX*) may be Associated with Female major Depressive Disorder in the Japanese Population

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Abstract Several investigations have reported that the translin-associated factor X gene (*TSNAX*)/disrupted-in-schizophrenia-1 gene (*DISC1*) was associated with major psychiatric disorders including schizophrenia, bipolar disorder (BP), and major depressive disorder (MDD). *TSNAX* is located immediately upstream of *DISC1*, and has been shown to undergo intergenic splicing with *DISC1*. It thus may also be influenced by translocation. To our knowledge, there are no reported gene-based association analyses between *TSNAX* and mood disorders in the Japanese population. We conducted a case–control study of Japanese samples (158 bipolar patients, 314 major depressive disorder patients, and 811 controls) with three tagging SNPs in *TSNAX*, selected using HapMap database. In addition, we performed an association analysis between *TSNAX* and the efficacy of fluvoxamine treatment in 120 Japanese patients with MDD. The MDD patients in this study had scores of 12 or higher on the 17 items of the Structured Interview Guide for Hamilton Rating Scale for Depression (SIGH-D). We defined a clinical response as a decrease of more than 50% in baseline SIGH-D within 8 weeks, and clinical remission as an SIGH-D score of less than 7 at 8 weeks. We found an association between rs766288 in *TSNAX* and female MDD in the allele/genotype analysis. However, we did not find any association between *TSNAX* and BP or the fluvoxamine therapeutic response in MDD in the allele/genotype analysis or haplotype analysis. Our results suggest that rs766288 in *TSNAX* may play a role in the pathophysiology of female MDD in the Japanese population. A replication study using larger samples may be required for conclusive results, since our sample size was small.

Keywords Major depressive disorder · Bipolar disorder · Translin-associated factor X gene (*TSNAX*) · Disrupted-in-schizophrenia-1 gene (*DISC1*) · Linkage disequilibrium · Tagging SNP

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Introduction

The translin-associated factor X gene (*TSNAX*) and disrupted-in-schizophrenia-1 gene (*DISC1*) are located at 1q 42. These genes are associated with major psychiatric disorders, such as schizophrenia, bipolar disorder (BP), and major depressive disorder (MDD).

TSNAX (OMIM * 602964, 7 exons in this genomic region spanning 38.672 bp and 1q42), is located immediately upstream of *DISC1*, and has been shown to undergo

intergenic splicing with *DISC1* (Millar et al. 2000). This genomic region has been shown to be closely related to susceptibility for BP (Curtis et al. 2003; Macgregor et al. 2004). It may thus also be influenced by translocation. Hennah et al.'s (2003) haplotype transmission analysis showed that *TSNAX* was associated with schizophrenia. Palo et al. (2007) reported that *TSNAX* was associated with female psychotic disorder. Thomson et al. (2005) also showed an association between *TSNAX* and male Scottish BP patients. These studies that have found association with *TSNAX* have used Caucasian populations for which the underlying linkage disequilibrium (LD) spans *TSNAX* into the first portion of the *DISC1*. In the Japanese population the two genes are on distinct LD regions according to HapMap database (release#23a/phase II, March 2008, www.hapmap.org, population: Japanese Tokyo). However, Zhang et al. (2005) reported that *TSNAX* was not associated with schizophrenia in Japanese patients. *TSNAX* was associated with impaired spatial working memory, increased reaction time to visual targets, and reduced gray matter predominantly in the superior and middle frontal gyri (Cannon et al. 2005). There are no reported gene-based association analyses between *TSNAX* and mood disorders in the Japanese population. Therefore, we conducted a case–control study with Japanese mood disorder samples. Two recent studies reported that MDD and SSRI response in MDD have common susceptibility genes. Lekman et al. (2008) reported that *FKBP5* was associated with MDD and the citalopram therapeutic response in the White non-Hispanic population. Tsai et al. (2008) also reported significant associations between plasminogen activator inhibitor type 1 gene (*SERPINE1*) and Chinese MDD patients and the SSRI therapeutic response. We therefore performed an association analysis between *TSNAX* and the efficacy of fluvoxamine treatment in Japanese patients with MDD.

Materials and Methods

Subjects

The subjects in the association analysis were 314 MDD patients (155 males and 159 females; mean age ± standard deviation 47.3 ± 14.9 years), 158 BP patients (81 males and 77 females; 99 patients with bipolar I disorder and 59 patients with bipolar II disorder; 47.9 ± 14.2 years), and 811 healthy controls (352 males and 459 females; 37.2 ± 15.9 years). Of the 314 MDD patients, 120 (59 males and 61 females; 42.0 ± 17.2 years) were treated with fluvoxamine and 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). The remaining MDD 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. Fluvoxamine was taken two or three times a day for 8 weeks. The initial total dose was 50–100 mg per day, and the dosage was then increased gradually to a maximum of 150 mg, depending on the patients' condition. Patients with insomnia and severe anxiety were prescribed benzodiazepine drugs, but no other psychotropic drugs were permitted during the study. All subjects were unrelated to each other, ethnically Japanese, and lived in the central area of Japan.

All healthy controls were also psychiatrically screened based on unstructured interviews. None had severe medical complications such as cirrhosis, renal failure, heart failure, or other Axis-I disorders according to DSM-IV. 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 and Nagoya University School of Medicine.

Data Collection

The 120 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. 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.

SNP Selection and LD Evaluation

We first consulted the HapMap database (release#23a/phase II, March 2008, www.hapmap.org, population: Japanese Tokyo, minor allele frequencies (MAFs) of more than 0.05) and included 30 SNPs covering *TSNAX* (5′-flanking regions including about 55 kb from the initial exon and about 10 kb bp downstream (3′) from the last exon: HapMap database contig number chr 1q42.1: 229673505.229774037). Three 'tagging SNPs' in *TSNAX* were then selected with the criteria of r^2 threshold greater than 0.8 in 'pair-wise tagging only' mode using the 'Tagger' program (Paul de Bakker, http://www/broad.mit.edu/mpg/tagger) in Haploview for the following association analysis (Barrett et al. 2005).

