OPEN

Molecular Psychiatry (2018) 23, 639–647

www.nature.com/mp

ORIGINAL ARTICLE

A genome-wide association study identifies two novel susceptibility loci and trans population polygenicity associated with bipolar disorder

M Ikeda[1,33], A Takahashi[2,3,33], Y Kamatani[2,33], Y Okahisa[4], H Kunugi[5], N Mori[6], T Sasaki[7], T Ohmori[8], Y Okamoto[9], H Kawasaki[10], S Shimodera[11], T Kato[12], H Yoneda[13], R Yoshimura[14], M Iyo[15], K Matsuda[16], M Akiyama[2], K Ashikawa[17], K Kashiwase[18], K Tokunaga[19], K Kondo[1], T Saito[1], A Shimasaki[1], K Kawase[1], T Kitajima[1], K Matsuo[20], M Itokawa[21], T Someya[22], T Inada[23], R Hashimoto[24], T Inoue[25], K Akiyama[26], H Tanii[27], H Arai[28], S Kanba[29], N Ozaki[23], I Kusumi[30], T Yoshikawa[31], M Kubo[32], N Iwata[1] and for the advanced Collaborative Study of Mood Disorder (COSMO) team

Genome-wide association studies (GWASs) have identified several susceptibility loci for bipolar disorder (BD) and shown that the genetic architecture of BD can be explained by polygenicity, with numerous variants contributing to BD. In the present GWAS (Phase I/II), which included 2964 BD and 61 887 control subjects from the Japanese population, we detected a novel susceptibility locus at 11q12.2 (rs28456, $P = 6.4 \times 10^{-9}$), a region known to contain regulatory genes for plasma lipid levels (*FADS1/2/3*). A subsequent meta-analysis of Phase I/II and the Psychiatric GWAS Consortium for BD (PGC-BD) identified another novel BD gene, *NFIX* ($P_{best} = 5.8 \times 10^{-10}$), and supported three regions previously implicated in BD susceptibility: *MAD1L1* ($P_{best} = 1.9 \times 10^{-9}$), *TRANK1* ($P_{best} = 2.1 \times 10^{-9}$) and *ODZ4* ($P_{best} = 3.3 \times 10^{-9}$). Polygenicity of BD within Japanese and trans-European-Japanese populations was assessed with risk profile score analysis. We detected higher scores in BD cases both within (Phase I/II) and across populations (Phase I/II and PGC-BD). These were defined by (1) Phase II as discovery and Phase I as target, or *vice versa* (for 'within Japanese comparisons', $P_{best} \sim 10^{-29}$, $R^2 \sim 2\%$), and (2) European PGC-BD as discovery and Japanese BD (Phase I/II) as target (for 'trans-European-Japanese comparison,' $P_{best} \sim 10^{-13}$, $R^2 \sim 0.27\%$). This 'trans population' effect was supported by estimation of the genetic correlation using the effect size based on each population (liability estimates ~ 0.7). These results indicate that (1) two novel and three previously implicated loci are significantly associated with BD and that (2) BD 'risk' effect are shared between Japanese and European populations.

Molecular Psychiatry (2018) **23,** 639–647; doi:10.1038/mp.2016.259; published online 24 January 2017

INTRODUCTION

Bipolar disorder (BD) is a common psychiatric disorder characterized by mood swings between positive manic/hypomanic and negative/depressive states, with a lifetime prevalence of $>1\%$.[1] Family, twin and adoption studies have yielded heritability estimates of ~80%.[2]

Recent European ancestry-based genome-wide association studies (GWASs), including a meta-analysis of GWASs by the

[1]Department of Psychiatry, Fujita Health University School of Medicine, Toyoake, Japan; [2]Laboratory for Statistical Analysis, RIKEN Center for Integrative Medical Sciences, Yokohama, Japan; [3]Laboratory for Omics Informatics, Omics Research Center, National Cerebral and Cardiovascular Center, Osaka, Japan; [4]Department of Neuropsychiatry, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan; [5]Department of Mental Disorder Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan; [6]Department of Psychiatry and Neurology, Hamamatsu University School of Medicine, Hamamatsu, Japan; [7]Laboratory of Health Education, Graduate School of Education, the University of Tokyo, Tokyo, Japan; [8]Department of Psychiatry, Course of Integrated Brain Sciences, Medical Informatics, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima, Japan; [9]Department of Psychiatry and Neurosciences, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan; [10]Department of Psychiatry, Fukuoka University, Faculty of Medicine, Fukuoka, Japan; [11]Department of Neuropsychiatry, Kochi Medical School, Kochi University, Nankoku, Japan; [12]Laboratory for Molecular Dynamics of Mental Disorders, RIKEN Brain Science Institute, Wako, Japan; [13]Department of Neuropsychiatry, Osaka Medical College, Takatsuki, Japan; [14]Department of Psychiatry, University of Occupational and Environmental Health, Kitakyusyu, Japan; [15]Department of Psychiatry, Chiba University Graduate School of Medicine, Chiba, Japan; [16]Laboratory of Clinical Sequence, Graduate School of Frontier Sciences, The University of Tokyo, Tokyo, Japan; [17]Laboratory for Genotyping Development, Center for Integrative Medical Sciences, RIKEN, Japan; [18]Japanese Red Cross Kanto-Koshinetsu Block Blood Center, Tokyo, Japan; [19]Department of Human Genetics, Graduate School of Medicine, University of Tokyo, Tokyo, Japan; [20]Division of Neuropsychiatry, Department of Neuroscience, Yamaguchi University Graduate School of Medicine, Ube, Japan; [21]Center for Medical Cooperation, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan; [22]Department of Psychiatry, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan; [23]Department of Psychiatry, Nagoya University, Graduate School of Medicine, Nagoya, Japan; [24]Molecular Research Center for Children's Mental Development, United Graduate School of Child Development, Osaka University, Suita, Japan; [25]Department of Psychiatry, Tokyo Medical University, Tokyo, Japan; [26]Department of Biological Psychiatry and Neuroscience, Dokkyo Medical University School of Medicine, Mibu, Japan; [27]Department of Neuropsychiatry, Mie University, Graduate School of Medicine, Tsu, Japan; [28]Department of Psychiatry and Behavioral Sciences, Juntendo Graduate School of Medicine, Tokyo, Japan; [29]Department of Neuropsychiatry, Kyushu University, Graduate School of Medical Sciences, Fukuoka, Japan; [30]Department of Psychiatry, Hokkaido University Graduate School of Medicine, Sapporo, Japan; [31]Laboratory for Molecular Psychiatry, RIKEN Brain Science Institute, Wako, Japan and [32]RIKEN Center for Integrative Medical Sciences, Yokohama, Japan. Correspondence: Professor N Iwata, Department of Psychiatry, School of Medicine, Fujita Health University, 1-98 Dengakugakubo, Kutsukake-Cho, Toyoake, Aichi 470-1192, Japan.
E-mail: nakao@fujita-hu.ac.jp
[33]These authors contributed equally to this work.
The members of for the advanced Collaborative Study of Mood Disorder (COSMO) team are listed before references.
Received 13 April 2016; revised 29 November 2016; accepted 13 December 2016; published online 24 January 2017

