Faculty of Health: Medicine, Dentistry and Human Sciences

School of Biomedical Sciences

2017-01-10

Evidence for genetic heterogeneity between clinical subtypes of bipolar disorder

Charney, AW

http://hdl.handle.net/10026.1/9202

10.1038/tp.2016.242 Translational Psychiatry Springer Science and Business Media LLC

All content in PEARL is protected by copyright law. Author manuscripts are made available in accordance with publisher policies. Please cite only the published version using the details provided on the item record or document. In the absence of an open licence (e.g. Creative Commons), permissions for further reuse of content should be sought from the publisher or author.

www.nature.com/tp

ORIGINAL ARTICLE Evidence for genetic heterogeneity between clinical subtypes of bipolar disorder

AW Charney¹, DM Ruderfer^{1,2}, EA Stahl^{1,2}, JL Moran³, K Chambert³, RA Belliveau³, L Forty⁴, K Gordon-Smith⁵, A Di Florio^{4,6}, PH Lee^{3,7,8}, EJ Bromet⁹, PF Buckley¹⁰, MA Escamilla¹¹, AH Fanous^{12,13}, LJ Fochtmann⁹, DS Lehrer¹⁴, D Malaspina¹⁵, SR Marder¹⁶, CP Morley^{17,18,19}, H Nicolini^{20,21}, DO Perkins⁶, JJ Rakofsky²², MH Rapaport²², H Medeiros²³, JL Sobell²³, EK Green²⁴, L Backlund^{25,26}, SE Bergen^{3,27}, A Juréus²⁷, M Schalling²⁶, P Lichtenstein²⁷, P Roussos^{1,2,28}, JA Knowles^{23,29}, I Jones⁴, LA Jones⁵, CM Hultman^{1,27}, RH Perlis³⁰, SM Purcell^{1,2,28}, SA McCarroll^{3,31}, CN Pato^{23,29}, MT Pato^{23,29}, N Craddock⁴, M Landén^{27,32}, JW Smoller^{3,7,8,33} and P Sklar^{1,2,28}

We performed a genome-wide association study of 6447 bipolar disorder (BD) cases and 12 639 controls from the International Cohort Collection for Bipolar Disorder (ICCBD). Meta-analysis was performed with prior results from the Psychiatric Genomics Consortium Bipolar Disorder Working Group for a combined sample of 13 902 cases and 19 279 controls. We identified eight genome-wide significant, associated regions, including a novel associated region on chromosome 10 (rs10884920; $P = 3.28 \times 10^{-8}$) that includes the brain-enriched cytoskeleton protein adducin 3 (*ADD3*), a non-coding RNA, and a neuropeptide-specific aminopeptidase P (*XPNPEP1*). Our large sample size allowed us to test the heritability and genetic correlation of BD subtypes and investigate their genetic overlap with schizophrenia and major depressive disorder. We found a significant difference in heritability of the two most common forms of BD (BD I SNP- $h^2 = 0.35$; BD II SNP- $h^2 = 0.25$; P = 0.02). The genetic correlation between BD I and BD II was 0.78, whereas the genetic correlation was 0.97 when BD cohorts containing both types were compared. In addition, we demonstrated a significantly greater load of polygenic risk alleles for schizophrenia and BD in patients with BD I compared with patients with BD II, and a greater load of schizophrenia risk alleles in patients with the bipolar type of schizoaffective disorder compared with patients with either BD I or BD II. These results point to a partial difference in the genetic architecture of BD subtypes as currently defined.

Translational Psychiatry (2017) 7, e993; doi:10.1038/tp.2016.242; published online 10 January 2017

INTRODUCTION

Bipolar disorder (BD) is a mental illness characterized by episodes of mania and depression. Over the past century, the diagnostic criteria for this condition have evolved. First, 'manic-depressive insanity' was split from the condition that is today known as schizophrenia (SCZ).¹ It was then split from what we now label major depressive disorder (MDD), and renamed BD.²

Subsequently, BD was formally divided into two clinical subtypes: bipolar I disorder (BD I), characterized by manic episodes; and bipolar II disorder (BD II), characterized by hypomanic episodes and recurrent depressive episodes.³ The initial BD I and BD II distinction was based primarily on a different longitudinal course, as differences in family loading³ and lithium responsiveness⁴ were not observed. The exact prevalence of each clinical subtype

¹Department of Psychiatry, Icahn School of Medicine at Mount Sinai, One Gustave L. Levy Place, New York, NY, USA; ²Institute for Genomics and Multiscale Biology, Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, One Gustave L. Levy Place, New York, NY, USA; ³Stanley Center for Psychiatric Research, Broad Institute of Harvard and MIT, Cambridge, MA, USA; ⁴MRC Centre for Psychiatric Genetics and Genomics, Cardiff Unviersity, Cardiff, UK; ⁵Department of Psychological Medicine, University of Worcester, Worcester, UK; ⁶Department of Psychiatry, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA; ⁷Department of Psychiatry, Harvard Medical School, Boston, MA, USA; ⁸Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA, USA; ⁹Department of Psychiatry, Stony Brook University, Stony Brook, NY, USA; ¹⁰Department of Psychiatry, Georgia Regents University Medical Center, Augusta, GA, USA; ¹¹Center of Excellence in Neuroscience, Department of Psychiatry, Texas Tech University Health Sciences Center at El Paso, El Paso, TX, USA; ¹²Department of Psychiatry, Veterans Administration Medical Center, Washington, DC, USA; ¹³Department of Psychiatry, Georgetown University, Washington, DC, USA; ¹⁴Department of Psychiatry, Wright State University, Dayton, OH, USA; ¹⁵Department of Psychiatry, New York University, New York, NY, USA; ¹⁶Department of Psychiatry, University of California, Los Angeles, Los Angeles, CA, USA; ¹⁷Department of Psychiatry and Behavioral Science, State University of New York, Upstate Medical University, Syracuse, NY, USA; ¹⁸Departments of Family Medicine, State University of New York, Upstate Medical University, Syracuse, NY, USA; ¹⁹Department of Public Health and Preventive Medicine, State University of New York, Upstate Medical University, Syracuse, NY, USA; ²⁰Center for Genomic Sciences, Universidad Autónoma de la Ciudad de México, Mexico City, Mexico; ²¹Department of Psychiatry, Carracci Medical Group, Mexico City, Mexico; ²²Department of Psychiatry and Behavioral Sciences, Emory University, Atlanta, GA, USA; ²³Department of Psychiatry and the Behavioral Sciences, University of Southern California, Keck School of Medicine, Los Angeles, CA, USA; ²⁴School of Biomedical and Health Sciences, Plymouth University Peninsula Schools of Medicine and Dentistry, Plymouth University, Plymouth, UK; ²⁵Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden; ²⁶Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden; ²⁷Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden; 28 Friedman Brain Institute, Department of Neuroscience, Icahn School of Medicine at Mount Sinai, One Gustave L. Levy Place, New York, NY, USA; 29Zilkha Neurogenetic Institute, University of Southern California, Keck School of Medicine, Los Angeles, CA, USA; 30Center for Experimental Therapeutics, Massachusetts General Hospital, Boston, MA, USA; ³¹Department of Genetics, Harvard Medical School, Boston, MA, USA; ³²Institute of Neuroscience and Physiology, Sahlgenska Academy at the Gothenburg University, Gothenburg, Sweden and ³³Psychiatric and Neurodevelopmental Genetics Unit, Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA, USA. Correspondence: Dr P Sklar, Department of Psychiatry, Icahn School of Medicine at Mount Sinai, One Gustave L. Levy Place, New York 10029, NY, USA,