Table 1 Clinical characteristics of the patients in both definition groups

	N			Age (mean ± SD)	Baseline SIGH-D (avg ± SD)	Fluvoxamine dose at 8 weeks (mg/day) (avg ± SD)	Number of previous episode (avg ± SD)
	Total	Male	Female				
Overall	120	59	61	42.0 ± 17.2	20.3 ± 5.88	122 ± 3.84	1.39 ± 0.658
Clinical response group[a]							
Responders	61	31	30	42.2 ± 16.2	21.4 ± 6.14	119 ± 40.8	1.36 ± 0.570
Nonresponders	59	28	31	41.7 ± 18.5	19.1 ± 5.39	125 ± 40.7	1.44 ± 0.783
P value	0.712			0.895	0.0274	0.433	0.849
Clinical remission group[b]							
Remitters	47	22	25	40.1 ± 15.1	19.5 ± 5.01	115 ± 43.6	1.36 ± 0.110
Nonremitters	73	37	36	43.0 ± 18.3	20.7 ± 6.36	127 ± 38.2	1.42 ± 0.107
P value	0.678			0.510	0.271	0.114	0.697

[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

SNP Genotyping

We used TaqMan assays (ABI: Applied Biosystems, Inc., Foster City, CA,) for all SNPs. One allelic probe was labeled with FAM dye and the other with fluorescent VIC dye. The plates were heated at 50°C for 2 min and 95°C for 10 min, followed by 45 cycles of 95°C for 15 s and 58°C for 1 min. Please refer to ABI for the primer sequence. Detailed information is available on request.

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). Marker-trait association analysis was used to evaluate allele- and genotype association with the Chi-square test (SAS/Genetics, release 8.2, SAS Japan Inc, Tokyo, Japan), and haplotype association analysis was evaluated with a likelihood ratio test using the COCAPHASE2.403 program (Dudbridge 2003). In the haplotype analysis, we determined that the cutoff for testing haplotype frequency was 0.05. We used the permutation test option as provided in the haplotype analysis to avoid spurious results and correct for multiple testing. Permutation test correction was performed using 1000 iterations (random permutations). In addition, Bonferroni's correction was used to control inflation of the type I error rate in the single marker association analysis and in the explorative analysis. For Bonferroni correction, we employed the following numbers of multiple tests: 3 for each sample set in allele- and genotype analysis (3 tagging SNPs in *TSNAX*); and 6 for the explorative analysis by sex (2 × 3 tagging SNPs). We had already performed a permutation test in the haplotype analysis. Power calculation was performed using a genetic power calculator (Purcell et al. 2003). The significance level for statistical tests was 0.05.

Results

The LD structure as determined from the HapMap database can be seen in Fig. 1. Genotype frequencies of all SNPs were in HWE. We did not detect any associations between *TSNAX* and mood disorders in the allele/genotype or haplotype analyses (Tables 2 and 3). It is known that there are sex differences in the pathophysiology of mood disorders (Currier et al. 2006; Faraone et al. 1987). Therefore, we performed an explorative analysis of subjects divided by sex. We found an association between rs766288 in *TSNAX* and female MDD in the allele/genotype analysis (Table 4). This significance remained after Bonferroni's correction. However, we did not find any association between *TSNAX* and BP in the allele/genotype analysis or haplotype analysis (Tables 4 and 5).

With regard to the clinical characteristics of patients, only one difference was detected between responders and nonresponders in baseline SIGH-D scores (P value = 0.0274) (Table 1). In addition to fluvoxamine treatment in this cohort, one patient each was prescribed alprazolam, loflazepate, and etizolam. Two patients each were prescribed lorazepam, brotizolm, flunitrazepam, and zopiclone. We did not find any association between *TSNAX* and the fluvoxamine therapeutic response in MDD patients in allele/genotype (Table 6) or haplotype analysis (Response: P value = 0.797 and Remission: P value = 0.773).

In the power analysis, we obtained more than 80% power for the detection of association when we set the genotype relative risk at 1.26–1.30 and 1.41–1.48 in MDD and BP, respectively, for *TSNAX* under a multiplicative model of inheritance.

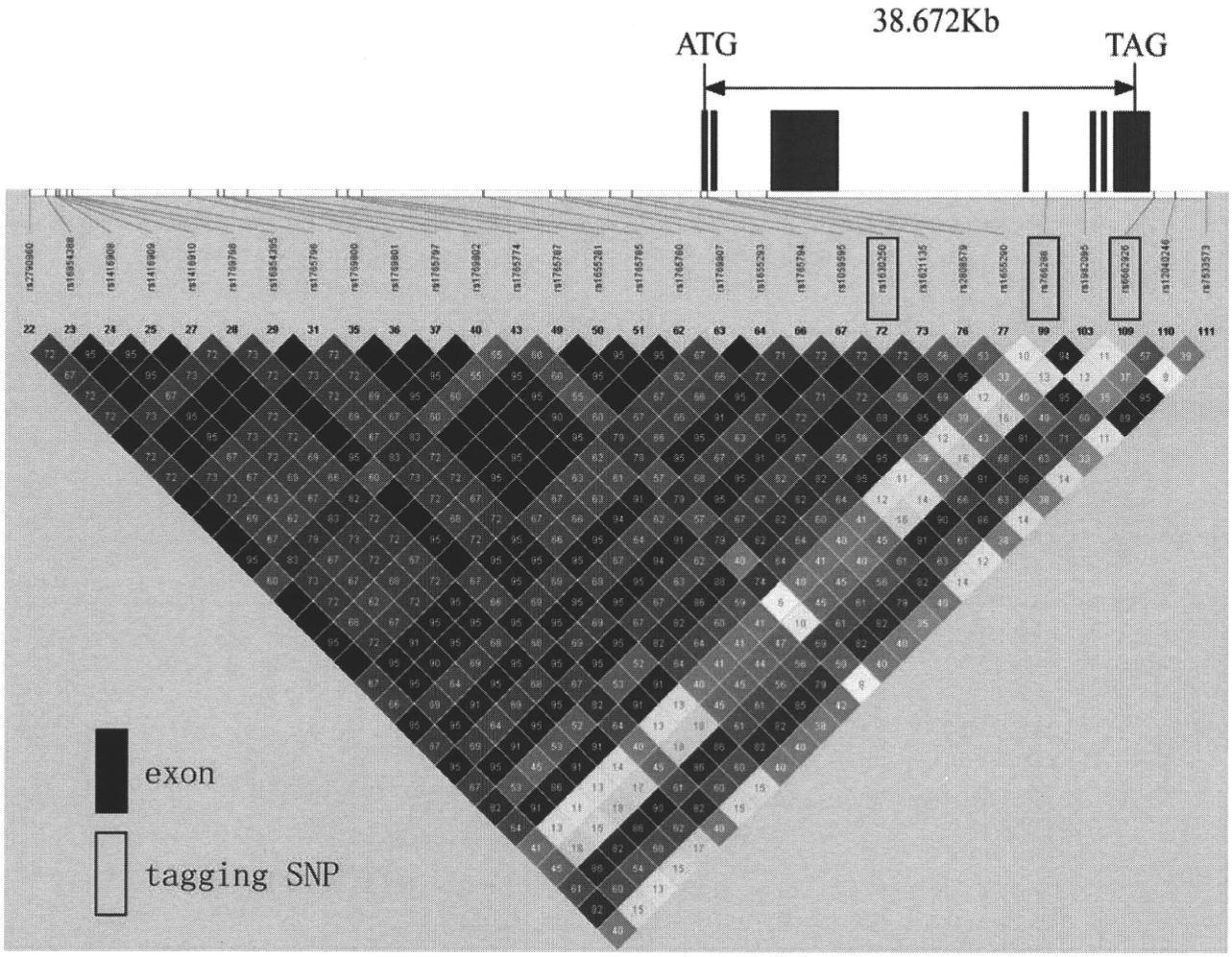

Fig. 1 LD evaluation and tagging SNPs in *TSNAX* ATG is the start codon and TAG is the stop codon. *Vertical bars* represent exons. Tagging SNPs selected from the HapMap database are represented by *black boxes*. Color scheme is based on r^2 value. Other information can be seen at the Haploview website