Psychiatric GWAS Consortium Bipolar Disorder Working Group (PGC-BD), have highlighted a number of susceptibility loci for BD.[3–7] However, many BD loci may be unidentified because their effect sizes are so small that most studies to date are not adequately powered to detect them individually.[2]

Based on these results, BD can be considered a polygenic disorder with susceptibility, which is the result of the accumulative genetic effect of numerous variants with small effect size. Schizophrenia has been studied with great success using polygenic models such as risk profile score (RPS) analysis.[8–10] RPS analysis has also shown that BD risk can be explained with polygenicity.[11] In this model, trans population analysis is important because it can provide more information about the shared genetic 'risk' that exists across populations than can analyses that compare the results only according to the same ancestry. However, studies of BD subjects with Asian ancestry have tended to be smaller in size, affording less power to detect small effects[12,13] and making it difficult to examine the trans population effects of the BD 'risk' variants.

In this study, we conducted a GWAS of BD in the Japanese population and meta-analysis using data acquired from the PGC-BD with the aim of identifying novel BD susceptibility genes. Further, we aimed to assess whether there was evidence for polygenicity of BD (that is, to assess whether there was evidence that BD susceptibility is influenced by a large number of single nucleotide polymorphisms (SNPs) distributed across the genome) by examining within the Japanese samples and between subjects with Japanese and European ancestries (as the trans population analysis).

MATERIALS AND METHODS

Subjects

We conducted two GWASs for BD within the Japanese population because of the different sample collection periods and the use of different DNA chips (Supplementary Table 1). These were subsequently meta-analyzed.

BD subjects. The Phase I GWAS (Illumina HumanOmniExpressExome v1.0 chip) initially included 1612 BD subjects, with 1545 BD cases remaining after genotype quality control (QC); and the Phase II GWAS (Illumina HumanOmniExpressExome v1.2 chip) initially included 1604 BD subjects, with 1419 BD cases remaining after QC (Supplementary Table 1). The diagnosis for each subject followed the DSM-IV-TR criteria for BD and schizoaffective disorder and was reached by the consensus of at least two experienced psychiatrists, based on unstructured interviews with the subject and their family, as well as a review of the subject's medical records. Subjects were excluded if they had also been diagnosed with an intellectual disability.

Non-psychiatric controls. As controls, we used GWAS data for subjects in the BioBank Japan project. For the Phase I study, these comprised 7408 subjects who were genotyped (Illumina HumanOmniExpress v1 chip) in a previous GWAS as case subjects for five non-psychiatric disorders (cerebral aneurysm, esophageal cancer, endometrial cancer, chronic obstructive pulmonary disease and glaucoma) or as healthy volunteers (Supplementary Table 1). The controls for the Phase II study included 54 479 subjects who had also been genotyped (Illumina HumanOmniExpressExome v1.2 chip) as case subjects for 14 non-psychiatric disorders: nephrotic syndrome, cancers (stomach, lung, colorectal, prostate and breast), glaucoma, chronic periodontitis, type 2 diabetes (T2D), dyslipidemia, arrhythmia, cerebral infarction, epilepsy and nephrolithiasis, or as healthy controls (Supplementary Table 1). The controls were not psychiatrically evaluated.

Genotyping, QC and imputation

We genotyped the BD subjects using the Illumina HumanOmniExpressExome v.1.0/v.1.2 BeadChips (Supplementary Table 1). Detailed QC, including population stratification (Supplementary Figure 1), is presented in the Supplementary Texts. We then performed genotype imputation using a subset of the 1000 Genomes Project Phase I dataset as a reference, comprising the Japanese in Tokyo (JPT), Han Chinese in Beijing (CHB), and Southern Han Chinese (CHS) populations[14] (Supplementary Texts). Cases and controls for each phase were imputed in the same run. After imputation, we included only SNPs with an imputation quality score $R^2 \geqslant 0.3$ and minor allele frequency $>1\%$.

Statistical analysis

GWA analysis and meta-analysis. For the SNP-based association analysis, logistic regression with co-variation of the first two eigenvectors was applied and the combined *P* values from GWAS Phases I and II and/or the PGC-BD data were calculated using a fixed-effect model using an inverse-variance method (Supplementary Texts). Regional association plots were generated using LocusZoom.[15] The significance level was set at 5×10^{-8} (two-sided).

RPS analysis. For the RPS analysis,[8–10] we used the statistical analysis software package PRSice v1.23.[16] The *P* threshold (P_T) for selecting the 'risk' SNPs was set sequentially at 0.1, 0.2, 0.3, 0.4 and 0.5; SNPs were selected if their *P* values were between 0 and the chosen value of P_T. As including SNPs in the MHC region could inflate the score because of high linkage disequilibrium (LD), we removed SNPs in this region by setting the 'remove.mhc' flag in the software. The eligible SNPs for RPS were then selected based on LD clumping (used by the default setting of the software). The variance explained for the RPS was estimated using Nagelkerke's R^2 from a logistic regression model.

To calculate the RPS within the Japanese samples, we set Phase II (with a larger sample size) as the discovery and Phase I as the target (Phase II/Phase I: discovery/target) and analyzed this set, and repeated this with the discovery and target reversed (that is, Phase I/Phase II: discovery/target). Next, to evaluate the trans population effect of the 'risk' SNPs from the PGC-BD (subjects of European ancestry), we set the PGC-BD as discovery and (Phase I+II) as the target (PGC-BD/Phase I+II: discovery/target).

There may have been a possible bias in this analysis because our control subjects comprised people with a number of diseases, as described earlier. To check for any heterogeneity caused by this, and to use these results as a negative control, we performed leave-one-disease-out analysis for each disease. For example, a proportion of the Phase I control subjects had cerebral aneurysm, and so we performed RPS for target samples excluding these cerebral aneurysm subjects from the 'control' and by defining cerebral aneurysm as the 'case' for the negative control as follows:

$$\begin{aligned}&\text{Target set : "Case" (cerebral aneurysm subjects) vs "control"}\\&[(\text{Phase I control, all}) - (\text{Phase I control cerebral aneurysm subjects})\\&\quad +(\text{All of Phase II control})]\end{aligned}$$

Each of the 21 disease groups, including two sets of healthy volunteers, was removed in turn from the target 'control' samples, and we calculated the polygenic score defined by PGC-BD for each non-psychiatric control.

The significance level for the RPS analysis was set at 0.001, a conservative threshold described by Euesden *et al.*[16]

LD score regression. We used LD score regression to examine SNP heritability[17] for the meta-analysis (Phases I and II: imputation $R^2 \geqslant 0.3$) and/or PGC-BD (imputation INFO $\geqslant 0.3$), and to assess whether there was population stratification.[18] We used pre-computed LD scores for East Asian (for Japanese results) and European (for PGC results) populations based on 1000 Genome Project data listed on the website (https://data.broadinstitute.org/alkesgroup/LDSCORE/eas_ldscores.tar.bz2). We filtered the SNPs to HapMap3 SNPs ('--merge-alleles' flags), and set the population prevalence of BD at 0.01 ('--pop-prev 0.01' flag) and the sample prevalence at appropriate values (='number of cases'/'number of total subjects') in each dataset ('--samp-prev' flag).

