Genome-wide association and meta-analysis of bipolar disorder in individuals of European ancestry


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Bipolar disorder (BP) is a disabling and often life-threatening disorder that affects ~1% of the population worldwide. To identify genetic variants that increase the risk of BP, we genotyped on the Illumina HumanHap550 Beadchip 2,076 bipolar cases and 1,676 controls of European ancestry from the National Institute of Mental Health Human Genetics Initiative Repository, and the Prechter Repository and samples collected in London, Toronto, and Dundee. We imputed SNP genotypes and tested for SNP-BP association in each sample and then performed meta-analysis across samples. The strongest association P value for this 2-study meta-analysis was 2.4 × 10⁻⁶. We then imputed SNP and tested for SNP-BP association. Based on the publicly available Affymetrix 500K genotype data from the Wellcome Trust Case Control Consortium for 1,868 bipolar cases and a reference set of 12,831 individuals. A 3-study meta-analysis of 3,683 cases and 14,507 extended controls on 2.3 million genotyped and imputed SNPs resulted in 3 chromosomal regions with association P > 1.0 × 10⁻⁷: 1p31.1 (no known genes), 3p21 (30 genes), and 5q15 (84 genes). The most strongly associated nonsynonymous SNP rs10994336 in the nonoverlapping GSK London sample (OR = 1.19, P = 1.0 × 10⁻⁷) is in the ITH1 gene on chromosome 3, with other strongly associated nonsynonymous SNPs in ITIH3, NEK4, and ITIH5. Thus, these chromosomal regions harbor genes implicated in cell cycle, neurogenesis, neuropil plasticity, and neurosignaling. In addition, we replicated the reported ANK3 association results for SNP rs10994336 in the nonoverlapping GSK sample (OR = 1.37, P = 0.042). Although these results are promising, analysis of additional samples will be required to confirm that variant(s) in these regions influence BP risk.

Bipolar disorder (BP) is characterized by dramatic mood changes, with individuals experiencing alternating episodes of depression and mania interspersed with periods of normal function. BP is chronic, severely disabling, and life-threatening, with increased risk of suicide and estimated lifetime prevalence of ~1% (1).

BP has a substantial genetic component. Monozygotic twin concordance rate estimates range from 45 to 70% and sibling recurrence risk estimates from 5 to 10 (2). Nonetheless, the underlying genetic and neurobiological triggers of BP remain elusive. Numerous linkage and candidate gene studies have sought to identify BP linked regions and associated genes, but no loci have been convincingly identified. Several groups have recently reported results of BP genome-wide association studies (GWAS), using pooled (3) or individually genotyped (4–6) samples; these studies identified SNPs in CACNA1C (alpha 1C subunit of the L-type voltage-gated calcium channel), ANK3 (ankyrin 3), and DKGH (diacylglycerol kinase, eta) as potentially associated with BP.

To test for SNP-BP association in additional well-characterized samples, we analyzed data for >2.3 million genotyped and imputed SNPs on >3,700 individuals in 2 sample sets: (i) 1,177 bipolar I (BP I) cases and 772 controls from the National Institutes of Mental Health Genetic Initiative Repository and the Prechter Repository [National Institute of Mental Health (NIMH)/Pritzker], and (ii) 899 BP cases and 904 controls from London and Dundee, U.K., and, Toronto, Canada whose collection was sponsored by GlaxoSmithKline Research and Development (GSK). We analyzed the NIMH/Pritzker and GSK GWAS samples separately and performed a 2-study meta-analysis. We then imputed and analyzed the publicly available Affymetrix 500K genotype data from the Wellcome Trust Case Control Consortium (WTCCC) (4) for 1,865 BP cases and an expanded reference set of 12,831 individuals. The expanded reference set comprised a blood donor sample and 6 non-BP disease case groups. After removing from the GSK London sample 261 cases that overlapped with the WTCCC sample, we performed a 3-study meta-analysis of 3,683 cases and 14,507 extended reference set.
individuals. We identified 3 regions that harbored SNPs with association $P \approx 10^{-7}$.

