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ORIGINAL ARTICLE

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

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

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

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

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

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

MATERIALS AND METHODS

Subjects

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

Tagging SNP selection, SNP genotyping and quality control

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

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

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

Statistical analyses

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

RESULTS

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

DISCUSSION

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

Table 1 Linkage disequilibrium analysis of *USP46*

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

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

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

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

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

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

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

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

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

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

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

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

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