Although LD score regression can assess genetic correlation (if samples from the same population are targeted), it is difficult to calculate correlations between trans population datasets (such as between the Japanese and European populations). In the comparison between Japanese and European BD results, therefore, we used a recently developed program, *Popcorn* (version 0.9.6), which is based on a concept similar to LD score regression,[19] and examined (1) the trans population genetic effect correlation (the correlation coefficient for the per-allele SNP effect sizes, ρ_{ge}), and (2) the genetic impact correlation (the correlation coefficient for the population-specific allele variance normalized SNP effect sizes, ρ_{gi}).[19] For the calculation of the trans-population score, we used HapMap3 datasets, representing the European population by the CEU dataset and the Asian population by CHB+JPT datasets. Genetic

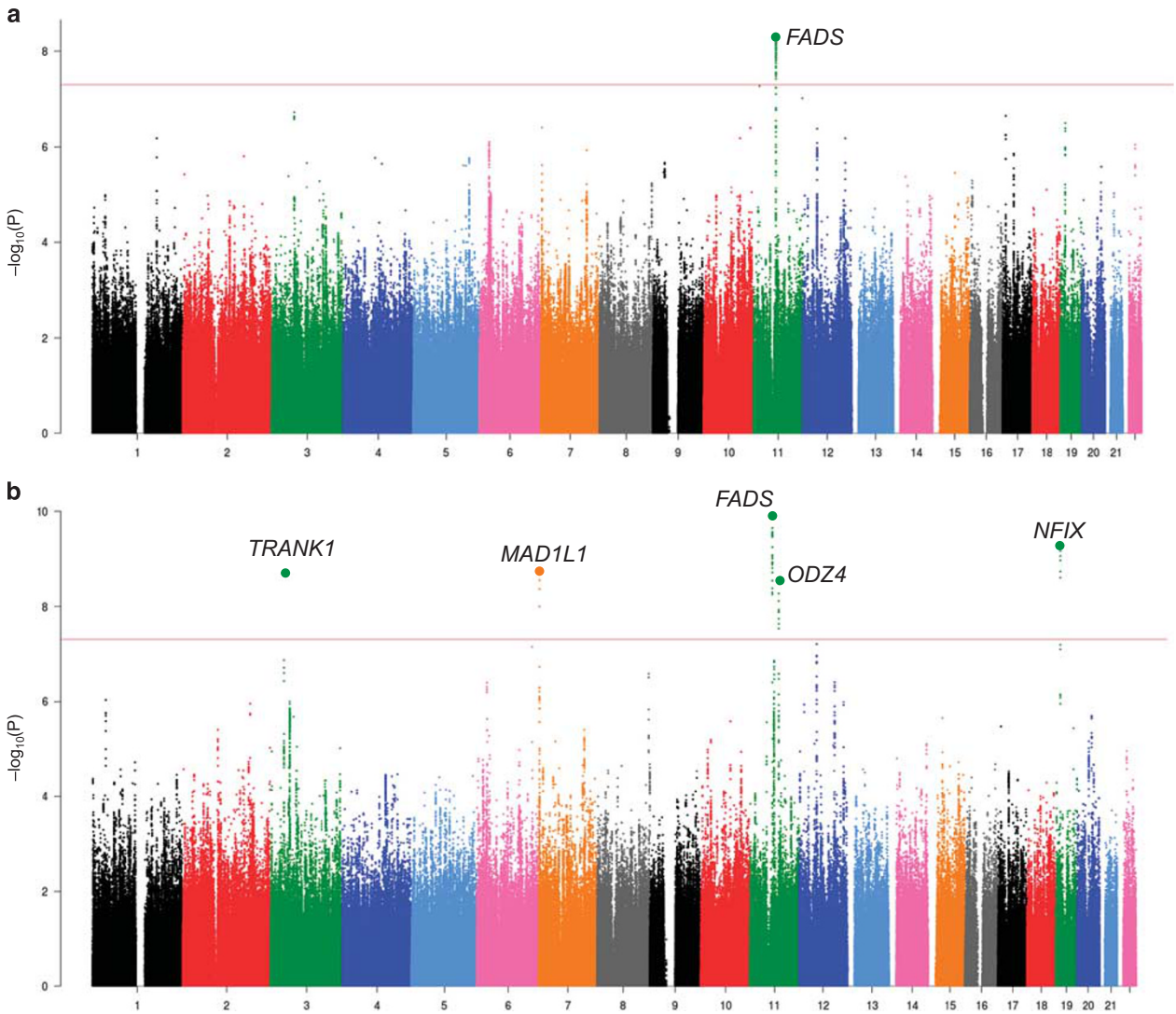

Figure 1. Manhattan plot of the meta-analyses for (**a**) the Japanese samples (Phases I and II) and (**b**) the Japanese samples (Phases I and II) and Psychiatric GWAS Consortium (PGC-BD). Horizontal line indicates threshold for genome-wide significance ($P < 5 \times 10^{-8}$). Manhattan plot for (**a**) the Japanese samples (Phases I and II) and (**b**) the Japanese samples (Phases I and II) and PGC-BD.

correlations were then calculated by the GWAS SNPs of the combined GWAS of the Japanese datasets (a meta-analysis of Phases I and II: imputation $R^2 \geqslant 0.3$) and PGC-BD (imputation INFO $\geqslant 0.3$) with the default setting, which removed SNPs below the minor allele frequency (MAF) cutoff of 5% and those with A/T or G/C alleles.

RESULTS

Combined GWAS of the two Japanese datasets (Phases I and II)

A total of 6 195 093 imputed SNPs in 2964 BD and 61 887 non-psychiatric subjects passed our stringent QC. From these, we generated the Manhattan and quantile-quantile (QQ) plots shown in Figure 1 and Supplementary Figure 2. The genomic inflation factor (λ_{GC}) for the meta-analysis appeared at 1.047. In addition, the intercept from the LD score regression analysis was 1.054, indicating that most of the inflation was due to polygenicity rather than bias.[18]

One region reached genome-wide significance (5×10^{-8}; Figure 1), namely the association at a SNP on 11q12.2 of rs28456 in the fatty acid desaturase 2 gene (*FADS2*) ($P = 6.4 \times 10^{-9}$, odds ratio (OR) = 1.18; Table 1). This had hits in multiple genes, including *FADS1/2/3* (Table 1 and Supplementary Table 2). It is of interest that (1) this region is a known locus for the regulation of plasma blood lipid traits[20] and n-3/n-6 polyunsaturated fatty acids[21–23] and (2) the top SNP (rs28456) has an expression quantitative trait locus (eQTL) effect on *FADS1* in the brain (cerebellum) samples ($P = 7.8 \times 10^{-10}$, beta = −0.62 as the effect of the minor allele 'G (risk for BD)' relative to major allele 'A') according to the Genotype-Tissue Expression (GTEx) database (http://www.gtexportal.org/home/, Data Source: GTEx Analysis Release V6p (dbGaP Accession phs000424.v6.p1). In addition, other datasets (BRAINEAC: http://www.braineac.org/) supported the eQTL effect in the brain samples (*FADS2* in temporal cortex, $P_{best} = 1.7 \times 10^{-5}$; *FADS1* in cerebral cortex, $P_{best} = 1.3 \times 10^{-5}$).