Results

For the NIMH/Pritzker GWAS, 1,177 BP I cases (473 sibling pairs and 231 unrelated individuals) from the NIMH and Prechter Repositories and 772 controls from the NIMH Repository (Table 1) were genotyped on the Illumina HumanHap 550K chip; 512,844 autosomal SNPs passed QC (see Methods) and had minor allele frequency (MAF) $\geq 1\%$. For the GSK GWAS, 899 BP cases and 904 controls were genotyped (Table 1); 512,508 autosomal SNPs passed QC and had MAF $\geq 1\%$. We carried out genotype imputation, using these data together with estimated haplotypes for the HapMap CEU (Utah residents with ancestry from northern and western Europe) samples, resulting in 2,473,048 (NIMH/Pritzker) or 2,465,069 (GSK) genotyped or imputed autosomal SNPs with MAF $\geq 1\%$. Previous work has shown good concordance of imputed and experimental genotypes (7–9).

We tested for SNP-BP association in the WTCCC BP case and control samples (1,177 BP I cases and an extended reference set of 12,831 individuals comprised of the National Blood Service (NBS) controls and individuals with coronary artery disease, Crohn’s disease, hypertension, rheumatoid arthritis, type 1 diabetes, or type 2 diabetes (Table 1). Given the low population prevalence of BP (1%), use of an unscreened expanded reference set should result in little loss of power to detect BP association. These samples had been genotyped on the Affymetrix 500K chip; 397,653 autosomal SNPs passed WTCCC QC criteria and had MAF $\geq 1\%$. We carried out imputation as before, resulting in 2,431,899 genotyped or imputed SNPs that passed QC criteria and had MAF $\geq 1\%$.

To assess whether the non-BP disease cases could reasonably serve as part of an extended reference set for our BP association study, we first compared the non-BP disease cases to the NBS controls. We observed no strong SNP associations except in the HLA region. After removal of genotypes for autoimmune disease cases (Crohn’s disease, rheumatoid arthritis, type 1 diabetes) from the analysis of the HLA region, the genomic control value for non-BP cases vs. NBS controls was 1.03.

We tested for SNP-BP association in the WTCCC BP case and extended reference set under an additive model with the genotype-based PCs as covariates. Genomic control values for genotyped and imputed SNPs were both 1.15 (Fig. S1 and S2). Analysis without PCs resulted in genomic control values of 1.18 and 1.17, suggesting that differences between cases and controls were only partially captured by the PCs, consistent with observations by WTCCC (4) investigators.

We dropped 261 cases present in both the WTCCC and GSK samples from the GSK sample (Table 1 and Fig. S1 E and F), reanalyzed the remaining GSK samples, and applied genomic control to results from each of the 3 studies. We then performed a 3-study meta-analysis of 3,683 cases and 14,507 controls genotyped or imputed for 2,366,197 autosomal SNPs. The meta-analysis genomic control value was 1.04 (Figs. S2B and S3B). No SNP in the 3-study meta-analysis reached genome-wide significance at $P < 5 \times 10^{-8}$.

