TABLE 1. Diagnostic Criteria for Atypical Psychosis

A.	A sudden onset of psychotic symptoms (2 weeks or shorter from a mentally healthy state to a clearly psychotic state that fulfils criterion B)
B.	Symptoms from at least two of the following categories, occurring simultaneously
	1. Emotional turmoil[a]
	2. Perplexity and confusion of memory[b]
	3. Catatonic behavior[c] or hallucinations or delusions
C.	The total duration of the disorder does not exceed 3 months, and there is almost complete recovery to the premorbid level of functioning
	The diagnosis should be qualified as "provisional" until 3 months after the onset
D.	The disturbance is not due to the direct physiological effects of a substance or a general medical condition

[a]Characterized by intense feelings of happiness or ecstasy, overwhelming anxiety or marked irritability.
[b]Characterized by puzzlement over perceived disorganization of thought, misidentification of people or places, or incoherence of the train of thought, resulting in impairment in cognition of the environment and intellectual performance, leading to confused thoughts and behaviors.
[c]At least one of the following must be prominent: (1) Motoric immobility as evidenced by catalepsy or stupor. (2) Excessive motor activity. (3) Extreme negativism or mutism. (4) Peculiarities of voluntary movement as evidenced by posturing, stereotyped movement, prominent mannerisms or prominent grimacing. (5) Echolalia or echopraxia.

However, atypical psychosis, as the term indicates, has become a target for criticism because it is ambiguous as to which patients can be classified into this disorder. In response to this criticism, new clinical diagnostic criteria have been established by clinical specialists of atypical psychosis in Japan (Table 1). However, even according to these criteria, some data are still not available, such as morbidity, prevalence, and differences in the suicide rate, mortality rate, profit, and loss for the society and disability-adjusted life years between similar disorders such as SZ and BD.

Similarly, it essential to determine the etiology based on biological research. In particular, the susceptibility genes must be found because the estimated heritability in atypical psychosis and other similarities is slightly higher than for SZ and BD [Leonhard, 1957; Das et al., 2001; Marneros and Pillmann, 2004]. If the highly penetrant causative genes could be established in this particular patient group, there would be important consequences for etiological research on similar disorders. Thus, we explored the causative genes by employing the latest gene-chip platform for GWASs. In addition, comparison of the resulting GWAS data of similar disorders (i.e., SZ and BD) rendered it possible to determine the degree of overlap that exists between the three groups (i.e., atypical psychosis, SZ, and BD), or whether atypical psychosis is more similar to either SZ or BD.

MATERIALS AND METHODS

Participants

We selected 47 patients with atypical psychosis (males 35.4%) according to the diagnostic criteria (Table 1) and 882 psychiatrically unscreened healthy controls (males 48.9%) for GWAS analysis. All subjects were unrelated, living in Japan, and self-identified as Japanese. The subjects provided written informed consent to participate after receiving a complete description of the study. This study was approved by the ethics committees of each university participating in this project.

For the enrichment analyses, we used an independent Japanese sample of SZ GWAS (560 SZ and 548 controls) and BD GWAS (107 BD type I and 107 controls). The detailed data of the SZ cases are described elsewhere [Ikeda et al., 2011], and the GWAS dataset for BD was obtained from the "Japanese Genetics Initiative of Mood Disorders data of GWAS for Bipolar Disorder" (http://molpsych.brain.riken.jp/data.html) [Hattori et al., 2009].

GWAS and Quality Control

Genotyping was performed using the Affymetrix Genome-Wide Human single-nucleotide polymorphism (SNP) Array 6.0 (Santa Clara, CA) according to the manufacturer's protocol. After applying several quality control (QC) criteria [e.g., call rate $\geq$95%, autosomal chromosomes, Hardy–Weinberg equilibrium $\geq$0.0001 and minor allele frequency (MAF) $\geq$5%], the final GWAS comprised 929 samples (47 cases and 882 controls) and 545,513 SNPs (MAF $\geq$5%). Q-Q plots were generated on the basis of an allele-wise analysis of SNPs that passed QC criteria (Fig. 2); our observed value of λ (=1.027) was consistent with those generally reported in well-matched samples.

Gene-Based Comparison With SZ and BD Using Versatile Gene-Based Association Study (VEGAS) Software

Further investigation was conducted with the aid of Versatile Gene-based Association Study (VEGAS) software [Liu et al., 2010], which enables the comparison of different GWAS data with a higher power than individual SNP-based analyses. The software annotates SNPs according to their position in genes, produces a gene-based test statistic, and then uses simulation to calculate an empirical gene-based *P*-value. The HapMap Asian sample (Tokyo, Japan, and Beijing, China) was used as a reference and the 3′/5′ boundaries of the genes were set at 50 kb from the initial and last exons for each gene.

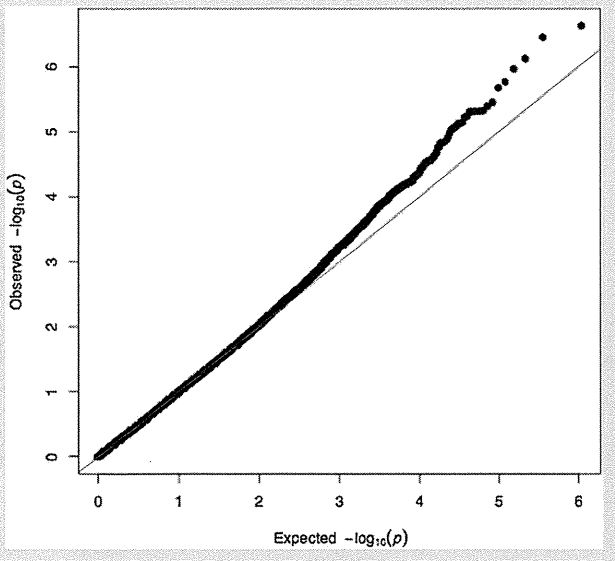

FIG. 2. Quantile-quantile (QQ) plot for association results (high-resolution image is also in Supplementary Information). The QQ plot shows the distribution of expected p values against the observed distribution. The empirical and theoretical distributions are shown as black dots and red line, respectively.

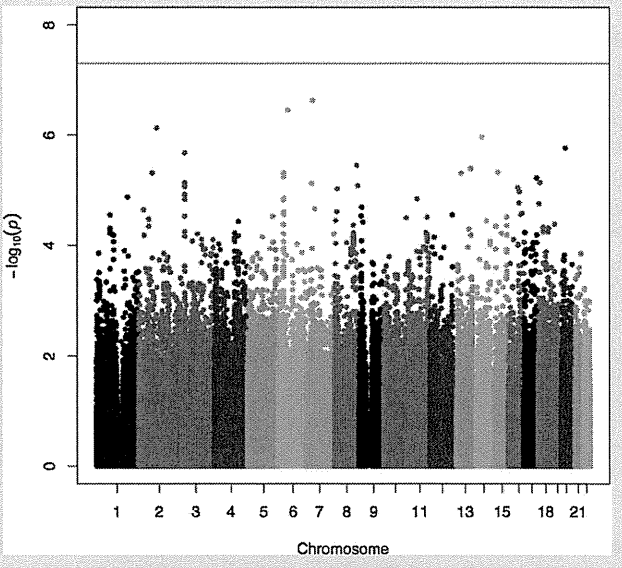

FIG. 3. Manhattan plot of atypical psychosis (high resolution image is also in Supplementary Information). The $-\log_{10}$ *P* values (y axis) of all SNPs in 47 cases with atypical psychosis and 882 healthy controls are shown relative to their chromosomal positions (x axis). The red horizontal line represents a genome-wide significance level ($P = 5 \times 10^{-8}$).

Gene enrichment was evaluated by hypergeometric analysis and the significance level was set at $P < 0.05$. This test enables us to evaluate whether suggested genes with *P*-values <0.05 in one disorder may correspond to the genes implicated in another disorder. The significance of the hypergeometric test represents the gene enrichment, with a higher number indicating more genes significantly matched than expected at random.

RESULTS

Single-Marker Association Analysis

Our atypical psychosis GWAS data did not detect an association with a genome-wide significance level (Fig. 3), although some susceptibility genes were suggested (Table 2). The top-ranked was *CHN2* (chimerin 2) [Hashimoto et al., 2005], which interacts with both *AKT1* [Ikeda et al., 2004] and *ERBB3* [Kanazawa et al., 2007] genes, which encode a protein with a phorbol-ester/DAG-type zinc finger (rs245914, $P = 1.6 \times 10^{-7}$). Another top-ranked *CPVL* (carboxypeptidase) exhibits strong sequence similarity to serine carboxypeptidases (rs12196860, $P = 2.5 \times 10^{-7}$), although the detailed function and its full-length nature have yet to be determined.

The third-ranked gene, *COL21A1* (collagen, type XXI, alpha 1), was significantly associated with an SZ GWAS analysis led by another group [Stefansson et al., 2009]. One of the highest peaks was detected on the major histocompatibility complex region (MHC), which is rs2736172 on Chromosome 6 ($P = 3.60 \times 10^{-6}$) closed to MICB, TNF, and HLA genes.

