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.

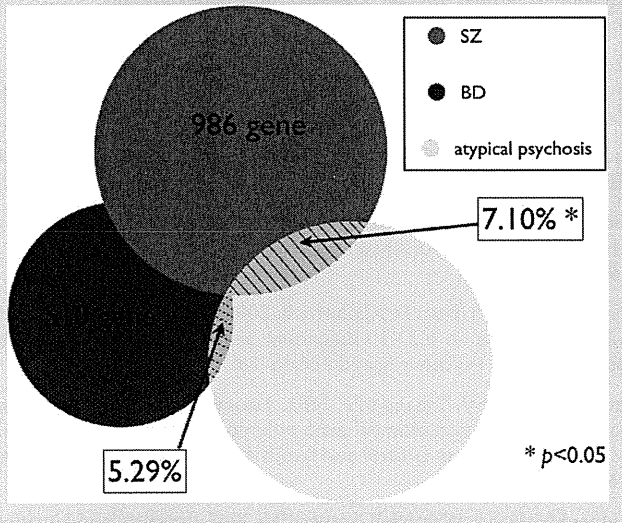

FIG. 4. The schema of overlapping percentages between three disorders ($P < .05$). Based on the result of VEGAS software analysis, the number of the genes with its *P*-value < 0.05 is shown in this schema (see Table 3). The overlapping percentage within Atypical psychosis is 7.10% (70/986) with SZ, and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

ACKNOWLEDGMENTS

The diagnostic criteria for "atypical psychosis" were established by a working group comprising Doctors Takaaki Abe, Hirohiko Harima, Akira Iwanami, Kosuke Kanemoto, Jun Koh, Kazuhiko Nakayama, Kaoru Sakamoto, Hiroshi Yoneda and Hidemichi Suga (the Chairman). Dr. Hirohiko Harima's insightful comments and suggestions regarding translation of the manuscript into English are particularly appreciated. Finally, we owe an intellectual debt to Professor Stephen V. Faraone and Professor Ming T. Tsuang, since this work could not have been published without their support.

REFERENCES

American Psychiatric Association. 2000. Diagnostic and statistical manual of mental disorders, 4th edition, DSM-IV-TR. Washington D.C. and London, England: American Psychiatric Publications.

Andreasen NC, Carpenter WT Jr. 1993. Diagnosis and classification of schizophrenia. Schizophr Bull 19:199–214.

Badner JA, Gershon ES. 2002. Meta-analysis of whole-genome linkage scans of bipolar disorder and schizophrenia. Mol Psychiatry 7: 405–411.

Berrettini W. 2004. Bipolar disorder and schizophrenia: Convergent molecular data. Neuromol Med 5:109–117.

Cardno AG, Rijsdijk FV, Sham PC, Murray RM, McGuffin P. 2002. A twin study of genetic relationships between psychotic symptoms. Am J Psychiatry 159:539–545.

Craddock N, Davé S, Greening J. 2001. Association studies of bipolar disorder. Bipolar Disord 3:284–298.

Craddock N, O'Donovan MC, Owen MJ. 2005. The genetics of schizophrenia and bipolar disorder: Dissecting psychosis. J Med Genet 42: 193–204.

Das SK, Malhotra S, Basu D, Malhotra R. 2001. Testing the stress-vulnerability hypothesis in ICD-10-diagnosed acute and transient psychotic disorders. Acta Psychiatr Scand 104:56–58.

Glatt SJ, Faraone SV, Tsuang MT. 2003. Association between a functional catechol O-methyltransferase gene polymorphism and schizophrenia: Meta-analysis of case-control and family-based studies. Am J Psychiatry 160:469–476.

Goldner EM, Hsu L, Waraich P, Somers JM. 2002. Prevalence and incidence studies of schizophrenic disorders: A systematic review of the literature. Can J Psychiatry 47:833–843.

Goldstein JM, Faraone SV, Chen WJ, Tolomiczencko GS, Tsuang MT. 1990. Sex differences in the familial transmission of schizophrenia. Br J Psychiatry 156:819–826.

Green EK, et al. 2009. The bipolar disorder risk allele at CACNA1C also confers risk of recurrent major depression and of schizophrenia. Mol Psychiatry 15:1016–1022.

Hashimoto R, Yoshida M, Kunugi H, Ozaki N, Yamanouchi Y, Iwata N, Suzuki T, Kitajima T, Tatsumi M, Kamijima K. 2005. A missense polymorphism (H204R) of a Rho GTPase-activating protein, the chimerin 2 gene, is associated with schizophrenia in men. Schizophr Res 73(2–3):383–385.

Hatotani N, Nomura J. 1983. Neurobiology of periodic psychoses. Tokyo, Japan: Igaku-Shoin Medical Publishers.

Hattori E, et al. 2009. Preliminary genome-wide association study of bipolar disorder in the Japanese population. Am J Med Genet Part B 150B:1110–1117.

Hayashi T, et al. 2001. Magnetic resonance imaging findings in schizophrenia and atypical psychoses. J Neural Transm 108:695–706.

Ikeda M, et al. 2004. Association of AKT1 with schizophrenia confirmed in a Japanese population. Biol Psychiatry 56:698–700.

Ikeda M, et al. 2011. Genome-wide association study of schizophrenia in a Japanese population. Biol Psychiatry 69:472–478.

International Schizophrenia Consortium, et al. 2009. Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. Nature 460:748–752.

Jeffreys AJ, Kauppi L, Neumann R. 2001. Intensely punctate meiotic recombination in the class II region of the major histocompatibility complex. Nat Genet 29:217–222.

Kanazawa T, et al. 2007. Schizophrenia is not associated with the functional candidate gene ERBB3: Results from a case-control study. Am J Med Genet Part B 144B:113–116.

Kasanin J. 1994. The acute schizoaffective psychoses. 1933. Am J Psychiatry 151:144–154.