In the 3-study meta-analysis, 53 SNPs from 3 independent regions had $P < 10^{-6}$ (Table 2 [top regional SNP] and Tables S2 and S3). On chromosome 5q15, we observed strongest association evidence in the genome with SNP rs17418283 (OR = 1.21, $P = 1.3 \times 10^{-7}$) located in an intron of MCTP1 (multiple C2 domains, transmembrane 1 isoform) (Fig. I). We observed a second signal at approximately 400 kb away in the first intron of MCTP1 at rs153291 (OR = 1.13, $P = 2.7 \times 10^{-4}$). Inclusion of rs17418283 as a covariate in the analysis did not change the evidence for association for rs153291 (OR = 1.12, $P = 2.8 \times 10^{-4}$) (Fig. S5). On chromosome 3p21, we observed strongest association evidence ($OR = 1.19, P = 1.8 \times 10^{-7}$) with rs1042779 in a large LD block from 52.2 to 53.2 Mb (Fig. I B). rs1042779 (Arg595Gln) and the nearby rs678 (Glu585Val) (OR = 1.19, $P = 2.5 \times 10^{-7}$) are nonsynonymous SNPs in ITIH1 (inter-alpha trypsin inhibitor, heavy chain 1). 33 SNPs in this region showed strong evidence for association ($P < 10^{-6}$) on chromosome 3p21, we observed strongest association evidence with rs472913 (OR = 1.18, $P = 2.0 \times 10^{-7}$) rs153291 (OR = 1.18, $P = 2.0 \times 10^{-7}$) at 800 kb from the closest known gene, NFIA (nuclear factor 1 A-type) (Fig. I C). In all 3 regions, the large WTCCC case/extended reference set showed the strongest association evidence, but there was no significant evidence of heterogeneity among the 3 studies (Table S2). On chromosomes 1 and 3, inclusion of the most strongly associated SNP as a covariate in the analysis diminished the evidence for association to $P > 10^{-2}$ for other SNPs in the region; these conditional analysis results are consistent with chromosome 1 and 3 association signals that reflect 1 BP-predisposing variant, or multiple BP-predisposing variants in high LD (Fig. S5).

To assess the sensitivity of our results to adjustment for PCs, we reanalyzed data from each study without PCs (Table S4). Meta-analysis $P$ values for the top SNPs with and without PC adjustment varied by up to approximately 1 order of magnitude (Table S5). The most notable change was that evidence for our most strongly associated nonsynonymous SNP, rs1042779 on chromosome 3, became stronger (OR = 1.20, $P = 2.7 \times 10^{-8}$ vs. OR = 1.19, $P = 1.8 \times 10^{-7}$), indicating there may have been some population stratification at this locus.
We next considered the impact on our top results of data imputation. On chromosome 3, the most strongly associated SNP was genotyped in NIMH/Pritzker and GSK; on chromosomes 5 and 1 the most strongly associated SNP was imputed in all 3 studies. Imputation quality of the most strongly associated SNP from each region was high (estimated \( r^2 > 0.8 \)) (Table S4); previous work demonstrates that results based on imputed data of this quality generally agree well with corresponding results based on direct genotyping (refs. 7 and 8 and Y. Li, C. J. Willer, J. Ding, P. Scheet, and G. R. Abecasis, personal communication). Further, in the chromosome 1 region, SNP rs1461356 (OR = 1.79, \( P = 3.3 \times 10^{-7} \)) was directly genotyped in all 3 studies, as was rs2289247 (OR = 1.16,
P = 1.7 × 10⁻⁶) in the chromosome 3 region. On chromosome 5, no completely genotyped SNP had P < 10⁻⁵. Because of the strong WTCCC contribution to the 3 top results, we further evaluated the use of the WTCCC extended reference set in our analysis. We tested for association between the WTCCC BP cases and the much smaller NBS-only control sample. With this much-reduced sample, we saw more modest evidence of association for each SNP, although similar effect sizes for the chromosome 1 and 3 SNPs: rs472913 (OR = 1.17, P = 0.0020 vs. OR = 1.20, P = 6.3 × 10⁻⁷) in the BP cases vs. extended reference set, rs1042779 (OR = 1.17, P = 0.0024 vs. OR = 1.16, P = 0.00012), and rs17418283 (OR = 1.11, P = 0.076 vs. OR = 1.25, P = 9.7 × 10⁻⁸) (Table S4C). We also tested for allele frequency differences between the NBS controls and the non-BP cases. SNPs rs472913 (P = 0.75) and rs1042779 (P = 0.56) on chromosomes 1 and 3 showed no evidence of a difference. For rs17418283, the allele frequencies in the BP cases, NBS controls, and non-BP cases were 0.31, 0.29, and 0.27, and the allele frequencies in the NBS and non-BP cases were significantly different (P = 0.0067), but this difference was considerably less significant than that observed in the BP/extended reference set analysis (P = 9.7 × 10⁻⁸).

These data quality and robustness analyses suggest that the evidence for BP association remains strong for the chromosome 1 and 3 regions and less so for the chromosome 5 region, although the presence of a second signal in the chromosome 5 region strengthens our interest in that region.

