sequence.[19] Each individual nucleotide of both the sense and the antisense DNA strands is interrogated with four 25-mer probes that differ only with respect to the central position (A, C, G, and T). According to Affymetrix's Custom-Seq Array Design Guide, we designed arrays covering all exon regions of *KALRN* and *EPHB1* (Ensembl release 52 [Human CCDS set]; Transcript: ENST00000360013, ENST00000240874, and ENST00000291478 for *KALRN*; ENST00000398015 for *EPHB1*). Because the principle of the resequencing arrays is based on hybridization, it is necessary to avoid cross-hybridization for accurate resequencing. For this purpose, we removed repetitive elements and highly homologous sequences from the array design.

Array-Based Resequencing

The experiments were conducted according to the manufacturer's instructions (supplementary figure S2). Genomic DNA was extracted from peripheral blood using standard methods. To generate enough target-enriched subject material for hybridization to the arrays, we generated 47 and 14 amplicons per sample for *KALRN* and *EPHB1*, respectively, using long-range polymerase chain reaction (PCR). The PCR conditions were as follows: 94°C for 2 minutes followed by 30 cycles consisting of 94°C for 15 seconds, 68°C for 3 minutes, followed by a final extension of 68°C for 8 minutes, using TaKaRa LA Taq™ (Takara Bio, Otsu, Shiga, Japan). Each PCR product was quantified using PicoGreen (Molecular Probes, Eugene, Oregon), pooled in an equimolar fashion. The PCR products were then purified, fragmented, labeled, and hybridized to the arrays, following the protocol. Finally, the arrays were washed and stained using the GeneChip Fluidics Station 450 (Affymetrix) and scanned using the GeneChip Scanner 3000 (Affymetrix). The data were analyzed using the GeneChip Operating Software (GCOS; Affymetrix), the GeneChip Sequence Analysis Software (GSEQ; Affymetrix), and SeqC (JSI Medical Systems, Kippenheim, Germany; http://www.jsi-medisys.de/html/products/SeqC/SeqC.htm) to automate the generation of sequence and genotype calls from the intensity data. In this study, around 17 kb was sequenced per sample, meaning that more than 5.4 Mb was sequenced in total. All missense mutations presented in this study were confirmed using both Sanger sequencing and Custom TaqMan SNP genotyping assays (Applied Biosystems, Foster City, California).

Association Analysis of Each Missense Mutation

Although the rare (<1%) missense mutations were originally discovered among 320 schizophrenic patients, it was possible that a portion of them might have neutral or protective effects.[5] In addition, it was necessary to reduce the number of statistical tests for multiple comparison problems. To accomplish this, we prioritized rare (<1%) deleterious variants for subsequent association analyses based on the frequencies in the second case-control sample set because rare deleterious variants relevant to schizophrenia can be assumed to have higher frequency in cases than in controls. The criteria for prioritization were as follows: (1) frequencies of mutations were <1% in controls and (2) frequencies of mutations were higher in cases (ie, OR > 1). Mutations not detected in the second sample set were not followed up in this analysis. The frequencies of such mutations can be so low (<0.0005) that the results of association analyses are unlikely to be statistically significant in our sample size. For mutations meeting the above criteria, we conducted association analyses with schizophrenia using the third sample set. Genotyping was conducted by Custom TaqMan SNP genotyping assays (Applied Biosystems). For quality control, samples with missing call rates of 10% or higher were excluded from the analyses.

Combined Association Analyses

In general, it is difficult to establish an association of a rare mutation with a phenotype because statistical power is limited by low population frequency and because the number of rare variants requires a strict multiple test correction. Therefore, we conducted combined association analyses across rare mutations observed in each gene in the third sample set, comparing the number of mutations in cases with the number in controls. The criteria for mutations included in these analyses were same as the above criteria with 1 exception: Mutations not detected in the second sample set were included in the combined association analyses.

In Silico Analysis

The potential influence of missense mutations was evaluated using PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) and[22] PMut (http://mmb2.pcb.ub.es:8080/PMut/)[23] softwares. PolyPhen-2 uses 8 sequence-based and 3 structure-based predictive features and compares a property of the wild-type allele and the corresponding property of the mutant allele. PolyPhen-2 trained on HumDiv datasets is reported to achieve true positive prediction rates of 92% with a false-positive rate of 20%.[22] A mutation is appraised qualitatively as benign, possibly damaging, or probably damaging based on naive Bayes posterior probability that a given mutation is damaging. PMut also allows the fast and accurate prediction (~80% success rate in humans) of the pathological character of missense mutations based on the use of neural networks. The final output is a pathogenicity index ranging from 0 to 1 (indexes >0.5 signal pathological mutations).

We also examined evolutionary conservation of the mutated residues and surrounding amino acids. Multiple sequence alignment of human *KALRN* or *EPHB1* with 6 orthologs was performed for this purpose.

Power Calculation

Power calculation was performed with a power calculator called CaTS (http://www.sph.umich.edu/csg/abecasis/CaTS/).[24] Power was estimated under the following parameter assumptions with respect to association test statistics: genetic relative risk = 2, prevalence of disease = 0.01, risk allele frequency = the values frequency observed in controls, and $\alpha = .05$; a multiplicative model was used.

Statistical Analysis

For the association analysis of each variant, Fisher exact test was used to examine whether rare deleterious variants were significantly overrepresented in the patient group rather than the control group.

A combined association test was performed following a previous study.[7] In brief, to account for variable sample size, sample size was adjusted to $N=n/(\sum(1/Ni))$, where Ni is the sample size at the ith variant, and n is the number of variants. The number of observed variants was adjusted as $\sum(pi)\times N$, where pi is the frequency of the ith variant. Fisher exact test was used in this test as well to examine an overrepresentation of rare deleterious missense mutations in the patient group rather than control group.

All statistical tests were 1 tailed, and a P value less than 0.05 was considered significant. Bonferroni correction was used for solving multiple testing problems.

Results

Discovery of Mutations

We detected 12 and 6 missense mutations with a frequency of <5% in *KALRN* and *EPHB1*, respectively, among 320 cases in the first sample set (table 1). All but 2 mutations (N2973S in *KALRN* and T981M in *EPHB1*) were novel. All mutations were validated by both Sanger sequencing and Custom TaqMan SNP genotyping assays. In the first sample set, 2 patients were compound heterozygotes for rare missense mutations in the 2 genes. One patient had R410H in *KALRN* and R905C in *EPHB1*. The other had A2382V in *KALRN* and D375N in *EPHB1*. There were no clinical characteristics shared between these patients. No nonsense mutations were identified in this study.

Association Analysis of Each Missense Mutation

In the prioritization phase using the second sample set, T1207M and P2255T in *KALRN* and R637C and R905C in *EPHB1* showed a higher frequency in cases than in controls (table 1). Seven missense mutations (R410H, Q770K, and A2382V in *KALRN* and F151S, D375N, D577N, and T981M in *EPHB1*) were not detected. The frequency of P1695Q was more than 4% both in cases and in controls. Based on our criteria, we selected 4 missense mutations (T1207M and P2255T in *KALRN* and R637C and R905C in *EPHB1*) for subsequent association analyses using the third sample set.

In the third phase, P2255T showed a nominally significant association with schizophrenia (OR = 2.09, P = .012) in the third sample set (table 2). This remained significant after correction for multiple testing of 4 variants (corrected P = .048). T1207M in *KALRN* and R637C and R905C in *EPHB1* were also more frequent in cases, although differences were not significant.

We excluded mutations not detected in the second sample set from this analysis. This was supported by a power analysis showing that the third sample set had only 10% power in analysis of very rare mutations.

Combined Association Analysis

In addition to 4 mutations (T1207M and P2255T in *KALRN* and R637C and R905C in *EPHB1*), 7 very rare mutations (R410H, Q770K, and A2382V in *KALRN* and F151S, D375N, D577N, and T981M in *EPHB1*), which were not detected in the second samples set, were included in the combined association analysis. A global comparison of the frequencies of 5 selected mutations in *KALRN* between cases and controls in the third sample set showed a significant increase in frequency in schizophrenic patients (OR = 2.07, P = .003) (table 3). This remained significant after correction for multiple testing (corrected P = .006). On the other hand, a global comparison of the frequencies of 6 selected mutations in *EPHB1* did not show a significant difference (OR = 1.09, P = .438).

In Silico Analysis

Results of *in silico* analysis are shown in table 4. All missense mutations but A2382V in *KALRN* were predicted to have functional relevance by PolyPhen-2 or PMut software.

A multiple alignment of the region of *KALRN* or *EPHB1* containing rare missense mutations with 6 orthologs is shown in table 4. Most of the rare missense mutations showed a high degree of amino acid conservation in different species.

Discussion

In this study, we conducted resequencing analyses for the 2 synaptogenic pathway genes (*KALRN* and *EPHB1*) in schizophrenia using a DNA microarray–based method. After resequencing more than 5.4 Mb, we discovered 17 rare (<1%) missense mutations in *KALRN* or *EPHB1* and detected a significant association between schizophrenia and P2255T in *KALRN*, as well as in the combined association analysis for *KALRN*. These findings are consistent with an estimation that most rare (<1%) missense mutations are mildly deleterious and are associated with a heterozygous fitness loss.[5]

Table 1. *KALRN* And *EPHB1* Missense Mutations Identified in The First Sample Set And Their Frequencies in The Second Sample Set

Gene	Genomic Position	Base Change	dbSNP Reference	AA Change	First Sample Set		Second Sample Set				
							Genotype Counts		Mutation Frequency		
					Homo	Hetero	SCZ	CONT	SCZ	CONT	OR >1
KALRN	125527659	G → A	ss250607852	R410H	0	1	0/0/701	0/0/541	0	0	
KALRN	125531474	T → A	ss250607853	L452Q	0	1	0/1/709	0/2/541	0.0007	0.0018	
KALRN	125600376	C → A	ss250607854	Q770K	0	1	0/0/706	0/0/544	0	0	
KALRN	125656787	C → T	ss250607855	T1207M	0	1	0/2/705	0/1/542	0.0014	0.0009	+
KALRN	125764534	C → A	ss250607856	P1695Q	0	1	0/59/636	1/44/492	0.0425	0.0428	
KALRN	125764599	A → T	ss250607857	M1717L	0	1	0/0/705	0/1/540	0	0.0009	
KALRN	125860927	G → A	ss250607858	R2049K	0	1	0/1/696	0/1/540	0.0007	0.0009	
KALRN	125873259	C → A	ss250607859	P2255T	0	7	1/14/684	0/7/536	0.0114	0.0064	+
KALRN	125873289	C → T	ss250607860	P2265S	1	0	0/6/701	0/7/533	0.0042	0.0065	
KALRN	125873382	G → T	ss250607861	G2296C	0	1	0/1/703	0/1/542	0.0007	0.0009	
KALRN	125876103	C → T	ss250607862	A2382V	0	1	0/0/697	0/0/540	0	0	
KALRN	125920964	A → G	rs16835896	N2973S	0	3	0/3/698	0/6/538	0.0021	0.0055	
EPHB1	136153231	T → C	ss252863894	F151S	0	1	0/0/710	0/0/543	0	0	
EPHB1	136334407	G → A	ss252863895	D375N	0	1	0/0/708	0/0/544	0	0	
EPHB1	136368508	G → A	ss252863896	D577N	0	1	0/0/707	0/0/544	0	0	
EPHB1	136394134	C → T	ss252863897	R637C	0	2	1/1/707	0/2/541	0.0021	0.0018	+
EPHB1	136450890	C → T	ss252863898	R905C	0	3	0/9/695	0/1/543	0.0064	0.0009	+
EPHB1	136460639	C → T	rs56186270	T981M	0	2	0/0/706	0/0/541	0	0	

Note: Genomic position based on NCBI build 36, chromosome 3. Amino acid changes based on NCBI Reference Sequence NP_001019831.2 (2986 aa) for KALRN and NP_004432.1 (984 aa) for EPHB1. All but N2973S (rs16835896) and T981M (rs56186270) are novel. AA change, amino acid change; dbSNP, Single Nucleotide Polymorphism Database; Homo, homozygote; Hetero, heterozygote; SCZ, schizophrenia; CONT, control; NCBI, National Center for Biotechnology Information.