The non-psychiatric controls of Phase II included phenotypes related to lipid and/or glucose metabolism ($N = 25\,228$), such as those for dyslipidemia and T2D. We therefore performed sensitivity analysis to exclude the possibility that this signal was derived from an association with these subjects. In this analysis, we confirmed that the effect size did not change substantially compared with the non-psychiatric controls excluding those with dyslipidemia or T2D (rs28456, OR = 1.15), although the *P* value was attenuated ($P = 7.4 \times 10^{-7}$; Supplementary Table 3).

Table 1. Summary of the association results in the Japanese BD samples

SNP	CHR	BP	gene	Effect allele	Reference allele	Meta-analysis				Phase I			Phase II		
						OR	95% CIs	P_{meta}	$P_{heterogeneity}$	AF (BD/control)	OR	P	AF (BD/control)	OR	P
rs28456	11	61589481	*FADS2*	G	A	1.18	1.11–1.24	6.40E-09	0.86	0.413/0.376	1.17	9.71E-05	0.419/0.380	1.18	1.50E-05
rs329674	11	133776948	*LOC646543*	A	G	1.22	1.13–1.31	9.59E-08	0.054	0.161/0.147	1.12	0.0335	0.185/0.149	1.29	1.45E-07
rs72874541	3	63133849	*LOC285401*	G	A	1.23	1.14–1.34	1.89E-07	0.92	0.865/0.840	1.23	3.45E-04	0.873/0.849	1.24	1.81E-04
rs76317718	17	8222777	*ARHGEF15*	G	T	1.19	1.12–1.28	2.24E-07	0.94	0.760/0.730	1.19	3.56E-04	0.762/0.731	1.20	1.80E-04
rs3931398	7	2031592	*MAD1L1*	A	G	1.35	1.20–1.51	3.92E-07	0.99	0.796/0.779	1.35	3.52E-04	0.800/0.783	1.35	3.17E-04
rs78089757	10	127112829	*RPS27P18*	A	G	1.41	1.23–1.61	3.99E-07	0.45	0.041/0.032	1.33	6.27E-03	0.050/0.035	1.47	1.44E-05
rs185880008	12	39201470	*CPNE8*	T	A	1.19	1.11–1.27	4.14E-07	0.84	0.707/0.677	1.20	2.51E-04	0.716/0.688	1.18	4.56E-04

Abbreviations: AF, allele frequency; BD, bipolar disorder; BP, base position based on hg19; CHR, Chromosome; CIs, confidence intervals; OR, odds ratio. Only top SNPs in the same region were shown. For example, 68 SNPs showed $P < 5 \times 10^{-7}$ in the 11q12.2 region in addition to rs28456. Full results ($P < 5 \times 10^{-7}$) can be seen in Supplementary Table 2.

To validate the previous findings, we checked the associations of susceptibility genes with genome-wide significance reported by the PGC-BD[3] and other individual GWASs (Supplementary Table 4 and Supplementary Figure 3).[4,6,7] Although, there were no significant associations (that is, with $P < 0.05$) of these indexed SNPs (except for the SNP in *ODZ4* rs12576775, $P = 0.042$) with BD in the Japanese population, the surrounding SNPs showed *P* values of < 0.05, particularly for *ODZ4* (known as *TENM4*, encoding teneurin transmembrane protein 4), *TRANK1* (encoding tetratricopeptide repeat and ankyrin repeat containing 1) and *DHH* (encoding desert hedgehog proteins). Specifically, the SNPs in these three genes showed a strengthened association after merging PGC-BD results (see below).

We calculated the SNP heritability of Phase I (979 751 SNPs), Phase II (959 428 SNPs), and the combined Japanese (Phase I/II, 903 223 SNPs) datasets by the LD score regression method. The SNP heritability on the total liability scale (h^2) was 0.263 (s.e. = 0.0642), 0.129 (s.e. = 0.0530) and 0.148 (s.e. = 0.0288) for Phase I, Phase II and the combined Phase I/II, respectively. The discrepancy of the h^2 between Phases I and II might be due to the smaller size in Phase I (1545 BD vs 7408 controls), therefore the $h^2 \sim 0.15$ was the best estimate for the BD in the Japanese population. Whereas, the h^2 for the PGC-BD was estimated around 0.25 (1 059 316 SNPs, $h^2 = 0.245$, s.e. = 0.0204), that was larger than that of Japanese sample. Based solely on the current results, we could not interpret the difference of the h^2 values between the Japanese samples and the PGC-BD; further replication with larger sample size will be required.

Meta-analysis of the Japanese (Phases I and II) and PGC-BD results

To maximize the sample size, we conducted a meta-analysis combining the Phases I, II and the PGC-BD datasets,[3] which produced a combined sample size of 10 445 cases and 71 137 controls. Manhattan and QQ plots are shown in Figure 1 and Supplementary Figure 2, respectively. Again, we found a significant association with 11q.12.2 (rs174576, $P = 1.34 \times 10^{-10}$, OR = 1.13; Figure 2, Table 2, and Supplementary Table 5). When we set 'control' samples excluding dyslipidemia/T2D subjects, rs174576 still reached genome-wide significance ($P = 4.9 \times 10^{-9}$; OR = 1.12, 95% confidence interval: 1.08–1.16), indicating that any possible bias derived from samples with dyslipidemia/T2D did not influence the significance. Notably, rs174576 has eQTL effect in GTEx dataset (*FADS1* in cerebrellum, $P = 2.3 \times 10^{-9}$; beta = − 0.59 as the effect of the minor allele 'A (risk for BD)' relative to major allele 'C') and BRAINEAC dataset (*FADS1* in cerebral cortex, $P_{best} = 2.5 \times 10^{-6}$; *FADS2* in temporal cortex, $P_{best} = 1.2 \times 10^{-5}$).

In this analysis, we found four additional regions that showed significant associations at the genome-wide level, one of which, *NFIX* (encoding nuclear family I/X, rs4926298, $P = 5.8 \times 10^{-10}$) is another novel BD susceptibility gene (Figure 2, Table 2 and Supplementary Table 5).