Gene-Based Comparison With SZ and BD

The gene-based analysis with employing the hypergeometric test revealed significant enrichment between SZ and atypical psychosis ($P = 0.014$), but not BD ($P = 0.93$; Fig. 4).

DISCUSSION

The scientific approach of beginning with an accurate classification, and ideally the precise nosology based on the symptomatology, represents a fundamental contribution to the scientific progress of clinical psychiatry. The feedback from new knowledge obtained through biological research improves clinical psychiatry, and brings crucial benefits to patients suffering from a psychiatric disorder. Since cases that are intermediate between SZ and BD are "amorphous", they are not usually a target for scientific research. Many researchers have struggled with this subgroup of patients, and no unequivocal evidence is available [Marneros and Tsuang, 1986; Andreasen and Carpenter, 1993; Marneros et al., 1995]. The objective selection of patients in the present study based on new atypical psychosis criteria, and the latest GWAS analysis and comparison with other similar disorders have revealed new clues as to the genetic features of these amorphous cases.

First of all, compared with the normal controls, the most-associated SNP did not reach a genome-wide significance level despite selected samples based on the relatively "pure" phenotype. Additionally, the SNPs in the *STAT6* genes positively suggested as a susceptible gene by the haplotype method were not positively associated with this GWAS analysis. However, it is of note that

TABLE 2. Top SNPs Based on GWAS of Atypical Psychosis

Rank	SNP	Chromosome	Position	Reference allele	*P*-value[a]	Closest gene[b]
1	rs245914	7	29218159	G	1.61×10^{-7}	*CHN2, CPVL*
2	rs12196860	6	55950374	A	2.45×10^{-7}	*COL21A1*
3	rs12105421	2	103576088	C	5.31×10^{-7}	
4	rs1959536	14	51446771	C	7.73×10^{-7}	*PYGL, TRIM9*
5	rs6081541	20	19212890	A	1.24×10^{-6}	*SLC24A3*
6	rs4619807	3	23193928	A	1.55×10^{-6}	*UBE2E2, RPL24P7*
7	rs16902460	8	128920197	G	2.61×10^{-6}	*PVT1*
8	rs1572591	13	102060076	A	2.99×10^{-6}	*ITGBL1, NALCN*
9	rs8029989	15	38733847	A	3.54×10^{-6}	*FAM98B, RASGRP1*
10	rs2736172	6	31590898	G	3.60×10^{-6}	*MICB, TNF*

SNP, single-nucleotide polymorphism; GWAS, Genome-Wide Association Study.The notational convention, such as Position and Reference allele, are in accordance with the National Center for Biotechnology Information SNP database.[a]*P*-value was calculated on the basis of the allele-wise test (two-tailed).
[b]Identified using the National Center for Biotechnology Information SNP database.

the SNPs in the major histocompatibility complex (MHC) region on chromosome 6 exhibited a nominal association with atypical psychosis (Supplementary Fig. 5). This area is well established as being associated with the robust risk of SZ by several GWAS, as well as more recently by mega-analysis conducted by the Psychiatric GWAS Consortium. However, the MHC region comprises large blocks with a very high linkage disequilibrium [Jeffreys et al., 2001]. Thus the particular "risk" gene being difficult to reliably identify at the present time.

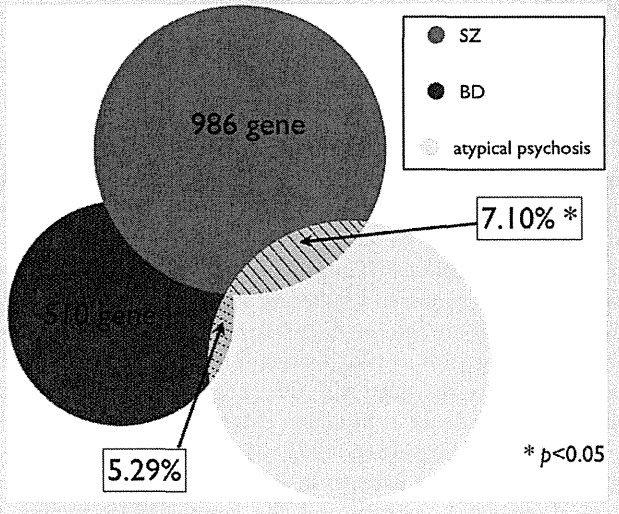

FIG. 4. The schema of overlapping percentages between three disorders ($P < .05$). Based on the result of VEGAS software analysis, the number of the genes with its *P*-value < 0.05 is shown in this schema (see Table 3). The overlapping percentage within Atypical psychosis is 7.10% (70/986) with SZ, and 5.29% (27/510) with BD. The enriched relationship of atypical psychosis with SZ was statistically significant ($P = 0.011$).

Comparison of our findings with independent GWAS datasets in the same population should only be conducted with the caveat that GWAS platforms differ between the groups. If there was no discrepancy between the platforms, the results from the different groups can be compared using polygenic component analysis [International Schizophrenia Consortium et al., 2009]. However, we used the latest version of the Affymetrics chip (version 6.0), even though the Japanese GWAS BD data available for comparison were obtained using another platform (Affymetrics 100 K). Since the SNPs on the arrays differed between atypical psychosis, SZ, and BD, it was not possible to make direct comparisons based on individual SNPs due to the use of different platforms (i.e., Affymetrics 6.0, 5.0, and 100 K, respectively). This meant that VEGAS software was applied [Liu et al., 2010], which calculates the *P* value for each gene even for data from different platforms (of course, results based on denser SNPs can generate more-accurate *P* values). The obtained data indicated that atypical psychosis is closer to SZ than to BD; 7.10% (70/986) of genes for which $P < 0.05$ overlapped with SZ, while 5.29% (27/510) of such genes overlapped with BD. The enriched relationship of atypical psychosis with SZ was statistically significant ($P = 0.011$) by hypergeometric analysis, although this is a nominal level of significance, it appears that both disorders (SZ and AP) may have a shared genetic risk (Table 3). It should be noted that a lower total number of genes in common with BD is partly due to the smaller BD sample size than SZ, therefore the failure to attain shared significance with BD could be partly explained by lower statistical power.

The present findings should be interpreted with caution, mainly because of the small sample, especially for atypical psychosis. The estimated prevalence of the disease is 11.4% within SZ and similar patients [Regier et al., 1994; Sartorius et al., 1995], which represents a prevalence of approximately 0.1% in the general population. The present study adopted specific criteria to determine the solid causative gene, which is why our sample was so limited in this preliminary study. Studies of more samples chosen based on the same diagnostic criteria will be essential for conclusive results. Moreover, larger samples of the Japanese BD cohort with a denser DNA chip are also required—this would enable direct comparisons

TABLE 3. Gene-Based Analysis of Atypical Psychosis Compared With SZ and BD

		atypical psychosis			
		$P < 0.05$	**$P \geq 0.05$**	**Total**	
SZ	$P < 0.05$	70	916	986	
	$P \geq 0.05$	858	15,371	16,229	
	Total	928	16,287	17,215	$P = 0.014$
		atypical psychosis			
		$P < 0.05$	**$P \geq 0.05$**	**Total**	
BD	$P < 0.05$	27	483	510	
	$P \geq 0.05$	628	11,029	11,657	
	Total	655	11,512	12,167	$P = 0.93$

SZ, schizophrenia; BD, bipolar disorder.Using the VEGAS software, a *P*-value was calculated from the sum of χ statistics of all GWAS SNPs in a certain gene. The number in the table is for the genes divided by *P*-value calculated by VEGAS software. The *P*-value on the bottom-right corner represents the result of the hypergeometric analysis for evaluating the gene enrichment between two disorders.

at the individual SNP level in addition to using polygenic component analysis.

Because of the newly established criteria for atypical psychosis, another limitation is the lack of inter- and intra-rater reliability. Moreover, as described in the Introduction section, we still need to determine the morbidity, prevalence, and differences in the suicide rate, mortality rate, profit and loss for the society and disability-adjusted life years between similar disorders such as SZ and BD, the replication work in different countries and areas is warranted.

In the GWAS era, a larger sample (~10,000) is essential in order to satisfy statistical requirements. However, we believe it is important to shed light on specific groups with certain phenotypes or courses, with the realization that it will be difficult to obtain a sufficient large number of patients that fit the criteria for those specific groups. The current method adopted in mainstream research under the name of SZ or BD may overlook the importance of clinical nosology. To see the patients in detail in order to make the appropriate diagnosis and in order to give the suitable treatment is another essential part of psychiatry, and these two disorders appear to be empirically heterogeneous. At the very least, the subgroups of SZ and BD should be considered before sampling. Data obtained in future investigations similar to the current study will provide clues that will reduce the heterogeneity of both SZ and BD.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Figure 5 Scatter plot of MHC region of GWAS analysis of atypical psychosis. This figure was constructed by SNAP (http://www.broadinstitute.org/mpg/snap/ldplot.php). Several SNP including rs2336172 ($p = 3.6e^{-6}$) showed high significance in the MHC region. Population panel: JPT(Japanese Tokyo) + CHB(Chinese Beijing). SNP dataset: Hapmap 3.