Discussion
We carried out genome-wide association analyses of 2 new and 1 previously published (WTCCC) BP GWAS, and then performed metaanalyses of the 2 new studies with and without the WTCCC study. The sample size of the 3-study meta-analysis was 3,683 cases and 14,507 controls. No SNP reached genome-wide significance. For the most strongly associated SNP from each of the 7 regions with P < 10⁻⁵ in the 2-study meta-analysis, none had P < 0.1 in the WTCCC data or <0.0001 in the 3-study meta-analysis (Table S1B). However, 6 of these 7 associations went in the same direction in the WTCCC data.

In the 3-study meta-analysis, we identified 3 regions with P = 10⁻⁷: 1p31.1, 3p21, and 5q15. The most strongly associated SNP genome-wide is rs17418283, located in an intron of MCTP1 on chromosome 5. MCTP1 is an extensively spliced, highly conserved membrane protein that binds Ca²⁺ with high affinity in the absence of phospholipids (12) and is highly expressed in the brain (http://symatlas.gnf.org/SymAtlas/). This gene may have 2 independent association signals, because SNPs in the first intron of MCTP1 are also moderately and independently associated with BP. Our finding is intriguing because Ferreira et al. (6) recently implicated another Ca²⁺-related gene, the L-type calcium channel subunit gene, CACNA1C, in BP (P = 7.0 × 10⁻⁸). The chromosome 5 region also contains ANKR3D2 (ankyrin repeat domain 3), which encodes an uncharacterized ankyrin-repeat-containing protein. There is no known functional relationship between ANKR3D2 and the ANK3 gene implicated by Ferreira et al. (6).

We observed the strongest nonsynonymous SNP-BP association genome-wide for rs1042779 on chromosome 3 at 52.8 Mb. There are 10 genes in the most strongly associated 243 kb region, and ≈25 genes in the ≈1 Mb larger region bounded by flanking recombination hot spots (Fig. 1B). rs1042779 is an Arg595Gln SNP in ITIH1. We also observed association with the nonsynonymous Glu585Val SNP rs678 in the same exon of ITIH1 (r² = 0.96 for rs678 and rs1042779 in HapMap CEU). Four additional nonsynonymous SNPs in this region had P < 10⁻⁵: rs2289247 (Val367Met) and rs11177 Arg27Gln in GNL3 (guanine nucleotide binding like-3), rs1029871 (Pro225Ala) in NEK4, and rs3617 (Gln315Lys) in ITIH3. For the Gln585Val and Pro225Ala variants, Gln and Pro are the conserved alleles in mammals, and the Val and Ala alleles are functionally nonconservative changes. ITIH1 encodes a serine protease inhibitor highly expressed in liver (13). The family of inter-alpha trypsin inhibitors is thought to have anti-proteolytic activities and to play an anti-inflammatory role (14). GNL3 encodes nucleostemin, which was isolated from rat CNS; is expressed in the nucleolus of stem cells, and is thought to be a critical regulator of the cell cycle. Expression of GNL3 rapidly declines upon neuronal cell differentiation, and both over- and under-expression lead to decreased stem cell proliferation in the CNS (15). Aberrant regulation of nucleostemin would be consistent with the neurotrophic hypothesis of mood disorders, which posits that stem-cell proliferative potential in the brain modulates BP risk (16). This association signal is 11 Mb proximal to a BP linkage peak (LOD = 2.01) reported for the families that are the source of the NIMH/Pritzker BP cases analyzed here (17).

On chromosome 1 at 60.8 Mb, there is no well annotated gene within 500 kb of the most strongly associated SNP, rs472913. rs472913 is in moderately high LD with rs2989476 (r² = 0.74), the most strongly associated BP SNP in the WTCCC BP case/extended reference set analysis (4). This SNP is in an intron of a possible transcript annotated by Unigene as P3 NTera2D1 teratocarcinoma, and defined by multiple expressed sequence tags, including one expressed in brain.