ODZ4, *MAD1L1* (encoding MAD1 mitotic arrest deficient-like 1) and *TRANK1*, are known BD/psychosis susceptibility genes; these enhanced the association of this meta-analysis of the combined Japanese datasets and the PGC-BD dataset (Figure 2, Table 2 Supplementary Table 5), supporting previous evidence. It is of note that the *P* value for *MLL2* (encoding lysine (K)-specific methyltransferase 2D, located near *DHH*) did not quite reach that for genome-wide significance, but was just below this level (rs10875914, $P = 6.3 \times 10^{-8}$; Figure 2, Table 2 and Supplementary Table 5).

Trans population effect of SNPs on BD

To evaluate any trans population effect, we first checked the SNP-wise correlation of the effect size between the Phase I/II and the PGC-BD datasets for the top hits based on the final meta-analysis (Phase I/II and PGC-BD). As expected, we found a good correlation

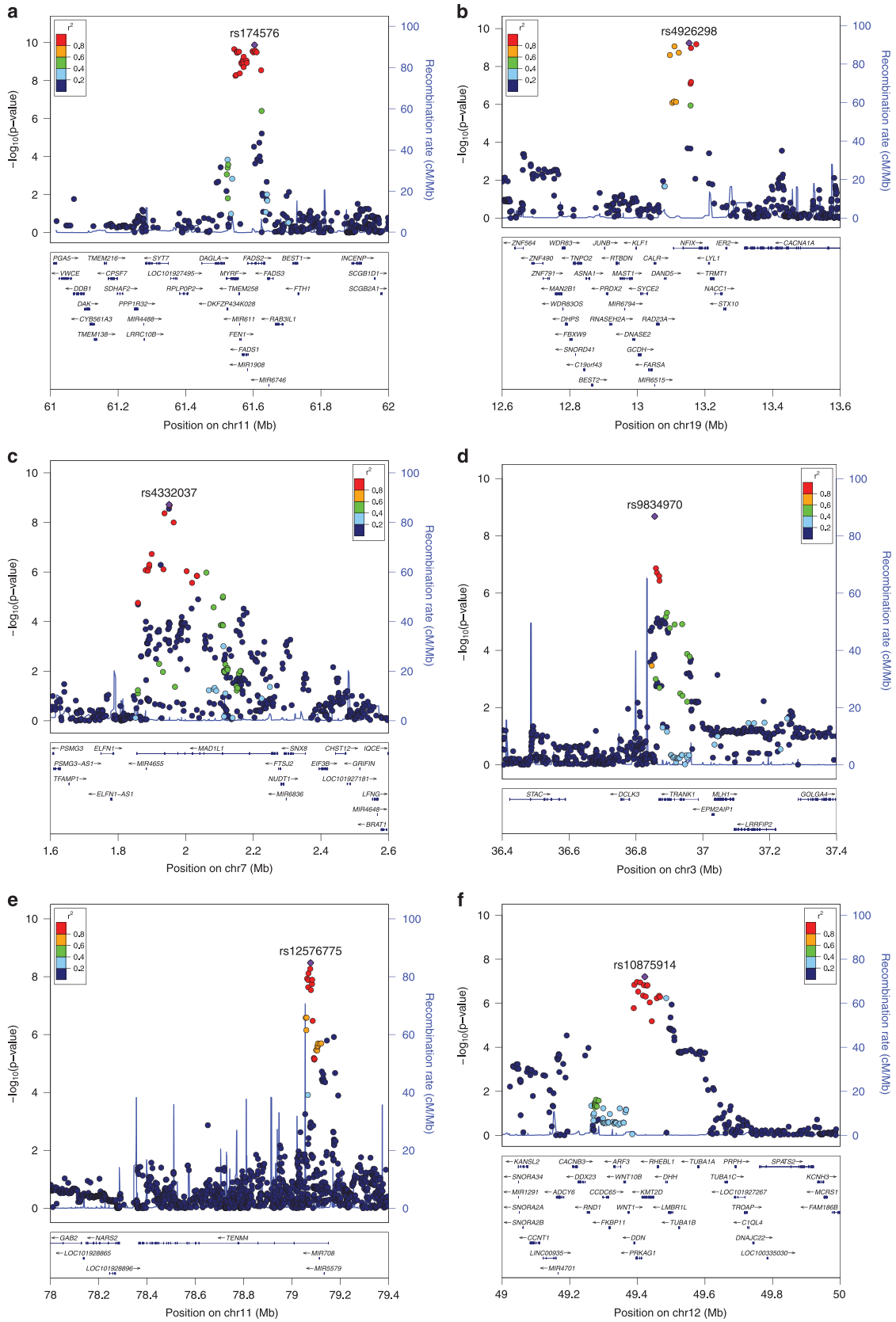

Figure 2. Regional plots of the top hit in the association results based on the meta-analysis of Japanese (Phases I and II) and the PGC-BD results. Blue lines indicate the recombination rate for the ASN population in the 1000 Genome Project. The Y axis is $-\log_{10}$(*P*-values) of the SNPs and the X axis is chromosomal position (hg19). The linkage disequilibrium (r^2) between the top and the remaining SNPs is indicated by color. (**a**) *FADS* gene cluster, (**b**) *NFIX*, (**c**) *MAD1L1*, (**d**) *TRANK1*, (**e**) *ODZ4* (known as *TENM4*), (**f**) *MLL2*∼*DHH*. PGC-BD, Psychiatric GWAS Consortium for BD; SNPs, single nucleotide polymorphism.

Table 2. Summary of the meta-analysis combining the Japanese BD samples and PGC-BD datasets ($P < 1 \times 10^{-7}$)

SNP	CHR	BP	Gene	Effect Allele	Non-Effect Allele	Meta-analysis (Phase I+II+PGC-BD)				Phase I			Phase II			PGC-BD		
						OR	95% CIs	P_{meta}	$P_{heterogeneity}$	AF (BD/control)	OR	P	AF (BD/control)	OR	P	AF (CEU)	OR	P
rs174576	*11*	*61603510*	*FADS2*	*A*	*C*	*1.13*	*1.09–1.17*	*1.34E-10*	*0.12*	*0.414/0.379*	*1.16*	*1.81E-04*	*0.423/0.383*	*1.19*	*9.36E-06*	*0.345*	*1.09*	*5.16E-04*
rs4926298	*19*	*13153035*	*NFIX*	*G*	*A*	*1.13*	*1.09–1.18*	*5.78E-10*	*0.17*	*0.378/0.363*	*1.06*	*0.155*	*0.408/0.369*	*1.17*	*7.72E-05*	*0.650*	*1.15*	*8.36E-07*
rs4332037	*7*	*1950809*	*MAD1L1*	*T*	*C*	*1.17*	*1.11–1.23*	*1.91E-09*	*0.73*	*0.102/0.089*	*1.17*	*0.0211*	*0.109/0.093*	*1.22*	*0.00137*	*0.142*	*1.15*	*4.28E-06*
rs9834970	*3*	*36856030*	*TRANK1*	*C*	*T*	*1.12*	*1.08–1.16*	*2.08E-09*	*0.72*	*0.668/0.636*	*1.15*	*9.41E-04*	*0.659/0.638*	*1.10*	*0.0202*	*0.549*	*1.11*	*6.19E-06*
rs12576775	*11*	*79077193*	*ODZ4*	*G*	*A*	*1.18*	*1.12–1.25*	*3.32E-09*	*0.075*	*0.032/0.022*	*1.42*	*0.0023*	*0.023/0.023*	*0.96*	*0.751*	*0.177*	*1.18*	*2.66E-08*
rs10875914	*12*	*49422094*	*MLL2*	*G*	*A*	*1.11*	*1.07–1.15*	*6.28E-08*	*0.10*	*0.387/0.366*	*1.09*	*0.0325*	*0.372/0.363*	*1.03*	*0.404*	*0.375*	*1.14*	*8.27E-08*
rs4523096	*6*	*152793412*	*SYNE1*	*A*	*C*	*1.15*	*1.10–1.22*	*7.17E-08*	*0.16*	*0.992/0.990*	*1.29*	*0.276*	*0.987/0.989*	*0.845*	*0.322*	*0.647*	*1.16*	*3.40E-08*