Kawashige S, et al. 2008. An association study of the signal transducer and activator of transcription 6 gene with periodic psychosis. Psychiatry Investig 5:41–44.

Kraepelin E. 1921. Manic depressive insanity paranoia. J Nerv Ment Dis 53:350.

Kraepelin E. 1971. Dementia praecox and paraphrenia. Florida: Krieger Publishing Company.

Leonhard K. 1957. Aufteilung der endogenen Psychosen. Berlin: Akademie-Verlag.

Leonhard K. 1961. Cycloid psychoses—Endogenous psychoses which are neither schizophrenic nor manic-depressive. Br J Psychiatry 107: 633–648.

Liu JZ, et al. 2010. A versatile gene-based test for genome-wide association studies. Am J Hum Genet 87:139–145.

Magnan V. 1893. Leçon Cliniques Sur les Maladies Mentales à l'Asile Clinique Sainte-Anne, 2nd edn. Paris: Bataille.

Marneros A, Pillmann F. 2004. Acute and transient psychoses. Cambridge, UK: Cambridge University Press.

Marneros A, Tsuang MT. 1986. Schizoaffective psychoses. Berlin, Germany: Springer.

Marneros A, Andreasen NC, Tsuang MT. 1995. Psychotic continuum. Berlin, Germany: Springer.

Merikangas KR, et al. 2007. Lifetime and 12-month prevalence of bipolar spectrum disorder in the National Comorbidity Survey replication. Arch Gen Psychiatry 64:543–552.

Mitsuda H. 1950. On a pedigree of atypical psychoses. Folia Psychiatr Neurol Jpn 4:115–122.

Mitsuda H. 1965. The concept of "atypical psychoses" from the aspect of clinical genetics. Acta Psychiatr Scand 41:372–377.

Mitsuda H, Sakai T. 1968. Comparative studies with monozygotic twins discordant for typical and atypical schizophrenia. Jinrui Idengaku Zasshi 13:183–188.

Murray RM, et al. 2004. A developmental model for similarities and dissimilarities between schizophrenia and bipolar disorder. Schizophr Res 71:405–416.

Owen MJ, Craddock N, Jablensky A. 2007. The genetic deconstruction of psychosis. Schizophr Bull 33:905–911.

Potash JB, et al. 2003. Suggestive linkage to chromosomal regions 13q31 and 22q12 in families with psychotic bipolar disorder. Am J Psychiatry 160:680–686.

Regier DA, Kaelber CT, Roper MT, Rae DS, Sartorius N. 1994. The ICD-10 clinical field trial for mental and behavioral disorders: Results in Canada and the United States. Am J Psychiatry 151:1340–1350.

Rice DP. 1999. The economic impact of schizophrenia. J Clin Psychiatry 60 (Suppl. 1):4–6; discussion 28–30.

Saha S, Chant D, McGrath J. 2008. Meta-analyses of the incidence and prevalence of schizophrenia: Conceptual and methodological issues. Int J Methods Psychiatr Res 17:55–61.

Sartorius N, Ustün TB, Korten A, Cooper JE, van Drimmelen J. 1995. Progress toward achieving a common language in psychiatry, II: Results from the international field trials of the ICD-10 diagnostic criteria for research for mental and behavioral disorders. Am J Psychiatry 152:1427–1437.

Stefansson H, et al. 2009. Common variants conferring risk of schizophrenia. Nature 460:744–747.

Sullivan PF, Daly MJ, O'Donovan M. 2012. Genetic architectures of psychiatric disorders: The emerging picture and its implications. Nat Rev Genet 13:537–551.

Tsuang MT, Winokur G. 1975. The Iowa 500: Field work in a 35-year follow-up of depression, mania, and schizophrenia. Can Psychiatr Assoc J 20:359–365.

Tsuang MT, Winokur G, Crowe RR. 1980. Morbidity risks of schizophrenia and affective disorders among first degree relatives of patients with schizophrenia, mania, depression and surgical conditions. Br J Psychiatry 137:497–504.

Tsuang MT, Faraone SV, Johnson PDC. 1997. Schizophrenia: The facts. Oxford, UK: Oxford University Press.

Van Snellenberg JX, de Candia T. 2009. Meta-analytic evidence for familial coaggregation of schizophrenia and bipolar disorder. Arch Gen Psychiatry 66:748–755.

Williams HJ, et al. 2011a. Fine mapping of ZNF804A and genome-wide significant evidence for its involvement in schizophrenia and bipolar disorder. Mol Psychiatry 16:429–441.

Williams HJ, et al. 2011b. Most genome-wide significant susceptibility loci for schizophrenia and bipolar disorder reported to date cross traditional diagnostic boundaries. Hum Mol Genet 20:387–391.

World Health Organization. 1993. The ICD-10 Classification of Mental and Behavioural Disorders: Diagnostic Criteria for Research. Geneva, Switzerland: World Health Organization.

Wyatt RJ, Henter I, Leary MC, Taylor E. 1995. An economic evaluation of schizophrenia–1991. Soc Psychiatry Psychiatr Epidemiol 30: 196–205.

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.

Neuropsychopharmacology (2013), 1–7

www.neuropsychopharmacology.org

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

Masashi Ikeda[1], Yuko Okahisa[2], Branko Aleksic[3], Mujun Won[4,5], Naoki Kondo[4,6], Nobuya Naruse[4,7], Kumi Aoyama-Uehara[8], Ichiro Sora[4,9], Masaomi Iyo[4,10], Ryota Hashimoto[11,12], Yoshiya Kawamura[13], Nao Nishida[14], Taku Miyagawa[14], Masatoshi Takeda[11], Tsukasa Sasaki[15], Katsushi Tokunaga[14], Norio Ozaki[3,4], Hiroshi Ujike[2,4] and Nakao Iwata*[,1,4]