Table 2 Tagging SNPs and association analysis of *TSNAX*

SNP ID[a]	Phenotype[b]	MAF	*N*	Genotype distribution			*P* value[c]		
				M/M	M/m	m/m	HWE	Genotype	Allele
rs1630250	Controls	0.442	811	245	415	151	0.287		
5′ flanking region	MDD	0.475	314	85	160	69	0.700	0.356	0.165
C>G	BP	0.446	158	43	89	26	0.0789	0.493	0.892
rs766288	Controls	0.362	811	340	350	116	0.137		
Intron 4	MDD	0.322	314	141	144	29	0.367	0.0742	0.0727
C>T	BP	0.370	158	57	85	16	0.0535	0.0572	0.778
rs6662926	Controls	0.497	811	207	402	202	0.807		
3′ flanking region	MDD	0.463	314	87	163	64	0.438	0.270	0.153
C>G	BP	0.468	158	44	80	34	0.833	0.628	0.353

[a] Major allele > minor allele

[b] *MDD* Major depressive disorder, *BP* bipolar disorder, *MAF* minor allele frequency, *M* major allele, *m* minor allele

[c] Hardy–Weinberg equilibrium

Table 3 Haplotype analysis of tagging SNPs in *TSNAX*

TSNAX common haplotypes rs1630250-rs766288-rs6662926	Phenotype[a]	Individual haplotype frequency	Individual *P* value[b]	Phenotype[a]	Global *P* value
C–C–G	Control	0.263			
	MDD	0.236	0.280		
	BP	0.252	0.712		
C–T–G	Control	0.296		MDD	0.143
	MDD	0.266	0.258	BP	0.724
	BP	0.321	0.421		
G–C–C	Control	0.441			
	MDD	0.498	**0.0481**		
	BP	0.427	0.677		

[a] *MDD* Major depressive disorder, *BP* bipolar disorder

[b] Bold numbers represent significant *P* value

Table 4 Tagging SNPs and association analysis of *TSNAX* by sex

SNP ID[a]	Phenotype[b]	MAF	*N*	Genotype distribution			*P* value[c,d]			Corrected *P* value[d,e]	
				M/M	M/m	m/m	HWE	Genotype	Allele	Genotype	Allele
rs1630250	Male controls	0.440	352	107	180	65	0.482				
5′ flanking region	Male MDD	0.455	155	46	77	32	0.983	0.848	0.669		
C>G	Male BP	0.469	81	20	46	15	0.207	0.567	0.506		
	Female controls	0.443	459	138	235	86	0.425				
	Female MDD	0.494	159	39	83	37	0.577	0.286	0.120		
	Female BP	0.422	77	23	43	11	0.204	0.607	0.623		
rs766288	Male controls	0.362	352	148	153	51	0.266				
Intron 4	Male MDD	0.368	155	62	72	21	0.989	0.822	0.866		
C>T	Male BP	0.364	81	29	45	7	0.0726	0.110	0.962		
	Female controls	0.362	459	192	202	65	0.315				
	Female MDD	0.277	159	79	72	8	0.0980	**0.00661**	**0.00586**	**0.0397**	**0.0352**
	Female BP	0.377	77	28	40	9	0.351	0.429	0.721		
rs6662926	Male controls	0.492	352	92	174	86	0.835				
3′ flanking region	Male MDD	0.471	155	41	82	32	0.442	0.630	0.547		
C>G	Male BP	0.444	81	25	40	16	1.00	0.561	0.280		
	Female controls	0.501	459	115	228	116	0.889				
	Female MDD	0.456	189	46	81	32	0.735	0.363	0.166		
	Female BP	0.494	77	19	40	18	0.731	0.920	0.862		

[a] Major allele > minor allele

[b] *MDD* Major depressive disorder, *BP* bipolar disorder, *MAF* minor allele frequency, *M* major allele, *m* minor allele

[c] Hardy–Weinberg equilibrium

[d] Bold represents significant *P* value

[e] Calculated by Bonferroni's correction

Discussion

We first performed a gene-based association analysis between *TSNAX* and mood disorders including BP and MDD in the Japanese population. We found almost no association between *TSNAX* and mood disorders. However, we detected a significant association between *TSNAX* and Japanese female MDD in the Japanese population. This significant association remained after Bonferroni's correction was used to control inflation of the type I error rate due to multiple testing. *TSNAX* was associated with impaired spatial working memory, increased reaction time to visual targets, and reduced gray matter predominantly in the superior and middle frontal gyri (Cannon et al. 2005). This evidence may be involved in the pathophysiology of female MDD.

Table 5 Haplotype analysis of tagging SNPs in *TSNAX* by sex

TSNAX common haplotypes rs163 0250–rs766288–rs6662926	Phenotype[a]	Individual haplotype frequency	Individual *P* value[b]	Phenotype[a]	Global *P* value
C–C–G	Male controls	0.282			
	Male MDD	0.227	0.134		
	Male BP	0.258	0.596		
	Female controls	0.249		Male MDD	0.751
	Female MDD	0.245	0.903	Male BP	0.751
	Female BP	0.246	0.937		
C–T–G	Male controls	0.284		Female MDD	0.0744
	Male MDD	0.304	0.508	Female BP	0.885
	Male BP	0.317	0.477		
	Female controls	0.304			
	Female MDD	0.231	**0.0400**		
	Female BP	0.325	0.628		
G–C–C	Male controls	0.424			
	Male MDD	0.469	0.393		
	Male BP	0.425	0.864		
	Female controls	0.447			
	Female MDD	0.524	**0.0488**		
	Female BP	0.429	0.703		