Abbreviations: AF, allele frequency; BD, bipolar disorder; BP, base position based on hg19; CEU, HapMap CEU; CHR, Chromosome; CIs, confidence intervals; OR, odds ratio; PGC-BD, Psychiatric GWAS Consortium for BD; SNP, single nucleotide polymorphism. Only top SNPs in the same region are shown. For example, eight SNPs showed $P < 5 \times 10^{-7}$ in the *NFIX* region including rs4926298. Full results for all SNPs ($P < 5 \times 10^{-7}$) are presented in Supplementary Table 5.

Figure 3. Risk profile score (RPS) analysis. Y axis indicates the variance-explained (Nagaelkerke's R^2). Three discovery/target sets were included: (**a**) Discovery: Phase II, Target: Phase I, (**b**) Discovery: Phase I, Target: Phase II, and (**c**) Discovery: PGC-BD, Target: Phase I+II. *$P < 10^{-10}$, **$P < 10^{-20}$.

between the effect sizes of the two datasets (Supplementary Figure 4A). However, the top SNPs selected based on the combined Japanese datasets only (Supplementary Figure 4B) or the PGC-BD dataset only (Supplementary Figure 4C) did not show strong correlations, particularly the SNPs selected from the combined Japanese datasets. Again, we speculate that this may be due to the lower sample size for the Japanese samples resulting in less statistical power.

To analyze the polygenic architecture represented by numerous 'risk' SNPs, we calculated the RPSs defined within the Japanese sample as follows. 'risk' SNPs were defined according to the association results for Phase II (the dataset with the larger sample size), and we examined whether cases with BD had a higher RPS than controls in Phase I (Phase II/Phase I: discovery/target pair); we then repeated this with the dataset swapped (i.e., Phase I/Phase II: discovery/target pair). In these analyses of the Japanese case-control samples, we found a significant higher RPS in the case (*P* values 10^{-20}–10^{-29}), explaining ~0.7%–2.3% of the variance (Figure 3, Supplementary Figures 5, 6, and Supplementary Table 6). We then performed a trans population analysis between the European and the Japanese populations. A larger sample size improves the discovery results defining the 'risk' SNPs, because more accurate results may be obtained; we therefore set the PGC-BD as discovery and Phase I+II as target. In this comparison, we found a significant higher RPS defined by the European BD in the target Japanese BD samples (*P* values: 10^{-12}–10^{-13}). However, the variance explained (~0.27%) was smaller than that for the Japanese pair (Figure 3, Supplementary Figures 5, 6, and Supplementary Table 6).

To determine whether such results were detected only in BD patients, we performed leave-one-disease-out analyses, removing each disease group from the 'control' and treating them as 'cases.' These results indicated that most of the profile scores predicted almost no variance (~10^{-5}) in BD risk. The highest variance explained was found in the analysis for nephrotic syndrome (Phase II, $N = 677$: best $R^2 = 0.00087$) (Supplementary Figure 6 and Supplementary Table 7); however, this result was probably due to the small sample size of subjects with nephrotic syndrome

($N = 677$), as the P value for nephrotic syndrome was 0.0128. We therefore concluded there were no differences in RPSs based on the BD 'risk' SNPs between the disease groups making up the controls (Supplementary Figure 6 and Supplementary Table 7). This implies that (1) our controls were minimally biased by sample heterogeneity associated with a polygenic effect, and (2) BD risk based on PGC-BD was shared only by subjects with BD (but not other diseases) in the Japanese population.

Lastly, we estimated trans population genetic correlations using the effect sizes of the SNPs.[19] In this analysis of 733 574 SNPs, we detected a significant trans population genetic effect correlation between our Japanese samples and PGC-BD (ρ_{ge}: liability = 0.724, $P = 5.40 \times 10^{-3}$) as well as a population genetic impact correlation that accounted for the allele frequencies of the SNPs (ρ_{gi}: liability = 0.684, $P = 9.17 \times 10^{-4}$). Notably, these liabilities were higher than those for rheumatoid arthritis ($\rho_{ge}/\rho_{gi} = 0.46/0.46$) and T2D ($\rho_{ge}/\rho_{gi} = 0.62/0.61$) reported in the original method paper.[19]

DISCUSSION

We identified two novel loci with significant associations with BD: SNPs in the *FADS* gene cluster and *NFIX*. Our findings for *ODZ4*, *MAD1L1* and *TRANK1* supported the associations detected previously.

Our meta-analysis found a significant association with BD in a region that included genes encoding fatty acid desaturase (*FADS*) (Figure 2), where robust associations have been established with blood lipid traits[20] and with n-3/n-6 polyunsaturated fatty acids (PUFA).[21–23] The LD matrices with the index BD SNP (rs174576) revealed that all QTL SNPs for lipid traits (HDL/LDL/TG/T-Chol, n-3/n-6 PUFAs) around the *FADS* region were in strong LD with the index SNP (Supplementary Table 8). It suggested that SNPs located around this region are in the LD block, and most QTL SNPs exhibited significant association with BD. Therefore, we speculate that the lipid abnormality may be involved in the pathophysiology of BD. Notably, epidemiological surveys have revealed an increased incidence of hyperlipidemia in BD patients, at ~5.6% higher than that for the general population (OR = 1.75),[24] and an approximately two-fold greater risk of metabolic syndrome, including dyslipidemia.[25,26] Although the medication, specifically second-generation antipsychotics, for BD symptoms is the definitive risk for dyslipidemia (which was not taken into account in the studies just cited[24–26]), the risk of metabolic abnormality may overlap that of BD. This is supported by surveys, albeit of a small sample size, that have shown an increased prevalence of metabolic syndrome in the first-degree relatives of psychosis subjects, including those with BD,[27] and in drug-naive patients with BD.[28] As yet, there has been no evidence of a causal relationship of lipid abnormality on BD; further epidemiological or genetic studies are therefore warranted to establish such a relationship.