E-mail: pamela.sklar@mssm.edu

Received 13 February 2016; revised 28 September 2016; accepted 28 September 2016

remains uncertain, with the most recent large epidemiological study reporting lifetime prevalence of 0.6% for BD I and 0.4% for BD II,⁵ similar to a recent systematic review⁶ but lower than previously reported.^{7,8}

Recurrence risk ratios for BD of 7–10 for first-degree relatives are observed, and several family studies have aimed to evaluate whether there is shared etiology for BD I and BD II. Some studies have shown familial co-aggregation of BD and MDD,⁹ but investigation of the specificity of familial aggregation of BD subtypes and their relationship to MDD to determine whether BD subtypes share etiology have yielded inconclusive results.^{10,11} More recently, contemporary family studies have found familial aggregation of mania and major depressive episodes but not hypomania,¹² and, similarly, of BD I and MDD but not BD II.¹³

In addition to BD I, a manic episode is required for the DSM-V diagnosis of schizoaffective disorder bipolar type (SAB). Since its initial description in 1933¹⁴ there has been debate regarding whether schizoaffective disorder (SA) is a form of SCZ, affective disorder, a combination of the two, or a separate entity altogether. BD and SCZ have historically been regarded as genetically distinct;^{12,15} however, more recent studies have shown a significant shared genetic component of these disorders. A large population-based study of > 35 000 patients with SCZ, 40 000 patients with BD and their family members found an increased risk of BD in first-degree relatives of SCZ probands, and vice versa.^{16,17} Compared with SCZ and BD, there is evidence that SA shows weaker disorder-specific familial aggregation, with relatives of SA probands having relatively equivalent risk for SA, SCZ and BD.^{15,18}

Genetic studies of common variation in BD have identified multiple genome-wide significant associations between disease status and single nucleotide polymorphisms (SNPs).¹⁹⁻²⁷ In 2011, the Psychiatric Genomics Consortium Bipolar Disorder Working Group (PGCBD) reported four loci meeting genome-wide significance, including regions in close proximity to genes *ODZ4*, *ANK3* and *SYNE1*.²⁴ Subsequently two studies performed metaanalyses incorporating the PGCBD data and their own new samples: Chen et al.²⁶ identified novel associations near the genes TRANK1 (LBA1), LMAN2L and PTGFR; Muhleisen et al.²⁷ identified two new risk loci near ADCY2 and a region between MIR2113 and POU3F2. As demonstrated in other disorders, in particular SCZ,²⁸ increasing sample size led to the identification of additional associated loci. Although each genome-wide association study (GWAS) only found a handful of genome-wide significant associations, it has been convincingly demonstrated that BD is polygenic; there are many common DNA variants whose effects are too small to detect individually, but when summed together, are contributing to BD risk.

Traditional family studies focus on relatives to determine the proportion of variance of liability to disease that is attributable to inherited genetic factors. In the absence of molecular data, these studies provide no information about the number, frequency or effect sizes of any genes or associated variants involved. With the emergence of genome-wide data for multiple psychiatric disorders, several methods have been developed for comparing conditions to one another at the genetic level. Three commonly used analytic approaches are comparative GWAS, polygenic scoring²⁹ and SNP-based heritability estimation.³⁰⁻³² Crossdisorder analysis of individual SNPs in SCZ and mood disorders (BD I, BD II, MDD) reported a genome-wide significant locus on chromosome 11 that appeared to be specific to BD II.³³ Because there are many small-effect DNA variants, polygenic scoring methods were developed that derive a disease risk score for each individual in a data set by counting the number of previously identified risk alleles present. Polygenic scores derived from both SCZ- and MDD-risk alleles have discriminant ability between BD cases and controls.^{17,34} SNP-based heritability methods, as opposed to utilizing genetic data only from loci previously implicated in disease, make genetic relatedness calculations by comparing all possible pairs of individuals in a data set at all genetic markers. When this information has been determined for two independent case–control data sets, the proportion of variance in phenotype explained by SNPs (labeled the SNP-based heritability, or SNP- h^2) for each case trait and the genetic correlation $(r_g)^{[ref. 35]}$ between the case traits can be determined. Using this approach, SNP- h^2 has been estimated to range between 0.20 and 0.25 for BD, SCZ and MDD, whereas the BD-MDD and BD-SCZ genetic correlations have been estimated at 50% and 70%, respectively.³⁰ The effect of BD subtype composition on these estimates has not been investigated.

In order to markedly increase the available sample size for a GWAS and improve our power to identify risk loci and discern genetic differences across BD subtypes, we established the International Cohort Collection for Bipolar Disorder (ICCBD) with investigators from the United States (US), United Kingdom (UK) and Sweden. We performed a GWAS on the ICCBD sample, as well as a meta-analysis with the PGCBD for a total of 13 902 cases and 19 279 controls. Further, we took advantage of the size and composition of our sample to explicitly survey the genetic relationship across the clinical subtypes of BD (BD I, BD II and SAB). Specifically, we compare SNP-based measures of heritability and genetic correlation for BD I and BD II, and assess the polygenic loading of BD subtypes for risk alleles identified previously through large-scale GWAS of three psychiatric disorders (BD, SCZ and MDD).

MATERIALS AND METHODS

Subject ascertainment and sample collection

All procedures were approved by ethical committees at the Karolinska Institutet, University of Southern California and Cardiff University. All subjects provided written informed consent (or legal guardian consent and subject assent).

The ICCBD includes BD cases and unaffected controls from the Sweden Bipolar Disorder Cohort (SWEBIC), the Bipolar Disorder Research Network (BDRN) in the United Kingdom, and the Genomic Psychiatry Consortium (GPC) from the University of Southern California. Inter-rater reliability across sites was performed and showed agreement between trained clinicians on case status (Fleiss' Kappa statistic for multiple raters κ = 0.72 for distinguishing BD from other disorders based on case notes; see Supplementary Note). GWAS results have not been reported on 85.7% of the ICCBD case subjects. All of the SWEBIC and BDRN control subjects have been reported in prior GWAS of BD, schizophrenia and other disorders^{36,37}

SWEBIC controls are from the Swedish Schizophrenia Consortium.³⁶ SWEBIC cases were identified through four channels: two national registries and two catchment areas. The Swedish National Quality Assurance Registry for Bipolar Disorder (BipoläR) led to the ascertainment of 1304 BD cases and the Swedish Hospital Discharge Register (HDR) yielded 233 BD cases. The HDR case subjects have been previously reported.³⁸ Additional cases were obtained via two catchment areas: 271 cases were recruited via physician's referral from the Affective Center at St. Göran Hospital in Stockholm and 493 cases were recruited from the greater Stockholm County region. SWEBIC controls were collected via the HDR and have been reported elsewhere.³⁶ All BD diagnoses for the SWEBIC sample were made according to the DSM-IV criteria (Supplementary Note). Genotype analyses have been previously reported for a portion of the SWEBIC cases.³⁸

Sample ascertainment strategies for the BDRN cases and controls have been previously reported^{37,39} (Supplementary Note). BDRN controls are from the Wellcome Trust Case Control Consortium 2 (WTCCC2) control cohort.³⁷ Case participants were interviewed using the Schedules for Clinical Assessment in Neuropsychiatry (SCAN).⁴⁰ Psychiatric and general practice case-notes, where available, were also reviewed. On the basis of these data best-estimate lifetime diagnoses were made according to DSM-IV criteria and key clinical variables, such as age at onset and number of episodes, were rated. In cases where there was doubt, diagnostic and clinical ratings were made by at least two members of the research team blind to each other's rating. Team members involved in the interview, rating and diagnostic procedures were all research psychologists or psychiatrists. Green *et al.*³⁹ reported 1218 BDRN cases for 3106 SNPs with immunological annotations.