Schizophrenia is a genetically heterogeneous disorder, with both very rare variants with a high effect size (eg, CNVs in 1q21.1, 15q13.3) and common variants with a low effect size (eg, rs1344706 in *ZNF804A*) involved in its genetic architecture. In this frequency-effect size spectrum, P2255T (OR: ~2, risk allele frequency in controls: ~0.005) is located between the CNV in 1q21.1 (OR: ~10, frequency in controls: ~0.0001)[25] and rs1344706[T] in *ZNF804A* (OR: ~1.1, risk allele frequency in controls: ~0.6),[26] both of which have been recently associated with schizophrenia. The relatively modest effect size of P2255T compared with that of the above CNVs can be attributable to the difference in the effect of each variant on gene(s): Although CNVs strongly influence the expression of multiple genes, missense mutations in *KALRN* are presumed to have limited effects on KALRN function. P2255T is located in the evolutionally conserved proline-rich region between the C-terminal GEF and SH3 domains[27] and is surrounded by 2 nearby phosphorylation sites (S2237 and S2262), according to Human Protein Reference Database (http://www.hprd.org/index_html)[28] (figure 1). *In silico* analysis with PhosphoMotif Finder[29] shows that T2255 itself can be recognized and phosphorylated by many kinases, suggesting functional implications of P2255T (figure 1). In addition, *in silico* analysis predicts that phosphorylation of T2255 will induce that of nearby S2253. Thus, P2255T may greatly change the phosphorylation status in a narrow

Table 2. Association Analyses of Each Missense Mutation in the Third Sample Set

	AA Change	Third Sample Set					
		Genotype Counts		Mutation Frequency			
		SCZ	CONT	SCZ	CONT	OR	*P* Value
KALRN	T1207M	0/7/1477	0/3/1482	0.0024	0.0010	2.34	.171
KALRN	P2255T	0/31/1448	0/15/1473	0.0105	0.0050	2.09	.012
EPHB1	R637C	0/4/1477	0/4/1478	0.0014	0.0014	1.00	.636
EPHB1	R905C	0/15/1458	0/12/1466	0.0051	0.0041	1.26	.347

Note: Abbreviations are explained in the first footnote to table 1. *P* values were calculated by Fisher exact test (1 tailed).

Table 3. Combined Association Analysis in The Third Sample Set

Gene	AA Change	Third Sample Set				Combined Analysis	
		Genotype Counts		Mutation Frequency		Gene Based	
		SCZ	CONT	SCZ	CONT	OR	*P* value
KALRN	R410H	0/0/1481	0/0/1484	0	0	2.07	.003
KALRN	Q770K	0/0/1486	0/0/1490	0	0		
KALRN	T1207M	0/7/1477	0/3/1482	0.0024	0.0010		
KALRN	P2255T	0/31/1448	0/15/1473	0.0105	0.0050		
KALRN	A2382V	0/7/1473	0/4/1480	0.0024	0.0013		
EPHB1	F151S	0/0/1478	0/0/1484	0	0	1.09	.438
EPHB1	D375N	0/0/1483	0/0/1490	0	0		
EPHB1	D577N	0/0/1486	0/2/1483	0	0.000673		
EPHB1	R637C	0/4/1477	0/4/1478	0.0014	0.0014		
EPHB1	R905C	0/15/1458	0/12/1466	0.0051	0.0041		
EPHB1	T981M	0/5/1481	0/4/1484	0.0017	0.0013		

Note: Abbreviations are explained in the first footnote to table 1. *P* values were calculated by Fisher exact test (1 tailed).

region between the C-terminal GEF and SH3 domain. A protein with multiple phosphorylated sites like KALRN can be assumed to have an exponential number of phospho-forms, and individual phospho-forms may have distinct biological effects. The diffuse distribution of these phospho-forms at steady state enables the phosphoproteome to encode information and flexibly respond to varying demands.[30] Thus, it is conceivable that P2255T may influence such plasticity in KALRN by changing the number of phosphorylated sites. Interestingly, detailed examination of clinical information from the first sample set, which was uniquely available to us, revealed that congenital or early-onset vascular disease was observed in 5 of 7 cases with P2255T (supplementary table S1). Because *KALRN* may represent a candidate gene for vascular diseases,[31,32] it is tempting to speculate that P2255T may be a potential risk factor for vascular disease.

In addition to P2255T, we detected multiple rare (<1%) missense mutations in *KALRN* or *EPHB1*. Such variants are not sufficiently frequent to be covered by GWAS nor do they have sufficiently large effect sizes to be detected by linkage analysis in family studies. For modest effect sizes, it is suggested that association testing may require composite tests of overall mutational load,

Table 4. Results of *In Silico*/Conservation Analysis

KALRN							
Analysis		R410H	Q770K	T1207M	P2255T	A2382V	
PolyPhen-2		Probably damaging	Probably damaging	Probably damaging	Benign	Benign	
PMut		Pathological	Neutral	Pathological	Pathological	Neutral	
Conservation analysis	Human (NP_001019831.2)	LDERSTI	IFLQLRI	IHATEIR	RSQPARL	SILAPLT	
	Chimpanzee (XP_516703.2)	LDERSTI	IFLQLRI	IHATEIR	RSQPARL	SILAPLT	
	Dog (XP_535768.2)	LDERSTI	IFLQLRI	IHATEIR	RSQPSRV	SVLAPLT	
	Cattle (XP_001790302.1)	LDERSTI	IFLQLRI	IHATEIR	RSQPARV	SILTPLT	
	Mouse (XP_001481079.1)	LDERSTI	IFLQLRI	IHATEIR	RSQPPRV	SILAPLA	
	Rat (NP_114451.1)	LDERSTI	IFLQLRI	IHATEIR	RSQPPRV	SILAPLT	
EPHB1							
Analysis		F151S	D375N	D577N	R637C	R905C	T981M
PolyPhen-2		Benign	Probably damaging	Possibly damaging	Probably damaging	Probably damaging	Probably damaging
PMut		Pathological	Neutral	Neutral	Pathological	Pathological	Pathological
Conservation analysis	Human (NP_004432.1)	QVDFGGR	RCDDNVE	VYSDKLQ	YKGRLKL	LLDRSIP	QSPTAMA
	Chimpanzee (XP_001150963.1)	QVDFGGR	RCDDNVE	LLVEQWQ	YKGRLKL	LLDRSIP	QSPTAMA
	Dog (XP_542791.2)	QVDFGGR	RCDDNVE	VYSDKLQ	YKGRLKL	LLDRSIP	QSPTTMA
	Cattle (XP_614602.4)	QVDFGGR	RCDDNVE	VYSDKLQ	YKGRLKL	LLDRSIP	QSPTAMA
	Mouse (NP_775623.2)	QVDFGGR	RCDDNVE	AYSDKLQ	YKGRLKL	LLDRSIP	QSPSVMA
	Rat (XP_217250.1)	QVDFGGR	RCDDNVE	VYSDKLQ	YKGRLKL	LLDRSIP	QSPSVMA

Note: The bold are the mutated amino acids.

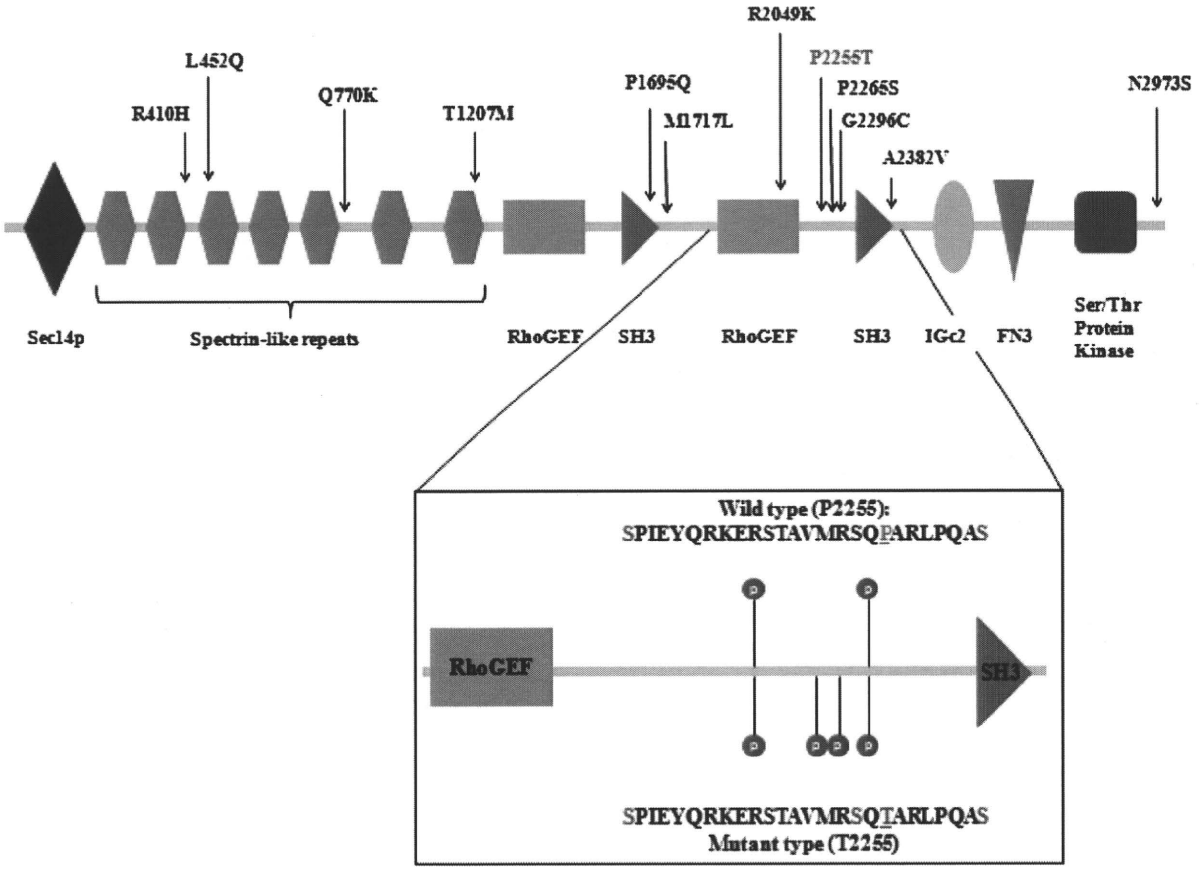

Fig. 1. Rare Missense Mutations in *KALRN* and Change in Phosphorylation Status by P2255T.

