

IMMEDIATE COMMUNICATION**Whole-genome association study of bipolar disorder**

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We performed a genome-wide association scan in 1461 patients with bipolar (BP) 1 disorder, 2008 controls drawn from the Systematic Treatment Enhancement Program for Bipolar Disorder and the University College London sample collections with successful genotyping for 372 193 single nucleotide polymorphisms (SNPs). Our strongest single SNP results are found in myosin5B (*MYO5B*; $P=1.66 \times 10^{-7}$) and tetraspanin-8 (*TSPAN8*; $P=6.11 \times 10^{-7}$). Haplotype analysis further supported single SNP results highlighting *MYO5B*, *TSPAN8* and the epidermal growth factor receptor (*MYO5B*; $P=2.04 \times 10^{-8}$, *TSPAN8*; $P=7.57 \times 10^{-7}$ and *EGFR*; $P=8.36 \times 10^{-8}$). For replication, we genotyped 304 SNPs in family-based NIMH samples ($n=409$ trios) and University of Edinburgh case-control samples ($n=365$ cases, 351 controls) that did not provide independent replication after correction for multiple testing. A comparison of our strongest associations with the genome-wide scan of 1868 patients with BP disorder and 2938 controls who completed the scan as part of the Wellcome Trust Case-Control Consortium indicates concordant signals for SNPs within the voltage-dependent calcium channel, L-type, alpha 1C subunit (*CACNA1C*) gene. Given the heritability of BP disorder, the lack of agreement between studies emphasizes that susceptibility alleles are likely to be modest in effect size and require even larger samples for detection.

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Introduction

Bipolar (BP) disorder is characterized by profound mood symptoms that include episodes of mania, hypomania and depression. Although family and twin studies unequivocally demonstrate a strong contribution of inherited genetic variation to the risk for BP,² traditional linkage mapping and prior

candidate gene studies have failed to identify genes that increase risk in a consistent manner.

Advances in gene mapping created by the International HapMap Project,^{3,4} as well as highly parallel genotyping technology, have enabled investigation of the hypothesis that common variation may play a role in the liability to BP. This strategy is supported by recent successes in other common, complex disease studies as well as previous lack of success in BP genetics. Rare, highly penetrant, Mendelian forms of BP disorder have not been identified. Linkage data have largely identified areas with modest evidence of linkage to BP disorder that are not well localized, even when the data are pooled.^{5,6} Candidate gene studies while implicating several genes,⁷ have only been able to focus on a minority of genes in the

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genome, and the interpretation of replication of individual findings has been difficult. Furthermore, strong support for applying a whole genome approach to BP disorder comes from the success in finding novel, strong and consistent susceptibility loci in type 2 diabetes,^{8–10} prostate cancer,¹¹ Crohn's disease,¹² breast cancer¹³ and coronary artery disease¹⁴ using similar whole genome approaches. Like BP disorder, these diseases have a clear genetic component. The underlying types of genetic susceptibility to these disorders have been variable. For example, age-related macular degeneration is thus far found to have a modest number of risk genes and alleles that have relatively large effect sizes,¹⁵ while others are likely to have many smaller risk alleles. As yet there is no obvious relationship between the heritability of a particular disorder and the number or strength of the observed effects. For at least two disorders, type 2 diabetes⁸ and breast cancer,¹³ while susceptibility alleles were identified, very large sample sizes ($n > 10\,000$) were required to establish consistency of results.

In this paper, we present data from a whole-genome scan of BP disorder, two independent replication samples and a comparison of our most associated results with previous whole-genome association studies.^{1,16}

Materials and methods

Study samples

Cases and controls included in the final analysis were obtained from several sources. DNA samples from bipolar I (BPI) patients ($n = 955$) were obtained from the Systematic Treatment Enhancement Program for Bipolar Disorder (STEP-BD). Additional BPI cases ($n = 506$) were obtained from University College London, United Kingdom (UCL). Control samples were obtained from two sources in the United States through the National Institute of Mental Health (NIMH) Genetics Repository ($n = 1498$) and from the UK ($n = 510$, matched to the UCL cases). To achieve maximal power to detect common variants of modest effect, our primary analyses were of all samples combined, estimating a common odds ratio (OR) but controlling for any systematic differences between samples (see Association analysis section below).