[1]Department of Psychiatry, Fujita Health University School of Medicine, Aichi, Japan; [2]Department of Neuropsychiatry, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan; [3]Department of Psychiatry, Nagoya University Graduate School of Medicine, Nagoya, Japan; [4]Japanese Genetic Initiative for Drug Abuse (JGIDA), Japan; [5]Kojin Hospital, Nagoya, Aichi, Japan; [6]Seimei Hospital, Fuji, Shizuoka, Japan; [7]Saitama Prefectural Psychiatric Hospital, Saitama, Japan; [8]Serigaya Hospital, Yokohama, Kanagawa, Japan; [9]Department of Biological Psychiatry, Tohoku University Graduate School of Medicine, Sendai, Japan; [10]Department of Psychiatry, Chiba University Graduate School of Medicine, Chiba, Japan; [11]Department of Psychiatry, Osaka University Graduate School of Medicine, Osaka, Japan; [12]Molecular Research Center for Children's Mental Development, United Graduate School of Child Development, Osaka University, Kanazawa University and Hamamatsu University School of Medicine, Osaka, Japan; [13]Department of Psychiatry, Sakae Seijinkai Hospital, Yokohama, Kanagawa, Japan; [14]Department of Human Genetics, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan; [15]Graduate School of Education and Division for Counseling and Support, The University of Tokyo, Tokyo, Japan

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.
Neuropsychopharmacology advance online publication, 12 June 2013; doi:10.1038/npp.2013.94

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

*Correspondence: Dr N Iwata, Department of Psychiatry, Fujita Health University School of Medicine, Toyoake, Aichi 470-1192, Japan, Tel: +81 562 93 9250, Fax: +81 562 93 1831, E-mail: nakao@fujita-hu.ac.jp
Received 20 December 2012; revised 25 March 2013; accepted 26 March 2013; accepted article preview online 12 April 2013

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*) ($P=3.6\times10^{-6}$,

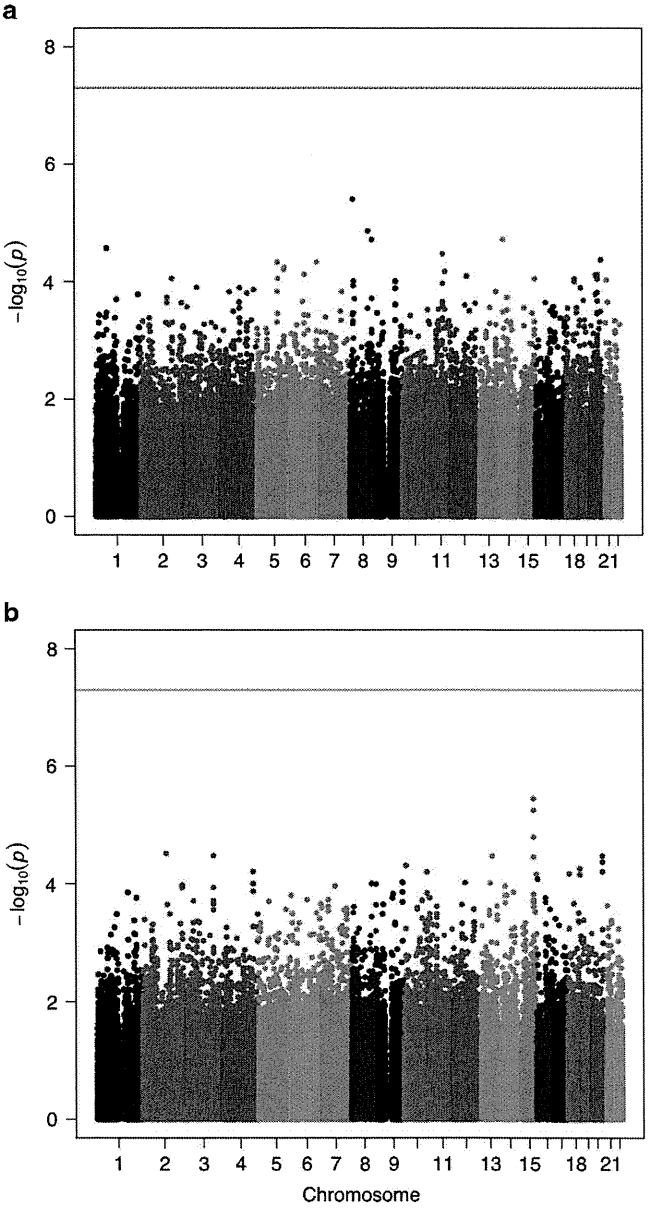

Figure 1 The Manhattan plots for (a) METH dependence and (b) METH-induced psychosis GWASs. METH, methamphetamine.

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	14864473 1	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