comparing frequencies of mutations of potentially similar functional effect in cases and controls. Thus, we also performed combined association analyses for *KALRN* or *EPHB1* and found evidence that multiple rare (<1%) missense mutations in *KALRN* as a whole are associated with schizophrenia. This finding is supported by *in silico* analyses showing that most of the mutations are predicted as being of functional relevance and that they are located in evolutionally conserved regions. In contrast, there were no significant differences in the cumulative frequencies of rare missense mutations in *EPHB1*. This might be due to a type II error. The cumulative frequency of rare mutations of *EPHB1* in controls is almost same as the one of *KALRN* in controls (0.0075 vs 0.0073), indicating that cumulative effect size of rare missense mutations in *EPHB1* may be smaller than the one in *KALRN*. In the mammalian genome, there are 5 different EphB receptors (EphB1, EphB2, EphB3, EphB4, and EphB6), with a high similarity at the amino acid level. Analysis of double and triple knockout mice lacking EphB1, EphB2, and EphB3 in different combinations revealed that EphBs have functional redundancy even though all these EphBs are responsible for spine morphogenesis and synapse formation to varying degrees.[33] This is in contrast with the drastic phenotypes observed in *KALRN*-knockout mice.[16] Therefore, biological effects of rare missense mutations in *EPHB1* may be compensated for by other intact *EPHBs*. This might lower the ORs of rare missense mutations in *EPHB1*. Given that all the mutations detected in *EPHB1* were predicted to have pathogenicity by PolyPhen-2 or PMut, a larger-scale case-control study with sufficient power may provide a significant result in a combined analysis for *EPHB1*.

One important aspect of the present study is that we found rare mutations associated with schizophrenia in the *KALRN* gene, in which GWASs detected association signals for schizophrenia. Several studies have recently reported the 1 gene may harbor both rare and common variants associated with the same diseases, including schizophrenia,[34] type 2 diabetes,[35] and hypertriglyceridemia.[36] Given that the cost of whole-genome sequencing is still high to search for rare mutations, resequencing analyses for genes with support from GWAS might be a better strategy for detection of rare mutations with larger effect size.

There are several limitations to this study. First, we could not conduct segregation analyses for mutations due to limited access to family members. Furthermore, given the modest risk (OR ~2), these mutations would show incomplete penetrance. In fact, it is reported that penetrance estimates of CNVs at 1q21.1 and 15q13.3,

both of which show higher ORs, are 0.061 and 0.074, respectively.[25] Therefore, a population-based study is a better choice to evaluate genetic associations for missense mutations with modest risk.[37] The second limitation is population stratification. Although a Japanese population is considered relatively homogenous, small population stratifications may have influenced our findings.[38] However, we believe that the recruitment of subjects in local regions minimized this concern. Third, we did not conduct functional analyses for detected missense mutations. The detailed effects of these mutations on the pathophysiology of schizophrenia need to be examined in a future study. Fourth, our resequencing analyses were not comprehensive in terms of the kind of variants and the number of genes. In other words, the present study did not cover indels or CNVs because of the methodological limitation of the DNA microarray–based method. Because these classes of variants could have a more profound effect on protein function, their genetic contribution to schizophrenia might be revealed in future studies. Also, as shown in *EPHB1*, it is assumed that a variety of molecules or pathways have a role in spine formation or synapse plasticity, which are impaired in patients with schizophrenia, to compensate for each other. A combined analysis of a large number of genes relevant for synaptic function might provide more robust evidence that rare missense mutations as a whole contribute to pathomechanisms of schizophrenia.

In conclusion, we provide the first evidence that multiple rare (<1%) missense mutations in *KALRN* may be genetic risk factors for schizophrenia. Further studies will be needed to examine the pathogenicity of these mutations from a biologic point of view.

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Supplementary Material

Supplementary material is available at http://schizophreniabulletin.oxfordjournals.org.

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Serotonin 1A receptor gene, schizophrenia and bipolar disorder: An association study and meta-analysis

Taro Kishi [a,*], Tomo Okochi [a], Tomoko Tsunoka [a], Takenori Okumura [a], Tsuyoshi Kitajima [a], Kunihiro Kawashima [a], Yoshio Yamanouchi [a], Yoko Kinoshita [a], Hiroshi Naitoh [a], Toshiya Inada [b], Hiroshi Kunugi [c], Tadafumi Kato [d], Takeo Yoshikawa [e], Hiroshi Ujike [f], Norio Ozaki [g], Nakao Iwata [a]

[a] Department of Psychiatry, Fujita Health University School of Medicine, Toyoake, Aichi 470-1192, Japan
[b] Seiwa Hospital, Institute of Neuropsychiatry, Tokyo, Japan
[c] National Center of Neurology and Psychiatry, Mental Disorder Research, National Institute of Neuroscience, Tokyo, Japan
[d] Laboratory for Molecular Dynamics of Mental Disorders, RIKEN Brain Science Institute, Saitama, Japan
[e] Laboratory for Molecular Psychiatry, RIKEN Brain Science Institute, Saitama, Japan
[f] Department of Neuropsychiatry, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Science, Okayama, Japan
[g] Department of Psychiatry, Nagoya University Graduate School of Medicine, Nagoya 466-8850, Japan

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ABSTRACT

Several investigations have reported associations between serotonin 1A (5-HT1A) receptor and major psychiatric disorders, such as schizophrenia and bipolar disorder (BP), making the 5-HT1A receptor gene (*HTR1A*) a good candidate gene for the pathophysiology of schizophrenia and BP. To evaluate the association between *HTR1A* and schizophrenia and BP, we conducted a case-control study of Japanese population samples with two single- nucleotide polymorphisms (SNPs), including rs6295 (C-1019G) in *HTR1A*. In addition, we conducted a meta-analysis of rs6295, which has been examined in other studies. Using one functional single- nucleotide polymorphism (SNP; rs6295) and one tagging SNP (rs878567), we conducted a genetic association analysis of case-control samples (857 schizophrenic patients, 1028 BP patients and 1810 controls) in the Japanese population. Two association studies for schizophrenia and three association studies for BP, including this study, met our criteria for the meta-analysis of rs6295. We found an association between *HTR1A* and Japanese BP in a haplotype-wise analysis, the significance of which remained after Bonferroni correction. In addition, we detected an association between rs6295 and BP in the meta-analysis (fixed model: $P(Z) = 0.000400$). However, we did not detect an association between *HTR1A* and schizophrenia in the allele/genotype-wise, haplotype-wise or meta-analysis. *HTR1A* may play an important role in the pathophysiology of BP, but not schizophrenia in the Japanese population. In the meta-analysis, rs6295 in *HTR1A* was associated with BP patients.

1. Introduction

Altered serotonergic neural transmission is hypothesised to be a susceptibility factor for schizophrenia (Meltzer et al., 2003; Geyer and Vollenweider, 2008).

The serotonin 1A (5-HT1A) receptor is present in various regions of the brain, including the cortex, hippocampus, amygdala, hypothalamus and septum (Barnes and Sharp, 1999; Aznar et al., 2003; Varnas et al., 2004; Le Francois et al., 2008), and several post-mortem studies reported increased 5-HT1A receptor in the prefrontal cortex of schizophrenic patients (Hashimoto et al., 1991; Hashimoto et al., 1993; Burnet et al., 1996; Simpson et al., 1996; Sumiyoshi et al., 1996). Some antipsychotic drugs, such as aripiprazole, clozapine and perospirone, have partial agonist effects on 5-HT1A receptors (Meltzer et al., 2003; Meltzer and Sumiyoshi, 2008; Sumiyoshi et al., 2008).

Sumiyoshi and colleagues conducted several studies of the effects of the addition of tandospirone, a 5-HT1A receptor agonist, on cognitive function in patients with schizophrenia being treated with antipsychotics (Sumiyoshi et al., 2001a,b). The addition of tandospirone (30 mg day^{-1}), but not placebo, to antipsychotic drugs for 4–6 weeks, was found to improve executive function in one study and verbal learning and memory in another (Sumiyoshi et al., 2007).

Mason and Reynolds reported that one of the major pharmacological therapeutic targets of clozapine is 5-HT1A receptors on cortical glutamatergic neurons (Mason and Reynolds, 1992). These authors suggested that clozapine binding to 5-HT1A receptors may contribute to the mechanism of the unique efficacy of clozapine in schizophrenic patients (Mason and Reynolds, 1992). Recent pharmacogenetics studies

* Corresponding author. Tel.: +81 562 93 9250; fax: +81 562 93 1831.
E-mail address: tarok@fujita-hu.ac.jp (T. Kishi).

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reported that a SNP (C-1019G: rs6295) in the promoter region of the 5-HT1A receptor gene (*HTR1A*), which regulates *HTR1A* transcription (Lemonde et al., 2003; Le Francois et al., 2008), is associated with improved response in negative symptoms with antipsychotics such as risperidone (Reynolds et al., 2006; Wang et al., 2008; Mossner et al., 2009).