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Evidence for Shared Genetic Risk Between Methamphetamine-Induced Psychosis and Schizophrenia

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Methamphetamine (METH) use can provoke psychotic reactions requiring immediate treatment, namely METH-induced psychosis. Although the distinction between METH-induced and primary psychosis is important for understanding their clinical courses, we do not have clear diagnostic procedure by their symptoms. Not only are there similarities between the clinical features of METH-induced psychosis and schizophrenia (SCZ), but there is also epidemiological evidence of a shared genetic risk between 'METH-related' disorders and SCZ, which makes the differentiation of these two conditions difficult. In this study, we conducted a genome-wide association study (GWAS) targeting METH-dependent patients. The METH sample group, used in the METH-dependence GWAS, included 236 METH-dependent patients and 864 healthy controls. We also included a 'within-case' comparison between 194 METH-induced psychosis patients and 42 METH-dependent patients without psychosis in a METH-induced psychosis GWAS. To investigate the shared genetic components between METH dependence, METH-induced psychosis, and SCZ, data from our previous SCZ GWAS (total $N = 1108$) were re-analyzed. In the SNP-based analysis, none of the SNPs showed genome-wide significance in either data set. By performing a polygenic component analysis, however, we found that a large number of 'risk' alleles for METH-induced psychosis are over-represented in individuals with SCZ ($P_{best} = 0.0090$). Conversely, we did not detect enrichment either between METH dependence and METH-induced psychosis or between METH dependence and SCZ. The results support previous epidemiological and neurobiological evidence for a relationship between METH-induced psychosis and SCZ. These also suggest that the overlap between genes scored as positive in these data sets can have higher probability as susceptibility genes for psychosis.
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Keywords: substance-induced psychosis; genome-wide association study; schizophrenia; methamphetamine; substance use disorder; polygenic component analysis

INTRODUCTION

Illicit drug use, a major concern worldwide, can place a large burden both on individuals and on society. Methamphetamine (METH) use, in particular, is a growing problem; recent evidence from the United Kingdom suggests that METH is one of the most harmful drugs, with its overall harm (harm to users plus harm to others) ranking fourth out of 20 drugs (Nutt *et al*, 2010). It is of note that harm from METH is mainly associated with harm to the users (self-harm), and one reason for this finding is that METH can provoke psychotic reactions (METH-induced psychosis) requiring immediate medical treatment (Nutt *et al*, 2010).

The distinction between METH-induced psychosis and primary psychosis is critical for understanding the clinical courses of these disorders and planning appropriate treatment; however, we do not fully understand why some

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METH abusers develop schizophrenia (SCZ)-like psychosis (Grelotti *et al*, 2010) and others do not. Moreover, these two conditions are clinically similar partly because some patients do not remit psychotic symptoms for weeks or months after METH exposure, which suggests a specific phenotype induced by METH (ie, 'prolonged type' of METH-induced psychosis) (Ujike and Sato, 2004). Clinical investigators in Japan have long suggested that exposure to METH may cause persistent SCZ-like psychosis, whereas this possibility is discounted in the Western literature: the 'prolonged type' of METH-induced psychosis is recognized as a pre-existing psychotic state, such as SCZ (Callaghan *et al*, 2012).

Not only are there similarities in the clinical features between these conditions, but there are also several epidemiological studies that suggest a shared genetic risk in 'METH-related' disorders (ie, METH use disorder, METH dependence, and/or METH-induced psychosis) and SCZ, which makes the distinction between the two conditions complex. A family study revealed an increased risk of SCZ in the relatives of METH-induced psychosis patients compared with non-psychotic METH abusers or the general population (Chen *et al*, 2005). More recently, although the authors left open the question regarding the clear classification of SCZ and the 'prolonged type' of METH-induced psychosis, an epidemiological survey suggested that METH abuse patients were at the highest risk of developing SCZ compared with the users of other psychoactive drugs (eg, cocaine, alcohol, opioids, and cannabis; Callaghan *et al*, 2012).

In this investigation, we conducted a genome-wide association study (GWAS) of METH dependence to explore the relationship between METH dependence, METH-induced psychosis, and SCZ, by including a re-analysis of the following: (a) the METH data set by dividing it according to the presence of psychosis in each participant and (b) a previously reported Japanese SCZ GWAS (Ikeda *et al*, 2011).

MATERIALS AND METHODS

Samples

A total of 236 patients with METH dependence (185 males, 51 females), most of whom were analyzed in a previous GWAS using the pooling method (Uhl *et al*, 2008), and 864 healthy controls (410 males, 454 females) were included in this study (we will refer to this case-control analysis as the 'METH-dependence GWAS'). These controls had been used as the comparison subjects in other GWASs for narcolepsy (Miyagawa *et al*, 2008), panic disorder (Otowa *et al*, 2009), and SCZ (Hashimoto *et al*, unpublished data).

The METH-dependence GWAS data set was re-analyzed within case samples based on the presence (or absence) of psychotic symptoms (we will refer to this analysis as the 'METH-induced psychosis GWAS'). This METH-induced psychosis sample group consisted of 194 METH-dependent patients with psychosis (METH-induced psychosis: 155 males and 39 females) and 42 METH-dependent patients without psychosis (METH non-psychosis: 30 males and 12 females).

All subjects were unrelated Japanese subjects and were recruited from the same relatively small geographical area of Japan. Consensus diagnoses were made by at least two experienced psychiatrists according to ICD-10 criteria on the basis of unstructured interviews with patients and their families, as well as a review of medical records. Patients were excluded if they had a history of SCZ, bipolar disorder, or known intellectual disability. The Japan SCZ sample consisted of 560 SCZ cases and 548 controls, and results from the GWAS of this sample were published previously ('SCZ GWAS') (Ikeda *et al*, 2011). Healthy controls reported no personal history of mental disorders, but they were not screened using standard diagnostic procedures.

After providing a complete description of the study to the subjects, written informed consent was obtained. This study was approved by the ethics committees of each university participating in this project.

Genotyping and Quality Control (QC)

Genotyping for the METH-dependence GWAS was performed using the Affymetrix Genome-Wide Human SNP Array 5.0 or 6.0 (Affymetrix, Santa Clara, CA) according to the manufacturer's protocol. Of the 236 subjects with METH dependence, 169 of them (all METH-induced psychosis) were genotyped using the 5.0 chip, whereas 67 subjects (25 METH-induced psychosis and 42 METH non-psychosis) were genotyped using the 6.0 chip. The healthy controls were genotyped using the Affymetrix 6.0 chip. Genotypes were called from the CEL files using the BRLMM-P algorithm for the 5.0 chip and Birdseed v2 for the 6.0 chip implemented in the Genotyping Console software (Affymetrix). To correct for hidden confounding factors introduced by different genotyping platforms, only SNPs that are in common were selected (total of 436 213 SNPs). We then applied the following QC criteria to exclude samples: (1) arrays with a low QC ($<86\%$ for 5.0 chip or <0.4 for 6.0 chip) according to the BRLMM-P or Birdseed v2 algorithm ($n=0$) and (2) samples for which $<95\%$ of genotypes were called ($n=0$). Next, we excluded SNPs that (1) had low call rates (<0.95), (2) were duplicated, (3) localized to sex chromosomes, (4) deviated from Hardy–Weinberg equilibrium in controls ($P<0.0001$), or (5) had low minor allele frequencies (<0.05). Finally, 244 224 QC-ed SNPs were used in the subsequent analyses.

To test for the presence of genetic structure in the data, we performed a principal component analysis (PCA) using EIGENSTRAT 3.0 (Price *et al*, 2006). Ten Eigenvectors were calculated. Genotype information from the JPT, CHB, CEU, and YRI in HapMap phase III was compared with our data set to check for population stratification (Supplementary Figure S1). All Japanese samples from our case-control sample were in a separate cluster from the non-Japanese HapMap samples; however, two samples lay outside the main Japanese cluster, and those samples were excluded. Therefore, the final analysis consisted of 234 METH-dependent (193 METH-induced psychosis and 41 non-psychosis) subjects and 864 healthy comparison subjects.

The genotyping platform for the Japanese SCZ Study was Affymetrix 5.0, and SNPs were evaluated using the same procedures used in our previous study (Ikeda *et al*, 2011).

Statistical Analysis

To assess the association between individual SNPs, we used genomic control (GC)-adjusted *P*-values derived from

allele-wise analysis based on median chi-square statistics (Purcell *et al*, 2007).

We performed a polygenic component analysis following the method described by the International Schizophrenia Consortium (ISC) (Purcell *et al*, 2009). This analysis is based on the idea that common SNPs collectively contribute to a substantial proportion of the heritability of complex diseases. To test this hypothesis, in essence, the authors defined sets of putative 'risk' alleles as being those that surpassed more liberal thresholds for association (eg, $P<0.5$) in a discovery case-control sample set ('discovery' sample). These sets (ie, putative 'risk' alleles) were used to construct polygenic scores, which represent the number of 'risk' alleles carried by individuals in a second test data set ('target' sample). The analysis showed that the cases had on average significantly higher polygenic scores than controls. After discounting the influence of potential sources of bias, the authors in ISC concluded that the findings were best explained by the existence of polygenic component to the disorder comprised of a large number of common 'risk' alleles with small and cumulative effect.