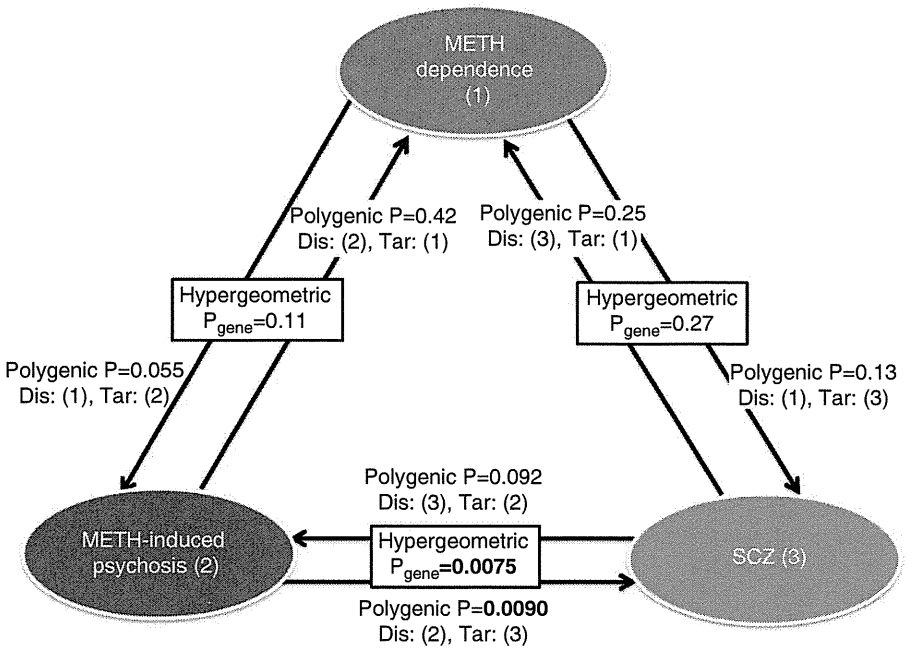

Figure 2 Relationship between METH-dependence (1), METH-induced psychosis (2), and schizophrenia (3). METH, methamphetamine; SCZ, schizophrenia; Dis, Discovery sample; Tar, Target sample. 'Polygenic *P*' indicates the best *P*-value calculated by logistic regression analysis in the polygenic component 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, $P = 3.6 \times 10^{-6}$) 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 ($R^2 \sim 0.7\%$), and it showed similar magnitude reported in the comparison of SCZ between Japanese and the UK populations ($R^2 \sim 0.8\%$, 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.

ACKNOWLEDGEMENTS

This work was supported by research grants from Grants-in-Aid for Scientific Research of the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan; Grants-in-Aid for Scientific Research on Innovative Areas (Comprehensive Brain Science Network) from MEXT of Japan; Ministry of Health, Labor and Welfare of Japan; Academic Frontier Project for Private Universities, Comparative Cognitive Science Institutes; Core Research for Evolutional Science and Technology; Uehara Memorial Foundation; SENSHIN Medical Research Foundation; Takeda Science Foundation; the Novartis Foundation, Japan; Naito Foundation, Japan; and Strategic Research Program for Brain Sciences of the MEXT of Japan.

DISCLOSURE

The authors declare no conflict of interest.

REFERENCES

Bousman CA, Glatt SJ, Everall IP, Tsuang MT (2009). Genetic association studies of methamphetamine use disorders: a systematic review and synthesis. *Am J Med Genet B Neuropsychiatr Genet* **150B**: 1025–1049.

Callaghan RC, Cunningham JK, Allebeck P, Arenovich T, Sajeev G, Remington G *et al* (2012). Methamphetamine use and schizophrenia: a population-based cohort study in California. *Am J Psychiatry* **169**: 389–396.

Chen CK, Lin SK, Sham PC, Ball D, Loh el W, Murray RM (2005). Morbid risk for psychiatric disorder among the relatives of methamphetamine users with and without psychosis. *Am J Med Genet B Neuropsychiatr Genet* **136B**: 87–91.

Cirulli ET, Goldstein DB (2010). Uncovering the roles of rare variants in common disease through whole-genome sequencing. *Nat Rev Genet* **11**: 415–425.

Clark SL, Adkins DE, Aberg K, Hettema JM, McClay JL, Souza RP *et al* (2012). Pharmacogenomic study of side-effects for antidepressant treatment options in STAR*D. *Psychol Med* **42**: 1151–1162.

Grelotti DJ, Kanayama G, Pope HG Jr. (2010). Remission of persistent methamphetamine-induced psychosis after electroconvulsive therapy: presentation of a case and review of the literature. *Am J Psychiatry* **167**: 17–23.

Huang J, Perlis RH, Lee PH, Rush AJ, Fava M, Sachs GS *et al* (2010). Cross-disorder genomewide analysis of schizophrenia, bipolar disorder, and depression. *Am J Psychiatry* **167**: 1254–1263.

Ikeda M, Aleksic B, Kinoshita Y, Okochi T, Kawashima K, Kushima I *et al* (2011). Genome-wide association study of schizophrenia in a Japanese population. *Biol Psychiatry* **69**: 472–478.

Ikeda M, Aleksic B, Yamada K, Iwayama-Shigeno Y, Matsuo K, Numata S *et al* (2012). Genetic evidence for association between NOTCH4 and schizophrenia supported by a GWAS follow-up study in a Japanese population. *Mol Psychiatry* (in press).

Kristiansen LV, Beneyto M, Haroutunian V, Meador-Woodruff JH (2006). Changes in NMDA receptor subunits and interacting PSD proteins in dorsolateral prefrontal and anterior cingulate cortex indicate abnormal regional expression in schizophrenia. *Mol Psychiatry* **11**: 737–747 705.

Lavedan C, Licamele L, Volpi S, Hamilton J, Heaton C, Mack K *et al* (2009). Association of the NPAS3 gene and five other loci with response to the antipsychotic iloperidone identified in a whole genome association study. *Mol Psychiatry* **14**: 804–819.

Liu JZ, McRae AF, Nyholt DR, Medland SE, Wray NR, Brown KM *et al* (2010). A versatile gene-based test for genome-wide association studies. *Am J Hum Genet* **87**: 139–145.

Machiyama Y (1992). Chronic methamphetamine intoxication model of schizophrenia in animals. *Schizophr Bull* **18**: 107–113.

Macintyre G, Alford T, Xiong L, Rouleau GA, Tibbo PG, Cox DW (2010). Association of NPAS3 exonic variation with schizophrenia. *Schizophr Res* **120**: 143–149.

MacLaren EJ, Charlesworth P, Coba MP, Grant SG (2011). Knockdown of mental disorder susceptibility genes disrupts neuronal network physiology in vitro. *Mol Cell Neurosci* **47**: 93–99.

Miyagawa T, Kawashima M, Nishida N, Ohashi J, Kimura R, Fujimoto A *et al* (2008). Variant between CPT1B and CHKB associated with susceptibility to narcolepsy. *Nat Genet* **40**: 1324–1328.

Nutt DJ, King LA, Phillips LD (2010). Drug harms in the UK: a multicriteria decision analysis. *Lancet* **376**: 1558–1565.