[a] *MDD* Major depressive disorder, *BP* bipolar disorder

[b] Bold numbers represent significant *P* value

Table 6 Genotype and allele distributions of *TSNAX* in both definition groups

SNP ID[a]	Phenotype[b]	MAF	*N*	Genotype distribution			*P* value[c]		
				M/M	M/m	m/m	HWE	Genotype	Allele
rs1630250	Responders	0.500	61	15	31	15	0.898		
5′ flanking region	Nonresponders	0.551	59	14	25	20	0.270	0.507	0.430
C>G	Remission	0.510	47	12	22	13	0.664		
	Nonremission	0.534	73	17	34	22	0.584	0.942	0.721
rs766288	Responders	0.311	61	26	32	3	0.0815		
Intron 4	Nonresponders	0.271	59	31	24	4	0.823	0.429	0.492
C>T	Remission	0.266	47	23	23	1	0.0824		
	Nonremission	0.308	73	34	33	6	0.608	0.380	0.482
rs6662926	Responders	0.426	61	20	30	11	0.966		
3′ flanking region	Nonresponders	0.339	59	18	29	12	0.959	0.936	0.720
C>G	Remission	0.404	47	16	24	7	0.680		
	Nonremission	0.459	73	22	35	16	0.768	0.628	0.405

[a] Major allele > minor allele

[b] *MDD* Major depressive disorder, *BP* bipolar disorder, *M* major allele, *m* minor allele, *MAF* minor allele frequency

[c] Hardy–Weinberg equilibrium

It is known that there are sex differences in the pathophysiology of mood disorders (Currier et al. 2006; Faraone et al. 1987). We detected an association between rs766288 in intron 4 in *TSNAX* and female Japanese MDD patients. Several other investigations have also reported sex differences in associations between *TSNAX* and psychiatric disorders. Thomson et al. (2005) showed an association between several SNPs, including rs766288, in *TSNAX* and male Scottish BP patients in a haplotype analysis. Palo et al. (2007) reported that *TSNAX* (single marker association analysis: rs1655285 and haplotype analysis, including rs1655285) was associated with female psychotic disorder. However, because SNP composites for these haplotypes were "MAFs = 0" or raw data were not presented in the

HapMap database, we did not perform an association analysis for this SNP in the current study. Other genes have demonstrated gender differences in association to mood disorders. Szczepankiewicz et al. reported an association between a diagnosis of BP II in females and the glycogen synthase kinase-3 β gene (*GSK3B*) (Szczepankiewicz et al. 2006). Our previous study reported an association between prokineticin 2 receptor gene (*PROKR2*) and female BP and MDD (Kishi et al. 2009c). Several sex differences are observed in mood disorders, with the prevalence of MDD being higher in females. Hormonal variations in females, such as during the menstrual cycle, pregnancy and menopause, affect the onset and course of mood disorders, and it is possible that the etiology of mood disorders differs somewhat in females and males. Since our findings show significant associations between *TSNAX* and MDD in female Japanese patients, our results may support the supposition that the etiology of mood disorders differs somewhat in females and males.

According to HapMap database, rs1630250's MAFs in the Japanese population appear to be smaller than in Caucasians. On the other hand, rs6662926's MAFs in Caucasians were smaller than in Japanese. Also, rs766288 was the almost same MAFs in both Japanese and Caucasians. Schosser et al. (2009) reported that rs766288 was not associated with BP or MDD in the UK population. Although they selected only one SNP (rs766288) in *TSNAX*, their study was a case–control study using larger samples than our study (Schosser et al. 2009). Hennah et al. haplotype transmission analysis showed that SNPs in intron 4 in *TSNAX* (rs1615344, rs1615409, and rs766288) was associated with schizophrenia. Zhang et al. reported that *TSNAX* was not associated with Japanese schizophrenic patients. In this study, they selected rs1630250, rs1621135, and rs1655284 in *TSNAX*. We used rs1630250, and considered other SNPs that were in LD with tagging SNPs according to the HapMap database (release#23a/phase II, March 2008, www.hapmap.org, population: Japanese Tokyo). For MAF of rs1630250, our findings were almost same as those of Zhag and et al.' study (2005). In the Finnish population, Kilpinen et al. (2008) did not detect an association between *TSNAX* and autism or Asperger syndrome.

Because testing for HWE is commonly used for quality control in large-scale genotyping and is one of the few ways to identify systematic genotyping errors in unrelated individuals (Wittke-Thompson et al. 2005), we estimated HWE and confirmed the genotyping quality in this study. Genotype frequencies were in HWE for the SNPs in this study.

A few points of caution should be mentioned with respect to our results. Firstly, an association of *TSNAX* with female MDD patients may be due to biased samples, such as small sample sizes or unmatched age. In the power analysis, we obtained power of more than 80% for the detection of association when we set the genotype relative risk at 1.26–1.30 and 1.41–1.48 in MDD and BP, respectively, under a multiplicative model of inheritance. Because our samples were small, the 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. 2008b, 2009a; Stensland et al. 2008). 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. 2008a, b, 2009b). Secondly, we did not perform a mutation scan of *TSNAX*. 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. Thirdly, HapMap data has been updated to release #27 to date.

In conclusion, our results suggest that *TSNAX* probably plays a role in female MDD in the Japanese population. 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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The *chitinase 3-like 1* gene and schizophrenia: Evidence from a multi-center case–control study and meta-analysis

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ABSTRACT

The *chitinase 3-like 1* (*CHI3L1*) gene acts as a cellular survival factor in response to several environmental and psychosocial stresses. The expression level of *CHI3L1* was increased in the hippocampus and prefrontal cortex regions of patients with schizophrenia. Genetic variants of the *CHI3L1* gene have been significantly associated with schizophrenia in two distinct ethnic groups, the Chinese and Irish populations. The aims of this study are to confirm the association between the *CHI3L1* gene and schizophrenia in a Japanese population using the largest sample size to date (1463 cases and 1795 controls) and perform a meta-analysis of the combined samples (3005 cases, 3825 controls and 601 trios). We found significant associations between single nucleotide polymorphism (SNP) 4/rs4950928 ($p=0.009$), which is located in the promoter region of the *CHI3L1* gene, and haplotypes including this SNP and schizophrenia (the most significant global $p<0.001$). As the meta-analysis of the combined samples showed significant heterogeneity among studies of SNP3/rs10399805 ($p=0.026$) and SNP4 ($p<0.001$), we performed meta-analyses separately in the Japanese (2033 cases and 2365 controls) and Chinese populations (412 cases, 464 controls and 601 trios), the major groups analyzed in association studies of the *CHI3L1* gene. The meta-analysis in Japanese populations showed stronger evidence for the association of schizophrenia with SNP4 ($p=0.003$), while the meta-analysis in Chinese populations showed an association with a different variant (SNP3) ($p=0.003$). We conclude that the genetic variants in the *CHI3L1* gene have ethnic heterogeneity and confer a susceptibility to schizophrenia in Asian populations.

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1. Introduction

Schizophrenia (OMIM 181500) is a common and complex psychiatric disease. The lifetime morbidity rate is 0.5–1.0% across distinct populations. Family, twin and adoption studies of schizophrenia have indicated that there are strong genetic factors with an estimated heritability of approximately 80% (Cardno and Gottesman, 2000). Many genes have been implicated in the pathogenesis of schizophrenia (Sun et al., 2008).