These findings suggest a crucial relationship between the 5-HT1A receptor and schizophrenia, and that *HTR1A* is a good candidate for the aetiology of schizophrenia. *HTR1A* (OMIM * 109760, one exon in this genomic region spanning 2.069 kb) is located on 5q11. This genomic region has been shown to be a susceptibility region for schizophrenia (McGuffin et al., 1990; Amos et al., 1991; Hallmayer et al., 1992; Macciardi et al., 1992; Kalsi et al., 1999). Huang and colleagues reported that rs6295 in *HTR1A* was associated with Caucasian schizophrenia patients (108 schizophrenic patients and 107 controls) (Huang et al., 2004). However, their study had a small number of samples. We calculated the statistical power in this research using a genetic power calculator (Purcell et al., 2003), and obtained more than 80% power for the detection of association when we set the genotype relative risk at 2.4 in schizophrenia for rs6295 in *HTR1A* under a multiplicative model of inheritance. On the other hand, Kawanishi and colleagues reported no association between *HTR1A* and Japanese schizophrenic patients (Kawanishi et al., 1998). This study also had a small number of samples (61 schizophrenic patients and 100 controls). In addition, they performed a mutation scan with *HTR1A* and an association analysis between rare variants and schizophrenia. In a power analysis, we obtained more than 80% power for the detection of association when we set the genotype relative risk at 4.8–7.4 in schizophrenia for *HTR1A* under a multiplicative model of inheritance. Thus, it is difficult to evaluate the association of such extremely rare variants from the viewpoint of power (Kawanishi et al., 1998). Several whole genome association studies (GWAS) reported no association between *HTR1A* and schizophrenia in the Caucasian population (O'Donovan et al., 2008; Ng et al., 2009). However, to obtain adequate statistical power in GWAS between common variants and common complex disease, it is thought that more than 10 000 cases and control samples are necessary (Kong et al., 2009; Manolio et al., 2009). Therefore, we examined the association between *HTR1A* and Japanese schizophrenic patients using a sample larger than that in the two original studies (Kawanishi et al., 1998; Huang et al., 2004).

Several investigations reported that the translin-associated factor X gene (*TSNAX*)/disrupted-in-schizophrenia-1 gene (*DISC1*) was associated with schizophrenia and bipolar disorder (BP) (Hennah et al., 2003; Hennah et al., 2005; Thomson et al., 2005; Zhang et al., 2005; Hashimoto et al., 2006; Palo et al., 2007; Schosser et al., in press). We considered that BP and schizophrenia might have common susceptibility genes. Schizophrenia and BP have approximately 80% heritability. Recent whole genome studies have showed that a number of susceptibility regions overlap in schizophrenia and BP (1q32, 10p11-15, 13q32, 18p11.2 and 22q11-13). Schizoaffective disorder is known to be a disorder with both characteristics of schizophrenia and BP. The evidence for this is discussed in more detail in four reviews (Ivleva et al., 2010; Moskvina et al., 2009; O'Donovan et al., 2009; Purcell et al., 2009). Recent GWAS reported that zinc Wnger binding protein 804A (*ZNF804A*) and calcium channel, voltage-dependent, L type, alpha 1C subunits (*CACNA1C*) were associated with schizophrenia and BP (Consortium, 2007; O'Donovan et al., 2008; Green et al., in press; Moskvina et al., 2009; Purcell et al., 2009). This evidence is discussed in more detail in a review by O'Donovan (O'Donovan *et al.*, 2009). A recent GWAS reported that BP and schizophrenia have common susceptibility genes (Moskvina et al., 2009). Another GWAS using Japanese BP samples did not include *HTR1A* (Hattori et al., 2009). When GWAS between common variants and common complex disease are performed, it is thought that more than 10 000 cases and control samples are necessary to obtain adequate statistical power (Kong et al., 2009; Manolio et al., 2009). Because the main problem of these past association studies between *HTR1A* and schizophrenia and BP was small sample sizes, we conducted an analysis of the association of *HTR1A* with schizophrenia and BP using the recently recommended strategy of 'gene-based' association analysis (Neale and Sham, 2004) and larger samples than the original studies (Huang et al., 2004; Sullivan et al., 2009). Recently, it has been suggested that meta-analysis, in which larger samples are examined, is required for conclusive results in genetic studies (O'Donovan et al., 2008). Therefore, we conducted a meta-analysis of rs6295, which has been examined in other genetic research.

2. Materials and methods

2.1. Subjects

715 schizophrenic patients and 1017 BP patients were diagnosed according to Diagnostic and Statistical Manual for Mental Disorders (DSM)-IV criteria with the consensus of at least two experienced psychiatrists on the basis of unstructured interviews and a review of medical records. As many as 142 schizophrenic patients and 11 BP patients underwent the Structured Clinical Interview for DSM-IV disorders (SCID-1). Schizophrenic patients were grouped according to the following DSM-IV subtypes of schizophrenia: paranoid type ($n=221$), disorganised type ($n=224$), Catatonic type ($n=29$), residual type ($n=143$) and undifferentiated type ($n=125$). A total of 1633 controls were also diagnosed according to DSM-IV criteria with the consensus of at least two experienced psychiatrists on the basis of unstructured interviews, of which 46 and 131 controls underwent the Mini-International Neuropsychiatric Interview (MINI) and SCID-1, respectively. None had severe medical complications such as liver cirrhosis, renal failure, heart failure or other Axis-I disorders according to DSM-IV. Controls included hospital staff and medical students. 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); however, another recent study confirmed that there is no population stratification in our control samples (Ikeda et al., 2010). However, our control samples may not be representative of the general population. The study was described to subjects and written informed consent was obtained from each. This study was approved by the Ethics Committee at Fujita Health University and Nagoya University School of Medicine.

2.2. SNPs selection and linkage disequilibrium (LD) evaluation

We first consulted the HapMap database (release#23.a.phase2, Mar 2008, www.hapmap.org, population: Japanese Tokyo: minor allele frequencies (MAFs) of more than 0.05) and included three SNPs (rs6449693, rs878567 and rs1423691) covering *HTR1A* (5′-flanking regions including about 1 kb from the initial exon and about 2 kb downstream (3′) from the last exon: HapMap database contig number chr5: 63287418–63291774). Then, one tagging SNP was selected with the criteria of an 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) of the HAPLOVIEW software (Barrett et al., 2005).

HTR1A has also been reported to have one biologically functional SNP (C-1019G: rs6295) (Albert et al., 1996; Lemonde et al., 2003; Albert and Lemonde, 2004). Rs6295 (C-1019G) in the promoter region regulates *HTR1A* transcription (Lemonde et al., 2003; Le Francois et al., 2008). The C allele is a part of a 26-letter palindrome that connects transcription factors (Deaf-1, Hes1 and Hes5) by nuclear deformed epidermal autoregulatory factor (NUDR), whereas the G allele abolishes repression by NUDR (Lemonde et al., 2003; Le Francois et al., 2008). This would lead to elevated levels of 5-HT1A receptor in the presynaptic raphe nucleus in GG genotypes, compared with CC genotypes (Lemonde et al., 2003; Le Francois et al., 2008). Since no information about rs6295 was shown in the HapMap database, we included this SNP. These two SNPs were then used for the following association analysis.

2.3. SNPs genotyping

We used TaqMan assays (ABI: Applied Biosystems, Inc., Foster City, CA, USA) for all SNPs. One allelic probe was labelled 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, 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.

2.4. Statistical analysis

2.4.1. Case-control study

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-wise association with the chi-square test (SAS/Genetics, release 8.2, SAS Japan Inc., Tokyo, Japan). The distribution of patient characteristics in the schizophrenia group, BP group and healthy control group was analysed using a *t* test or a chi-square test. We found significant differences in gender distribution among these groups ($P_{schizophrenia}=0.00110$ and

$P_{BP}=0.512$); however, there was no difference in age among them ($P_{schizophrenia}<0.001$ and $P_{BP}<0.001$). We therefore performed logistic regression analysis to compare the phenotype of each of the examined SNPs genotypes to adjust for possible confounding. The phenotype (each disorder or healthy control) was the dependent variable, and gender, age at the time of recruitment and each of the examined SNP genotypes were set as the independent variables. The statistical package JMP for Windows was used for logistic regression analysis (JMP 5.0. 1 J, SAS Japan Inc., Tokyo, Japan). A haplotype-wise association analysis was done with a likelihood ratio test using the COCAPHASE2.403 program (Dudbridge, 2003). This software uses the expectation-maximisation (EM) algorithm to estimate the haplotype frequencies of unphased genotype data and standard unconditional logistic regression analysis, applying the likelihood-ratio test under a log-linear model to compare haplotype frequencies between cases and healthy controls. In order to avoid misleading results caused by rare haplotypes, all haplotypes with a frequency ≤5% in both the cases and the controls were declared rare and clumped together for a test of the null hypothesis, using the command line option 'rare 0.05'. This analysis was adjusted for age and gender. To avoid spurious results and correct for multiple testing, we used the permutation test option as provided in the haplotype-wise analysis. Permutation test correction was performed using 10 000 iterations (random permutations). In addition, Bonferroni's correction was used to control inflation of the type I error rate in the marker trait association analysis. For Bonferroni correction, we employed the following numbers of multiple tests: three for each sample set in allele, genotype and haplotype-wise analysis (two examined SNPs), and two for marker-trait association analysis (chi-square test and logistic regression analysis). Therefore, we performed 12 Bonferroni correction tests ($3\times2\times2$) to all *P* values. 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: 0.01 in schizophrenia and BP, and user-defined: 0.025 (Two SNPs were examined in this study. Bonferroni's correction was used to control inflation of the type I error rate). When we calculated the statistical power using the genetic power calculator, we substituted MAFs of cases and healthy controls and number of cases and healthy controls (the MAFs used to calculate the statistical power are shown in Table 1). The significance level for statistical tests was 0.05.

2.4.2. Meta-analysis

To identify studies eligible for the meta-analysis, we searched PubMed citations through March 2009 using the terms '*HTR1A*,' 'serotonin 1A receptor gene,' 'schizophrenia,' 'bipolar disorder,' or 'BP' as keywords. In cases when we could not obtain detailed information about allele frequencies in the article, we referred to the 'SzGene database' (http://www.schizophreniaforum.org/res/sczgene/default.asp) (Allen et al., 2008).

We used the following criteria for selection of eligible studies: (1) be published in peer-reviewed journal, (2) contain independent data, (3) have distribution of genotypes in the control population that was in HWE, (4) have schizophrenia or BP patients diagnosed according to DSM and (5) use healthy individuals as controls in case–control studies.

Cochran's chi-square-based *Q*-statistic test was applied to assess between-study heterogeneity. The significance of the pooled odds ratio (OR) was determined using a *Z*-test. Overall ORs and their 95% confidence intervals (CIs) were estimated under both the Mantel–Haenszel fixed-effects (Mantel and Haenszel, 1959) and DerSimonian–Laird random-effects models (DerSimonian and Laird, 1986). The random-effects model is more conservative than the fixed-effects model and produces a wider CI. When there is no evidence of heterogeneity, the random-effects model will give results similar to the fixed-effects model. Therefore, if it is confirmed that there was no heterogeneity, we could calculate pooled ORs and *P*-values according to the Mantel–Haenszel fixed-effects model. If there was evidence of heterogeneity, we could calculate pooled ORs and *P*-values according to the DerSimonian and Laird random-effects model. Publication bias was evaluated using a funnel plot asymmetry with Egger's test. The statistical significance was set at 0.05. All data were analysed using Comprehensive Meta-Analysis (Ver 2.0). More detailed information about the meta-analysis method is given in our previous articles (Kawashima et al., 2009; Okochi et al., 2009). The significance level for all statistical tests was 0.05.