STEP-BD cases

The Systematic Treatment Enhancement Program for Bipolar Disorder was a national, longitudinal cohort study designed to examine the effectiveness of treatments and their impact on the course of BP disorder¹⁷ that enrolled 4361 participants across the United States who met the Diagnostic and Statistical Manual of Mental Disorders-IV (DSM-IV) criteria for BPI, BPII, BP NOS, schizoaffective manic or BP type, or cyclothymic disorder on the basis of diagnostic interviews. Assessment of DSM-IV psychopathology was performed using two semistructured diagnostic

interviews: (1) the Affective Disorders Evaluation, which includes mood and psychosis modules adapted from the Structured Clinical Interview for DSM-IV,¹⁸ was administered by treating psychiatrists who were trained and certified in the administration of the interview and (2) the Mini International Neuropsychiatric Interview,¹⁹ a validated structured diagnostic interview, was administered by trained clinical specialists. Both interviews use DSM-IV criteria to establish diagnoses.

From the parent study, 2089 individuals who were over 18 years of age and consented to the collection of blood samples for DNA and cell lines for genetic studies were enrolled in the STEP-Genetic Repository for Participants (Co-Directors: JW Smoller, VL Nimgaonkar). Of the 2089 STEP-Genetic Repository for Participants 62% had a consensus diagnosis of BPI on both the Affective Disorders Evaluation and the Mini International Neuropsychiatric Interview. BPI was chosen for the phenotype in this study for the following reasons: (1) diagnostic accuracy is higher than other forms of mood disorder with high inter-rater reliability,^{20–22} (2) heritability of BPI has been repeatedly demonstrated² and (3) psychosis, a common feature of BP disorder, has been shown to be familial.^{23–25}

UCL cases

The UCL sample comprised Caucasian individuals who were ascertained and received clinical diagnoses of BP disorder according to UK National Health Service (NHS) psychiatrists at interview using the categories of the International Classification of Disease version 10. In addition, BP subjects were included only if both parents were of English, Irish, Welsh or Scottish descent and if three out of four grandparents were of the same descent. One grandparent was allowed to be of Caucasian European origin but not of Jewish or non-European Union ancestry, on the basis of the European Union countries before the 2004 enlargement. These data were recorded in an Ancestry Questionnaire. They were then interviewed by one of three research psychiatrists (JL, NJB, AA) using a structured interview and defined as having BP 1 disorder. Research subjects were volunteers from UK NHS psychiatric services and from the UK Manic Depression Fellowship, a self-help organization for patients with BP disorder. The interviews were conducted between October 1991 and June 2006 using the Schizophrenia and Affective Disorders Schedule—Life Time version, which provides diagnoses according to the probable level of the Research Diagnostic Criteria and also according to the US DSM-III-R criteria.^{26,27}

Further clinical information was collected using the 90-item Operational Criteria checklist.²⁸ All volunteers read an information sheet approved by the Metropolitan Medical Research Ethics Committee who also approved the project for all NHS hospitals. Written informed consent was obtained from each volunteer.

Both the STEP-BD and UCL samples comprised Caucasian BPI individuals, and, as shown in Supplementary Table 1, were similar in terms of other clinical characteristics except for a somewhat higher proportion of cases with psychotic symptoms in the UCL sample.

NIMH control samples

Two groups of control samples were obtained from the NIMH Genetics Initiative through the NIMH Center for Collaborative Studies (<http://zork.wustl.edu/nimh/>) from the Rutgers's Repository (<http://rucdr.rutgers.edu/>). The first control sample comprised 454 DNA samples derived from US Caucasian anonymous cord blood donors.²⁹ The second control sample comprised 1044 US Caucasian controls who completed an online self-administered psychiatric screen and were ascertained by Knowledge Networks (<http://www.knowledgenetwork.com>), a survey and market research company whose panel contains approximately 60 000 households (>120 000 unrelated adults). Households were selected via random digit dialing. Knowledge Networks provides financial incentives to its panel members for participation in web-based surveys. The panel provides a weighted probability sample, representative of the US population. The online screen included questions regarding demographics, ethnic ancestry and DSM-IV criteria for depression and anxiety disorders. In addition, participants were queried about any history of schizophrenia, psychosis or BP disorder using a three-part questionnaire: 'Have you ever received treatment for, or been diagnosed with, any of the following conditions: (a) schizophrenia or schizoaffective disorder; (b) hearing voices that others could not hear or believing things that others said were not true (such as that people were trying to harm you); (c) bipolar disorder (manic depression).' Controls were included only if they answered 'no' to all three of these questions. In addition, controls who met lifetime criteria for recurrent major depressive disorder with functional impairment on the basis of their responses to depression items were excluded. The second control sample and the control samples utilized in Baum *et al.*¹⁶ were selected from the same larger group of controls collected by KN and may overlap considerably.