Otowa T, Yoshida E, Sugaya N, Yasuda S, Nishimura Y, Inoue K *et al* (2009). Genome-wide association study of panic disorder in the Japanese population. *J Hum Genet* **54**: 122–126.

Pickard BS, Christoforou A, Thomson PA, Fawkes A, Evans KL, Morris SW *et al* (2009). Interacting haplotypes at the NPAS3 locus alter risk of schizophrenia and bipolar disorder. *Mol Psychiatry* **14**: 874–884.

Pieper AA, Wu X, Han TW, Estill SJ, Dang Q, Wu LC *et al* (2005). The neuronal PAS domain protein 3 transcription factor controls FGF-mediated adult hippocampal neurogenesis in mice. *Proc Natl Acad Sci USA* **102**: 14052–14057.

Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D (2006). Principal components analysis corrects for stratification in genome-wide association studies. *Nat Genet* **38**: 904–909.

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D *et al* (2007). PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* **81**: 559–575.

Purcell SM, Wray NR, Stone JL, Visscher PM, O'Donovan MC, Sullivan PF *et al* (2009). Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. *Nature* **460**: 748–752.

Stefansson H, Ophoff RA, Steinberg S, Andreassen OA, Cichon S, Rujescu D *et al* (2009). Common variants conferring risk of schizophrenia. *Nature* **460**: 744–747.

Sullivan PF, Lin D, Tzeng JY, van den Oord E, Perkins D, Stroup TS *et al* (2008). Genomewide association for schizophrenia in the CATIE study: results of stage 1. *Mol Psychiatry* **13**: 570–584.

Uhl GR, Drgon T, Liu QR, Johnson C, Walther D, Komiyama T *et al* (2008). Genome-wide association for methamphetamine dependence: convergent results from 2 samples. *Arch Gen Psychiatry* **65**: 345–355.

Ujike H, Sato M (2004). Clinical features of sensitization to methamphetamine observed in patients with methamphetamine dependence and psychosis. *Ann N Y Acad Sci* **1025**: 279–287.

Yang J, Lee SH, Goddard ME, Visscher PM (2011). GCTA: a tool for genome-wide complex trait analysis. *Am J Hum Genet* **88**: 76–82.

Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)

PLOS ONE

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

Kenji Kondo[1], Masashi Ikeda[1]*, Yusuke Kajio[1], Takeo Saito[1], Yoshimi Iwayama[2], Branko Aleksic[3], Kazuo Yamada[2], Tomoko Toyota[2], Eiji Hattori[2], Hiroshi Ujike[4], Toshiya Inada[5], Hiroshi Kunugi[6], Tadafumi Kato[7], Takeo Yoshikawa[2], Norio Ozaki[3], Nakao Iwata[1]

1 Department of Psychiatry, Fujita Health University School of Medicine, Toyoake, Aichi, Japan, **2** Laboratory for Molecular Psychiatry, RIKEN Brain Science Institute, Wako, Saitama, Japan, **3** Department of Psychiatry, Nagoya University Graduate School of Medicine, Nagoya, Aichi, Japan, **4** Department of Neuropsychiatry, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Science, Okayama, Okayama, Japan, **5** Department of Psychiatry, Seiwa Hospital, Institute of Neuropsychiatry, Shinjuku, Tokyo, Japan, **6** Department of Mental Disorder Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Kodaira, Tokyo, Japan, **7** Laboratory for Molecular Dynamics of Mental Disorders, RIKEN Brain Science Institute, Wako, Saitama, Japan

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 ($P = 5 \times 10^{-8}$) only for BD ($P = 9.4 \times 10^{-9}$) and psychosis ($P = 2.0 \times 10^{-10}$). Although the association of rs2709722 in Sp8 transcription factor (*SP8*) was suggestive in the Asian population ($P = 2.1 \times 10^{-7}$ for psychosis), this signal weakened when the samples size was increased by including data from a Caucasian population ($P = 4.3 \times 10^{-3}$).

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.

Citation: Kondo K, Ikeda M, Kajio Y, Saito T, Iwayama Y, et al. (2013) Genetic Variants on 3q21 and in the Sp8 Transcription Factor Gene (*SP8*) as Susceptibility Loci for Psychotic Disorders: A Genetic Association Study. PLoS ONE 8(8): e70964. doi:10.1371/journal.pone.0070964

Editor: Ryota Hashimoto, United Graduate School of Child Development, Osaka University, Japan

Received June 9, 2013; **Accepted** June 17, 2013; **Published** August 13, 2013

Funding: This work was supported by research grants from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan, the Ministry of Health, Labor and Welfare of Japan, the Academic Frontier Project for Private Universities, Comparative Cognitive Science Institutes, the Uehara Memorial Foundation, the SEISHIN Medical Research Foundation, the Takeda Science Foundation and the Strategic Research Program for Brain Sciences of the MEXT of Japan. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: Tadafumi Kato and Takeo Yoshikawa are PLOS ONE Editorial Board members. This does not alter the authors' adherence to all the PLOS ONE policies on sharing data and materials.

* E-mail: ikeda-ma@fujita-hu.ac.jp

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	ITHI3	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.

doi:10.1371/journal.pone.0070964.t001

(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-caluculated P values based upon their results.
d: BD: bipolar disorder, SCZ: schizophrenia.
e: the effect is with respect to the A1 allele.
doi:10.1371/journal.pone.0070964.t002

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)

Acknowledgments

We thank Ms. M. Miyata, Ms. M. Aizawa, Ms. Y. Umekage and Ms. M. Uchida for their technical support.

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.