3. Results

3.1. Case-control study

715 schizophrenic patients, 1017 BP patients and 1633 healthy controls did not undergo structured interviews (more detailed characteristic information about subjects can be seen in Section 2.1.). 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 misdiagnosis in a patient, we promptly excluded the misdiagnosed case to maintain the precision of our sample. Because the diagnosis of one patient in our BP sample was changed to schizoaffective disorder, we excluded this patient from the BP sample. There were no schizophrenia patients whose diagnoses were changed. Detailed information on our samples was provided in previous articles (Kishi et al., 2008a, b, 2009a).

We added 5 randomly selected samples that were genotyped again as a measure of genotyping quality control, and the genotype consistency rates for all two SNPs were 100%.

The LD from rs6449693, rs878567 and rs1423691 was tight in from the HapMap database samples ($r^2=1.00$). However, the LD structure of rs6295 (functional SNP) and rs878567 (tagging SNP) in our healthy control samples was not tight ($r^2=0.160$). Further, the MAFs in our healthy control samples were similar to those in the HapMap database. The LD of rs6295 and rs878567 in our BP samples was looser than in the healthy controls and schizophrenia samples (r^2 value: healthy controls = 0.160, schizophrenia = 0.101 and BP = 0.00600).

3.1.1. Schizophrenia

Genotype frequencies of all SNPs were in HWE. We detected an association between rs878567 and schizophrenia in the allele-wise analysis (Table 1). However, this significance disappeared after multiple testing (Table 1). We did not detect a significant association between *HTR1A* and schizophrenia in the genotype-wise analysis or haplotype-wise analysis with logistic regression adjusted for age and gender (Table 2 and 3). In the power analysis, we obtained power of more than 80% for the detection of association when we set the genotype relative risk at 1.25–1.33 in schizophrenia for *HTR1A*, under a multiplicative model of inheritance.

3.1.2. Bipolar disorder

Genotype frequencies of all SNPs were in HWE. We detected a significant association between *HTR1A* and BP in the allele/genotype-

Table 1
Association analysis of *HTR1A* with schizophrenia and bipolar disorder.

SNP[a]	Phenotype	MAFs[b]	N	Genotype distribution[c]			*P*-value[d,e]			Corrected *P*-value[e,f]	
				M/M	M/m	m/m	HWE	Genotype	Allele	Genotype	Allele
rs6295	Controls	0.247	1810	1024	678	108	0.762				
C>G	Schizophrenia	0.229	857	518	286	53	0.113	0.120	0.146		
	Bipolar disorder	0.283	1028	524	427	77	0.433	**0.0116**	**0.00337**	**0.139**	**0.0404**
rs878567	Controls	0.174	1810	1242	506	62	0.240				
C>T	Schizophrenia	0.149	857	619	220	18	0.764	0.0606	**0.0238**		0.286
	Bipolar disorder	0.225	1028	621	350	57	0.407	**0.00000183**	**0.00000212**	**0.0000220**	**0.0000254**

[a] Major allele>minor allele.
[b] MAFs: minor allele frequencies.
[c] M: major allele, m: mimor allele.
[d] Hardy–Weinberg equilibrium.
[e] Bold numbers represent significant *P*-value.
[f] Calculated by Bonferroni correction (12 times).

Table 2
Logistic regression analysis of single markers in *HTR1A* with schizophrenia and bipolar disorder.

SNP[a]	Genotype	Schizophrenia			Bipolar disorder		
		OR[b]	95% CI[c]	*P*-value	OR[b]	95% CI[c]	*P*-value
rs6295	CC (reference)						
C>G	CG	2.09	0.932–4.59	0.0682	1.03	0.706–1.50	0.886
	GG	1.34	0.534–3.18	0.518	1.08	0.742–1.57	0.702
rs878567	CC (reference)						
C>T	CT	1.98	0.105–1.20	0.0667	1.00	0.648–1.54	0.993
	TT	2.91	0.589–16.3	0.201	1.90	0.930–3.89	0.0782

Reference genotypes are common genotype. Adjustment for age and gender.
[a] Major allele>minor allele.
[b] OR: odds ratio.
[c] CI: confidence interval.

wise analysis with the chi-square test (Table 1), but not with logistic regression adjusted for age and gender (Table 2). In addition, we found an association between *HTR1A* and BP in the haplotype-wise analysis adjusted for age and gender (Table 3). In the power analysis, we obtained power of more than 80% for the detection of association when we set the genotype relative risk at 1.20–1.26 in BP for *HTR1A*, under a multiplicative model of inheritance.

3.2. Meta-analysis

3.2.1. Schizophrenia

In the meta-analysis, two association studies, including our study, met our criteria for rs6295 (Table 4). We found significant heterogeneity among ORs ($P(Q) = 0.000142$). The pooled OR derived from all studies comprising 965 patients and 1964 healthy control subjects did not indicate a significant association (random model: pooled OR = 0.793, 95% CI = 0.387–1.623, $P(Z) = 0.526$) (Fig. 1).

3.2.2. Bipolar disorder

In the meta-analysis, three association studies, including our study, met our criteria for rs6295 (Table 4).We did not find significant heterogeneity among ORs ($P(Q) = 0.789$). The pooled OR derived from all studies comprising 1148 patients and 1964 healthy control subjects indicated a significant association (fixed model: pooled OR = 0.794, 95% CI = 0.641–0.983, $P(Z) = 0.0344$) (Fig. 1). No publication bias was found ($t = 0.656$, $p = 0.536$).

4. Discussion

Although we detected an association between rs878567 and schizophrenia in the allele-wise analysis, this significance disappeared after multiple testing. We did not detect a significant association between *HTR1A* and schizophrenia in the genotype-wise analysis or haplotype-wise analysis with logistic regression adjusted for age and gender (Tables 2 and 3). Therefore, our results suggest that *HTR1A* does not play a role in the pathophysiology of schizophrenia in the Japanese population. On the other hand, in the single-marker association study, we detected a significant association between *HTR1A* and BP with the chi-square test. However, this association may have been due to biased samples, which were unmatched for gender. We therefore performed a logistic regression analysis to compare the phenotypes of each of the examined SNP genotypes, using several clinical factors as other independent variables to adjust for possible confounding. Although we did not detect an association between the two SNP genotypes in *HTR1A* and BP with logistic regression analysis, we found an association between *HTR1A* and BP in the haplotype-wise analysis adjusted for age and gender. Our results, therefore, suggest that *HTR1A* plays a role in the pathophysiology of BP in the Japanese population.

We detected an association between *HTR1A* and BP, but not schizophrenia. Ivleva and colleagues suggested that genes, which are associated with schizophrenia but not BP, may play a major role in the pathophysiology of psychosis. Genes associated with BP, but not schizophrenia, may also play a major role in the pathophysiology of mood dysregulation (Ivleva et al., 2010). Considering the above, *HTR1A* was considered to have an influence in mood regulation. However, we reported that *HTR1A* was associated with methamphetamine-induced psychosis in the Japanese population (Kishi et al., 2009c). We also detected a marginal association between *HTR1A* and schizophrenia in the Japanese population. Considering the neurodevelopmental model of the pathophysiology of both disorders, *HTR1A* may relate to neurodevelopment (Ivleva et al., 2010). It will be necessary to conduct further studies, including environmental factors.

The LD of rs6295 and rs878567 in our BP samples was looser than in controls and schizophrenia samples (r^2 value: controls = 0.160, schizophrenia = 0.101 and BP = 0.00600). Although we detected no association between *HTR1A* and BP in the single-marker association analysis, it may be that the difference in LD reflects the haplotype-wise analysis.

We detected an association between rs6295 and BP in the meta-analysis. The studies of Huang and colleagues and Sullivan and colleagues found no association between rs6295 in *HTR1A* and BP (Huang et al., 2004; Sullivan et al., 2009). However, in our meta-analysis, we detected an association between *HTR1A* and BP. The following may be causes for these different results: First, because the samples in the two original studies were small, there is a possibility of type II errors in their studies. Second, although we did not detect significant heterogeneity among ORs ($P(Q) = 0.789$), the MAFs of the studies included in the meta-analysis were each different. Third, because there are few samples to use in meta-analysis, the significant associations between *HTR1A* and BP in the case-control study and the meta-analysis also may have been due to type I errors. Further, different screening methods were used in each study for the samples included in this meta-analysis. Rs 6295 is associated with disorders, including major depressive disorder (Lemonde et al., 2003; Parsey et al., 2006; Anttila et al., 2007; Kraus et al., 2007; Neff et al., 2009),

Table 3
Haplotype-wise analysis of *HTR1A*.

Haplotype rs6295–rs878567	Phenotype	Number of subjects	Individual haplotype frequency	OR[a]	95% CI[b]	Individual *P*-value[c]	Phenotype	Global *P*-value[c]	Corrected Global *P*-value[c,d]
C-C (reference)	Controls	1372	0.814						
	Schizophrenia	635	0.788	1.20	0.971–1.47	0.0921			
	Bipolar disorder	736	0.761	1.38	1.19–1.59	**0.0000176**	Schizophrenia	0.0935	
G-C	Controls	314	0.186				Bipolar disorder	**0.0000203**	**0.000244**
	Schizophrenia	171	0.212	1.20	0.971–1.47	0.0921			
	Bipolar disorder	231	0.239	1.38	1.19–1.59	**0.0000176**			

Adjustment for age and gender.
[a] OR: odds ratio.
[b] CI: confidence interval.
[c] Bold numbers represent significant *P*-value.
[d] Calculated by Bonferroni correction (12 times).

Table 4
Studies included in meta-analysis for rs6295.