Although several approaches are now available to examine the polygenic effects on the complex diseases/phenotypes (eg, GCTA: Yang *et al*, 2011), the polygenic component analysis which we used in this study is suitable to evaluate the genetic overlap within the same phenotype (eg, in the ISC, they extracted 'risk' from their 'discovery' SCZ GWAS and applied these to the independent 'target' SCZ GWASs to examine whether cases in the target samples had higher polygenic score) or between different diseases (extracted risk from their 'discovery' SCZ GWAS and applied to the 'target' bipolar disorder GWASs). Therefore, we applied this method to evaluate the genetic risk overlap with relaxed thresholds in METH dependence, METH-induced psychosis, and SCZ. The same criteria used by the ISC (Purcell *et al*, 2009) for LD pruning were applied to METH samples (r^2 threshold of 0.25 and window size of 200 SNPs) and 64 815 LD-independent SNPs based on linkage equilibrium were analyzed. The polygenic score was calculated using PLINK ver.1.07 (Purcell *et al*, 2007). Nagelkerke's pseudo R^2 was calculated by logistic regression with covariation for 'non-missing SNPs'. A one-tailed test was applied under a directional hypothesis that expects higher scores to be associated with an increased risk of disease.

We also performed gene-based testing of the associations, because this method can provide more statistical power than single SNP-based analysis (Liu *et al*, 2010). To conduct the analysis, the software package VEGAS (Liu *et al*, 2010) was used by applying individual SNP-based *P*-values of the GC adjustment. VEGAS can perform analyses by taking into account not only gene size but also linkage disequilibrium patterns based on the HapMap East Asia (JPT and CHB) panel. SNPs were assigned to one or more defined boundaries of autosomal genes by extending the genomic sequence corresponding to each gene by 50 kb in the 5′ and 3′ directions. Gene-based *P*-values for all genes were calculated in this analysis, and 17 049 genes were assigned for both disorders (genomic positions were based on hg18). To include genes with nominal associations, we set the type I error rate for the gene-based test to 0.05. The enrichment was assessed by hyper-geometric analysis.

RESULTS

SNP-Based Association Results of 'METH-Dependence' and 'METH-Induced Psychosis' GWASs

The Manhattan plots for the 'METH-dependence' and 'METH-induced psychosis' GWASs are shown in Figure 1, and the Q-Q plots are shown in Supplementary Figures S2 and S3. The GC inflation factors (λ) were 1.024 and 1.016 for the 'METH-dependence' and 'METH-induced psychosis' GWASs, respectively. We did not detect any SNPs with genome-wide significance (5×10^{-8}), which is widely used as a benchmark of association, in either data set. The strongest association with METH dependence was observed for the rs4427170 SNP in the sarcoglycan zeta gene (*SGCZ*) ($P=3.9\times10^{-6}$, two-tailed test; Table 1), and the strongest association with METH-induced psychosis was observed for rs12591257, an intronic SNP in ATP/GTP binding protein-like1 (*AGBL1*) ($P=3.6\times10^{-6}$,

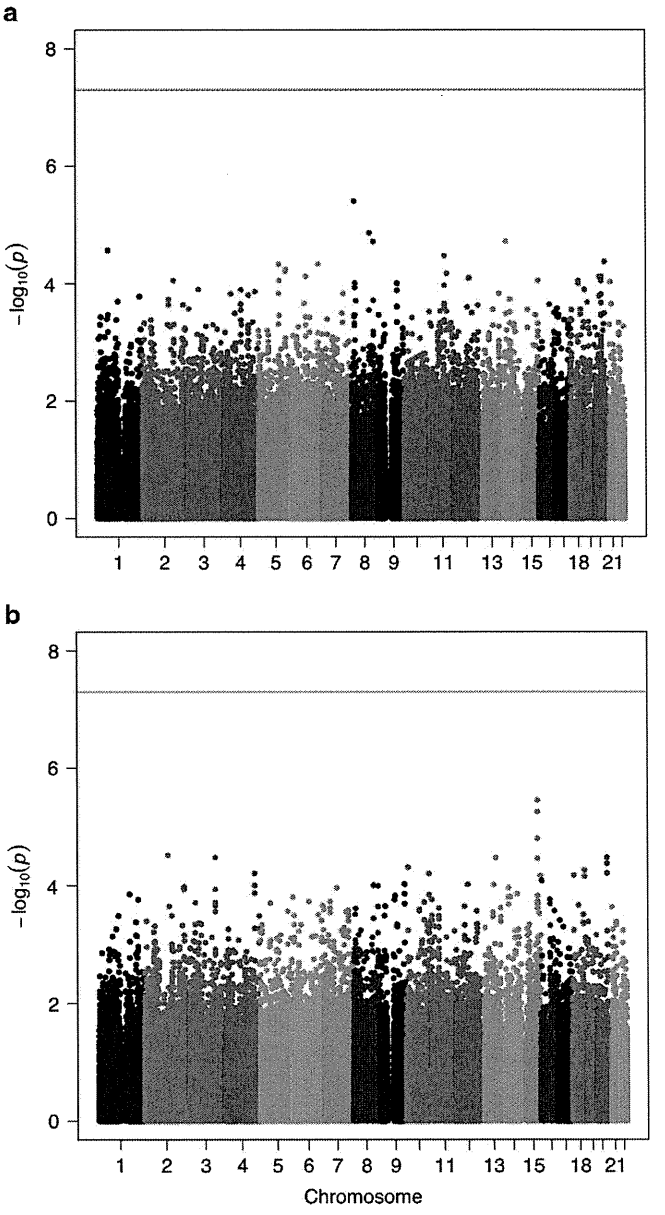

Figure 1 The Manhattan plots for (a) METH dependence and (b) METH-induced psychosis GWASs. METH, methamphetamine.

Table 1 Individual SNP-Based Analyses of METH-Dependence and METH-Induced Psychosis GWASs

Phenotype	SNP	Rank	CHR[a]	BP[b]	A1[c]	Frequency of A1 in case	Frequency of A1 in control	A2[d]	OR[e]	P_{GC}[f]	Gene[g]
METH-dependence GWAS	rs4427170	1	8	14853781	T	0.515	0.395	A	1.63	3.9×10^{-6}	SGCZ
	rs7826857	2	8	99436341	A	0.081	0.162	G	0.45	1.4×10^{-5}	KCNS2
	rs12894058	3	14	33532016	A	0.222	0.140	G	1.75	1.9×10^{-5}	NPAS3
	rs2326193	4	8	120029709	A	0.338	0.239	C	1.63	1.9×10^{-5}	
	rs4915748	5	1	61954169	T	0.150	0.242	C	0.55	2.7×10^{-5}	
	rs617231	6	11	84837898	C	0.131	0.070	T	1.99	3.4×10^{-5}	DLG2
	rs6022102	7	20	51425077	C	0.209	0.133	T	1.73	4.2×10^{-5}	
	rs6940190	8	6	148644731	A	0.280	0.383	T	0.63	4.6×10^{-5}	SASH1
	rs2416305	9	5	112483604	G	0.197	0.293	A	0.59	4.7×10^{-5}	MCC
	rs17111695	10	5	150432446	C	0.186	0.115	T	1.77	5.7×10^{-5}	TNIP1
METH-induced psychosis GWAS[h]	rs12591257	1	15	87064089	C	0.041	0.183	A	0.19	3.6×10^{-6}	AGBL1
	rs2346713	2	15	87078337	C	0.067	0.232	A	0.24	5.6×10^{-6}	AGBL1
	rs16977267	3	15	86993464	T	0.073	0.232	G	0.26	1.6×10^{-5}	AGBL1
	rs13414154	4	2	129012073	A	0.145	0.342	G	0.33	3.1×10^{-5}	HS6ST1
	rs6767236	5	3	143758399	G	0.189	0.402	T	0.35	3.3×10^{-5}	
	rs6091985	6	20	53445939	C	0.044	0.171	G	0.22	3.3×10^{-5}	
	rs7333069	7	13	82458858	A	0.394	0.646	C	0.36	3.4×10^{-5}	
	rs8026683	8	15	86825255	A	0.098	0.268	C	0.30	3.5×10^{-5}	AGBL1
	rs6064117	9	20	53458592	G	0.047	0.175	A	0.23	4.2×10^{-5}	
	rs2768428	10	10	12523129	T	0.052	0.183	C	0.24	4.9×10^{-5}	CAMK1D

[a]CHR: chromosome.
[b]BP: base position based on hg19.
[c]A1: minor allele name based on whole sample.
[d]A2: major allele name.
[e]OR: odds ratio (for A1: A2 is reference).
[f]P_{GC}: *P*-value-adjusted genomic control (two-sided).
[g]Gene: gene (±20 kb).
[h]This comparison was based on the presence (or absence) of psychotic symptoms in the METH-dependence samples.

two-tailed test; Table 1). The 100 SNPs most associated with these disorders are shown in Supplementary Tables S1 and S2 (Figure 1 and Table 1).