2



GWAS

Subtype analyses

Figure 1. ICCBD sample description and analysis pipeline. The top panel (table) shows the composition of the ICCBD data set by site, genotyping platform and phenotype. The three ICCBD sites are represented by color, and the BD subtypes by shade. The bottom panel uses this representation of the dataset to diagram the workflow of the study and the portions of the ICCBD data set included in each analysis presented in the manuscript. Thickened borders designate the included subset, while excluded subsets are colored in grey. The coloring of text in the bottom panel is intended to illustrate how the same method (e.g., SNP-based heritability) was utilized for multiple purposes (i.e., ensuring similarity to previous GWAS, and comparing BD I to BD II). BD, bipolar disorder; GWAS, genome-wide association studies; ICCBD, International Cohort Collection for Bipolar Disorder; SNP, single-nucleotide polymorphism.

GPC cases and controls were collected via the University of Southern California healthcare system, as previously described.⁴¹ Using a combination of focused, direct interviews and data extraction from medical records, diagnoses were established using the OPCRIT.⁴² Age and gender-matched controls were ascertained from the University of Southern California health system and assessed using a validated screening instrument and medical records.

Genotyping

For all ICCBD sites, DNA was extracted from peripheral blood samples that had been collected and stored at -20 °C. Samples were then genotyped at the Broad Institute. Genotypes were called using Birdsuite (Affymetrix, Santa Clara, CA, USA) or BeadStudio (Illumina, San Diego, CA, USA). Genotypes were generated as sufficient numbers of samples accumulated from field work (Supplementary Note).

Quality control

Data were processed by a quality control (QC) pipeline modeled after the central analysis pipeline of the PGCBD study.²⁴ For each site, the goal was to create a set of genotyped SNPs of high and uniform quality maximizing the number of individuals retained. We first harmonized the SNP names, position and strand, then removed duplicated SNPs and individuals. SNPs with data missing in >5% of the sample were removed. Next, individuals with heterozygosity rate >15%, missingness rate >2%, or whose genotype-determined gender was ambiguous (0.25 < F < 0.75) were removed. SNPs on sex chromosomes were removed from analysis, and those remaining SNPs with data missing in >2% of the sample, minor allele frequency (MAF) < 1%, or deviation from Hardy–Weinberg equilibrium ($P < 5 \times 10^{-5}$) were removed. For data sets containing both case and control individuals, SNPs with controls ($P < 1 \times 10^{-3}$) or differential missingness in cases compared with controls ($P < 1 \times 10^{-3}$)

missingness based on haplotype ($P < 1 \times 10^{-10}$). After these initial QC checks, SNPs were pruned based on linkage disequilibrium (window size = 100, window shift = 50 SNPs and VIF threshold = 2), and multidimensional scaling (MDS) analysis on the N × N matrix of genome-wide identity-by-state pairwise distances was performed. Outlier individuals were identified using the ten most significant MDS components and excluded from the analysis so that cases and controls were appropriately matched. Relatedness between individuals in the population (defined as PIHAT value > 0.1) was also evaluated, with one of the members being removed from the analysis when such relationships were identified. After all QC, there remained 6447 cases (1378 SWEBIC Illumina, 923 SWEBIC Affymetrix, 2609 BDRN, 1537 GPC) and 12 639 controls (3716 SWEBIC Illumina, 2215 SWEBIC Affymetrix, 5413 BDRN, 1295 GPC; Figure 1 and Supplementary Note).

Phasing and imputation

After QC was completed on each of the ICCBD cohorts, phasing and imputation was performed. The data were phased using SHAPEIT⁴³ and imputed using IMPUTE2,⁴⁴ with phased 1000 Genomes world panel as reference. Each data set was imputed separately, splitting the data sets into 5MB imputation chunks. Following imputation, SNPs were filtered for MAF > 0.01 and imputation quality score > 0.3. After applying these filters to each cohort, meta-analysis was performed and only those SNPs with high-quality data in all cohorts were retained for analysis. This resulted in 8 886 502 SNPs for the ICCBD GWAS and 8 837 380 SNPs for the ICCBD-PGCBD meta-analysis.

Sign test

We performed sign tests on a modified ICCBD sample where duplicates or related individuals between ICCBD and PGCBD had been removed (26 BDRN cases and 2610 BDRN controls). Sign tests were performed using

Genetics of bipolar disorder in an international cohort AW Charney et al



^bp-values reported in references where region was reported to meet genome-wide significant for BD. Since the p-values we report are not corrected for genomic inflation, the p-values in this column are also the unco

x SNP in LD with the ICC8D-PGC8D index SNP was also reported as genome-wide significant in reference 26 (rs4948418, R²=0.45, p-value=2.99 x 10⁻¹⁰) and reference 24 (rs10994299, R²=1.00, p-value=5.50 x 10⁻¹⁰

SNP name and p-value for the index SNP in the PGCBD as determined from our re-analysis of the PGCBD data using 1000 genomes imputed dosages. Association results for this SNP (or SNPsin LD with this SNP) have not been reported for BD in previous publications

Figure 2. Association results for the ICCBD-PGCBD meta-analysis (13 902 cases, 19 279 controls). Horizontal axis shows chromosome and position. Vertical axis shows the -log10 P-value for association with BD. The table shows detailed statistics of the index SNP from each independent locus. The color of chromosome and SNP in the table corresponds the color of points for that locus in the Manhattan plot. BD, bipolar disorder; ICCBD, International Cohort Collection for Bipolar Disorder; PGCBD, the Psychiatric Genomics Consortium Bipolar Disorder Group; SNP, single-nucleotide polymorphism.

index SNPs from the PGCBD data in approximate linkage equilibrium with association P-values below four thresholds (0.001, 1×10^{-4} , 1×10^{-5} and 1×10^{-6}).

Polygenic scoring

We carried out polygenic scoring analyses²⁹ on the ICCBD data set using the PGCBD discovery data set. Ouantitative scores were computed for each ICCBD subject based on the set of SNPs with P-values less than predefined P-value thresholds (pT) in the discovery data set. For each SNP set defined by pT, we calculated the proportion of variance explained (Nagelkerke's R^2) by subtracting the Nagelkerke's R^2 attributable to ancestry covariates alone from the Nagelkerke's R^2 for polygenic scores plus covariates.