Author	Yesr	Ethnic	Diagnostic system	N[a]			C allele[a]			G allele[a]		
				SCZ	BP	CON	SCZ	BP	CON	SCZ	BP	CON
Kishi		Asian	DSM-IV	857	1028	1810	1036	1475	2726	392	581	894
Huang	2004	Caucasian	DSM-IV	108	88	107	86	81	118	130	95	96
Sullivan	2009	Caucasian	DSM-IV	-	32	47	-	31	57	-	31	35

a BP: bipolar disorder SCZ: schizophrenia CON: controls.

panic disorder (Strobel et al., 2003) and antidepressant response in major depressive disorder (MDD) (Lemonde et al., 2004; Serretti et al., 2004; Arias et al., 2005; Hong et al., 2006; Parsey et al., 2006; Yu et al., 2006). Recently, we reported that *HTR1A* was associated with MDD in a meta-analysis (Kishi et al., 2009b). In addition, we reported that *HTR1A* was associated with Japanese methamphetamine-induced psychosis patients (Kishi et al., 2009c). Our previous studies selected the same variant as this study. Huang and colleagues reported that rs6295 was associated with schizophrenia (Huang et al., 2004). However, we could not replicate the association between rs6295 and schizophrenia found in their study (Huang et al., 2004). We also found no association between rs6295 and schizophrenia in the meta-analysis. Recent studies reported that rs6295 was associated with improvement in negative symptoms from anti-psychotics such as risperidone (Reynolds et al., 2006; Wang et al., 2008; Mossner et al., 2009) and that 5-HT1A receptor agonists such as tandospirone produced improvements in cognitive impairment in schizophrenia (Sumiyoshi et al., 2001a, 2007; Meltzer and Sumiyoshi, 2008). We assume that quantitative traits, including negative symptoms and cognitive symptoms for schizophrenic patients, will be key features in assessing the genetic contribution of *HTR1A* to schizophrenia.

The heterogeneity in this meta-analysis for schizophrenia may have resulted from: (1) different ancestries (Asian population vs. Caucasian population), (2) the small size of the overall sample included in the meta-analysis (851 schizophrenic patients and 911 control subjects) and (3) the inclusion of different samples in the screening method in the meta-analysis (Shi et al., 2008).

A few points of caution should be mentioned with respect to our results. First, our results in the case-control study and meta-analysis may be due to biased samples, such as unmatched age and gender samples, or to small sample sizes such as case-control genetic association studies (Shi et al., 2009). Therefore, the significant associations between *HTR1A* and BP in the case-control study and meta-analysis may be due to type I errors. However, we performed a logistic regression analysis to compare the phenotypes of each of the examined SNP genotypes, using several clinical factors as other independent variables to adjust for possible confounding. Second, we did not perform a mutation scan of *HTR1A*. Because we consider it to be difficult to evaluate the association of such extremely rare variants from the viewpoint of statistical power, a replication study using a larger sample is required for conclusive results. Third, we did not include GWAS data regarding rs6295 in this meta-analysis. Fourth, we combined Asian and Caucasian populations in this meta-analysis. However, we did not detect significant heterogeneity for BP samples. In addition, we included the different samples of the screening method in the meta-analysis.

5. Conclusion

In conclusion, our results suggest that *HTR1A* may play a role in the pathophysiology of Japanese BP, but not Japanese schizophrenia. Further, in the meta-analysis, *HTR1A* was associated with BP patients.

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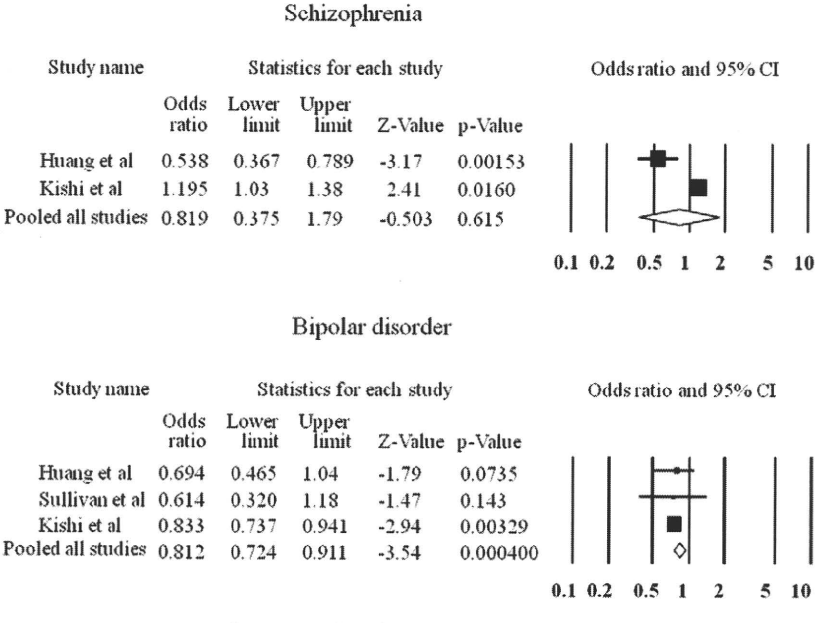

Fig. 1. Forest plots of OR with 95% CI for rs6295.

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Full length article

Serotonin 6 receptor gene is associated with methamphetamine-induced psychosis in a Japanese population

Taro Kishi[a,*], Yasuhisa Fukuo[a], Tomo Okochi[a], Tsuyoshi Kitajima[a], Kunihiro Kawashima[a], Hiroshi Naitoh[a], Hiroshi Ujike[b,c], Toshiya Inada[b,d], Mitsuhiko Yamada[b,e], Naohisa Uchimura[b,f], Ichiro Sora[b,g], Masaomi Iyo[b,h], Norio Ozaki[b,i], Nakao Iwata[a,b]

[a] Department of Psychiatry, Fujita Health University School of Medicine, Toyoake, Aichi 470-1192, Japan
[b] Japanese Genetics Initiative for Drug Abuse, Japan
[c] Department of Neuropsychiatry, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama 700-8558, Japan
[d] Department of Psychiatry, Seiwa Hospital, Institute of Neuropsychiatry, Tokyo 162-0851, Japan
[e] National Institute of Mental Health, National Center of Neurology and Psychiatry, Ichikawa 272-0827, Japan
[f] Department of Neuropsychiatry, Kurume University School of Medicine, Kurume 830-0011, Japan
[g] Department of Psychobiology, Department of Neuroscience, Tohoku University Graduate School of Medicine, Sendai 980-8576, Japan
[h] Department of Psychiatry, Chiba University Graduate School of Medicine, Chiba 260-8677, Japan
[i] Department of Psychiatry, Nagoya University Graduate School of Medicine, Nagoya 466-8850, Japan

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ABSTRACT

Background: Altered serotonergic neural transmission is hypothesized to be a susceptibility factor for psychotic disorders such as schizophrenia. The serotonin 6 (5-HT6) receptor is therapeutically targeted by several second generation antipsychotics, such as clozapine and olanzapine, and D-amphetamine-induced hyperactivity in rats is corrected with the use of a selective 5-HT6 receptor antagonist. In addition, the disrupted prepulse inhibition induced by D-amphetamine or phencyclidine was restored by 5-HT6 receptor antagonist in an animal study using rats. These animal models were considered to reflect the positive symptoms of schizophrenia, and the above evidence suggests that altered 5-HT6 receptors are involved in the pathophysiology of psychotic disorders. The symptoms of methamphetamine (METH)-induced psychosis are similar to those of paranoid type schizophrenia. Therefore, we conducted an analysis of the association of the 5-HT6 gene (*HTR6*) with METH-induced psychosis.
Method: Using five tagging SNPs (rs6693503, rs1805054, rs4912138, rs3790757 and rs9659997), we conducted a genetic association analysis of case–control samples (197 METH-induced psychosis patients and 337 controls) in the Japanese population. The age and sex of the control subjects did not differ from those of the methamphetamine dependence patients.
Results: rs6693503 was associated with METH-induced psychosis patients in the allele/genotype-wise analysis. Moreover, this association remained significant after Bonferroni correction. In the haplotype-wise analysis, we detected an association between two markers (rs6693503 and rs1805054) and three markers (rs6693503, rs1805054 and rs4912138) in *HTR6* and METH-induced psychosis patients, respectively.
Conclusion: *HTR6* may play an important role in the pathophysiology of METH-induced psychosis in the Japanese population.

1. Introduction

Abnormalities in serotonergic neural transmission are hypothesized to be a susceptibility factor for psychotic disorders, including schizophrenia and drug-related conditions such as methamphetamine (METH)-induced psychosis (Geyer and Vollenweider, 2008; Meltzer et al., 2003). Recently, we reported that the serotonin 1A receptor gene (*HTR1A*) was associated with Japanese METH-induced psychosis patients (Kishi et al., 2010a). The serotonin 6 (5-HT6) receptor is targeted by several second generation antipsychotics, such as clozapine and olanzapine (Bymaster et al., 2001). Therefore, the 5-HT6 receptor is known as a therapeutic target in psychotic disorders. The 5-HT6 receptor is expressed in the frontal cortex, hippocampus, amygdala and striatum, where it is prevalent on gamma-amino butyric acid (GABA)-ergic neurones and activation indirectly regulates a variety of neurotransmitters,

* Corresponding author. Tel.: +81 562 93 9250; fax: +81 562 93 1831.
E-mail address: tarok@fujita-hu.ac.jp (T. Kishi).

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including serotonin (5-HT), acetylcholine, glutamate and dopamine (Chalmers and Watson, 1991; Fone, 2008; King et al., 2008). 5-HT6 receptor antagonist was shown to produce an increase in amphetamine-induced locomotor activity and also to augment amphetamine-induced increases in extracellular dopamine within the rat prefrontal cortex (Frantz et al., 2002). Also, 5-HT6 antisense injections in the rat brain decreased 5-HT release in the prefrontal cortex (Yoshioka et al., 1998a,b). In addition, 5-HT6 receptor antagonist has been reported to enhance glutamatergic, noradrenergic, and dopaminergic neurotransmission in the rat striatal and frontal cortex (Dawson et al., 2000). These altered neurotransmitters suggest that 5-HT6 might be involved in the pathophysiology of psychotic disorders (Lang et al., 2007). D-amphetamine-induced hyperactivity in rats was corrected with the use of a selective 5-HT6 receptor antagonist. In addition, disrupted prepulse inhibition induced by D-amphetamine or phencyclidine (PCP) in rats was restored by 5-HT6 receptor antagonist. These animal models were considered to reflect the positive symptoms of schizophrenia. Dawson and colleagues also suggested that the 5-HT6 receptor has a regulatory function on several neurotransmitters, when dopaminergic neurotransmission in the rat brain is enhanced by amphetamine. Patients with schizophrenia show cognitive deficits in verbal memory, working memory, attention, processing speed and other areas. Several studies have reported that the influence on cognitive function differs with the kind of antipsychotic (Carter et al., 2008; Crespo-Facorro et al., 2009; Cuesta et al., 2009; Davidson et al., 2009; Keefe et al., 2006; Lindenmayer et al., 2007; Purdon et al., 2000, 2003; Riedel et al., 2007; Woodward et al., 2005). Rodefer and colleagues reported that abnormalities in executive function induced by PCP in rats were restored by the 5-HT6 receptor antagonist SB 271046 but not risperidone, clozapine, olanzapine, or the 5-HT2A receptor antagonist M100907. 5-HT6 receptors are located primarily in the striatum, olfactory tubercles, nucleus accumbens and hippocampus. It is known that functions of serotonin 6 receptors include modulation of cholinergic and dopaminergic neurotransmission. They suggested that the combination of 5-HT6 antagonistic activity and the absence of antimuscarinic activity may play an important role in the pharmacological profile for improved antipsychotic drugs for cognitive dysfunction in schizophrenia. Tsai et al. reported that the 5-HT6 receptor gene (*HTR6*) was associated with schizophrenia in the Chinese population. These facts suggest a crucial relationship between the 5-HT6 receptor and schizophrenia, and that *HTR6* is an adequate candidate for the etiology of schizophrenia. *HTR6* (OMIM * 601109, 2 exons in this genomic region spanning 15.076 kb) is located on 1p36-35.