Polygenic Component Analysis and Gene-Based Analysis

In the polygenic component analysis, we detected statistically significant enrichment of alleles scored in the 'discovery' METH-induced psychosis GWAS sample in the 'target' SCZ GWAS sample at *P*-thresholds (P_Ts) of <0.3, 0.4, and 0.5 ($P_{best} = 0.0090$, Supplementary Figure S4; Figure 2). However, the variances in SCZ liability explained by the 'risk' SNPs of METH-induced psychosis were smaller ($R^2 \sim 0.7\%$, Supplementary Figure S4) than those found in previous studies when only SCZ samples were used in the polygenic component analysis ($R^2 \sim 3\%$) (Purcell *et al*, 2009; Ikeda *et al*, 2011). The reciprocal analysis (discovery/target SCZ/METH-induced psychosis pair) revealed a non-significant *P*-value of statistical enrichment of the 'risk' SCZ alleles in the 'target' METH-induced psychosis samples ($P_{best} = 0.092$, Supplementary Figure S5). Although statistical evidence was not obtained from this analysis (likely due to the small 'target' sample), it is of note that the variances explained were higher ($R^2 \sim 1.2\%$, Supplementary Figure S5) than that detected in the discovery/target METH-induced psychosis/SCZ pair, suggesting that larger METH-induced psychosis sample sizes will be essential for obtaining conclusive results. By contrast, no statistical overlap was observed among other combinations of discovery/target pairs (Figure 2, Supplementary Figures S6–S9).

We also performed gene-based testing of the associations, because this method can provide more statistical power than individual SNP-based analysis (Liu *et al*, 2010). Seventy-four of the 17 049 genes showed significant associations at $P_{gene} < 0.05$, both in METH-induced psychosis and SCZ, suggesting significant enrichment by hypergeometric analysis ($P = 0.0075$: Supplementary Table S3). Supplementary Table S4 lists the genes that overlapped. Notably, *NOTCH4*, a promising candidate gene for SCZ (Stefansson *et al*, 2009; Ikeda *et al*, 2012) was found to be associated with both disorders (Figure 2).

DISCUSSION

In the current study, we detected shared genetic risk between METH-induced psychosis and SCZ but failed to

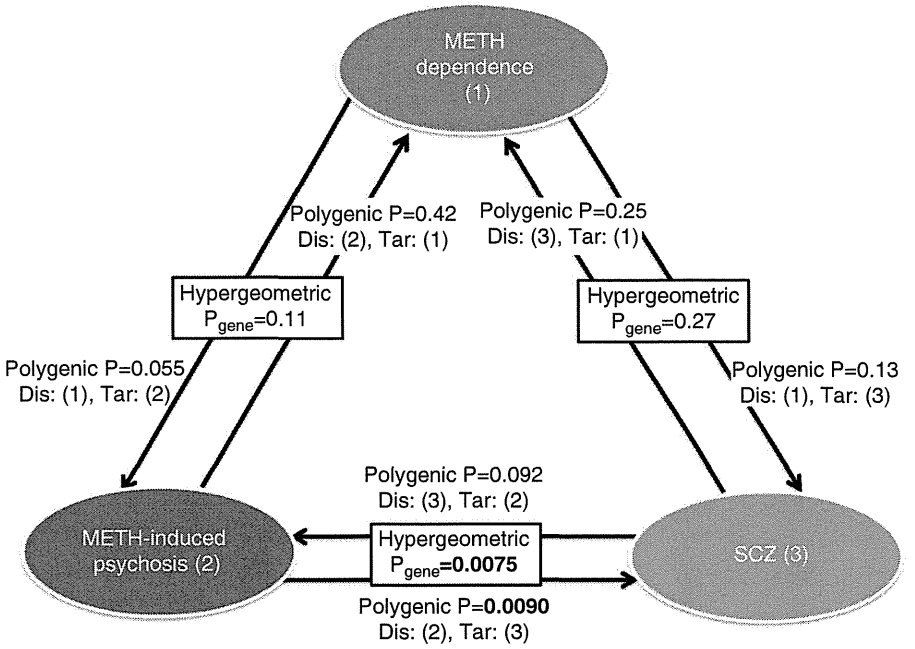

Figure 2 Relationship between METH-dependence (1), METH-induced psychosis (2), and schizophrenia (3). METH, methamphetamine; SCZ, schizophrenia; Dis, Discovery sample; Tar, Target sample. 'Polygenic *P*' indicates the best *P*-value calculated by logistic regression analysis in the polygenic component analysis. 'Hyper-geometric *P*' indicates the *P*-value calculated by hyper-geometric analysis to assess the enrichment of the risk genes that showed significance in both the two conditions (based on gene-based analysis).

detect an overlap between METH dependence and SCZ. These results highlight that the comparison between the 'presence' and 'absence' of psychosis within METH-dependent subjects, in our case, is preferable and important (even for polygenic component analysis) because the effect size of genetic markers associated with drug response (ie, psychosis as a response to METH *exposure*) is considered to be larger than that expected in the susceptibility to common and complex diseases (ie, SCZ and METH *dependence*) (Cirulli and Goldstein, 2010). Although the division of the METH-dependent subjects according to psychotic status will result in lowering the statistical power due to the reduced sample size, this type of research, especially in the psychiatry field where substantial disease heterogeneity is assumed, may benefit from the larger effect size following by this pharmacogenetic/genomic concept (Bousman *et al*, 2009; Cirulli and Goldstein, 2010).

However, our SNP-based analysis, even for METH-induced psychosis, did not show any associations with genome-wide significance. The presence of type II errors in this study are inevitable given our sample size; it is of note, however, that *AGBL1* (rs12591257, $P=3.6\times10^{-6}$) showed a trend for an association with METH-induced psychosis. *ABGL1* was one of the candidate genes for SCZ based on the CATIE GWAS (Sullivan *et al*, 2008), as well as for the side effect by antidepressant treatment in the STAR*D study (Clark *et al*, 2012). In addition, several reports suggested an association between SCZ and the genes listed in our top hits, such as *NPAS3* (Pieper *et al*, 2005; Lavedan *et al*, 2009; Pickard *et al*, 2009; Huang *et al*, 2010; Macintyre *et al*, 2010) and *DLG2* (Kristiansen *et al*, 2006; MacLaren *et al*, 2011). Therefore, although we failed to find associations in this study, the non-genome-wide level of statistical significance should be interpreted with caution and be validated by independent replication study.

To address the issue of the small sample size in the current study, we applied the powerful method of polygenic component analysis described by ISC, which showed that common SNPs collectively contribute to a substantial proportion of the heritability of common complex diseases. Our main finding supports previous epidemiological evidence linking between METH-induced psychosis and SCZ (Chen *et al*, 2005). Nevertheless, the variance-explained connecting these conditions was modest ($R^2\sim0.7\%$), and it showed similar magnitude reported in the comparison of SCZ between Japanese and the UK populations ($R^2\sim0.8\%$, Ikeda *et al*, 2011). This suggests that at least some of the liberal 'risk' alleles of the METH-induced psychosis GWAS are likely to be SCZ risk alleles, but these disorders have specific risk alleles as well. Furthermore, the results obtained by gene-based analysis, which showed a significant enrichment of the risk genes between these two conditions, are also in agreement with the results of the polygenic component analysis (Figure 2). Therefore our results based on the polygenic component and the gene-based analyses are the first molecular genetic evidence for overlap between METH-induced psychosis and SCZ in humans: this, in turn, supports the proposed role of the METH exposure, where mice treated by METH have been used as a mouse model of SCZ (Machiyama, 1992). In the gene-based analysis, although it involves multiple comparison issues, it is also of note that we detected a significant association of *NOTCH4* (Stefansson *et al*, 2009; Ikeda *et al*, 2012) both with the METH-induced psychosis and SCZ samples. Other overlap genes are possible candidate genes for 'psychosis', thus these genes should be examined with different sets of

samples, especially for SCZ, because our results indicate a higher previous likelihood that these genes are susceptibility genes for SCZ.

Several limitations should be noted to interpret our results. Firstly, the polygenic score aggregates evidence from multiple weakly associated genomic loci, which is important in the situation where most of the true-positive association signals fall below the genome-wide association threshold due to a lack of statistical power. In this context, there are several confounding problems associated with the interpretation of polygenic score analysis results. One critical factor is population stratification. To check for the possibility of this effect, we performed 'discovery/target METH-induced psychosis/SCZ' analysis with the major four principle components included as covariates. In this analysis, however, we still found statistically significant enrichment at $P_T < 0.3$, 0.4, and 0.5, despite the decreased variance described above (Supplementary Figure S10). Another concern is the difference between cases and controls in terms of gender ratio, which is biased substantially towards males in the METH-dependent subjects ($>3:1$). We have not conducted the analysis of X-linked loci, as there is no gold standard so far established in analyzing the genotypes on chromosome X but included the 'discovery/target METH-induced psychosis/SCZ' analysis using gender-adjusted *P*-values for discovery statistics (Supplementary Figure S11). Again no large change was observed in this explorative analysis.