We additionally performed polygenic scoring analyses for three different target BD subtypes within the ICCBD cohort: BD I; BD II; and SAB (Figures 1 and Supplementary Table 1). Polygene scores were calculated using three discovery datasets: non-overlapping sets derived from GWAS of SCZ (9087 cases, 12 171 controls; cases included a small fraction of SAB),⁴⁵ BD (6704 cases, 9381 controls; cases were 85% BD I, 11% BD II and 4% SAB)²⁴ and MDD (9041 cases, 9381 controls).⁴⁶ The same set of controls was used in each of the target sets, and targets sets were filtered such that there were no overlaps with any of the discovery data sets. This yielded 3323 BD I cases, 1340 BD II cases, 570 SAB cases and 7814 controls for analysis. Quantitative scores were computed for each ICCBD subject at the defined pT and for each SNP set we calculated the proportion of variance explained (R^2) by subtracting the Nagelkerke's R^2 attributable to ancestry covariates alone from the R^2 for polygenic scores plus covariates. Next, using the same three discovery data sets, we performed the same polygene scoring procedure on target sets that, rather than containing ICCBD cases and controls, contained two ICCBD subtype cohorts. Three comparisons were performed in this manner: BD I vs BD II (3323 BD I cases, 1340 BD II cases), BD I vs SAB (3323 BD I cases, 570 SAB cases), and BD II vs SAB (1340 BD II cases, 570 SAB cases).

Translational Psychiatry (2017), 1-9

Association analyses

All association analyses were conducted using logistic regression in PLINK.³² MDS was performed on the entire data set, and each collection wave was analyzed separately using as covariates those MDS components in the top 10 that were significantly correlated with phenotype. Results were then combined by meta-analysis in PLINK,³² and the heterogeneity Pvalues reported are those for the Cochrane's Q statistic. Association analyses for BD were conducted using the 1000 Genomes imputed data. We used a genome-wide significance threshold of $P < 5 \times 10^{-8}$. Prior to performing the ICCBD meta-analysis with the PGCBD, we reanalyzed the PGCBD data set (7481 cases and 9250 controls)²⁴ using 1000 Genomes Project imputation, identifying no new genome-wide significant hits.

Heritability analyses

Using methods previously described,³⁰⁻³² we estimated the variance in liability explained by SNPs (SNP- h^2) in the full ICCBD cohort and each individual site for BD and its subtypes (Figure 1 and Supplementary Tables 1-3). From the set of 8 886 502 SNPs included in the ICCBD GWAS, we further filtered to retain only SNPs that had imputation R^2 of >0.8 and MAF>0.01 in all ICCBD samples, resulting in 7 252 417 SNPs upon which to calculate genome-wide similarity relationships between all pairs of individuals. For all SNP- h^2 estimates reported, individuals were excluded to ensure that no pairs of individuals had a genome-wide similarity relationship >0.05. This procedure removes ancestry outliers in addition to those already removed in the standard GWAS quality control pipeline.

To fully dissect whether previous observations of SNP- h^2 heterogeneity in BD³⁰ were driven by subtype or site, further filtering was required to ensure that the composition of the case cohorts being compared were balanced in terms of these variables. Any subtype for which >75% of cases derived from a single ICCBD site were thus excluded from these analyses, limiting SNP- h^2 subtype comparisons to BD I and BD II as 80% of SAB cases were from GPC (Figure 1 and Supplementary Table 1). Next, any site for which the relative contribution to the BD I and BD II case cohorts

b

SNP-based heritability (SNP-h²)

d

Density 50

0.3

0.2

0 1

0

30

10

07

BD I

r_{g-I/II} r_{a-miz}



1 To calculate SNP-h² for a BD subtype, the full set of cases with that subtype are compared to the full set of controls.

- 2 The genetic correlation between BD I and BD II ($r_{g_{el/II}}$) is calculated by splitting the controls into 2 independent groups, one of which is compared to the full set of BD I cases and the other which is compared to the full set of BD II cases. A total of 100 permutations of the control splitting procedure were carried out, such that $r_{g_{el/II}}$ was estimated 100 times.
- (3) The genetic correlation between two BD case groups that each contain a mixture of BD I and BD II ($r_{g_{mik}}$) is performed by pooling all of the cases, randomly splitting them, and comparing each subset to a subset of the controls. This case splitting procedure is performed 100 times for each of the 100 control splitting permutations, resulting in 10,000 estimates of $r_{g_{emik}}$.

Figure 3. SNP heritability and genetic correlation estimations for BD I and BD II. We estimated the SNP-h² for BD I (n = 2,811 cases) and BD II (n = 1398 cases), as well as the genetic correlation (r_g) explained by SNPs between BD I and BD II ($r_{g-I/II}$) and between random subsets of BD cases (r_{g-mix}). (a) Sample splitting procedures for SNP-based heritability and genetic correlation estimation. (b) SNP-based heritability estimates for BD I and BD II. We observed a significant difference in the SNP-h² of BD I and BD II (BD I SNP-h² = 0.35, SE = 0.02; BD II SNP-h² = 0.25, SE = 0.04; two-sided *t*-test P = 0.02). (c) SNP-based heritability estimates are presented. A significant difference between the distributions was observed ($P = 6.25 \times 10^{-45}$ for a two-sided *t*-test of mean difference between the empirical $r_{g-I/II}$ and r_{g-mix} distributions). BD, bipolar disorder; BDRN, Bipolar Disorder Research Network; SNP, single-nucleotide polymorphism; SWEBIC, Swedish Bipolar Disorder Cohort.

was grossly imbalanced was excluded. This removed GPC from the $SNP-h^2$ subtype analyses (Figure 1 and Supplementary Table 1).

Having applied these site/subtype filters, BD I and BD II SNP- h^2 were compared in the SWEBIC-BDRN cases using the full SWEBIC-BDRN control set to calculate each subtype SNP- h^2 (Supplementary Table 3). We then applied a bivariate extension of these methods³⁵ in order to estimate the genetic correlation (r_g) explained by SNPs between BD I and BD II cases ($r_{g-1/II}$), and to test whether it differed from r_g between cohorts containing a mix of BD I and BD II cases (r_{g-mix}).

To avoid inflation of $r_{\rm g}$ estimates, the SWEBIC-BDRN controls used in the SNP- h^2 calculations were randomly split into two evenly sized nonoverlapping groups, one each for pairing with the case subsets being compared. We calculated 100 estimates of $r_{\rm g-I/II}$, each using the same BD I (n = 2811) and BD II (n = 1,398) case groups but a unique permutation of the control splitting procedure. To calculate $r_{\rm g-mix}$ for each of the 100 control group permutations used in calculating $r_{\rm g-I/II}$ we combined the BD I and BD II cases into a single pool (n = 4,209 cases) that was then randomly divided into two non-overlapping mixed case groups (that is, cohorts containing both BD I and BD II cases) sized according to the BD I and BD II case groups used in the $r_{\rm g-I/II}$ estimates. We performed this case splitting procedure 100 times for each of the 100 control group permutations, for a total of 10 000 calculations of $r_{\rm g-mix}$. To determine whether the distributions of $r_{\rm g-I/II}$ and $r_{\rm g-mix}$ estimates differed from one another, a *t*-test was performed.