After the 3 loci with the strongest associations on chromosomes 1, 3, and 5, there were additional notable observations. On chromosome 2, we identified rs13409348 (OR = 1.20, P = 2.7 × 10⁻⁶) (Table S2) located in intron 4 of the gene CTNN2A2 (encoding alpha N catenin 2). CTNN2A2 is expressed almost exclusively in distinct neuronal populations in primates (18), alpha N-catenin is thought to be a key regulator of the stability of synaptic contacts and the motility of dendritic spines, a key aspect of neuronal plasticity (19). Its deficiency in mice causes axon migration defects (20) and an abnormal startle response (21), a murine behavior indicative of dysregulation of sensorimotor gating that is often considered an endophenotype of psychosis in humans (22). The gene LRRTM1 (Leucine-rich repeat transmembrane neuronal 1) is located in an intron of CTNN2A2. We observed associations of a haplotype near LRRTM1 with schizophrenia (P = 0.0014 in 1,002 families) and handedness (P = 0.0002) (23). This risk haplotype is best tagged by the rs1446109 A allele, which is ~1 Mb from rs13409348 and shows modest evidence of association in our meta-analysis (A risk allele, OR = 1.08, P = 0.08). We also identified rs2537859 (OR = 1.16, P = 4.2 × 10⁻⁵) (Table S2) on chromosome 4, 42 kb upstream of KIT (v-kit Hardy-Zuckerman 4 feline sarcoma viral). KIT encodes a cytokine receptor of the tyrosine kinase family, expressed in multiple cells including neurons (24). KIT binds stem cell factor and plays a role in cell survival, proliferation, and differentiation (25). Nonsynonymous mutations and deletions in KIT are associated with multiple diseases and KIT overexpression in the brain can induce gliomas (26).

In the 2- and 3-study meta-analyses we identified a region on chromosome 1 at ≈195 Mb with 2 strong but distinct association signals ~300 kb apart and separated by a strong recombination hotspot. rs2813164 (Table S1) emerged in the 2-study meta-analysis and is located between NEK7 and ATP6V1G3 (Fig. S4B). rs15268099 emerged in the 3-study meta-analysis (Table S2) and is located 25 kb downstream of PTPRC (Fig. S4C); there was also evidence for association around NEK7. Within the region, ATP6V1G3 is of particular interest as it encodes a component of vacuolar proton-pumping ATPase involved in organelle and synaptic vesicle acidification (27).

Previous genome-wide association studies and subsequent meta-analyses of BP have identified SNPs that reached genome-wide significance or had support across multiple studies; for a description of the overlap between our sample and those of other studies, see SI Materials and Methods. The Ferreira et al. (6) meta-analysis identified SNPs with strong evidence of association in the regions of CACNA1C (rs1006737, OR = 1.18, P = 7.0 × 10⁻⁸) and ANK3 (rs10994336, OR = 1.45, P = 9.1 × 10⁻⁸). Schulze et al. (28)
reported support for association with ANK3 in a German sample (rs10994336, OR = 1.70, P = 0.0001) and stronger evidence from meta-analysis with NIMH case and control samples (OR = 1.70, P = 1.7 × 10^{-3}). In the GSK samples that did not overlap the sample of Ferreira et al. (6), we observed modest replication of rs10994336 (OR = 1.37, P = 0.042) and slightly stronger evidence when we included the NIMH samples overlapping those of Shultze et al., but not Ferreira et al. (OR = 1.39, P = 0.012); a fixed-effects meta-analysis of the results of Ferreira et al., the German Schulze et al. sample, and the nonoverlapping GSK and NIMH samples, yielded compelling evidence for association (OR = 1.47, P = 1.1 × 10^{-10}). The ANK3-encoded protein, Ankyrin G, is a cytoskeleton-binding molecule located at the Nodes of Ranvier and involved in the assembly of a variety of membrane proteins, including voltage-gated sodium (29) and potassium (30) channels and cell adhesion molecules (reviewed in ref. 31). We did not observe association with rs1006737 (OR = 1.01, P = 0.81). The Baumb et al. (3) meta-analysis identified a strongly associated SNP in DGKH (rs1012053, OR = 1.59, P = 1.5 × 10^{-5}); we did not observe association in the independent GSK sample (OR = 1.01, P = 0.76). The lack of confirmation of association in our independent NIMH/Pfitzker and GSK samples for these loci may reflect the need for larger sample sizes to detect small effects, differences in sample inclusion criteria including heterogeneity in BP cases, or false positive results in the original studies.