The *chitinase 3-like 1* gene [*CHI3L1*, (OMIM 601525)] consists of 10 exons and spans approximately 8 kb of genomic DNA. The protein was named YKL-40 based on its three N-terminal amino acids, tyrosine (Y), lysine (K) and leucine (L), and its molecular mass of 40 kDa (Johansen et al., 1992). The protein has several names, including chitinase 3-like 1, human cartilage glycoprotein-39 (HC gp39), breast regressing protein 39 (brp-39), 38-kDa heparin-binding glycoprotein (gp38k), chondrex and 40-kDa mammary gland protein (MGP-40). In this study, to avoid confounding these terms, the gene is referred to as *CHI3L1* and the protein is referred to as YKL-40.

This gene acts as a cellular survival factor in responses to a variety of adverse environments, including various types of physiologic stress, such as inflammation, hypoxia and nutrient deprivation. These stresses may induce high expression of *CHI3L1* (Junker et al., 2005; Recklies et al., 2005). YKL-40 is secreted by activated macrophages and neutrophils in different tissues during inflammation and during increased remodeling of the extracellular matrix (Kirkpatrick et al., 1995; Rehli et al., 1997; Volck et al., 1998). YKL-40 initiates mitogen-activated protein (MAP) kinase and phosphoinositide 3-kinase (PI3K) signaling cascades in fibroblasts. Signaling leads to the phosphorylation of both the extracellular signal-regulated kinase (ERK)-1/2 MAP kinase- and the protein kinase B (AKT)-mediated signaling cascades, which are associated with the control of mitogenesis (Recklies et al., 2002). The PI3K pathway and the downstream phosphorylation of AKT in particular are strongly associated with cell survival (Bakkenist and Kastan, 2004), which suggests a role for YKL-40 as an anti-apoptotic protein.

The synthesis of YKL-40 is induced by the inflammatory cytokines IL-1, IL-6 and TNF-α (Ling and Recklies, 2004; Recklies et al., 2005; Johansen et al., 2006). The genetic variants of the *CHI3L1* gene and high serum levels of YKL-40 are associated with several inflammatory diseases, including sarcoidosis, asthma and inflammatory bowel diseases (Kruit et al., 2007; Kucur et al., 2007; Ober et al., 2008). The role of YKL-40 in the nervous system is unclear. YKL-40 is elevated in the cerebrospinal fluid (CSF) of patients with spinal diseases in which the neural tissue has been damaged or stressed, including cervical myelopathy, lumbar canal stenosis and lumbar disc herniation (Tsuji et al., 2002). High levels of YKL-40 in the CSF have also been reported in patients with purulent meningitis (Ostergaard et al., 2002). The *CHI3L1* gene expression analyses demonstrated higher postmortem mRNA levels in the hippocampus and prefrontal cortex of patients with schizophrenia than in the respective tissues of controls (Chung et al., 2003; Arion et al., 2007). It has been hypothesized that YKL-40 protects cells from undergoing apoptosis and plays a role in inflammatory processes in patients with schizophrenia.

The *CHI3L1* gene is located on chromosome 1q32.1 and shows evidence of modest linkage with schizophrenia (Shaw et al., 1998; Jang et al., 2007), although recent genome-wide association studies have not identified any variant of this gene that is associated with schizophrenia (O'Donovan et al., 2008). Zhao et al. (2007) have detected genetic associations between schizophrenia and three single nucleotide polymorphisms (SNPs; rs6691378, rs10399805 and rs4950928) within the promoter region of *CHI3L1* in two independent Chinese cohorts. They found that an allele at rs4950928 impaired MYC/MAX-regulated transcriptional activation of *CHI3L1* by altering the transcription factor consensus sequences. Yang et al. (2008) subsequently indicated significant associations between schizophrenia and two SNPs in an Irish cohort. One was the same SNP (rs10399805) in the promoter that was reported in the original study and the other SNP (rs2275351) was within the gene at intron 7. These findings suggest that the *CHI3L1* gene is likely involved in predisposition to schizophrenia. However, the two studies were not replicated in two more recent studies, one conducted with Chinese trio samples and Japanese case–control samples (Yamada et al., 2008) and the other studying a small Bulgarian population (Betcheva et al., 2009). To further investigate this controversial issue, we first investigated whether the *CHI3L1* gene is associated with schizophrenia in a large Japanese population. Second, we performed meta-analyses on the overall population and separately in Japanese and Chinese populations.

2. Methods

2.1. Subjects

The subjects in our genetic association study consisted of 1463 unrelated patients with schizophrenia [54.6% males (799/664), mean age ± SD; 47.3 ± 15.0 years] and 1795 unrelated healthy controls [51.3% males (920/875), mean age ± SD; 45.5 ± 20.1 years]. The sex ratio did not differ significantly between groups ($\chi^2 = 3.7$, $p = 0.06$), while the mean age differed significantly between groups ($z = -5.1$, $p < 0.001$). These subjects were independent of those used by Yamada et al. (2008). All subjects were biologically unrelated Japanese and were recruited at three geographic regions in Japan: Osaka, Aichi and Tokushima (Yamaguchi-Kabata et al., 2008; Ohi et al., 2009). Cases were recruited from both outpatients and inpatients at university hospitals and psychiatric hospitals. Each schizophrenic research subject had been diagnosed and assessed by at least two trained psychiatrists according to the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) criteria, based on an unstructured clinical interview. Controls, including the hospital and institutional staff, were recruited through local advertisements. Psychiatrically healthy controls were evaluated using unstructured interviews to exclude individuals who had current or past contact with psychiatric services. Written informed consent was obtained for all subjects after the procedures had been fully explained. This study was carried out in accordance with World Medical Association's Declaration of Helsinki and approved by the Research Ethical Committee of Osaka University, Fujita Health University, Nagoya University and Tokushima University.

2.2. SNP selection and SNP genotyping

We designed our replication study by selecting six SNPs in the *CHI3LI* gene and the flanking regions. Five of the six SNPs were identical to the SNPs used in the original study: rs2364574 (SNP1), rs6691378 (SNP2), rs10399805 (SNP3), rs4950928 (SNP4) and rs880633 (SNP5). The designations of these SNPs in parentheses are according to Zhao et al. (2007). The remaining SNP (rs2275351) was chosen from the following study as it showed evidence for association with schizophrenia (Yang et al., 2008). Venous blood was collected from the subjects and genomic DNA was extracted from whole blood according to standard procedures. These SNPs were genotyped using the TaqMan 5′-exonuclease allelic discrimination assay (Applied Biosystems, Foster City, California, USA), as described previously (Hashimoto et al., 2006, 2007; Ohi et al., 2009). Detailed information on the PCR conditions is available upon request. Genotyping call rates were 99.0% (SNP1), 95.0% (SNP2), 99.2% (SNP3), 99.6% (SNP4), 99.8% (SNP5) and 97.7% (rs2275351). SNP2 was excluded from the present study because this variant was not clearly discriminated as a result of a lower call rate. No deviation from Hardy–Weinberg equilibrium (HWE) in the examined SNPs was detected in the controls ($p>0.05$), while the genotypic frequencies of two SNPs deviated from HWE in the schizophrenia patients (SNP1; $p=0.016$, rs2275351; $p<0.001$). The positions of the five SNPs analyzed in the present study are indicated in Fig. 1.