RESULTS

ICCBD GWAS and comparability to previous BD samples

Genome-wide SNP data of 6447 BD cases and 12 639 controls matched for ancestry were analyzed from a previously unreported population-based sampling in Sweden, the United Kingdom and the United States (Supplementary Figure 1). We performed logistic regression of case status on imputed dosages of 8 886 502

autosomal SNPs including sample and ancestry as covariates (see Materials and Methods). The resulting genomic inflation factor $(\lambda)^4$ was 1.11 (Figure 1 and Supplementary Figure 2). Two regions met a genome-wide significance threshold of $P < 5 \times 10^{-8}$: a 2 base-pair deletion on chromosome 9 (position 129209201, risk allele = TC, $P = 2.48 \times 10^{-8}$, odds ratio (OR) = 1.14), and a locus on chromosome 10 (position 111774807, rs10884920, risk allele = A, $P = 1.20 \times 10^{-8}$ OR = 1.17; Supplementary Table 4 and Supplementary Figure 3). Neither variant has been previously reported to have an association with BD, MDD, or SCZ. Comparability of this new cohort was assessed in three ways. First, sign tests found that most ICCBD SNPs had the same direction of effect as the index PGCBD SNP at P < 0.001 (66.73% in same direction, $P = 1.86 \times 10^{-28}$; Supplementary Figure 4). Second, polygenic risk scoring found higher BD risk scores in cases in the full ICCBD sample ($P = 5 \times 10^{-89}$; Supplementary Figure 5). Third, we estimated the BD SNP-based heritability $(SNP-h^2)$ using genome-wide complex trait analysis $(GCTA)^{48}$ and found a SNP- h^2 of 0.24 (s.e. = 0.01; Supplementary Table 2), consistent with previous estimates from the PGCBD sample.³⁰

0.8

Genetic Correlation (r_)

ICCBD-PGCBD meta-analysis

We combined the ICCBD cohort with PGCBD for a meta-analysis of 13 902 BD cases and 19 279 controls. Applying the same filters used in the ICCBD GWAS yielded 8 032 748 SNPs for analysis. There were 117 SNPs in 8 genomic regions that surpassed genome-wide significance (Figure 2 and Supplementary Figure 3). Of these, one region is novel; the chromosome 10 locus identified in the ICCBD GWAS (chromosome 10, position 111774807, rs10884920, $P = 3.28 \times 10^{-8}$, heterogeneity P = 0.27, OR = 1.12). Among the genes in strong linkage disequilibrium with this locus



0.9

1.0

5

Polygenic scoring of BD subtypes



Figure 4. Comparison of BD subtypes with one another and controls using bipolar disorder (BD), schizophrenia (SCZ) and major depressive disorder (MDD) polygenic scores. Regression analyses of phenotype (top: BD subtype vs controls; bottom: BD subtype vs BD subtype) on polygenic scores derived from three previous GWAS (blue—BD, red—SCZ and green—MDD) were performed using MDS components and study site as covariates. The *t*-statistic plotted on the *x* axis is the ratio of the coefficient of the polygenic score variable and its standard error from the generalized linear model regression equation. The direction of the plotted bars indicates the phenotype in the comparison with the higher polygenic scores. The *P*-values for whether scores differed significantly between phenotypes are shown at the far right. Nagelkerke's R^2 values were derived from a corresponding logistic regression analysis performed for each phenotype comparison, and are used as to estimate the variance in phenotype explained by the polygenic score. GWAS, genome-wide association studies.

are adducin3 (*ADD3*), which codes for a ubiquitously expressed cytoskeletal protein implicated in cerebral palsy,⁴⁹ and aminopeptidase P (*XPNPEP1*), which has been found to be involved in the degradation and maturation of neuropeptides.⁵⁰ Of the seven other genome-wide significant signals, four were reported in the PGCBD²⁴ and an additional three by Chen *et al.*²⁶ (Figure 2 and Supplementary Table 5). When these eight loci were tested for association with BD subtypes in the ICCBD cohort, the threshold for nominal significance (P < 0.05) was surpassed for all loci in BD I, four loci in BD II and two loci in SAB (Supplementary Table 6). Moreover, the direction of effect was the same as in the ICCBD–PGCBD for all eight loci with respect to SAB (Supplementary Table 6).

Heritability of BD I and BD II

We next compared BD subtypes with one another at the genetic level by estimating SNP-based heritability $(SNP-h^2)^{[refs 30-32]}$ with GCTA.⁴⁸ Traditional estimates of heritability infer the total genetic contribution to the variance of a given phenotype through familial relationships. In contrast, SNP-based estimation has the advantage of harnessing actual genotype data, estimating the variance in liability to disease that can be attributed to SNPs across the whole genome. Reliable BD subtype SNP-h² estimation required the ICCBD sample analyzed be balanced with respect to disease subtype and site of collection (see Materials and Methods). This was found to be the case when considering BD I and BD II in the SWEBIC and BDRN cohorts (2811 SWEBIC-BDRN BD I cases; 1398

SWEBIC-BDRN BD II cases; 11 164 SWEBIC-BDRN controls; Supplementary Tables 1–3). We observed a significant difference in the SNP- h^2 of BD I and BD II (BD I SNP- h^2 = 0.35, s.e. = 0.02; BD II SNP- h^2 = 0.25, s.e. = 0.04; two-sided *t*-test *P*-value = 0.02; Figure 3 and Supplementary Table 3).

We next estimated the genetic correlation between BD I and BD II $(r_{\alpha-I/II})^{[ref. 35]}$ in SWEBIC–BDRN to directly assess their degree of genetic overlap. In general, this method requires that each case group be compared with an independent control group. In order to compare the BD I cases (n = 2813) to the BD II (n = 1397) cases in SWEBIC-BDRN, the 11 164 controls were therefore split into two groups. We estimated $r_{q-l/ll}$ to be 0.78, the mean of 100 estimates calculated using 100 permutations of the splitting procedure used to create independent control groups (range = 0.63-1.00, s. d. = 0.07; see Materials and Methods; Figure 3). Positive r_q values are observed when case groups share the same risk alleles relative to controls. In principle, this value should equal one when the two case groups are random samples of the same case population. Indeed, when genetic correlations were estimated between case groups containing random mixtures of BD subtypes (r_{g-mix}; see Materials and Methods), a mean value of 0.97 was observed (range = 0.64–1.00, s.d. = 0.05). The distribution of r_{g-mix} estimates was significantly higher than the distribution of $r_{q-l/ll}$ estimates, suggesting the genetic heterogeneity observed between BD I and BD II is non-random and not simply a function of BD being a complex genetic disease ($P = 6.25 \times 10^{-45}$ for *t*-test of mean difference between the empirical $r_{q-I/II}$ and r_{q-mix} distributions; Figure 3).