Our GWAS results and others reported to date suggest there are few, if any, common SNP variants with large effects on BP risk. BP differs from complex diseases such as macular degeneration (32), Crohn’s disease (4), and type 1 diabetes (4), which are influenced by one or more loci of large effect. Type 2 diabetes appears intermediate in that it has a single locus (TCF7L2) of moderate effect together with loci of substantially smaller effects requiring large samples for detection (33). BP may be due to a combination of many common variants with weak effects, potentially including those identified above, and rarer variants with a range of effect sizes. For common variants, detection and verification will require larger GWA and follow-up samples. The strong familial component of BP and the lack of common variants of large effect suggest that rarer SNP variants along with other types of genetic differences such as copy number variations (CNVs) or epigenetic modifications may play a substantial role. Sequencing of BP cases and controls will help unravel the role of rare SNPs and copy number variants. Detection of BP risk variants anywhere in the alke frequency spectrum has the potential to lead to greater understanding of the biology that underlies BP, the sources of heterogeneity among BP cases, and to the subsequent development of new BP treatments.

In summary, we have identified chromosomal regions and specific genes that may harbor variants that contribute to BP risk and merit further study. Several of the genes encoded in these chromosomal regions are consistent with the notion that subtle differences in neural development and/or neuroplasticity may underlie mood disorders. Further work is required to determine whether these loci contain causal variants that could be more strongly associated with BP risk. If so, it would be of great interest to study the neural localization, expression, and regulation of these genes in human and animal brain, particularly in circuits relevant to mood disorders.

Methods

Sample Ascertainment and Selection. Subjects for all participating studies gave informed consent and study protocols were approved by the relevant institutional review board or ethics committee.

NIMH/Pfitzker. We selected samples for previously studied individuals from 2 sources: 1,130 Bipolar I (BP I) cases and 772 controls from the NIMH Human Genetics Initiative Repository (http://zork.wustl.edu/nimh/) and 47 BP I cases from the University of Michigan Prechter Repository. NIMH Repository cases were collected as part of a multistate study of individuals with BP and their families (34–35). Cases were diagnosed according to DSM-III or DSM-IV criteria, using the Diagnostic Interview for Genetic Studies (DIGS) (36) (n = 1,081) or Family Inter-view for Genetic Studies (FIGS) (37) and medical record review (n = 67); we excluded cases with low confidence diagnoses. From each available non-Ashkenazi European-origin family, when possible we selected 2 BP I siblings including the proband if available (n = 946 individuals in 473 sibling pairs); otherwise we selected a single BP I case (n = 184).

We selected an additional 47 non-Ashkenazi European-origin BP I cases who met DSM-IV criteria from the University of Michigan Prechter Bipolar Genetics Repository. Subjects were recruited using flyers distributed at the University of Michigan Depression Center and on the Center website and interviewed using the DIGS.

We selected as controls 772 non-Ashkenazi European-origin individuals aged 20–70 years from the NIMH Human Genetics Initiative Repository who reported they had not been diagnosed with or treated for BP or schizophrenia, and had not heard voices that others could not hear. We excluded individuals with suspected major depression based on answers to questions related to depressed mood.

To minimize population stratification, we selected individuals reporting primarily European ancestry and matched NIMH controls to NIMH cases based on self-reported ancestry in the DIGS.