2.3. Power analysis

We performed power calculations using the Power Calculator for Two Stage Association Studies [http://www.sph.umich.edu/csg/abecasis/CaTS/; (Skol et al., 2006)]. Power estimates were based on allele frequencies in patients ranging from 0.17 (SNP4) to 0.29 (SNP3), odds ratios ranging from 1.29 (SNP3) to 1.49 (SNP4) for each associated SNP, as indicated by Zhao et al. (2007), and an alpha level of 0.05. Power was calculated under a prevalence of 0.01 using a multiplicative model, assuming varying degrees of the marker allele frequency and the odds ratio.

2.4. Meta-analysis of the CHI3L1 association studies

The studies included in the meta-analysis were selected using the Schizophrenia Research Forum (http://www.schizophreniaforum.org) and PubMed with the search terms "CHI3L1" and "Schizophrenia." The analyzed data encompass all publications up to May 2009.

2.5. Statistical analyses

Statistical analyses were performed using SNPAlyze V5.1.1 Pro software (DYNACOM, Yokohama, Japan) and SPSS 16.0J software (SPSS Japan Inc., Tokyo, Japan). Differences in clinical characteristics between patients and controls were

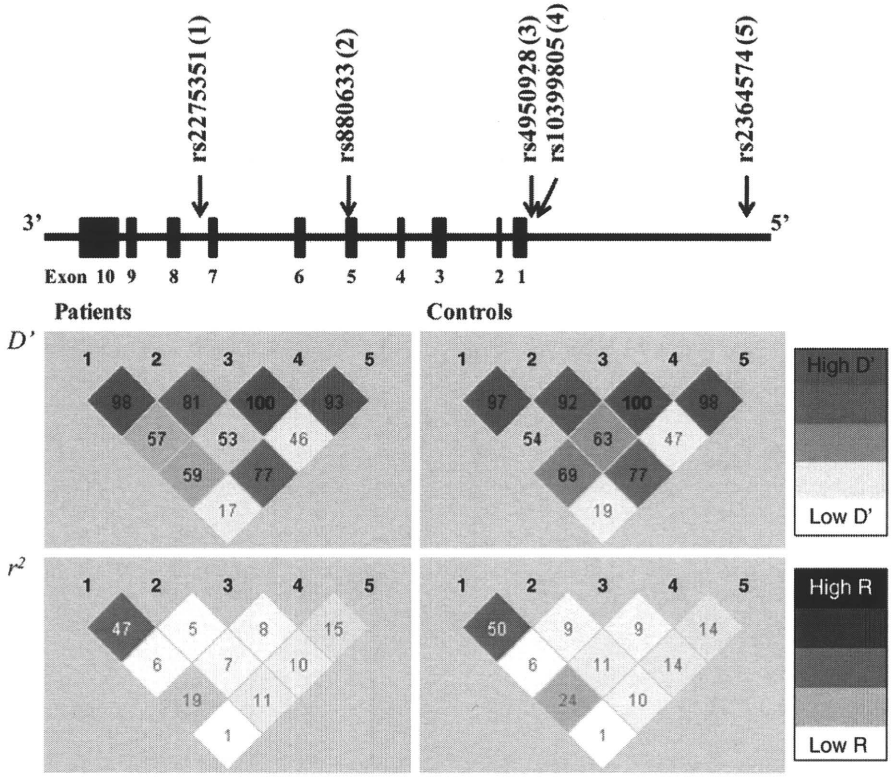

Fig. 1. Genomic structure of *CHI3L1*, including locations of the five SNPs studied, and linkage disequilibrium of these five SNPs in the patient and control groups. Based on an entry in the Entrez Gene database (National Center for Biotechnology Information), the genomic structure of *CHI3L1* is shown above. The locations of SNPs analyzed in this study are indicated by arrows. Numbers indicated in parentheses refer to numbering of the SNPs in the linkage disequilibrium (LD) diagram. The distances of exons–introns and intermarkers are drawn to scale. The LD between pairwise SNPs, using D' and r^2 values, are shown at the bottom of the map of gene structure separately for cases and controls. High levels of LD are represented by red (D') and black (r^2) coloring with increasing color intensity from 0 to 100, as shown by color bars.

analyzed using χ^2 tests for sex and the Mann–Whitney *U*-test for age. Deviation from HWE was tested separately in cases and controls using χ^2 tests for goodness of fit. The allelic and genotypic distributions of *CHI3L1* polymorphisms between patients and controls were analyzed using χ^2 tests. The number of effective independent SNPs assayed was estimated by the spectral decomposition method of Nyholt using SNPSpD software (Nyholt, 2004). Pairwise linkage disequilibrium (LD) analyses, expressed by D' and r^2, were applied to detect the intermarker relationship in each group using Haploview 4.1 software (http://www.broad.mit.edu/mpg/haploview/contact.php). Haplotype frequencies were estimated by the method of maximum likelihood using genotyping data through the use of the expectation–maximization algorithm. Rare haplotypes found in less than 3% of both patients and controls were excluded from the haplotypic association analysis. We performed 10,000 permutations for most significant tests to determine empirical significance. We used a 2- to 5-window fashion analysis.

The meta-analyses were performed using the case–control and TDT meta-analysis package (catmap) for the R-project program (Version 2.8.0, http://www.r-project.org.), which implements fixed- and random-effect pooled estimates for case–control and the transmission disequilibrium method, allowing for the use of genetic association data across study types (Nicodemus, 2008). Cochran's χ^2 based *Q* statistical test was performed in order to assess possible heterogeneity among the individual studies and, thus, to ensure that each group of studies was suitable for meta-analysis. The catmap was configured so that the random-effect model described by DerSimonian and Laird was applied in the presence of heterogeneity of the genetic effects ($p \leq 0.32$), while the fixed-effect model described by Mantel–Haenszel was applied in the absence of heterogeneity ($p > 0.32$). The significance of the pooled ORs and the heterogeneity of the group of ORs were assessed using a χ^2 test. The significance level for statistical tests was set at two-tailed $p < 0.05$.