In summary, a large number of 'risk' SNPs selected from a METH-induced psychosis GWAS are enriched in individuals with SCZ. This result suggests that the overlap between genes scored as positive in both the data sets can have higher probability as susceptibility genes for psychosis. In the future, the shared genetic risk component between these disorders may provide insights into disease processes and the diagnosis and may open up new avenues for drug development in terms of pharmacological modeling of psychosis. Based on the current study, however, it is difficult to clarify the question of whether the 'transient' type (remitting a psychiatric state immediately or a couple of weeks after METH exposure) or 'prolonged' type of psychosis has more genetic similarity with SCZ. To obtain conclusive results, further studies with much larger samples are required.

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DISCLOSURE

The authors declare no conflict of interest.

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PLOS ONE

Genetic Variants on 3q21 and in the Sp8 Transcription Factor Gene (*SP8*) as Susceptibility Loci for Psychotic Disorders: A Genetic Association Study

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Abstract

Background: Recent genome-wide association studies (GWASs) investigating bipolar disorder (BD) have detected a number of susceptibility genes. These studies have also provided novel insight into shared genetic components between BD and schizophrenia (SCZ), two major psychotic disorders. To examine the replication of the risk variants for BD and the pleiotropic effect of the variants associated with BD, we conducted a genetic association study of single nucleotide polymorphisms (SNPs) that were selected based upon previous BD GWASs, which targeted psychotic disorders (BD and SCZ) in the Japanese population.

Methods: Forty-eight SNPs were selected based upon previous GWASs. A two-stage analysis was conducted using first-set screening (for all SNPs: BD = 1,012, SCZ = 1,032 and control = 993) and second-set replication samples (for significant SNPs in the screening analysis: BD = 821, SCZ = 1,808 and control = 2,149). We assessed allelic association between BD, SCZ, psychosis (BD+SCZ) and the SNPs selected for the analysis.

Results: Eight SNPs revealed nominal association signals for all comparisons ($P_{uncorrected}<0.05$). Among these SNPs, the top two SNPs (associated with psychosis: $P_{corrected}=0.048$ and 0.037 for rs2251219 and rs2709722, respectively) were further assessed in the second-set samples, and we replicated the signals from the initial screening analysis (associated with psychosis: $P_{corrected}=0.0070$ and 0.033 for rs2251219 and rs2709722, respectively). The meta-analysis between the current and previous GWAS results showed that rs2251219 in Polybromo1 (*PBRM1*) was significant on genome-wide association level ($P=5\times10^{-8}$) only for BD ($P=9.4\times10^{-9}$) and psychosis ($P=2.0\times10^{-10}$). Although the association of rs2709722 in Sp8 transcription factor (*SP8*) was suggestive in the Asian population ($P=2.1\times10^{-7}$ for psychosis), this signal weakened when the samples size was increased by including data from a Caucasian population ($P=4.3\times10^{-3}$).

Conclusions: We found 3p21.1 (including *PBRM1*, strong linkage disequilibrium made it difficult to pinpoint the risk genes) and *SP8* as risk loci for BD, SCZ and psychosis. Further replication studies will be required for conclusive results.

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Introduction

Bipolar disorder (BD) is a severe mental condition, and the main symptom is associated with abnormal affective status (i.e., a patient's mood will swing from manic to depression or *vice-versa*). The prevalence of BD worldwide is greater than 1% [1,2], but the precise molecular mechanism is largely unknown. Nevertheless, epidemiological surveys have suggested that genetic factors contribute substantially compared with the environmental factors, and heritability has been estimated at 80% [3].

The results of genetic association studies, particularly genome-wide association studies (GWASs), have identified an increasing number of risk genes for susceptibility to BD. The initial meta-analysis of the GWASs detected a single nucleotide polymorphism

Table 1. Association analysis in the first-set screening samples (SNPs with nominal significant association).

				First-set screening										Second-set replication						
Chr	SNP	closest GENE	BP[a]	A1[b]	A2[c]	Phenotype[d]	F_A[e]	F_U[f]	P[g]	$P_{corrected}$	OR[h]	SE[i]	direc-tion[j]	F_A	F_U	P[g]	$P_{corrected}$	OR	SE	direction
1	rs472913	NF1A	61095558	G	C	BD	0.485	0.499	0.20	1	0.945	0.066	+							
						SCZ	0.469		**0.036**	1	0.888	0.066	+							
						Psychosis	0.477		0.063	1	0.916	0.058	+							
3	rs2251219	PBRM1	52584787	A	G	BD	0.490	0.452	0.011	0.39	1.17	0.067	+	0.485	0.460	0.042	0.085	1.11	0.059	+
						SCZ	0.500		**0.0018**	0.064	1.21	0.066	+	0.489		**0.0051**	**0.010**	1.13	0.046	+
						Psychosis	0.495		**0.0013**	**0.048**	1.19	0.058	+	0.488		**0.0035**	**0.0070**	1.12	0.042	+
3	rs1042779	ITIH1	52821011	A	G	BD	0.498	0.465	0.025	0.92	1.14	0.067	+							
						SCZ	0.503		**0.012**	0.43	1.16	0.066	+							
						Psychosis	0.500		**0.0076**	0.28	1.15	0.058	+							
3	rs736408	ITIH3	52835354	A	G	BD	0.450	0.480	0.034	1	0.885	0.067	+							
						SCZ	0.446		**0.021**	0.75	0.874	0.066	+							
						Psychosis	0.448		**0.013**	0.46	0.879	0.058	+							
7	rs10240470	MADIL1	1916497	G	T	BD	0.107	0.085	0.014	0.49	1.29	0.11	+							
						SCZ	0.098		0.090	1	1.17	0.11	+							
						Psychosis	0.102		**0.022**	0.79	1.23	0.10	+							
7	rs2709736	SP8	20862302	A	G	BD	0.305	0.345	0.0055	0.20	0.835	0.071	+							
						SCZ	0.314		**0.025**	0.88	0.871	0.070	+							
						Psychosis	0.310		**0.0046**	0.16	0.853	0.061	+							
7	rs2709722	SP8	20867808	T	C	BD	0.311	0.350	0.0063	0.23	0.838	0.071	+	0.303	0.331	0.023	0.046	0.880	0.064	+
						SCZ	0.307		**0.0024**	0.085	0.820	0.070	+	0.314		**0.049**	0.098	0.922	0.049	+
						Psychosis	0.309		**0.0010**	**0.037**	0.829	0.061	+	0.311		**0.017**	**0.033**	0.909	0.045	+
10	rs10994336	ANK3	62179812	T	C	BD	0.362	0.346	0.16	1	1.07	0.070	+							
						SCZ	0.380		**0.017**	0.62	1.16	0.069	+							
						Psychosis	0.371		**0.036**	1	1.11	0.060	+							

a: BP: base position based upon hg19.
b: A1: minor allele based upon whole sample.
c: A2: major allele.
d: BD: bipolar disorder, SCZ: schizophrenia.
e: F_A: Frequency of A1 in affected subjects.
f: F_U: Frequency of A1 in unaffected subjects.
g: P values based upon one-tailed test.
h: OR: Odds ratio for A1 (i.e. A2 is reference).
i: SE: standard error.
j: direction: direction of the effect size based upon the original studies. +: same direction. −: opposite direction.
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(SNP) in the α subunit of the L-type voltage-gated calcium channel (*CACNA1C*) with suggestive statistical evidence for association ($P = 7 \times 10^{-8}$) [4]. This gene remains one of the most promising genes for BD, as the most recent mega-analysis in the Psychiatric GWAS Consortium (PGC) Bipolar Working Group provided stronger evidence of association with the SNPs in *CACNA1C* ($P = 1.5 \times 10^{-8}$) [5]. This mega-analysis also detected another risk SNP in Drosophila pair-rule gene ten-m (*ODZ4*) with genome-wide significance ($P = 4.4 \times 10^{-8}$), proposing a novel candidate gene for BD [5]. Other studies based upon genome-wide screening methodology identified several possible candidate genes for BD, such as ankyrin 3 (*ANK3*) [4] and neurocan (*NCAN*) [6].

Interestingly, genetic association studies of schizophrenia (SCZ), which is another major psychotic disorder, have also revealed that the BD risk SNPs, such as *CACNA1C* and *ANK3*, are associated with SCZ [7]. This result indicates that there is a shared genetic component between BD and SCZ. Furthermore, several independent lines of evidence converge to support this hypothesis: first, polygenic component analysis, in which the cumulative number of liberal risk alleles for SCZ or BD were enriched in the patients with BD or SCZ [8], and second, epidemiological studies, in which relatives of a proband with BD also had elevated risk for SCZ in addition to BD [9]. Therefore, combining BD and SCZ samples into a single psychosis group [10] can provide increased statistical power, specifically for the detection of overlapping risk SNPs. Several studies have used this concept also enhance detection of risk SNPs for either BD or SCZ [11,12].