GSK. We selected 899 BP cases (814 BP I and 85 BP II) and 904 controls from subjects recruited at 3 study sites: the Institute of Psychiatry (IOP) in London, U.K. (483 cases and 462 controls); the Centre for Addiction and Mental Health in Toronto, Canada (334 cases and 257 controls); and the University of Dundee, U.K. (82 cases and 84 controls). Cases were ascertained through hospital admissions, primary care physician offices, and patient support groups, were >18 years of age at interview, and reported Caucasian ethnicity. They were interviewed using the Schedules for Clinical Assessment in Neuropsychiatry (SCAN). BP diagnoses were established according to DSM-IV or ICD-10 criteria, using the computerized algorithm (CATEG) for the SCAN2.1 interview (WHO) (38). Cases were excluded if they received a diagnosis of i.v. drug dependency or reported i.v. drug use within the previous 12 months.

TCF7L2. We included cases with known psychotic symptoms or if manic episodes only occurred in conjunction with or as a result of alcohol, substance abuse, substance dependence, medical illnesses, or medications. 261 cases recruited at the IOP were present in the WTCCC BP sample. When we performed a 3-study meta-analysis, we excluded these overlapping cases from the GSK sample and used 638 cases and 904 controls as the GSK analysis sample (Table 1 and Table S1). Controls were >18 years of age, reported Caucasian ethnicity, and documented the presence of any psychiatric disorders in the DIGS.

We selected additional 47 non-Ashkenazi European-origin BP I cases who were recruited through advertisements in hospitals, recruited at 3 study sites: the Institute of Psychiatry (IOP) in London, U.K. (483 cases and 462 controls) and the University of Dundee, U.K. (82 cases and 84 controls). Cases were ascertained through hospital admissions, primary care physician offices, and patient support groups, were >18 years of age at interview, and reported Caucasian ethnicity. They were interviewed using the Schedules for Clinical Assessment in Neuropsychiatry (SCAN). BP diagnoses were established according to DSM-IV or ICD-10 criteria, using the computerized algorithm (CATEG) for the SCAN2.1 interview (WHO) (38). Cases were excluded if they received a diagnosis of i.v. drug dependency or reported i.v. drug use within the previous 12 months.

We conducted a 3-study meta-analysis of the WTCCC and the 270 HapMap samples. BP and related phenotypes were diagnosed using Research Diagnostic Criteria (4). 71% of cases were diagnosed as BP I, the remainder as schizoaffective disorder bipolar type (15%), BP II (9%), or manic disorder (5%). For our primary WTCCC control group, we included 12,831 individuals unscreened for BP: 1,458 National Blood Service controls together with a total of 11,373 individuals from the coronary artery disease (n = 1,926), Crohn’s disease (n = 1,748), hypertension (n = 1,952), rheumatoid arthritis (n = 1,860), type 1 diabetes (n = 1,963), and type 2 diabetes (n = 1,924) groups. We could not include the WTCCC 1958 Birth Cohort data owing to access constraints.

Sample Genotyping and Quality Control (QC). NIMH/Pfitzker. NIMH and Prechter samples were genotyped on the Illumina HumanHap550 Beadchip at Stanford University and the University of Michigan following manufacturer recommendations. Phase 1 (phase 2) comprised 999 (1,122) samples, including 29 (19) duplicate samples and 15 parent-offspring trios (1 parent-offspring duo, 1 parent-offspring trio, 8 parent-offspring quartets). In each phase, we first called genotypes using the Illumina default cluster file based on the HapMap CEU samples and excluded samples with success rate <90.0%. Genotypes were then reclustered based on our own genotyped samples; for each SNP, we retained the genotypes from the clustering method yielding higher genotyping success rate. Nine individuals with inconsistent reported and genotype-based sex were dropped as were 6 samples to eliminate cryptic relatedness. We removed 6 individuals with evidence for ancestry that differed from the rest of the sample (see below).

Of the 541,327 genotyped autosomal SNPs, we excluded 12,592 because of (i) total non-Mendelian and duplicate effects; (ii) data inconsistent with Hardy-Weinberg equilibrium (P < 10^{-6}) in unrelated cases and controls; or (iii) SNP call rate <95% in either phase. Genotyping success rate (99.9%), duplicate genotype concordance (99.99%), and parent-child Mendelian consistency rates (99.93%) were high. There was no evidence for differential quality parameters between phases 1 and 2. Among the 28,735 SNPs that passed QC, 512,844 had MAF ≥1% in the combined sample and were used in association analysis.