3. Results

3.1. Genetic association analysis

Our study size of 1463 cases and 1795 controls had sufficient power (>0.98) to detect an effect at an odds ratio of 1.29 or larger, as described in the initial report, for each SNP (Zhao et al., 2007). The genotype and allele frequencies of five SNPs located in the *CHI3L1* gene and the flanking regions are summarized in Table 1. Significant differences in the genotype and allele frequency between patients and controls were observed in SNP4, which is located within the promoter region (genotype; $\chi^2 = 7.9$, $p = 0.019$, allele; $\chi^2 = 6.7$, $p = 0.009$). The significant association remained even after SNPSpD correction for multiple tests (the effective number of independent marker loci: 4.47; $p = 0.040$). The G-allele frequency at SNP4 was higher in patients (85.9%) than in controls (83.6%). There was no allelic or genotypic association with schizophrenia for the other four SNPs. Haplotype analysis showed several significant associations with schizophrenia (the most significant global $p < 0.001$, SNP3–SNP4–SNP5 and SNP3–SNP4–SNP5–rs2275351) (Table 2). This evidence for association remained positive after correction for multiple tests (10 independent global tests, the haplotypic association: $p = 0.0010$ after Bonferroni correction). The differences in detailed haplotype frequencies between cases and controls are provided in Supplementary Table 1. The LD relationships between markers are provided in Fig. 1. The LD pattern observed in our controls was nearly identical to that among our patients, the previously reported Chinese samples and JPT HapMap samples, but was different from those reported for the CEU and YRI HapMap samples. The moderate LD patterns observed between SNP5–rs2275351 were observed in both groups ($0.25 < r^2 \leq 0.50$).

3.2. Meta-analysis

We selected four studies using the Schizophrenia Research Forum and MEDLINE (Zhao et al., 2007; Yamada et al., 2008; Yang et al., 2008; Betcheva et al., 2009). The four studies and the present study (five case–control studies and two family-based studies) included 3005 patients, 3825 controls and 601 trios. The demography of the combined studies is shown in Table 3. No association between any SNP and schizophrenia was revealed in the overall population (Table 4). There was no heterogeneity among studies in SNP1 or SNP5 in the overall population. We found evidence of heterogeneity among studies of SNP3 ($p = 0.026$), SNP4 ($p = 0.00035$) and rs2275351 ($p = 0.048$). Heterogeneity in the meta-analysis refers to variation in study outcomes among studies. Thus, we analyzed two subdivided ethnic groups, Japanese populations

Table 1
Genotype and allele distributions for SNPs in the *CHI3L1* gene between patients with schizophrenia and controls.

Marker				SCZ			CON			Genotypic *p*-value ($df=2$)	SCZ	CON	Allelic *p*-value ($df=1$)	OR
SNP IDs[a]	Position[b]	M/m[c]	Gene	M/M	M/m	m/m	M/M	M/m	m/m		MAF			
SNP1	201426329	T/C	5′	0.57	0.36	0.08	0.58	0.36	0.06	0.14	0.26	0.24	0.13	1.09
SNP3	201422621	C/T	5′	0.45	0.44	0.11	0.46	0.42	0.12	0.73	0.33	0.33	0.71	1.02
SNP4	201422505	G/C	5′ UTR	0.74	0.23	0.02	0.70	0.28	0.03	**0.019**	0.14	0.16	**0.009**	0.83
SNP5	201419424	A/G	Exon 5	0.43	0.45	0.13	0.42	0.45	0.13	0.89	0.35	0.36	0.65	1.02
rs2275351	201416696	G/A	Intron 7	0.30	0.45	0.25	0.27	0.48	0.25	0.08	0.47	0.49	0.22	0.94

SCZ, patients with schizophrenia; CON, healthy controls; m, minor allele; M, major allele; MAF, minor allele frequency; OR, odds ratio. Significant *p* values are shown as bold face and underline.

[a] The db SNP IDs equivalent to the SNP IDs designed by Zhao et al. (2007) are the following: SNP1 (rs2364574), SNP3 (rs10399805), SNP4 (rs4950928), SNP5 (rs880633).

[b] db SNP build 129.

[c] The first shown alleles are major allele. All the alleles are represented according to the minus strand DNA sequence to make them comparable with the previous published data.

Table 2
Haplotype analysis of *CHI3L1* gene between patients and controls.

	Haplotypic global *p* values			
	Window level			
db SNP IDs[a]	2	3	4	5
rs2364574 (SNP1)	0.25			
rs10399805 (SNP3)		**0.018**		
	0.037		**0.0037**	
rs4950928 (SNP4)		**0.00010**		**0.0040**
	0.0038		**0.00010**	
rs880633 (SNP5)		**0.0017**		
	0.18			
rs2275351				

Haplotypes with frequencies <3% in each group are excluded. Significant *p* values are shown as bold face and underline.
[a] The db SNP IDs equivalent to the SNP IDs designed by Zhao et al. (2007) are shown in parentheses.

Table 3
Demography of the combined studies.

Authors	Ethnicities	Patients	Controls
Case–control studies			
Zhao et al. (2007)	Chinese	412	464
Yang et al. (2008)	Irish	375	812
Yamada et al. (2008)	Japanese	570	570
Betcheva et al. (2009)	Bulgarian	185	184
Ohi et al. (present study)	Japanese	1463	1795
Family-based studies			
Zhao et al. (2007)	Chinese	308 probands	
Yamada et al. (2008)	Chinese	293 probands	

(2033 patients and 2365 controls) and Chinese populations (412 patients, 464 controls and 601 trios), which were major groups across the five studies (Table 4). There was no heterogeneity among studies for these SNPs in Japanese and Chinese populations individually, expect for SNP4 in Chinese populations ($p=0.012$). We detected a significant association between SNP4 and schizophrenia in Japanese populations [$p=0.003$, OR = 0.84 (0.75–0.94)], while we detected a significant association between SNP3 and schizophrenia in Chinese populations [$p=0.003$, OR = 0.85 (0.76–0.95)]. These results remained significant even after Bonferroni correction (independent tests of the four SNPs, SNP4; corrected $p=0.012$, SNP3; corrected $p=0.012$).

4. Discussion

In this study, we found that SNP4 in the *CHI3L1* gene was associated with schizophrenia in a large Japanese population. Second, we performed a meta-analysis of the overall combined populations of several studies. In the meta-analysis, significant heterogeneity among studies was observed in SNP3 and SNP4. Because of the significant heterogeneity, we stratified the studies by ethnicity. We found that schizophrenia was associated with distinct SNPs in the *CHI3L1* gene in the Japanese and the Chinese populations.