References

1. Merikangas KR, Jin R, He JP, Kessler RC, Lee S, et al. (2011) Prevalence and correlates of bipolar spectrum disorder in the world mental health survey initiative. Arch Gen Psychiatry 68: 241–251.
2. Craddock N, Sklar P (2009) Genetics of bipolar disorder: successful start to a long journey. Trends Genet 25: 99–105.
3. Smoller JW, Finn CT (2003) Family, twin, and adoption studies of bipolar disorder. Am J Med Genet C Semin Med Genet 123C: 48–58.
4. Ferreira MA, O'Donovan MC, Meng YA, Jones IR, Ruderfer DM, et al. (2008) Collaborative genome-wide association analysis supports a role for ANK3 and CACNA1C in bipolar disorder. Nat Genet 40: 1056–1058.
5. Psychiatric GWAS Consortium Bipolar Disorder Working Group (2011) Large-scale genome-wide association analysis of bipolar disorder identifies a new susceptibility locus near ODZ4. Nat Genet 43: 977–983.
6. Cichon S, Muhleisen TW, Degenhardt FA, Mattheisen M, Miro X, et al. (2011) Genome-wide association study identifies genetic variation in neurocan as a susceptibility factor for bipolar disorder. Am J Hum Genet 88: 372–381.
7. Williams HJ, Craddock N, Russo G, Hamshere ML, Moskvina V, et al. (2011) Most genome-wide significant susceptibility loci for schizophrenia and bipolar disorder reported to date cross-traditional diagnostic boundaries. Hum Mol Genet 20: 387–391.

8. Purcell SM, Wray NR, Stone JL, Visscher PM, O'Donovan MC, et al. (2009) Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. Nature 460: 748–752.
9. Lichtenstein P, Yip BH, Bjork C, Pawitan Y, Cannon TD, et al. (2009) Common genetic determinants of schizophrenia and bipolar disorder in Swedish families: a population-based study. Lancet 373: 234–239.
10. Craddock N, Owen MJ (2010) The Kraepelinian dichotomy – going, going... but still not gone. Br J Psychiatry 196: 92–95.
11. Hamshere ML, Walters JT, Smith R, Richards AL, Green E, et al. (2013) Genome-wide significant associations in schizophrenia to ITIH3/4, CACNA1C and SDCCAG8, and extensive replication of associations reported by the Schizophrenia PGC. Mol Psychiatry.
12. Smoller JW, Craddock N, Kendler K, Lee PH, Neale BM, et al. (2013) Identification of risk loci with shared effects on five major psychiatric disorders: a genome-wide analysis. Lancet 381: 1371–1379.
13. Matsunaga S, Ikeda M, Kishi T, Fukuo Y, Aleksic B, et al. (2012) An evaluation of polymorphisms in casein kinase 1 delta and epsilon genes in major psychiatric disorders. Neurosci Lett 529: 66–69.
14. Wellcome_Trust_Case_Control_Consortium (2007) Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 447: 661–678.
15. Baum AE, Akula N, Cabanero M, Cardona I, Corona W, et al. (2008) A genome-wide association study implicates diacylglycerol kinase eta (DGKH) and several other genes in the etiology of bipolar disorder. Mol Psychiatry 13: 197–207.
16. Lee MT, Chen CH, Lee CS, Chen CC, Chong MY, et al. (2011) Genome-wide association study of bipolar I disorder in the Han Chinese population. Mol Psychiatry 16: 548–556.
17. McMahon FJ, Akula N, Schulze TG, Muglia P, Tozzi F, et al. (2010) Meta-analysis of genome-wide association data identifies a risk locus for major mood disorders on 3p21.1. Nat Genet 42: 128–131.
18. Scott LJ, Muglia P, Kong XQ, Guan W, Flickinger M, et al. (2009) Genome-wide association and meta-analysis of bipolar disorder in individuals of European ancestry. Proc Natl Acad Sci U S A 106: 7501–7506.
19. Sklar P, Smoller JW, Fan J, Ferreira MA, Perlis RH, et al. (2008) Whole-genome association study of bipolar disorder. Mol Psychiatry 13: 558–569.
20. Smith EN, Bloss CS, Badner JA, Barrett T, Belmonte PL, et al. (2009) Genome-wide association study of bipolar disorder in European American and African American individuals. Mol Psychiatry 14: 755–763.
21. Nyholt DR (2004) A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. Am J Hum Genet 74: 765–769.
22. Li J, Ji L (2005) Adjusting multiple testing in multilocus analyses using the eigenvalues of a correlation matrix. Heredity (Edinb) 95: 221–227.
23. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, et al. (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 81: 559–575.
24. Sahara S, Kawakami Y, Izpisua Belmonte JC, O'Leary DD (2007) Sp8 exhibits reciprocal induction with Fgf8 but has an opposing effect on anterior-posterior cortical area patterning. Neural Dev 2: 10.
25. Purcell S, Cherny SS, Sham PC (2003) Genetic Power Calculator: design of linkage and association genetic mapping studies of complex traits. Bioinformatics 19: 149–150.

Journal of Human Genetics (2013), 1–3

www.nature.com/jhg

SHORT COMMUNICATION

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

Yusuke Kajio[1,8], Kenji Kondo[1,8], Takeo Saito[1], Yoshimi Iwayama[2], Branko Aleksic[3], Kazuo Yamada[2], Tomoko Toyota[2], Eiji Hattori[2], Hiroshi Ujike[4], Toshiya Inada[5], Hiroshi Kunugi[6], Tadafumi Kato[7], Takeo Yoshikawa[2], Norio Ozaki[3], Masashi Ikeda[1] and Nakao Iwata[1]

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.
Journal of Human Genetics advance online publication, 7 November 2013; doi:10.1038/jhg.2013.116

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

[1]Department of Psychiatry, Fujita Health University School of Medicine, Toyoake, Japan; [2]Laboratory for Molecular Psychiatry, RIKEN Brain Science Institute, Wako, Japan; [3]Department of Psychiatry, Nagoya University Graduate School of Medicine, Nagoya, Japan; [4]Department of Neuropsychiatry, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Science, Okayama, Japan; [5]Department of Psychiatry, Institute of Neuropsychiatry, Seiwa Hospital, Tokyo, Japan; [6]Department of Mental Disorder Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Kodaira, Japan and [7]Laboratory for Molecular Dynamics of Mental Disorders, RIKEN Brain Science Institute, Wako, Japan
[8]These authors contributed equally to this work.
Correspondence: Dr N Iwata, Department of Psychiatry, Fujita Health University School of Medicine, Toyoake, Aichi 470-1192, Japan.
E-mail: nakao@fujita-hu.ac.jp
Received 12 October 2013; accepted 15 October 2013