Polygenic scoring²⁹ was also used to assess the relationships between disease subtypes (see Materials and Methods). Risk scores were derived for each individual in the data set by counting the number of alleles present that have previously shown to increase disease risk across a range of P-value thresholds. Logistic regression was performed between diagnosis and risk score with site and the first 10 MDS components as covariates to test for significant correlation between BD subtype and SCZ, MDD, and BD genetic risk. For all subtypes tested, each discovery set produced a significantly higher polygenic risk score for cases compared with controls, with the most significant correlation being that between BD risk scores and diagnosis of BD I ($P = 2.75 \times 10^{-62}$, pseudo- $R^2 = 0.033$; Figure 4). MDD risk scores were the least significant across all subtypes (BD | $P = 8.92 \times 10^{-7}$, BD || $P = 4.34 \times 10^{-7}$, SAB P = 0.002). Next, we examined the ability of these risk scores to differentiate subtypes from each other. We again performed logistic regression on risk score except comparing BD subtypes to one another rather than to controls. BD risk scores had significant discriminant ability when comparing BD I and BD II, with more BD risk alleles found in BD I ($P = 5.45 \times 10^{-7}$). In addition, we identified significantly more SCZ risk alleles in BD I compared to BD II (P=0.001), SAB compared with BD I $(P=3.86 \times 10^{-4})$, and SAB compared with BD II ($P = 1.41 \times 10^{-4}$; Figure 4). MDD risk scores did not show significant discriminant ability between any pair of BD subtypes, though were higher in BD II compared to BD I (P = 0.06) and in BD I compared with SAB (P = 0.88; Figure 4).

DISCUSSION

We present a BD GWAS with over 5000 previously unreported cases (totaling 13 902 cases and 19 279 controls) identifying eight genome-wide significant loci, including a novel locus on chromosome 10. The new locus contains two coding genes, adducin 3 (ADD3) and aminopeptidase P (XPNPEP1) and a non-coding RNA also annotated as an antisense ADD3 RNA. Both genes are biologically interesting in relation to BD. First, ADD3 is a member of a family of cytoskeletal proteins responsible for capping the growing end of actin filaments and promoting the binding of spectrin to actin in the brain and elsewhere. Recently, adducins have been shown to form ladder-like structures in axons similar to those observed for actin and spectrin.⁵¹ Previously, genetic alterations in other components of the actin cytoskeleton have been suggested as risk factors for both BD and SCZ.⁵² Second. XPNPEP1 is an X-prolyl aminopeptidase that mediates the proteolytic cleavage of the N-terminal amino acid in peptides with proline. Proline containing neuropeptides include oxytocin, corticotropin releasing hormone, neuropeptide Y and substance P,53 each of which has been implicated with varying levels of support in BD. Heterozygous mice in which XPNPEP1 was deleted had smaller forebrains also consistent with a potential role in neuropsychiatric illness.54

Of the 12 loci previously reported genome-wide significant in at least 1 of the 3 largest BD GWAS, 9 have *P*-values below 10⁻⁶ in our data set and 7 of these surpass genome-wide significance (Supplementary Table 5). One straightforward explanation for our ability to support some, but not all prior BD loci, is the possibility of 'winner's curse'.⁵⁵ Given a polygenic model, the power will be low to detect a particular variant (due to the overestimation of the power to replicate individual results) at genome-wide significant threshold, though there will be many chance opportunities to identify at least one variant.⁵⁵ Thus any single lack of replication is not informative. In general, however, our data strongly support consistency of our samples with the prior literature as reflected by the sign test with the prior PGCBD samples (Supplementary Figure 4), significant polygenic risk prediction (Supplementary

7

Figure 5) and BD heritability estimates (Supplementary Table 2) consistent with previous estimates. $^{\rm 30}$

In this paper, we provide evidence of a molecular correlate for the division of BD into types I and II. SNP- h^2 was observed to be significantly higher in BD I compared with BD II. This observation led us to test the null hypothesis that BD I and BD II are the same genetic trait (that is, $r_{q, |I||} = r_{q, \text{mix}}$). The mean $r_{q, |I||}$ and $r_{q, \text{mix}}$ values were significantly different from one another, supporting the notion that BD I and BD II cohorts have a significantly lower genetic correlation than two BD cohorts composed of both subtypes. In addition to SNP- h^2 and r_q analyses, we found that polygenic score profiles of BD and SCZ risk variants had significant discriminant ability between BD I and BD II. The BD risk variants used for scoring were originally discovered in a cohort that is 85% BD I and 11% BD II,²⁴ thus the lower scores in the ICCBD BD II cohort is consistent with BD II having partially distinct risk loci. The discriminant ability of SCZ risk variants with higher scores in BD I is consistent with clinical phenomenology, as psychosis is far more commonly a feature of BD I than BD $\rm II.^{56}$ We hypothesized that MDD risk scores would be significantly higher in BD II compared with BD I due to the prominent depressive episodes required for a diagnosis of BD II. This was not found to be the case, though a non-significant trend in the predicted direction was observed.

The number of SAB cases in the ICCBD cohort allowed us to begin to assess its genetic overlap with BD I and BD II. Subtypespecific association results for the eight loci identified as significant in the ICCBD-PGCBD GWAS showed the expected direction of effect for all three subtypes at seven loci and for two of the three subtypes (BD I and BD II) at one locus (Supplementary Table 6). Thus, for these eight most significant loci, the association does not appear to be driven by a particular subtype of disease. Clinically, SAB is unique among BD subtypes in that psychosis in the absence of mania and depression is required for a diagnosis. As such, one may expect that the psychosis risk loci from studies of SCZ would demonstrate discriminant ability between SAB and other BD subtypes, which is indeed what we observe. We see a pattern of SCZ risk loci loading that follows the prominence of psychotic symptoms in the phenomenology of the three BD subtypes (SAB > BD I> BD II). One interpretation of these findings is that the genetic liability of psychosis influences the prominence of psychotic symptoms across diagnostic entities, ranging from psychosis being the sole feature and occurring only in the absence of mania or depression (that is, SCZ), to being a feature occurring in both the presence and absence of mania or depression (that is, SA), to being a common but not required feature occurring only in the presence of mania or depression (that is, BD I), to finally being a feature occurring rarely and only in the presence of depression (that is, BD II and MDD). However, it should be noted that the SCZ risk variants used for scoring were discovered in a dataset (PGCSCZ⁴⁵) that included SA cases. Of the 5 PGCSCZ cohorts that included SA, three explicitly reported the percentage of SA cases, which ranged from 6 to 17% of the total cohort sample (the percent of these cases with SAB is unavailable).⁴⁵ Therefore, the increased SCZ loading in SAB observed here could in part reflect genetic signal from SAB cases in the PGCSCZ discovery set in addition to (or, as opposed to) reflecting an increased loading for risk variants specific to psychosis. In order to further address these possibilities through genetics, larger SAB cohorts such as those that will be available in larger consortia are necessary.

This study has several limitations. Although our sample sizes for BD subtype comparisons are larger than those previously reported, we were still relatively underpowered to perform subtype-specific GWAS. As is common for most large-scale genetic studies in psychiatry, we were not able to assess higherdimensional phenotypes (that is, age of onset, lithium response) since this information is not currently available for all ICCBD subjects. Finally, heterogeneity between sites was observed in our analyses, most notably in that the GPC cohort demonstrated relatively decreased signal compared with the SWEBIC and BDRN cohorts. Such heterogeneity is not uncommon in genetic studies that pool samples from many different sites, but also raises the possibility that technical artifacts or subtle confounders (that is, cryptic relatedness) unable to be detected using current methods may be blunting the signal. When possible, the potential for such batch effects was taken into account (Supplementary Note).