The goal of the current study was to conduct replication and cross-phenotype analyses of the SNPs that were selected based upon BD GWAS findings with two psychotic disorders (BD and SCZ) in the Japanese population. We used a two-stage design (screening and replication samples) and a meta-analysis approach to obtain reliable results.

Materials and Methods

Ethics Statement

Written informed consent was obtained from each subject after the procedures had been fully explained. This study was performed in accordance with the World Medical Association's Declaration of Helsinki and approved by the ethics committees at Fujita Health University, RIKEN BSI and institutes participating in the Collaborative Study of Mood Disorder (COSMO) [13].

Subjects

We used two independent sample sets in this study. In the first-set screening analysis, we examined 1,012 patients with BD [51.8% female, age ± standard deviation (SD) = 50.7 ± 14.3 years, BD type I = 621, BD type II = 380, schizoaffective disorder (SA) = 7, BD type information not available = 4], 1,032 SCZ (48.3% female, mean ± SD = 46.8 ± 14.8 years) and 993 healthy controls (51.1% female, age ± SD = 49.7 ± 14.0 years).

For the two SNPs that showed a significant association in the screening analysis, we used an independent second-set of samples for replication analysis. This sample consisted of 821 patients with BD (54.6% female, age ± SD = 48.2±14.4, BD type I = 387, BD type II = 344, SA = 89, BD type information not available = 1), 1,808 patients with SCZ (45.1% female, age ± SD = 49.8±14.8 years) and 2,149 healthy controls (58.3% female, age ± SD = 42.3±14.2 years). A detailed description of our subjects, including a general characterization and psychiatric assessment, is described elsewhere [13].

Table 2. meta analysis of the two SNPs detected in the first-set screening analysis.

				P value of original study			current study (screeing+replication)			current study+PGC BP			current study+Lee et al.			current study+Lee et al.+PGC BP		
Chr	SNP	GENE	A1[a]	A2[b]	PGC BP	Lee et al.[c]	phenotype[d]	P	OR[e]	I^2	P	OR[e]	I^2	P	OR[e]	I^2	P	OR[e]	I^2
3	rs2251219	PBRM1	A	G	5.5E-07	-	BD	0.0048	1.13	0	9.4E-09	1.13	0						
							SCZ	0.00016	1.15	0	4.3E-10	1.13	0						
							Psychosis	8.0E-05	1.14	0	2.0E-10	1.13	0						
7	rs2709722	SP8	T	C	0.089	5.06E-05	BD	0.0016	0.86	0	0.0322	0.91	56.2	5.1E-07	0.84	0	0.0070	0.88	72.9
							SCZ	0.0030	0.89	46.6	0.0332	0.92	58.4	1.6E-06	0.86	42.2	0.0074	0.89	73.9
							Psychosis	0.00040	0.88	32.4	0.0212	0.91	64.6	2.1E-07	0.86	30.3	0.0043	0.89	74.7

a: A1: first allele code.
b: A2: second allele code.
c: Lee et al. reported the P values based upon dominant model. To conduct meta-analysis of allelic model, we re-calculated P values based upon their results.
d: BD: bipolar disorder, SCZ: schizophrenia.
e: the effect is with respect to the A1 allele.
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SNP selection and quality control

We selected 48 SNPs from BD GWAS data published prior to September 2011 [4,5,6,14,15,16,17,18,19,20]. Regarding SNP selection, we used the following inclusion criteria. The potential risk SNPs in autosomal chromosomes must have had a P-value less than 1×10^{-5} if the original GWAS was conducted using a Caucasian population. The P-value must have been less than 1×10^{-4} if the study was based upon Asian population or PGC [5]. The minor allele frequency (MAF) must not have been equal to zero based upon the HapMap JPT panel. We used a Sequenom iPLEX Gold System (Sequenom, San Diego, CA) genotyping platform. In the optimization step, two SNPs (rs10193871 and rs1012053) were excluded due to a primer design problem. Moreover, because a visual inspection of the clustering revealed that six SNPs did not yield acceptable genotyping calls, we designed new primers for their proxy SNPs (N = 8) based upon tight linkage disequilibrium (LD). However, at this stage, we could not obtain optimal clustering for three of these SNPs. In total, we analyzed 45 SNPs (Figure S1 and Table S1). The quality control (QC) was conducted based upon the following criteria: (1) the missing call rate per person (less than 10%); (2) the missing call rate per SNP (less than 5%); and (3) a Hardy-Weinberg Equilibrium (HWE) P>0.0001 threshold (Table S1).

Statistical analysis

We assessed the allelic association of the SNPs and the following three phenotypes: 1) BD (referred to as BD association); 2) SCZ (referred to as SCZ association); and 3) psychosis (BD+SCZ; referred to as psychosis association) (Figure S2 and S3).

A comparison between multiple variables is a major concern to be addressed in a genetic study in which multiple SNPs and phenotypes are analyzed. However, thus far, no gold standard has been established. Therefore, we used a two-stage analysis and stringent cut-off level for the type I error rate in the first-set screening sample. LD between SNPs selected for analysis was calculated by SNPSpD program [21,22] to establish an effective number of independent variables (N = 36.06). We used an adjusted statistical significance level (P<0.00138) based upon this number of independent variables. The associated SNPs from the first-set screening samples were followed-up and genotyped to replicate the association in the independent second sample set. In these analyses (first-set and second-set analyses), a one-tailed analysis was applied under a unidirectional hypothesis that risk alleles identified in the original studies were associated with risk in our dataset. We assumed this association because most of the original studies that we referred to used a larger number of subjects than those in our screening datasets [11].

A meta-analysis was conducted by combining the screening, the replication and/or the original datasets. It is worth noting that if the original dataset was involved in the PGC mega-analysis of BD, we used PGC results for the meta-analysis. A fixed model (if the I^2 heterogeneity index was less than 50) or random effect model (if the I^2 heterogeneity index was greater than 50) was applied in each analysis. All of the statistical procedures were calculated using PLINK version 1.07 [23].

Results

Replication analysis of the BD GWAS SNPs with BD, SCZ and psychosis in the Japanese population

After the QC calculations, 42 SNPs and 2,759 samples in the first-set screening samples were eligible for the association analysis (916 patients with BD, 946 patients with SCZ and 897 healthy controls). Table 1 lists the results, which indicated a nominal association signal (uncorrected P<0.05). Complete results are presented in Table S2. It is of note that all of the SNPs that had a nominal association with the BD sample (BD association), were also associated with SCZ (SCZ association), thus the P-values for psychosis (psychosis association) are more significant (Table 1).

In the analysis of BD association and psychosis association, the most significant association maps to the Sp8 transcription factor (*SP8*) locus (rs2709736: uncorrected P = 0.0055 for BD, and rs2709722, uncorrected P = 0.0010 for psychosis), which is the same direction as the original Taiwanese population-based study [16]. However, in the analysis of SCZ association, the strongest association signal maps to chromosome 3 (52–53 Mb) and rs2251219 (uncorrected P = 0.0018) in the polybromo 1 gene (*PBRM1*).

Only two SNPs (rs2709722 in *SP8* and rs2251219 in *PBRM1*) within the psychosis set remained significant after the multiple comparison correction (corrected P = 0.037 and 0.048 for rs2709722 and rs2251219, respectively). Thus, we performed a replication analysis of these two SNPs using an independent second set of samples with BD and SCZ. The associations of both SNPs were replicated, indicating a significant association with psychosis (corrected P = 0.033 and 0.0070 for rs2709722 and rs2251219, respectively). However, the *SP8* SNP (rs2709722) and *PBRM1* SNP (rs2251219) revealed only a nominal association level with BD or SCZ (0.01<corrected P<0.1, Table 1).

Meta-analysis of the significant SNPs detected in the first-set screening samples combining the results from our current study and the original study

In the meta-analysis, we combined our two datasets (the first-set screening samples and second-set replication samples) for the two SNPs (rs2709722 and rs2251219) to assess the association for only the Japanese population. We obtained stronger evidence of association in all of the sample sets (Table 2). Specifically, results from the psychosis sample had the most significant association ($P = 8.0\times10^{-5}$ for rs2251219 and $P = 4.0\times10^{-4}$ for rs2709722).

We then combined the results from the original study [16] and/or PGC [5] datasets. For rs2251219, the original study by McMahon et al. [17] reported that the association was included in the PGC [5]; thus, we only combined the PGC results. The original study by Lee et al. [16] showed significance for rs2709722 based upon a dominant model. To conduct this meta-analysis, we recalculated the allele-wise P-value based upon the allele frequency information. For rs2251219, we detected an association with a genome-wide significance level only in the BD ($P = 9.4\times10^{-9}$), SCZ ($P = 4.3\times10^{-10}$) and psychosis sets ($P = 2.0\times10^{-10}$). Stronger evidence for rs2709722 was obtained in the meta-analysis (the current study and Lee's results [16]) (BD only: $P = 5.1\times10^{-7}$, SCZ only: $P = 1.6\times10^{-6}$, psychosis: $P = 2.1\times10^{-7}$), although this signal weakened the sample size was increased by including data from a Caucasian population (results from the current, Lee et al. and PGC studies: BD only: P = 0.0070 SCZ only: P = 0.0074 and psychosis: P = 0.0043: Table 2).