We revealed a significant association of the G-allele of SNP4, which is located in the promoter region of the *CHI3L1* gene, with schizophrenia in a Japanese cohort (patients 85.9% vs. controls 83.6%). Our meta-analysis indicated a stronger association between SNP4 and schizophrenia in Japanese populations. Despite similar allele frequencies between cases and controls in the two Japanese cohorts, Yamada et al. (2008) reported no association between SNP4 and schizophrenia (patients 85.9% vs. controls 83.7%). This discrepancy might be attributed to the type II error for their small sample size (570 vs. 570) compared with our large sample size (1463 vs. 1795). In the meta-analysis of the overall combined population (Caucasian, Chinese and Japanese subjects), we found no association between these SNPs in the *CHI3L1* gene and schizophrenia. This result can be explained by the fact that the LD patterns in the HapMap data are different among each of these populations. For SNPs with heterogeneity among studies, we separately analyzed their association with schizophrenia in Japanese and Chinese populations. The meta-analyses showed that schizophrenia was associated with different variants (SNP3 and SNP4) in each population. Although the LD patterns between Asian populations were similar, the risk allele differed between Japanese and Chinese populations. It is unclear whether the difference resulted from subtle differences in LD patterns or allelic heterogeneity. It seems that an SNP might exist in this region that is more strongly associated with schizophrenia. This possibility could be addressed by re-sequencing or genotyping dense SNP mapping in this region and evaluating the association with schizophrenia.

It has been suggested that YKL-40 might be a potential biomarker for a cellular survival factor in an adverse microen-

Table 4
Meta-analysis of the genetic association studies for each SNP.

		Overall			Japanese			Chinese		
SNP ID	M/m	OR (95% CI)	$p(\chi)$	$p(Q)$	OR (95% CI)	$p(\chi)$	$p(Q)$	OR (95% CI)	$p(\chi)$	$p(Q)$
SNP1 (C)	T/C	(4)[a] 1.07 (0.99–1.15)	0.11[b]	0.74	(2)[a] 1.07 (0.97–1.18)	0.16[b]	0.53	(2)[a] 1.05 (0.93–1.19)	0.41[b]	0.37
SNP3 (T)	C/T	(6) 0.90 (0.80–1.01)	0.06	**0.026**	(2) 1.03 (0.94–1.12)	0.56[b]	0.79	(3) 0.85 (0.76–0.95)	**0.003**[b]	0.41
SNP4 (C)	G/C	(7) 1.03 (0.86–1.24)	0.72	**0.00035**	(2) 0.84 (0.75–0.94)	**0.003**[b]	0.90	(3) 1.29 (0.93–1.79)	0.13	**0.012**
SNP5 (G)	A/G	(4) 1.01 (0.94–1.08)	0.75[b]	0.80	(2) 1.00 (0.91–1.09)	0.98[b]	0.40	(2) 1.03(0.92–1.16)	0.58[b]	0.80
rs2275351 (A)	G/A	(2) 0.84 (0.65–1.09)	0.19	**0.048**	(1)–	–	–	(0)–	–	–

$p(\chi)$: chi-square test used determines the significance of the overall OR. Multiple testing corrections were not performed. Significant *p* values are shown as bold face and underline.
$p(Q)$: Cochran's *Q* test used to assess the heterogeneity. Random-effect model was applied in the presence of heterogeneity of the genetic effects ($p \leq 0.32$), while fixed-effect model was applied in the absence of heterogeneity ($p > 0.32$).
[a] The number of studies included in each meta-analysis is indicated in parentheses.
[b] This analysis was performed by fixed-effect model.

vironment because increased YKL-40 expression is found upon genotoxic and microenvironmental stress (i.e., hypoxia and ionizing radiation). It has been considered that a number of environmental stresses, such as fetal hypoxia and infection, in addition to genetic contributions, might induce susceptibility to schizophrenia (Palomo et al., 2004; Mittal et al., 2008). Patients with schizophrenia have shown increased levels of IL-6, IL-1RA and sIL-2R and a decrease in IL-2 (Potvin et al., 2008). YKL-40 is stimulated by IL-6 (Johansen et al., 2006), a multifunctional cytokine with varied system functions that plays a role in inflammatory processes and induces cell differentiation (Trikha et al., 2003). Cytokines play important roles in infection and inflammation and are crucial mediators of cross-talk between the brain and the immune system. Schizophrenia might be associated with an imbalance in inflammatory cytokines.

Elevated expression of the *CHI3L1* gene has been indicated in the hippocampus and prefrontal cortex in independent postmortem studies of patients who had schizophrenia (Chung et al., 2003; Arion et al., 2007). The G-allele at SNP4 has been associated with higher transcriptional activity according to a luciferase reporter assay and with higher *CHI3L1* mRNA levels in peripheral blood cells in patients with schizophrenia (Zhao et al., 2007). Interestingly, higher serum YKL-40 levels are involved in several inflammatory processes and tissue remodeling (Vind et al., 2003; Bergmann et al., 2005; Nordenbaek et al., 2005; Johansen, 2006; Kucur et al., 2007; Nojgaard et al., 2008). The G-allele at SNP4 occurred at a higher frequency in patients with asthma than in controls and was associated with higher serum YKL-40 levels (Ober et al., 2008). Our results suggest that the G-allele, which is enriched in patients with schizophrenia compared with controls, has a role in the etiology of schizophrenia. The risk *CHI3L1* genotype might be associated with serum YKL-40 levels in patients with schizophrenia. Further study of the possible association of *CHI3L1* genotype in patients with schizophrenia is required.

As schizophrenia is sensitive to environmental and psychological stresses (Leff, 1994; Howes et al., 2004), higher *CHI3L1* gene expression in patients with schizophrenia may be due to an excessive response to various stressors. SNP4, which is located within the promoter of the *CHI3L1* gene, might play a role in altering the expression and serum levels of YKL-40. In conclusion, we suggest that SNPs in the *CHI3L1* gene have ethnic heterogeneity and might contribute to the pathogenesis of schizophrenia in Asian populations. Further replication studies in other ethnic populations are required to confirm the possible relationship between *CHI3L1* and schizophrenia.

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Contributors

R. Hashimoto supervised the entire project, collected the data, wrote the manuscript, was critically involved in the design, analysis and interpretation of the data and was responsible for performing the literature review. K. Ohi was critically involved in the collection and analysis of the data, and contributed to the editing of the final manuscript and contributed intellectually to the interpretation of the data. Y. Yasuda, T. Yoshida, H. Takahashi, N. Iike, M. Iwase, K. Kamino, R. Ishii, H. Kazui, M. Fukumoto, H. Takamura, H. Yamamori, M. Azechi, K. Ikezawa, H. Tanimukai, S. Tagami, T. Morihara, M. Okochi, K. Yamada, S. Numata, M. Ikeda, T. Tanaka, T. Kudo, S. Ueno, T. Yoshikawa, T. Ohmori, N. Iwata, N. Ozaki and M. Takeda were heavily involved in the collection of the majority of the data and contributed intellectually to the interpretation of the data. All authors contributed to and have approved the final manuscript.

Conflict of interest

All authors declare that they have no conflicts of interest.

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Appendix A. Supplementary table

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.schres.2009.12.002.

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