The symptoms of METH-induced psychosis are similar to those of paranoid type schizophrenia (Sato et al., 1992), and it may be that METH-induced psychosis and schizophrenia have common susceptibility genes (Bousman et al., 2009). In support of this hypothesis, we reported that the V-act murine thymoma viral oncogene homologue 1 (AKT1) gene was associated with METH-induced psychosis (Ikeda et al., 2006) and schizophrenia (Ikeda et al., 2004) in the Japanese population. Therefore, we conducted an analysis of the association of *HTR6* with METH-induced psychosis, using the recently recommended strategy of 'gene-based' association analysis (Neale and Sham, 2004).

2. Materials and methods

2.1. Subjects

The subjects in the association analysis were 197 METH-induced psychosis patients (164 males (83.2%) and 33 females (16.8%); mean age ± standard deviation (SD) 37.6 ± 12.2 years) and 337 healthy controls (271 males (80.4%) and 66 females (19.6%); 37.6 ± 14.3 years). The age and sex of the control subjects did not differ from those of the methamphetamine dependence patients. All subjects were unrelated to each other, ethnically Japanese, and lived in the central area of Japan. All the patients examined in this study suffered not only from METH-induced psychosis (ICD-10-DCR criteria (F15.5)) but also METH dependence (ICD-10-DCR criteria (F15.2)). Consensus diagnoses of METH-induced psychosis were made by the trained psychiatrists according to the ICD-10-DCR criteria (F15.5) on the basis of interviews and medical records. The patients with methamphetamine psychosis in the present study usually showed predominant positive symptoms such as delusion and hallucination. We excluded cases in which the predominant symptoms were of the negative and/or disorganized type in order to maintain the homogeneity of the patient group. The patients were categorized by prognosis into two types, a transient type and a prolonged type, based on the duration of the psychotic state after METH discontinuance. The transient type of patient was defined as a patient whose symptoms improved within 1 month after METH discontinuance and the start of treatment with antipsychotic, and the prolonged type was defined as a patient whose psychosis continued for more than 1 month after METH discontinuance and the start of treatment with an antipsychotic. In this study, there were 112 patients (56.9%) with the transient type and 85 patients (43.1%) with the prolonged type patients of METH psychosis. Cannabinoids were the most frequency abused drugs (31.4%), followed by cocaine (9.09%), LSD (9.09%), opioids (7.69%), and hypnotics (7.69%). Subjects with METH use disorder were excluded if they had a clinical diagnosis of psychotic disorder, mood disorder, anxiety disorder, or eating disorder. More detailed characterizations of these subjects have been published elsewhere (Kishi et al., 2008a, 2010a). All healthy controls were also psychiatrically screened based on unstructured interviews including current and past psychiatric history. None had severe medical complications such as liver cirrhosis, renal failure, heart failure or other Axis-I disorders according to ICD-10DCR.

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 the Nagoya University School of Medicine, and by each participating member of the Institute of the Japanese Genetics Initiative for Drug Abuse (JGIDA).

2.2. SNPs selection and linkage disequilibrium (LD) evaluation

We first consulted the HapMap database (release#27. February 2009, www.hapmap.org, population: Japanese Tokyo: minor allele frequencies (MAFs) of more than 0.05) and included 10 SNPs covering *HTR6* (5′-flanking regions including about 4 kb from the initial exon and about 2 kb downstream (3′) from the last exon: HapMap database contig number Chr1:19,864,367.19878641). Then five tagging SNPs (rs6693503, rs1805054, rs4912138, rs3790757 and rs9659997) were selected with the criteria of an 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) of the HAPLOVIEW software (Barrett et al., 2005). These 5 tagging SNPs were then used for the following association analysis.

2.3. SNPs genotyping

We used TaqMan assays (Applied Biosystems, Inc., Foster City, CA,) for both SNPs. Detailed information, including primer sequences and reaction conditions, is available on request.

2.4. 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-wise associations with the Chi-square test (SAS/Genetics, release 8.2, SAS Japan Inc., Tokyo, Japan), and a haplotype-wise association analysis was conducted with a likelihood ratio test using the COCAPHASE2.403 program (Dudbridge, 2003). We used the permutation test option as provided in the haplotype-wise analysis to avoid spurious results and correct for multiple testing. Permutation test correction was performed using 10,000 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 individual haplotype-wise analysis. We had already performed a permutation test for global *P*-value in the haplotype-wise analysis. We also performed an explorative analysis of subjects divided by clinical diagnosis (transient type vs prolonged type). The significance level for all statistical tests was 0.05.

3. Results

The result of LD evaluation using our control samples can be seen Fig. 1. However, the LD structure was not tight in any of the tagging SNPs in our control samples. Genotype frequencies of all SNPs were in HWE (Table 1). Rs6693503 was associated with METH-induced psychosis patients in the allele/genotype-wise analysis (P allele = 0.00228 and P genotype = 0.000214) (Table 1). Moreover, the significance of these associations remained after Bonferroni correction (P allele = 0.00107 and P genotype = 0.0114) (Table 1). In addition, we detected an association

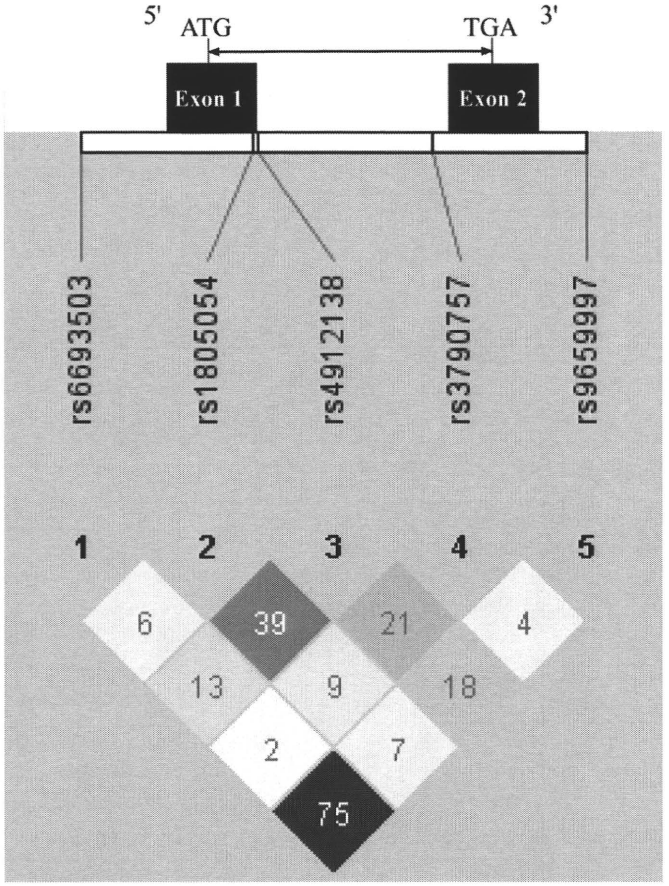

Fig. 1. LD evaluation and tagging SNPs in *HTR6*. ATG is the start codon and TGA is the stop codon. Vertical bars represent exons. Color scheme is based on r^2 value. Other information can be seen at the Haploview website.

between rs6693503 and rs1805054 in *HTR6* and METH-induced psychosis patients in the haplotype-wise analysis ($P=0.0314$) (Table 2). Rs6693503–rs1805054–rs4912138 was associated with METH-induced psychosis patients in the haplotype-wise analysis ($P<0.0001$) (Table 2). Haplotype analysis for rs6693503 and rs1805054 indicated three common haplotypes (A–C, A–T and G–C). Among them, the G–C haplotype was highly prevalent in subjects with METH-induced psychosis (corrected *P*-value = 0.0279) (Table 3). Haplotype analysis for rs6693503, rs1805054 and rs4912138 also indicated five common haplotypes (A–C–A, A–C–G, A–T–G, G–C–A and G–C–G). Among them, the G–C–A haplotype was highly prevalent in subjects with METH-induced psychosis (corrected *P*-value <0.0001) (Table 4).

Subcategory analyses were conducted on a clinical parameter (prognosis of psychosis) (Table 5). rs3790757 was associated with prognosis of psychosis in METH-induced psychosis patients in the allele-wise analysis (Table 5). However, the significance of this association disappeared after Bonferroni correction (Table 5). We also found no association between *HTR6* and the clinical features of patients with METH psychosis in the haplotype-wise analysis (Table 6).

4. Discussion

We found associations between rs6693503 in *HTR6*, which is located in a promoter region, and Japanese METH-induced psychosis patients in the allele/genotype-wise analysis. Therefore, we considered that the association between *HTR6* haplotype and METH-induced psychosis patients in this study might be reflected in rs6693503, and reasoned that *HTR6* may play an important role in the pathophysiology of METH-induced psychosis in the Japanese population. However, our samples are small. Although Bonferroni's correction was used to control inflation of the type I error rate, there is a possibility of type I error in these results.

Recently, we reported that *HTR1A* was associated with METH-induced psychosis in the Japanese population. To evaluate the interactions with each SNP in *HTR1A* and *HTR6* (197 METH-induced psychosis patients and 337 controls), we analyzed the gene–gene interactions with the use of the Multifactor Dimensionality Reduction (MDR) method (Hahn et al., 2003). This analysis, however, revealed no interactions with METH-induced psychosis (data not shown).

Several animal studies reported that the 5-HT6 receptor antagonist restored behavior that had become abnormal as a result of amphetamine (Dawson et al., 2000; Frantz et al., 2002; Yoshioka et al., 1998a,b). These findings suggest that altered serotonergic neural transmission caused by abnormalities in 5-HT6 receptors may be involved in the development of METH-induced psychosis. However, all the patients examined in this study suffered not only from METH-induced psychosis but also METH dependence. METH stimulates the release of dopamine in the mesolimbic system (Munzar et al., 2004), and dopamine is in turn involved in the reinforcing action of many addictive drugs such as METH (Vocci et al., 2005). The reciprocal action of these mechanisms may contribute to METH

Table 1
Association analysis of *HTR6* with methamphetamine-induced psychosis.