(Supplementary Information). We assessed the allelic associations (two-tailed) of the SNPs with three phenotypes: (1) SCZ vs control (referred to as a 'SCZ association'), (2) BD vs control (referred to as a 'BD association') and (3) 'psychosis' (SCZ + BD) vs control (referred to as a 'psychosis association') in the first and second sets of samples. A meta-analysis of the SNPs (nominally significant SNPs in the first set of samples and genotyped SNPs in the second set of replication samples) was conducted by combining the screening and the replication data sets. A fixed model (the I^2 heterogeneity index <50), or a random effect model ($I^2>50$), was applied in each analysis. All of the statistical procedures were calculated using PLINK 1.07 software.[9]

Following the quality control procedure, a total of 32 SNPs and 2675 samples in the first screening set remained eligible for association analysis (930 SCZ, 869 BD and 876 healthy controls). The results are listed in Table 1 and show the nominal association signals ($P_{uncorrected}<0.05$) found for all three phenotypes (SCZ, BD and psychosis). The full results can be found in Supplementary Table 2.

In the SCZ sample, only one SNP in *UBE2E2* (rs7612463) showed a nominal significant association ($P_{uncorrected}<0.05$). However, we observed that the direction of the effect of this SNP was opposite to the hypothesized direction (that is, that the risk for T2D shows the same direction as psychosis).

The BD associations exhibited nominal significance for SNPs in *BCL11A* (rs243021, $P_{uncorrected}=0.0031$) and *HMG20A* (rs7178572, $P_{uncorrected}=0.0043$), with the same direction of effect as T2D, and SNPs in *SLC30A8* (rs3802177, $P_{uncorrected}=0.042$) and *HNF4A* (rs4812829, $P_{uncorrected}=0.0082$), which showed the opposite direction of effect (Table 1).

When we corrected the multiple testing results using the Bonferroni method ($N=32$), none of the SNPs showed a significant association (Table 1). To avoid type II error, we examined the results of replication analysis of the top three SNPs with arbitrary and relaxed α-levels set at $P<0.01$ using an independent second set of replication samples. However, no significant association was revealed in these independent data sets (Table 1).

Finally, using a meta-analytical approach, we combined our two data sets (the first screening set and the second replication set) for the three SNPs (rs243021, rs7178572 and rs4812829) to maximize the sample size. Again, no association was observed (Table 2).

In this study, we did not find any significant association between risk SNPs for T2D with the SCZ, BD or psychosis phenotypes in the Japanese population. Our results therefore do not provide supportive evidence of shared genetic risk between T2D and SCZ/BD.[5,6] Interestingly, the results of the psychosis association analysis for the SNPs, which showed nominal associations only with SCZ or BD, did not enhance the significance, even when the sample size was doubled. Therefore, we speculate that the T2D SNPs examined in this study do

Table 2 Meta-analysis of the three SNPs detected in the first set of screening analysis

						Current study (screeing + replication)		
CHR	*SNP*	*GENE*	*A1*	*A2*	*Phenotype*	P-*value*	*OR*	I^2
2	rs243021	BCL11A	C	T	SCZ	0.63	0.97	60.6
					BD	0.30	0.90	78.9
					PSY	0.45	0.93	81.6
15	rs7178572	HMG20A	G	A	SCZ	0.95	1	58.3
					BD	0.17	0.89	68.8
					PSY	0.52	0.95	79.0
20	rs4812829	HNF4A	A	G	SCZ	0.97	1	38.3
					BD	0.22	0.91	68.1
					PSY	0.49	0.95	73.4

Abbreviations: A1, non-reference allele; A2, reference allele; BD, bipolar disorder; CHR, chromosome; SCZ, schizophrenia; SNP, single-nucleotide polymorphism; PSY, psychosis.

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

							Screening							*Replication*					
CHR	*SNP (original SNP)*	*GENE*	*BP*[a]	*A1*[b]	*A2*[c]	*Pheno-type*	*F_A*[d]	*F_U*[e]	P-*value*	$P_{corrected}$[f]	*OR*[g]	*s.e.*	*Direc-tion*[h]	*F_A*	*F_U*	P-*value*	*OR*	*s.e.*	*Direc-tion*[h]
2	rs243021	BCL11A	60584819	C	T	SCZ	0.311	0.335	0.12	1	0.89	0.071	+	0.314	0.309	0.59	1.03	0.048	−
						BD	0.288		**0.0031**	0.100	0.81	0.073	+	0.307		0.92	0.99	0.063	+
						PSY	0.300		**0.0095**	0.30	0.85	0.062	+	0.312		0.71	1.02	0.044	−
3	rs7612463	UBE2E2	23336450	A	C	SCZ	0.184	0.155	0.021	0.68	1.23	0.089	−						
						BD	0.154		0.92	1	0.99	0.094	+						
						PSY	0.170		0.19	1	1.11	0.080	−						
8	rs3802177[i] (rs13266634)	SLC30A8	118185025	T	C	SCZ	0.424	0.423	0.95	1	1.00	0.068	−						
						BD	0.457		0.042	1	1.15	0.068	−						
						PSY	0.440		0.24	1	1.07	0.059	−						
15	rs7178572	HMG20A	77747190	G	A	SCZ	0.404	0.423	0.25	1	0.93	0.068	+	0.420	0.408	0.28	1.05	0.045	−
						BD	0.376		**0.0043**	0.14	0.82	0.069	+	0.400		0.56	0.97	0.059	+
						PSY	0.390		0.023	0.72	0.87	0.059	+	0.414		0.57	1.02	0.041	−
20	rs4812829	HNF4A	42989267	A	G	SCZ	0.459	0.477	0.281	1.0	0.93	0.067	−	0.463	0.455	0.49	1.03	0.045	+
						BD	0.432		**0.0082**	0.26	0.84	0.068	−	0.450		0.72	0.98	0.058	−
						PSY	0.446		0.034	1	0.88	0.059	−	0.458		0.72	1.02	0.041	+