GSK. DNAs for 1,896 GSK individuals, including duplicated samples for 109 individuals, were genotyped on the Illumina HumanHap550 Beadchip at Illumina
following manufacturer recommendations. Genotype calls were generated as described in ref. 39 with minor modifications. Initial calls were made using the illumina CEU cluster file. 61 samples from 43 individuals with call rate >95% were dropped as were 11 individuals with inconsistent reported and genotype-based gender, 13 individuals to eliminate cryptic relatedness, and 26 outlier individuals in the stratification analysis (see below). SNPs with call frequency <99% were reclustered based on the GSK samples. 20,172 SNPs with (a) call frequency <95%; (b) call frequency 95–98% and cluster separation score <0.3, or heterozygote excess frequency >0.1 and less than 0.1; or (c) call frequency >98% and cluster separation <0.25 or heterozygote excess frequency >0.3 or less than 0.3 were dropped. Of the 521,990 SNPs that passed QC, 512,508 (full sample) and 512,668 (reduced sample) had MAF=0.01 and were used in association analysis. Duplicate genotype concordance was 99.99%.

WTCCC. Samples were genotyped on the Affymetrix GeneChip 500K Mapping Array Set as described in ref. 4. 397,653 SNPs passed WTCCC QC and had MAF>0.01.

Genotype Imputation. For each study, we imputed genotypes for up to ~2 million autosomal SNPs with MAF>0.01, using the genotypes from the Illumina Human-Hap550 Beadchip (NIHM/Pritzker, GSK) or the Affymetrix GeneChip 500K Mapping Array Set (WTCCC) and phased chromosomes for the 60 HapMap CEU founders. Genotypes were imputed using a Hidden Markov model as programmed in MACH (40) for SNPs not present in the genotyping platform or whose genotype data failed QC. We imputed WTCCC and Pritzker to Build 35 and GSK to Build 36. We retained imputed SNPs with estimated r² >0.3 and imputed MAF>0.01. The numbers of imputed SNPs used in analysis were 1,960,204, 1,952,561 (1,952,849), and 2,034,246 for NIHM/Pritzker, GSK full sample (reduced sample), and WTCCC, respectively.

The SI Materials and Methods and Materials contains an expanded version of the original sections.

Assessment of Stratification. In each set of study samples we performed principal components (PC) analysis based on a subset of the sample genotypes (40) in unrelated individuals. We excluded individuals in the NIHM/Pritzker and GSK samples with PC >6 SD from the mean of one or more of the top 10 PCs; to mirror the analysis used by the WTCCC (40), we did not exclude WTCCC samples.


GWA Analysis. We eliminated 694 SNPs with allele frequency differences >0.2 for any pair of studies. We analyzed the observed allele counts or imputed allele dosages, using logistic regression assuming an additive genetic model, with genotype-based PCs and study site (GSK only) as covariates, and then repeated the analysis without PCs. In the NIHM/Pritzker sample, we used a sandwich estimator (41) to adjust the estimated variances. For the WTCCC sample, we compared the BP cases to the extended reference set as our primary analysis, except in the HLA region on chromosome 6 from 27.2 to 34.0 Mb where we excluded the 5,571 autoimmune disease cases (type 1 diabetes, Crohn’s disease, rheumatoid arthritis) from the GWA analysis.

Meta-Analysis of GWA Samples. We performed a fixed effects meta-analysis, using the OR and 95% confidence intervals to combine the association evidence from the study-specific GWA analyses. We used association results for experimentally derived genotypes when available, and for imputed genotypes otherwise. 2,366,197 autosomal SNPs passed QC and had MAF>0.01 in all 3 samples. We adjusted for the genonic control values in each study separately for genotyped and imputed SNPs by increasing the standard error of the OR estimate to correspond to the genomic control Pvalue. Evidence for heterogeneity between ORs was assessed using Cochran’s Q statistic and I² (9).

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