Discussion

In this study, we conducted a two-stage association analysis of the promising risk SNPs based upon BD GWASs with BD, SCZ and psychosis samples from a Japanese population. Two SNPs were detected with significant associations in the all of the phenotypes from the first-set screening (if uncorrected for multiple comparison) and second-set replication samples, indicating that these SNPs may play a role as a common risk factor for both BD

and SCZ. Furthermore, we detected an association on a genome-wide significance level within the *PBRM1* locus (rs2251219) by combining the results from the recent mega-analysis, which used the largest sample size thus far [5].

The SNP rs2251219, which maps to the *PBRM1* locus, was originally reported in a meta-analysis of BD and major depressive disorders in a Caucasian population ($P = 1.7 \times 10^{-9}$) [17]. This SNP was also reported in the PGC BD as possessing a suggestive level of association ($P = 5.5 \times 10^{-7}$) [5]. Our meta-analysis supports this finding regarding BD because we detected a genome-wide significance ($P = 9.4 \times 10^{-9}$), even when the BD set was analyzed alone. It is also of note that the results of this meta-analysis merging our SCZ/psychosis sets and PGC BD showed genome-wide significance (SCZ and PGC BD: $P = 4.3 \times 10^{-10}$, psychosis and PGC BD: $P = 2.0 \times 10^{-10}$).

Interestingly, rs2251219 is in LD with another SNP (rs1042779, base position = 52.8 Mb) in inter-alpha-trypsin inhibitor heavy chain 1 (*ITIH1*), which was examined in our screening sample ($D' = 0.96$ and $r^2 = 0.84$ in an Asian population in the 1000 genome database as a reference panel) and revealed a nominal association (uncorrected $P < 0.05$ for all phenotypes). This region (3p21.1) is one of the most attractive loci as a candidate region for risk for psychotic disorders. A recent study by Hamshere et al. [11] reported that SNP (rs2239547) in inter-alpha-trypsin inhibitor heavy chain 3 and 4 (*ITIH3-4*) was significantly associated with BD and/or SCZ at a genome-wide significance level ($D' = 0.95$ and $r^2 = 0.58$ with rs2251219, Asian population in the 1000-genome database as a reference panel). The most recent study by the PGC Cross-Disorder Group (CDG) also reported that SNP (rs2535629) in *ITIH3-4* was associated with five psychiatric disorders, and the strongest association was observed in the BD set [12]. These SNPs located in and around *PBRM1*, *ITIH1* and *ITIH3-4* are in strong LD. Thus, all of the SNPs in this LD block are promising candidates for genetic risk for BD, SCZ or psychosis. This LD structure in turn indicates that it is difficult to narrow down the true susceptibility variants within this region (Figure S4).

A Taiwanese BD GWAS found a suggestive association signal that maps to the *SP8* locus ($P = 4.8 \times 10^{-7}$ in dominant model) [16]. Our result supports the association of this gene with BD, specifically for Asian populations. The meta-analysis of our results and Lee's results indicated a stronger association signal that was weakened when the sample size was increased by including data from a Caucasian population (Table 2). Considering the results of the heterogeneity test (in which the I^2 score significantly increased by combining PGC BD dataset), the *SP8* gene may play a role as a population-specific risk gene in individuals of Asian ancestry. *SP8* is a SP transcription factor and functions in neural development by interacting with Wnt/beta-catenin and fibroblast growth factor (FGF) signaling [24]. Because there are no studies that have examined the association between *SP8* and BD/SCZ, further research is needed to better understand the relationship between *SP8* and psychiatric disorders. Special attention is needed regarding the population-specific effect that rs2709722 might have.

SNPs located in other promising candidate genes were not significantly associated with BD, SCZ or psychosis in the Japanese population. Although a trans-population effect is a likely explanation, our sample size may not have sufficient power to observe the association compared with the estimated effect size (odds ratio (OR) of ~1.2). The analysis of the power of our study [25] indicated that our sample had 25% power for BD/SCZ and 40% for psychosis to detect significance (assuming an OR of 1.2) of risk with 25% MAF (average MAF in our examined SNPs in the control subjects) under an additive model (type I error rate = 0.00138). Therefore, a larger sample size in future work is essential.

In conclusion, we found two loci, *PBRM1* (and neighboring genes) and *SP8*, that were replicated in psychotic disorders in a Japanese population. Specifically, a SNP within *PBRM1* revealed genome-wide significance in the meta-analysis, suggesting promising candidate genes for BD. *SP8*, which was not significant on a genome-wide level, is still a candidate for a population-specific risk factor for BD.

Supporting Information

Figure S1 SNP selection strategy. JPT: HapMap Japanese Tokyo sample HWE: Hardy-Weinberg Equilibrium.
(TIF)

Figure S2 Sample numbers in the first-set screening samples. BD: Bipolar disorder SCZ: Schizophrenia.
(TIF)

Figure S3 Sample numbers in the second-set replication samples. BD: Bipolar disorder SCZ: Schizophrenia.
(TIF)

Figure S4 Linkage disequilibrium structure around 3q21 in the Asian population. Data are from HapMap JPT and CHB. The LD measure is based upon D'.
(TIF)

Table S1 SNPs selected based upon previous BD GWAS.
(XLSX)

Table S2 Association analysis for all SNPs.
(XLSX)

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Author Contributions

Conceived and designed the experiments: KK MI NI. Performed the experiments: KK MI YK TS YI BA KY TT EH. Analyzed the data: KK MI. Contributed reagents/materials/analysis tools: TI HK TK HU TY NO. Wrote the paper: KK MI BA NI.

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SHORT COMMUNICATION

Genetic association study between the detected risk variants based upon type II diabetes GWAS and psychotic disorders in the Japanese population

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Several epidemiological and genetic studies have suggested that the risk of type II diabetes (T2D) is likely to overlap with the susceptibility to psychotic disorders such as schizophrenia (SCZ) and bipolar disorder (BD). In this study, we aimed to examine the association of single-nucleotide polymorphisms (SNPs) detected in previous T2D genome-wide association studies (GWAS) with SCZ, BD and psychosis (SCZ plus BD). A total of 37 SNPs were selected from the literature. A two-stage analysis was conducted using a first set of screening samples (total $N=3037$) and a second set of replication samples ($N=4950$). None of the SNPs showed a significant association to the screening samples after correction for multiple testing. To avoid type II error, we genotyped the top three SNPs in *BCL11A*, *HMG20A* and *HNF4A* showing associations with any of the phenotypes ($P_{uncorrected}<0.01$) using independent samples to replicate the nominal associations. However, we were unable to find any significant associations based on the screening results ($P_{uncorrected}>0.05$). Our findings did not support the shared genetic risk between T2D and psychotic disorders in the Japanese population. However, further replication using a larger sample size is required.
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Keywords: bipolar disorder; schizophrenia; single-nucleotide polymorphism

An increasing rate of prevalence of metabolic abnormalities, including type II diabetes (T2D), has been observed in subjects with major psychiatric disorders such as schizophrenia (SCZ) and bipolar disorder (BD).[1,2] The hypotheses concerning the comorbidity between SCZ/BD and T2D include three categories of risk factors:[3] (1) adverse effects of antipsychotic drugs (gene × drug interaction); (2) a high-risk lifestyle arising from psychiatric symptoms (for example, bulimia); and (3) common etiological factors including shared genetic risk. Interestingly, the prevalence of T2D is higher in young patients with SCZ and BD, who are expected to be minimally influenced by antipsychotics, compared with healthy individuals.[4] This finding strongly suggests a shared genetic risk between T2D and SCZ and/or BD.

Based on genetic association studies, a number of researchers have reported that loci associated with T2D risk overlap with SCZ risk genes.[5,6] To test the hypothesis that there is shared genetic risk between T2D and psychotic disorders, and to replicate the previous findings, we examined whether T2D risk SNPs selected on the basis of previous GWASs are associated with SCZ and BD in the Japanese population.

Two independent sets of samples were used in this study. In the screening analysis involving the first set, we examined 1032 SCZ and 1012 BD patients, and 993 controls. For the three SNPs that showed a nominal significant association signal in the screening analysis, we used an independent second set sample for replication analysis (1808 SCZ, 821 BD and 2321 controls). A detailed description of the general characterization and psychiatric assessments of our subjects has previously been reported[7] and listed in the Supplementary Information. Written informed consent was obtained from each subject. The ethics committees of Fujita Health University, RIKEN Brain Science Institute (BSI) and the institutes participating in the Collaborative Study of Mood Disorder (COSMO) approved this study.[7,8]

We selected 37 SNPs from T2D GWAS data published before September 2011 (see Supplementary Information). All of the SNPs were genotyped using the Sequenom iPLEX GOLD system (Sequenom, San Diego, CA, USA) with stringent quality control

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