Abbreviations: BD, bipolar disorder; CHR, chromosome; PSY, psychosis; SNP, single-nucleotide polymorphism; SCZ, schizophrenia.
[a]BP, base position based upon hg19.
[b]A1, minor allele based upon whole samples.
[c]A2, major allele.
[d]F_A: allele frequency of A1 in case.
[e]F_U: allele frequency of A1 in controls.
[f]$P_{corrected}$, corrected P with Boferroni correction ($N=32$).
[g]OR, odds ratio (A2 is reference).
[h]Direction: + same direction of the effect with the original study '−' opposite direction of the effect.
[i]This SNP is proxy SNP with the original one.
Bold numerals: A P-value < 0.01.

not exhibit a pleiotropic effect on psychotic disorders even though recent evidence has indicated that several identified SCZ risk SNPs are associated with BD and *vice versa*, suggesting the existence of shared genetic risk between SCZ and BD.[10,11]

The SNPs examined in this study were selected based on the results of T2D GWASs conducted in Caucasian and Asian populations, including individuals of Japanese ancestry. Therefore, a population difference cannot fully explain our results showing a lack of an association. However, owing to the modest sample size in this study (see Supplementary Information), this negative finding does not completely negate the hypothesis that shared genetic components contribute to both psychotic disorders and T2D. To increase the statistical power for our results in the first set of screening samples, we included two additional analyses, a polygenic component analysis[12] and a sign test to check the association of the cumulative effect of the T2D 'risk' SNPs. Again it showed lack of the significant enrichment between T2D SNPs (or 'risk' allele) and SCZ/BD (Supplementary Tables 3 and 4).

In summary, our association analysis did not support the shared genetic risk between T2D and psychotic disorders in the Japanese population. However, a larger sample size will be required to obtain conclusive results.

ACKNOWLEDGEMENTS

We thank Ms M Miyata, Ms M Aizawa, Ms Y Umekage and Ms M Uchida for their technical support. This work was supported by research grants from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan; the Ministry of Health, Labor and Welfare of Japan; the Academic Frontier Project for Private Universities, Comparative Cognitive Science Institutes; the Uehara Memorial Foundation; the SEISHIN Medical Research Foundation; and the Takeda Science Foundation and Strategic Research Program for Brain Sciences of the MEXT of Japan.

1 Regenold, W. T., Thapar, R. K., Marano, C., Gavirneni, S. & Kondapavuluru, P. V. Increased prevalence of type 2 diabetes mellitus among psychiatric inpatients with bipolar I affective and schizoaffective disorders independent of psychotropic drug use. *J. Affect. Disord.* **70,** 19–26 (2002).
2 Bai, Y. M., Su, T. P., Chen, M. H., Chen, T. J. & Chang, W. H. Risk of d eveloping diabetes mellitus and hyperlipidemia among patients with bipolar disorder, major depressive disorder, and schizophrenia: A 10-year nationwide population-based prospective cohort study. *J. Affect. Disord.* **150,** 57–62 (2013).
3 Lin, P. I. & Shuldiner, A. R. Rethinking the genetic basis for comorbidity of schizophrenia and type 2 diabetes. *Schizophr. Res.* **123,** 234–243 (2010).
4 Jerrell, J. M., McIntyre, R. S. & Tripathi, A. A cohort study of the prevalence and impact of comorbid medical conditions in pediatric bipolar disorder. *J. Clin. Psychiatry.* **71,** 1518–1525 (2010).
5 Hansen, T., Ingason, A., Djurovic, S., Melle, I., Fenger, M., Gustafsson, O. *et al.* At-risk variant in TCF7L2 for type II diabetes increases risk of schizophrenia. *Biol. Psychiatry.* **70,** 59–63 (2011).
6 Alkelai, A., Greenbaum, L., Lupoli, S., Kohn, Y., Sarner-Kanyas, K., Ben-Asher, E. *et al.* Association of the type 2 diabetes mellitus susceptibility gene, TCF7L2, with schizophrenia in an Arab-Israeli family sample. *PLoS One* **7,** e29228 (2012).
7 Matsunaga, S., Ikeda, M., Kishi, T., Fukuo, Y., Aleksic, B., Yoshimura, R. *et al.* An evaluation of polymorphisms in casein kinase 1 delta and epsilon genes in major psychiatric disorders. *Neurosci. Lett.* **529,** 66–69 (2012).
8 Kondo, K., Ikeda, M., Kajio, Y., Saito, T., Iwayama, Y., Aleksic, B. *et al.* Genetic variants on 3q21 and in the Sp8 transcription factor gene (SP8) as susceptibility loci for psychotic disorders: A Genetic Association Study. *PLoS One* **8,** e70964 (2013).
9 Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A., Bender, D. *et al.* PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* **81,** 559–575 (2007).
10 Psychiatric GWAS Consortium Bipolar Disorder Working Group. Large-scale genome-wide association analysis of bipolar disorder identifies a new susceptibility locus near ODZ4. *Nat. Genet.* **43,** 977–983 (2011).
11 The Schizophrenia Psychiatric Genome-Wide Association Study (GWAS) Consortium.. Genome-wide association study identifies five new schizophrenia loci. *Nat. Genet.* **43,** 969–976 (2011).
12 Purcell, S. M., Wray, N. R., Stone, J. L., Visscher, P. M., O'Donovan, M. C., Sullivan, P. F. *et al.* Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. *Nature* **460,** 748–752 (2009).

Supplementary Information accompanies the paper on Journal of Human Genetics website (http://www.nature.com/jhg)