In summary, in addition to providing a new and interesting locus for BD, our findings begin the process of correlating molecular signatures with disorders on the bipolar spectrum of mental illness. Increasingly, it is realized that there is genetic overlap between psychiatric diagnoses. Here we add nuance to our understanding of this phenomenon, providing evidence that genetic overlap correlates with overlap in clinical phenomenology. Ultimately, these analyses could lead to refined distinctions with the potential to improve prognosis and treatment strategies. Whether there are other dimensional measures that may better reflect the underlying genetic architecture remains to be tested in larger consortia samples as increasingly refined phenotyping is available.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

We are grateful for the participation of all subjects contributing to this research, and to the collection team that worked to recruit them. We acknowledge funding support from National Institutes of Health (NIH)/National Institute of Mental Health (NIMH) grant R01MH085542 (AWC, JWS and PS), NIH/NIMH grant R01MH085545 (JWS), NIH/ NIMH grant R01MH085548 (AHE, CNP, CPM, DM, DOP, DSI, ELB, HM, HN, IAK, JIB, JIS, LJF, MAE, MHR, MTP, PFB and SRM), NIH/NIMH grant K99MH101367 (PHL), the Stanley Medical Research Institute (JLM, KC, RAB and SAM), philanthropic gifts from Kent and Elizabeth Dauten and Ted and Vada Stanley (JLM, KC, RAB and SAM), the Swedish Research Council 2013-3196 (CMH), the Swedish Medical Research Council grants K2014-62X-14647-12-51 and K2010-61 P-21568-01-4 (ML), the Swedish foundation for Strategic Research grant KF10-0039 (ML), the Swedish Federal Government under the LUA/ALF agreement grants ALF 20130032 and ALFGBG-142041 (ML), European Commission-Marie Curie Fellowship (AD), Wellcome Trust (IJ, KG, LAJ, LF and NC). Work at the Icahn School of Medicine at Mount Sinai was also supported by the Institute for Genomics and Multiscale Biology (including computational resources and staff expertise provided by the Department of Scientific Computing).

DISCLAIMER

The funders had no role in study design, execution, analysis or manuscript preparation.

REFERENCES

- 1 Kraepelin E. Manic-Depressive Insanity and Paranoia. E. & S. Livingstone, Edinburgh, 1921.
- 2 Leonhard K. Pathogenesis of manic-depressive disease. Der Nervenarzt 1957; 28: 271–272.
- 3 Dunner DL, Gershon ES, Goodwin FK. Heritable factors in the severity of affective illness. *Biol Psychiatry* 1976; **11**: 31–42.
- 4 Fieve RR, Kumbaraci T, Dunner DL. Lithium prophylaxis of depression in bipolar I, bipolar II, and unipolar patients. Am J Psychiatry 1976; 133: 925–929.
- 5 Merikangas KR, Jin R, He J-P, Kessler RC, Lee S, Sampson NA *et al.* Prevalence and correlates of bipolar spectrum disorder in the world mental health survey initiative. *Arch Gen Psychiatry* 2011; **68**: 241–251.
- 6 Ferrari AJ, Baxter AJ, Whiteford HA. A systematic review of the global distribution and availability of prevalence data for bipolar disorder. J Affect Disord 2011; 134: 1–13.
- 7 Angst J. The emerging epidemiology of hypomania and bipolar II disorder. J Affect Disord 1998; 50: 143–151.
- 8 Merikangas KR, Akiskal HS, Angst J, Greenberg PE, Hirschfeld RMA, Petukhova M et al. Lifetime and 12-month prevalence of bipolar spectrum disorder in the National Comorbidity Survey Replication. Arch Gen Psychiatry 2007; **64**: 543–552.
- Translational Psychiatry (2017), 1–9

- 9 Smoller JW, Finn CT. Family, twin, and adoption studies of bipolar disorder. Am J Med Genet C Semin Med Genet 2003; 123C: 48–58.
- 10 Gershon ES, Hamovit J, Guroff JJ, Dibble E, Leckman JF, Sceery W et al. A family study of schizoaffective, bipolar I, bipolar II, unipolar, and normal control probands. Am Med Assoc 1982; 39: 1157–1167.
- 11 Heun R, Maier W. The distinction of bipolar II disorder from bipolar I and recurrent unipolar depression: results of a controlled family study. *Acta Psychiatr Scand* 1993; **87**: 279–284.
- 12 Vandeleur CL, Merikangas KR, Strippoli MPF, Castelao E, Preisig M. Specificity of psychosis, mania and major depression in a contemporary family study. *Mol Psychiatry* 2013; **19**: 209–213.
- 13 Merikangas KR, Cui L, Heaton L, Nakamura E, Roca C, Ding J *et al.* Independence of familial transmission of mania and depression: results of the NIMH family study of affective spectrum disorders. *Mol Psychiatry* 2014; **19**: 214–219.
- 14 Kasanin J. The acute schizoaffective psychoses. Am J Psychiatry 1933; 90: 97-126.
- 15 Laursen TM, Labouriau R, Licht RW, Bertelsen A, Munk-Olsen T, Mortensen PB. Family history of psychiatric illness as a risk factor for schizoaffective disorder: a Danish register-based cohort study. Arch Gen Psychiatry 2005; 62: 841–848.
- 16 Lichtenstein P, Yip BH, Bjork C, Pawitan Y, Cannon TD, Sullivan PF et al. Common genetic determinants of schizophrenia and bipolar disorder in Swedish families: a population-based study. *Lancet* 2009; **373**: 234–239.
- 17 Ruderfer DM, Fanous AH, Ripke S, McQuillin A, Amdur RL et al. Schizophrenia Working Group of Psychiatric Genomics C Polygenic dissection of diagnosis and clinical dimensions of bipolar disorder and schizophrenia. *Mol Psychiatry* 2014; **19**: 1017–1024.
- 18 Bertelsen A. Gottesman II. Schizoaffective psychoses: genetical clues to classification. Am J Med Genet 1995; 60: 7–11.
- 19 Cichon S, Mühleisen TW, Degenhardt FA, Mattheisen M, Miró X, Strohmaier J et al. Genome-wide association study identifies genetic variation in neurocan as a susceptibility factor for bipolar disorder. Am J Hum Genet 2011; 88: 372–381.
- 20 Burton PR, Clayton DG, Cardon LR, Craddock N, Deloukas P, Duncanson A *et al.* Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* 2007; **447**: 661–678.
- 21 Scott LJ, Muglia P, Kong XQ, Guan W, Flickinger M, Upmanyu R et al. Genomewide association and meta-analysis of bipolar disorder in individuals of European ancestry. Proc Natl Acad Sci USA 2009; 106: 7501–7506.
- 22 Sklar P, Smoller JW, Fan J, Ferreira MAR. Whole-genome association study of bipolar disorder. *Mol Psychiatry* 2008; **13**: 558–569.
- 23 Smith EN, Bloss CS, Badner JA, Barrett T, Belmonte PL, Berrettini W et al. Genomewide association study of bipolar disorder in European American and African American individuals. *Mol Psychiatry* 2009; **14**: 755–763.
- 24 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* 2011; **43**: 977–983.
- 25 Schulze TG, Detera-Wadleigh SD, Akula N, Gupta A, Kassem L, Steele J et al. Two variants in Ankyrin 3 (ANK3) are independent genetic risk factors for bipolar disorder. *Mol Psychiatry* 2009; **14**: 487–491.
- 26 Chen DT, Jiang X, Akula N, Shugart YY, Wendland JR, Steele CJ *et al.* Genome-wide association study meta-analysis of European and Asian-ancestry samples identifies three novel loci associated with bipolar disorder. *Mol Psychiatry* 2013; 18: 195–205.
- 27 Muhleisen TW, Leber M, Schulze TG, Strohmaier J, Degenhardt F, Treutlein J *et al.* Genome-wide association study reveals two new risk loci for bipolar disorder. *Nat Commun* 2014; **5**: 3339.
- 28 Schizophrenia Working Group of the Psychiatric Genomics Consortium. Biological insights from 108 schizophrenia-associated genetic loci. *Nature* 2014; **511**: 421–427.
- 29 Purcell SM, Wray NR, Stone JL, Visscher PM, O'Donovan MC et al. International Schizophrenia Consortium Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. *Nature* 2009; 460: 748–752.
- 30 Lee SH, Ripke S, Neale BM, Faraone S V, Purcell SM, Perlis RH et al. Genetic relationship between five psychiatric disorders estimated from genomewide SNPs. Nat Genet 2013; 45: 984–994.
- 31 Lee SH, Wray NR, Goddard ME, Visscher PM. Estimating missing heritability for disease from genome-wide association studies. Am J Hum Genet 2011; 88: 294–305.
- 32 Lee SH, DeCandia TR, Ripke S, Yang J, Sullivan PF, Goddard ME *et al.* Estimating the proportion of variation in susceptibility to schizophrenia captured by common SNPs. *Nat Genet* 2012; **44**: 247–250.
- 33 Huang J, Perlis RH, Lee PH, Rush AJ, Fava M, Sachs GS et al. Cross-disorder genomewide analysis of schizophrenia, bipolar disorder, and depression. Am J Psychiatry 2010; 167: 1254–1263.
- 34 Cross-Disorder Group of the Psychiatric Genomics Consortium. Identification of risk loci with shared effects on five major psychiatric disorders: a genome-wide analysis. *Lancet* 2013; **381**: 1371–1379.