Another novel locus for BD detected in our GWAS is located near *NFIX*. In the meta-analysis of our GWAS involving the Japanese subjects (Phases I and II), SNPs in *NACC1*, downstream of *NFIX*, showed a stronger association. However, when PGC-BD data was also included, the association of *NFIX* strengthened, reaching genome-wide significance. *NFIX* is a member of the nuclear factor one (NF1) family and plays critical roles in transcription and replication.[29] Nonsense/frameshift mutations and copy number variants of *NFIX* cause Sotos syndrome 2 (SOTOS2: OMIM *6145753) or Marshall-Smith syndrome (MRSMS: OMIM *602535), which are characterized by intellectual disability. Although there is no evidence for an association between BD and *NFIX*, further studies are warranted.

The last locus highlighted was *MAD1L1*, which contributes to cell cycle control through the regulation of mitosis.[30] This gene represents a risk locus for psychosis in a combined population of schizophrenic and BD patients.[31] Our results indicate that this gene confers a risk for BD, as well as supporting a pleiotropic effect of variants in *MAD1L1*.

RPS analysis revealed evidence that the risk profile score defined by European-based BD 'risk' SNPs was higher in the BD subjects in the Japanese population. Interestingly, the level of variance explained in this trans population analysis was much lower than that for the 'within Japanese comparisons'. A previous study of schizophrenia analyzed the trans population effect using our Japanese samples and indicated that the contribution of the European-based score allele was one-half or one-third lower in the Japanese samples.[32] The sample size in the present study was larger than that of the schizophrenia GWAS (~500 cases vs ~500 controls),[32] but a similar reduction in the contribution (comparing the within-Japanese with the trans population samples) was observed in the two sample sets. This difference in the contribution may result from the combined influences of LD differences and/or a population with unique risk. However, it should be emphasized that BD 'risk' effect are shared across populations. A different analysis supported this result by estimating the trans-population genetic effect based on correlations of the effect size. In this analysis, we estimated that the genetic correlation coefficient for BD was ~0.7, which was higher than that for rheumatoid arthritis (~0.5) and T2D (~0.6).[19] This indicates that there might be a shared genetic basis in BD involving a higher proportion of subjects than for rheumatoid arthritis or T2D.

In addition, the genetic correlations calculated with and without consideration of the allele frequency ($\rho_{ge}/\rho_{gi} = 0.724/0.684$) had almost the same impact, similar to that in previous reports of rheumatoid arthritis ($\rho_{ge}/\rho_{gi} = 0.46/0.46$) and T2D ($\rho_{ge}/\rho_{gi} = 0.62/0.61$).[19] This indicates, and supports the previous speculation,[19] that differences in allele frequencies of common SNPs (observed in the Japanese and European populations) did not have a large impact on the trans-population phenotypic differences for BD, and probably for complex diseases. We further speculated that the population-specific effect could be assessed from our results. As an explorative analysis, we calculated the trans population genetic effect (ρ_{ge}) or impact (ρ_{gi}) within the Japanese population using the data from Phases I and II. As expected, we found nearly a perfect correlation (~1.0) in both ρ_{ge} and ρ_{gi}. The difference of ρ_{ge}/ρ_{gi} between the Japanese-European and within-Japanese comparisons was ~0.3. The reason for this difference remains to be explained, but we assume (1) environmental factors or gene-environment interaction have a larger impact on BD in the Japanese population, assuming we detected the correct estimates of SNP heritabilities, with h^2 in the Japanese population found to be lower than that in the European PGC-BD dataset or (2) the difference may derive from rare variants (low MAF SNPs (1–5%) or single nucleotide variants (SNVs: MAF < 1%)) only seen in one population, which were not selected in this analysis (MAF ⩾ 5%) because MAF in common SNPs does not have large impact, as mentioned above.

Our study had several limitations. First, the subjects we used as controls had not been psychiatrically screened. However, this is reasonable because the prevalence of BD is only ~1% (and may be only ~0.2% in Japan).[33] Although most of the control subjects had various non-psychiatric disorders, there were no clear associations between BD and these disorders. We assessed the influence of these diseases on the significant loci using analyses where one disease (non-psychiatric control) was excluded from the controls and compared with BD for the significant SNPs, and confirmed there was little possibility of bias (Supplementary Table 9). We further confirmed the minimal effect of the bias in the leave-one-disease-out RPS analysis (Supplementary Table 7). Second, our samples contained a higher proportion of subjects with BD type II than reported in other studies. The prevalence of BD type II varies across countries, and the proportions of BD I and BD II in our sample simply reflect the lifetime prevalence in Japan (0.1% each).[33] There were no significant differences in allele

frequencies between BD types I and II for all significant SNPs (that is, SNPs with $P_{meta} < 5 \times 10^{-8}$). Furthermore, the effect directions were the same for all significant SNPs (Supplementary Table 10). Thus, the proportions of BD types I and II appear to have had minimal influence on our findings. Third, the genotyping of case and control subjects had been performed on different platforms and at different times (Supplementary Table 1). To assess the influence of the batch effect, we checked the concordance of the genotyping determined by these chips (HumanOmniExpress v1, HumanOmniExpressExome v1.0/1.2); the concordance rates (concordance of genotypes between same individuals (three pairs between HumanOmniExpress v1 and HumanOmniExpressExome v1.0, four pairs between HumanOmniExpress v1 and HumanOmniExpressExome v1.2, and 23 pairs between HumanOmniExpressExome v1.0/1.2) on two platforms) in all comparisons were $>99.99\%$, suggesting minimal batch effects in our results.

In conclusion, we identified two novel susceptibility loci (*FADS* and *NFIX*) and confirmed loci identified in previous GWASs (*ODZ4*, *MAD1L1*, and *TRANK1*) as susceptibility genes or regions associated with BD. Based on the trans population analysis, we demonstrated a substantial effect of BD 'risk' genetic variants across populations. Further analysis will illuminate the shared risk among populations and the population-unique risk.

CONFLICT OF INTEREST

During the conduct of the study; Drs Ikeda, Takahashi, Kamatani, Okamoto, Yoshimura, Matsuo, Itokawa, Ozaki and Iwata have received research support from the Ministry of Education, Culture, Sports and Technology (MEXT) of Japan and Japan Agency for Medical Research and Development (AMED); Drs Kusumi, Yoshikawa and Kubo have received research support from MEXT of Japan; Dr Kanba has received research support from AMED and the Ministry of Health Labour and Welfare; Dr Kunugi has received research support from MEXT of Japan, AMED and the Ministry of Health Labour and Welfare; and Dr Kato has received research support from MEXT of Japan, AMED of Japan and Japan Science and Technology Agency. The sponcors had no role in design and conduct of the study; collection, management, analysis and interpretation of the data; and preparation, review or approval of the manuscript. The remaining authors declare no conflicts of interest.