SNP ID[a]	Phenotype[b]	MAF[c]	*N*	Genotype distribution[d]			*P*-value[e,f]			Corrected *P*-value[f,g]	
				M/M	M/m	m/m	HWE	Genotype	Allele	Genotype	Allele
rs6693503	Control	0.134	337	257	70	10	0.0602				
A>G	METH-induced psychosis	0.221	197	124	59	14	0.0688	**0.00228**	**0.000214**	**0.0114**	**0.00107**
rs1805054	Control	0.292	337	162	153	22	0.0738				
C>T	METH-induced psychosis	0.309	197	90	92	15	0.195	0.816	0.550		
rs4912138	Control	0.493	337	87	170	80	0.864				
A>G	METH-induced psychosis	0.484	197	55	93	49	0.441	0.764	0.879		
rs3790757	Control	0.191	337	220	105	12	0.903				
C>T	METH-induced psychosis	0.238	197	115	70	12	0.758	0.179	0.0672		
rs9659997	Control	0.157	337	243	82	12	0.132				
C>T	METH-induced psychosis	0.144	197	146	45	6	0.280	0.868	0.581		

[a] Major allele > minor allele.
[b] METH-induced psychosis: methamphetamine-induced psychosis patients.
[c] MAF, minor allele frequency.
[d] M, major allele; m, minor allele.
[e] HWE, Hardy–Weinberg equilibrium.
[f] Bold numbers represent significant *P*-value.
[g] Calculated by Bonferroni correction (5 tests).

Table 2
Haplotype-wise analysis between *HTR6* and methamphetamine-induced psychosis.

	Global *P*-value[a]			
	2 window	3 window	4 window	5 window
rs6693503				
	0.0314			
rs1805054		**<0.0001**		
	0.531		0.141	
rs4912138		0.203		0.138
	0.167		0.328	
rs3790757		0.201		
	0.180			
rs9659997				

[a] Bold numbers represent significant global *P*-value.

Table 3
Haplotype-wise analysis between rs6693503–rsl 805054 in HTR6 and methamphetamine-induced psychosis.

rs6693503–rsl805054	Phenotype[a]	Individual haplotype frequency	OR[b]	95% CI[c]	Individual *P*-value[d]	Corrected *P*-value[d,e]
A–C	Control	0.574	1.00	1.00–1.00	0.0824	
	METH-induced psychosis	0.519				
A–T	Control	0.292	1.09	0.813–1.46	0.863	
	METH-induced psychosis	0.287				
G–C	Control	0.134	1.61	1.13–2.29	**0.00931**	**0.0279**
	METH-induced psychosis	0.194				

[a] METH-induced psychosis: methamphetamine-induced psychosis patients.
[b] OR, odds ratio.
[c] 95% CI, 95% confidence interval.
[d] Bold numbers represent significant *P*-value.
[e] Calculated by Bonferroni's correction (3 tests).

Table 4
Haplotype-wise analysis between rs6693503–rsl805054–rs4912138 in HTR6 and methamphetamine-induced psychosis.

rs6693503–rsl805054–rs4912138	Phenotype[a]	Individual haplotype frequency	OR[b]	95% CI[c]	Individual *P*-value[d]	Corrected *P*-value[d,e]
A–C–A	Control	0.488	1.00	1.00–1.00	0.227	
	METH–induced psychosis	0.444				
A–C–G	Control	0.0862	0.948	0.574–1.57	0.351	
	METH-induced psychosis	0.0744				
A–T–G	Control	0.292	1.07	0.788–1.44	0.0772	
	METH-induced psychosis	0.284				
G–C–A	Control	0.00161	54.6	7.36–405	**<0.0001**	**<0.0001**
	METH-induced psychosis	0.0798				
G–C–G	Control	0.132	0.978	0.649–1.47	0.646	
	METH-induced psychosis	0.118				

[a] METH-induced psychosis: methamphetamine-induced psychosis patients.
[b] OR, odds ratio.
[c] 95% CI, 95% confidence interval.
[d] Bold numbers represent significant *P*-value.
[e] Calculated by Bonferroni's correction (5 tests).

Table 5
Association between HTR6 and clinically subcategorized METH-induced psychosis.

SNP ID[a]	Phenotype	MAF[b]	N	Genotype distribution[c]			*P*-value[d,e]			Corrected *P*-value[f]
				MM	M/m	m/m	HWE	Genotype	Allele	Allele
rs6693503	Transient type	0.223	112	70	34	8	0.187			
A > G	Prolonged type	0.217	85	54	25	6	0.209	0.989	0.895	
rs1805054	Transient type	0.299	112	52	5338	7	0.173			
C > T	Prolonged type	0.324	85	38	39	8	0.657	0.709	0.604	
rs4912138	Transient type	0.469	112	31	57	24	0.817			
A > G	Prolonged type	0.506	85	24	36	25	0.159	0.370	0.465	
rs3790757	Transient type	0.281	112	59	43	10	0.594			
C > T	Prolonged type	0.182	85	56	27	2	0.547	0.0650	**0.0225**	0.113
rs9659997	Transient type	0.156	112	80	29	3	0.849			
C > T	Prolonged type	0.129	85	66	16	3	0.129	0.491	0.453	

[a] Major allele > minor allele.
[b] MAF, minor allele frequency.
[c] M, major allele; m, minor allele.
[d] HWE, Hardy–Weinberg equilibrium.
[e] Bold numbers represent significant *P*-value.
[f] Calculated by Bonferroni correction (5 tests).

Table 6
Association between *HTR6* and clinically subcategorized METH-induced psychosis (transient type vs prolonged type).

	Global *P*-value			
	2 window	3 window	4 window	5 window
rs6693503				
	0.796			
rs1805054		0.942		
	0.756		0.426	
rs4912138		0.104		0.259
	0.0709		0.0918	
rs3790757		0.0783		
	0.0669			
rs9659997				

dependence. In addition, increased dopamine in the mesolimbic system is considered to produce psychotic symptoms such as hallucinations and delusions (Laviolette, 2007). Therefore, patients with METH dependence may develop psychotic symptoms in the long term revealed in the long term may cause the psychotic symptoms.

We reported that the AKT1 gene was associated with METH-induced psychosis (Ikeda et al., 2006) and schizophrenia (Ikeda et al., 2004) in the Japanese population. These findings may indicate that METH-induced psychosis and schizophrenia have common susceptibility genes (Bousman et al., 2009). In this study, we found an association between *HTR6* was associated with METH-induced psychosis in the Japanese population. Tsai et al. reported that *HTR6* was associated with schizophrenia in the Chinese population (Tsai et al., 1999a). However, other studies, including two studies using Japanese samples, showed no association (Chiu et al., 2001; Ohmori et al., 2001; Shinkai et al., 1999; Vogt et al., 2000). Past these association studies using the "candidate gene approach" had the serious problem of small samples (Chiu et al., 2001; Ohmori et al., 2001; Shinkai et al., 1999; Tsai et al., 1999a; Vogt et al., 2000). Therefore, a replication study of the association between *HTR6* and schizophrenia will be needed using the recently recommended strategy of 'gene-based' association analysis including rs1805054 (C267T) (Neale and Sham, 2004), and larger samples than the original studies (Chiu et al., 2001; Shinkai et al., 1999; Tsai et al., 1999a; Vogt et al., 2000).

Rs1805054 (C267T) is a silent mutation, which does not cause a change in the amino acid sequence (Kohen et al., 1996). Several studies have suggested that it may affect translation through the secondary structure and stability of the mRNA, or be in LD with a functional variant. Therefore, genetic association studies of psychiatric disorders such as schizophrenia (Chiu et al., 2001; Shinkai et al., 1999; Tsai et al., 1999a; Vogt et al., 2000), bipolar disorder (Hong et al., 1999), major depressive disorder (MDD) (Hong et al., 1999) and Alzheimer's disease (Alvarez-Alvarez et al., 2003; Kan et al., 2004; Liu et al., 2001; Tsai et al., 1999b), and pharmacogenic studies of the antipsychotic response in schizophrenia (Ikeda et al., 2008) have so far investigated only rs1805054 in *HTR6*. However, we found an association of rs6693503 but not rs1805054 (C267T) with METH-induced psychosis patients in this single marker association study.

Rs6693503 is located in the promoter region in *HTR6*, and two common haplotypes (rs6693503–rs1805054: G–C and rs6693503–rs1805054–rs4912138: G–C–A) were associated with METH-induced psychosis patients. Because of the possibility that rs6693503 or these haplotypes influence biological function in the brain, we suggest that functional analysis of these variants should be performed in future studies.

Patients with schizophrenia show cognitive deficits in verbal memory, working memory, attention, processing speed and other areas (Green, 1996). Cognitive impairment in these patients is significantly associated with insight, social skills, and delay in returning to normal life (Monteiro et al., 2008). Abnormalities in cognitive function are considered to be endophenotypes for schizophrenia (Walters and Owen, 2007). Such impairments may be partially mediated by serotonin receptors, including the 5-HT6 receptor. Several genetic studies reported that the *HTR6* was associated with Alzheimer's disease. Therefore, investigations on the relationship between cognitive function in schizophrenia and variants of *HTR6* are needed.

A few points of caution should be mentioned with respect to our results. Firstly, the positive association may be due to small sample size. Ideal samples for this study are METH use disorder samples with and without psychosis. Because we had only a few METH use disorder samples without psychosis, and we wanted to avoid statistical error, we did not perform an association analysis with these samples. Secondly, we did not include a mutation scan to detect rare variants. We designed the study based on the common disease-common variants hypothesis (Chakravarti, 1999). However, Weickert and colleagues have shown associations between a common disease such as schizophrenia and rare variants (Weickert et al., 2008). If the genetic background of METH-induced psychosis is described by the common disease-rare variants hypothesis, further investigation such as medical resequencing using larger samples will be required. However, statistical power is needed to evaluate the association of rare variants. Lastly, our subjects did not undergo structured interviews. 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 (Kishi et al., 2008a,b, 2009, 2010a,b). In addition, when we found misdiagnosis in a patient, we promptly excluded the misdiagnosed case to maintain the precision of our sample. To overcome these limitations, a replication study using larger samples or samples of other populations will be required for conclusive results.

In conclusion, our results suggest that *HTR6* may play a major role in the pathophysiology of METH-induced psychosis in the Japanese population. However, because we did not perform a mutation scan of *HTR6*, a replication study using a larger sample may be required for conclusive results.

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Conflict of interest

All authors declare that they have no conflict of interest.

Contributors

All authors contributed to and have approved the final manuscript.

Ethical approval

Written informed consent was obtained from all subjects. This study was approved by the ethics committees at Fujita Health University, Nagoya University Graduate School of Medicine and each participating member of the Institute of the Japanese Genetics Initiative for Drug Abuse (JGIDA).

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