- 35 Lee SH, Yang J, Goddard ME, Visscher PM, Wray NR. Estimation of pleiotropy between complex diseases using single-nucleotide polymorphism-derived genomic relationships and restricted maximum likelihood. *Bioinformatics* 2012; 28: 2540–2542.
- 36 Ripke S, O'Dushlaine C, Chambert K, Moran JL, Kähler AK, Akterin S et al. Genomewide association analysis identifies 13 new risk loci for schizophrenia. Nat Genet 2013; 45: 1150–1159.
- 37 Barrett JC, Lee JC, Lees CW, Prescott NJ, Anderson CA et al. UK IBD Genetics Consortium Genome-wide association study of ulcerative colitis identifies three new susceptibility loci, including the HNF4A region. Nat Genet 2009; 41: 1330–1334.
- 38 Bergen SE, O'Dushlaine CT, Ripke S, Lee PH, Ruderfer DM, Akterin S et al. Genomewide association study in a Swedish population yields support for greater CNV and MHC involvement in schizophrenia compared with bipolar disorder. *Mol Psychiatry* 2012; **17**: 880–886.
- 39 Green EK, Hamshere M, Forty L, Gordon-Smith K, Fraser C, Russell E et al. Replication of bipolar disorder susceptibility alleles and identification of two novel genome-wide significant associations in a new bipolar disorder casecontrol sample. *Mol Psychiatry* 2013; 18: 1302–1307.
- 40 Wing JK, Babor T, Brugha T, Burke J, Cooper JE, Giel R et al. SCAN. Schedules for clinical assessment in neuropsychiatry. Arch Gen Psychiatry 1990; 47: 589–593.
- 41 Pato MT, Sobell JL, Medeiros H, Abbott C, Sklar BM, Buckley PF et al. The Genomic Psychiatry Cohort: Partners in Discovery. Am J Med Genet B Neuropsychiatr Genet. 2013; 4: 306–312.
- 42 McGuffin P, Farmer A, Harvey I. A polydiagnostic application of operational criteria in studies of psychotic illness: development and reliability of the OPCRIT system. *Arch Gen Psychiatry* 1991; **48**: 764–770.
- 43 Delaneau O, Zagury JF, Marchini J. Improved whole-chromosome phasing for disease and population genetic studies. *Nat Methods* 2013; **10**: 5–6.
- 44 Howie B, Fuchsberger C, Stephens M, Marchini J, Abecasis GR. Fast and accurate genotype imputation in genome-wide association studies through pre-phasing. *Nat Genet* 2012; 44: 955–959.
- 45 Schizophrenia Psychiatric Genome-Wide Association Study Consortium. Genomewide association study identifies five new schizophrenia loci. *Nat Genet* 2011; 43: 969–976.
- 46 Major Depressive Disorder Working Group of the Psychiatric GWAS Consortium., Ripke S, Wray NR, Lewis CM, Hamilton SP, Weissman MM *et al.* A mega-analysis of genome-wide association studies for major depressive disorder. *Mol Psychiatry* 2013; **18**: 497–511.

- 47 Devlin B, Roeder K. Genomic control for association studies. *Biometrics* 1999; 55: 997–1004.
- 48 Yang J, Lee SH, Goddard ME, Visscher PM. GCTA: a tool for genome-wide complex trait analysis. Am J Hum Genet 2011; 88: 76–82.
- 49 Kruer MC, Jepperson T, Dutta S, Steiner RD, Cottenie E, Sanford L *et al.* Mutations in gamma adducin are associated with inherited cerebral palsy. *Ann Neurol* 2013; 74: 805–814.
- 50 Harbeck HT, Mentlein R. Aminopeptidase P from rat brain. Purification and action on bioactive peptides. *Eur J Biochem* 1991; **198**: 451–458.
- 51 Xu K, Zhong G, Zhuang X. Actin, spectrin, and associated proteins form a periodic cytoskeletal structure in axons. *Science* 2013; **339**: 452–456.
- 52 O'Dushlaine C, Kenny E, Heron E, Donohoe G, Gill M, Morris D *et al.* Molecular pathways involved in neuronal cell adhesion and membrane scaffolding contribute to schizophrenia and bipolar disorder susceptibility. *Mol Psychiatry* 2011; **16**: 286–292.
- 53 Vanhoof G, Goossens F, De Meester I, Hendriks D, Scharpe S. Proline motifs in peptides and their biological processing. *FASEB J* 1995; **9**: 736–744.
- 54 Yoon SH, Bae YS, Mun MS, Park KY, Ye SK, Kim E et al. Developmental retardation, microcephaly, and peptiduria in mice without aminopeptidase P1. Biochem Biophys Res Commun 2012; 429: 204–209.
- 55 Ioannidis JPA, Thomas G, Daly MJ. Validating, augmenting and refining genomewide association signals. *Nat Rev Genet* 2009; **10**: 318–329.
- 56 Parker G, Graham R, Hadzi-Pavlovic D, McCraw S, Hong M, Friend P. Differentiation of bipolar I and II disorders by examining for differences in severity of manic/ hypomanic symptoms and the presence or absence of psychosis during that phase. J Affect Disord 2013; 150: 941–947.

This work is licensed under a Creative Commons Attribution 4.0 International License. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit http://creativecommons.org/licenses/ by/4.0/

© The Author(s) 2017

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