ACKNOWLEDGMENTS

We thank all the individuals who participated in the study and the contributors of advanced COSMO and Biobank Japan. We also thank Dr Charles Yokoyama at RIKEN Brain Science Institute (BSI) and Dr Makoto Arita at RIKEN Center for Integrative Medical Sciences (IMS) for their critical comments. This work is the result of the Strategic Research Program for Brain Sciences (SRPBS) from Japan Agency for Medical Research and Development (AMED); part of the BioBank Japan Project from the Ministry of Education, Culture, Sports and Technology (MEXT) of Japan; JSPS Kakenhi Grant Numbers JP26117518, JP25293253, JP16H05378, JP26293266 and JP24689046: Grant-in-Aid for Brain/minds from AMED: Grant-in-Aid for Health Labour Sciences Research Grant from the Ministry of Health Labour and Welfare.

GROUP MEMBERS FOR THE ADVANCED COLLABORATIVE STUDY OF MOOD DISORDER (COSMO) TEAM

The advanced COSMO include authors and co-investigators Masashi Ikeda, MD, PhD, Kenji Kondo, MD, PhD, Takeo Saito, MD, PhD, Kunihiro Kawashima, MD, PhD, Yoshio Yamanouchi, MD, PhD, Tsuyoshi Kitajima, MD, PhD, Ayu Shimasaki, MD, Kohei Kawase, MD, Tomohiro Narita, MD, PhD, Taro Kishi, MD, PhD, Kosei Esaki, MD, PhD, Hiroshi Naitoh, MD, PhD, Nakao Iwata (PI), MD, PhD (Department of Psychiatry, Fujita Health University School of Medicine), Tomoko Toyota, MD, PhD, Kazuo Yamada, MD, PhD, Eiji Hattori, MD, PhD, Yoshimi Iwayama, MS, Takeo Yoshikawa (PI), MD, PhD (Laboratory for Molecular Psychiatry, RIKEN Brain Science Institute), Hiroshi Ujike, MD, PhD, Manabu Takaki, MD, PhD, Shinji Sakamoto, MD, Norihito Yamada, MD, PhD, Yuko Okahisa (PI), MD, PhD (Department of Neuropsychiatry, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences), Naoki Hashimoto, MD, PhD, Shin Nakagawa, MD, PhD, Yuki Kako, MD, Teruaki Tanaka, MD, PhD, Yutaka Fujii, MD, PhD, Yuji Kitaichi, MD, PhD, Ichiro Kusumi (PI), MD, PhD (Department of Psychiatry, Hokkaido University Graduate School of Medicine), Takeshi Inoue (PI), MD, PhD (Department of Psychiatry, Tokyo Medical University School of Medicine), Kotaro Hattori, MD, PhD, Noriko Yamamoto, Hiroaki Hori, MD, PhD, Toshiya Teraishi, MD, PhD, Miho Ota, MD, PhD, Hiroshi Kunugi (PI), MD, PhD (Department of Mental Disorder Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry), Branko Aleksic, MD, PhD, Itaru Kushima, MD, PhD, Shohko Kunimoto, PhD, Akira Yoshimi, PhD, Toshiya Inada (co-PI), MD, PhD, Norio Ozaki (PI), MD, PhD (Department of Psychiatry, Nagoya University, Graduate School of Medicine), Katsuaki Suzuki, MD, PhD, Yasuhide Iwata, MD, PhD, Norio Mori (PI), MD, PhD (Department of Psychiatry and Neurology, Hamamatsu University School of Medicine), Yoshio Minabe, MD, PhD (Department of Psychiatry and Neurobiology, Kanazawa University Graduate School of Medical Science), Takahiro A. Kato, MD, PhD, Satomi Katsuki, MD, PhD, Hiroshi Mitsuyasu, MD, PhD, Shigenobu Kanba (PI), MD, PhD (Department of Neuropsychiatry, Kyushu University, Graduate School of Medical Sciences), Hiroaki Kawasaki (PI), MD, PhD (Department of Psychiatry, Fukuoka University, Faculty of Medicine), Tsukasa Sasaki (PI), MD, PhD (Laboratory of Health Education, Graduate School of Education, the University of Tokyo), Mamoru Tochigi, MD, PhD (Department of Psychiatry, Teikyo University School of Medicine), Takeshi Otowa, MD, PhD (Department of NeuroPsychiatry, Graduate School of Medicine, the University of Tokyo), Tohru Ohnuma, MD, PhD, Hajime Baba, MD, PhD, Heii Arai (PI), MD, PhD, (Department of Psychiatry, Juntendo University, School of Medicine), Shusuke Numata, MD, PhD, Jun-ichi Iga, MD, PhD, Tetsuro Ohmori (PI), MD, PhD (Department of Psychiatry, Course of Integrated Brain Sciences, Medical Informatics, Institute of Health Biosciences, The University of Tokushima Graduate School), Motohiro Okada, MD, PhD, Yoshiaki Konishi, MS, Hisashi Tanii (PI), MD, PhD, (Department of Psychiatry, Mie University, Graduate School of Medicine), Shigeto Yamawaki, MD, PhD, Yasumasa Okamoto (PI), MD, PhD, (Department of Psychiatry and Neurosciences, Graduate School of Biomedical and Health Sciences, Hiroshima University), Satoshi Saito, MD, (Department of Psychiatry/ Department of Biological Psychiatry and Neuroscience, Dokkyo Medical University School of Medicine), Kazutaka Shimoda, MD, PhD (Department of Psychiatry, Dokkyo Medical University School of Medicine), Kazufumi Akiyama (PI), MD, PhD (Department of Biological Psychiatry and Neuroscience, Dokkyo Medical University School of Medicine), Hirokazu Fujita, MD, PhD, Shinji Shimodera (PI), MD, PhD (Department of Neuropsychiatry, Kochi Medical school, Kochi University), Masatoshi Takeda, MD, PhD, Ryota Hashimoto (PI), MD, PhD (Molecular Research Center for Children's Mental Development, United Graduate School of Child Development, Osaka University; Department of Psychiatry, Osaka University Graduate School of Medicine), Tadafumi Kato (PI), MD, PhD (Laboratory for Molecular Dynamics of Mental Disorders, RIKEN Brain Science Institute), Tetsufumi, Kanazawa, MD, PhD, Hiroshi Yoneda (PI), MD, PhD (Department of Neuropsychiatry, Osaka Medical College), Yuichiro Watanabe, MD, PhD, Toshiyuki Someya (PI), MD, PhD (Department of Psychiatry, Niigata University Graduate School of Medical and Dental Sciences), Reiji Yoshimura (PI), MD, PhD (Department of Psychiatry, University of Occupational and Environmental Health), Masanari Itokawa (PI), MD, PhD (Center for Medical Cooperation, Tokyo Metropolitan Institute of Medical Science), Masaomi Iyo (PI), MD, PhD (Department of Psychiatry, Chiba University Graduate School of Medicine) and Koji Matsuo (PI), MD, PhD (Division of Neuropsychiatry, Department of Neuroscience, Yamaguchi University Graduate School of Medicine). The contributors did not receive financial compensation.

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Supplementary Information accompanies the paper on the Molecular Psychiatry website (http://www.nature.com/mp)