UCL controls

The UCL control subjects were recruited from London branches of the National Blood Service, from local NHS family doctor clinics and from university student volunteers. All control subjects were interviewed with the Schizophrenia and Affective Disorders Schedule—Life Time version to exclude all psychiatric disorders including alcohol dependence according to Research Diagnostic Criteria, the US DSM-III-R criteria as well as drinking above the Royal College of Psychiatrists upper limit for safe drinking of 21 units per week for males and 14 units for females. The control subjects were further selected on the basis of not having a family history of BP disorder,

schizophrenia or alcoholism. The UCL supernormal control sample was matched to the UCL case sample using the same ancestry criteria listed above for the BPI volunteers.

Replication study subjects

NIMH genetics initiative. Parent-proband trios ($n=409$ from 256 nuclear families) were obtained from samples previously collected for linkage studies from the NIMH Genetics Initiative (NIMH-GI). The offspring in all trios were affected with BPI.

Edinburgh

This sample comprised Caucasian individuals contacted through the inpatient and outpatient services of hospitals in South East Scotland. A BPI diagnosis was based on an interview with the patient using the Schizophrenia and Affective Disorders Schedule—Life Time version supplemented by case note review and frequently by information from medical staff, relatives and care givers. Final diagnoses, on the basis of DSM-IV criteria³⁰ were reached by consensus between two trained psychiatrists. Ethnically matched controls from the same region were recruited through the South of Scotland Blood Transfusion Service. Controls were not directly screened to exclude those with a personal or a family history of psychiatric illness; however, the Blood Transfusion Service does not accept blood donations from subjects taking regular medication or with a history of a major illness. The study was approved by the Multi-Centre Research Ethics Committee for Scotland and patients gave written informed consent for the collection of DNA samples for use in genetic studies.

Genotyping

For all US samples, DNA was extracted from either whole blood, neonatal cord blood or from lymphoblastoid cell lines at the Rutgers Cell and DNA Repository. DNA samples were extracted from whole blood using standard protocols for all UCL samples. Case and control samples were randomized to 96-well plates. Individual plates did not contain mixtures of STEP-BD and UCL samples.

Genotyping was performed using the Affymetrix GeneChip Human Mapping 500K Array Set. This set comprises two high-density arrays, NspI and StyI. All genotyping was performed by the Genetic Analysis Platform at the Broad Institute of Harvard and MIT using standard protocols³¹ as previously described.⁸ Genotypes were called using the Bayesian Robust Linear Model with Mahalanobis distance classifier.³² A panel of 24 markers present on the whole genome product as well as 25 single nucleotide polymorphisms (SNPs) previously genotyped in the UCL samples were used as genetic fingerprints to detect sample switches.

Replication genotyping

Genotyping of replication SNPs were performed in the NIMH and Edinburgh samples by Sequenom MassArray.³³

Technical validation

Fifty-nine SNPs from among the most highly associated SNPs were selected for genotyping by an independent method (Sequenom MassArray³³). For the 56 SNPs with Sequenom genotyping rates greater than 90%, concordance with the Affymetrix data was 99.7% (based on 187 960 genotypes).

Data analysis and quality control

We performed all data analysis and quality control (QC) using the PLINK software package (<http://pngu.mgh.harvard.edu/purcell/plink/>).³⁴ Here we describe the individual and SNP exclusion criteria, methods to address population stratification, and the single SNP and haplotypic association analyses in the screening and replication samples.

Individual exclusions. Raw genotype data were available for 1800 cases (STEP $n=1247$; UCL $n=553$) and 2273 controls (cord blood $n=546$; NIMH-GI $n=1180$; UCL $n=547$). Prior to evaluating detailed genotyping quality per individual, we removed individuals with overall call rates of less than 85% (STEP cases $n=36$; UCL cases $n=17$; US controls $n=56$; UCL controls $n=17$), and SNPs with call rates of less than 90% or which mapped to multiple locations in the genome or were monomorphic ($n=10\,298$). All remaining individuals had call rates of greater than 95%. Seventy-five individuals (cases $n=50$; controls $n=25$) were excluded based on heterozygosity. The heterozygosity screen excluded outliers approximately three standard deviations from the mean estimated inbreeding coefficient, removing 55 individuals with many more heterozygote calls than expected and 20 individuals with more homozygote calls.

To address potential population stratification, the genome-wide proportion of alleles shared identical-by-state (IBS) for each pair of individuals was calculated. Gross outliers were first removed including individual who appeared to be close relatives, sample duplications or non-Caucasian (cases $n=84$; controls $n=39$). IBS clustering was then performed, which indicated that the vast majority of individuals belonged to a single cluster; 203 individuals who did not were removed (cases $n=113$; controls $n=90$). Finally, an additional 72 individuals (cases $n=39$; controls $n=33$) were removed for low-level relatedness, consistent with some degree of sample contamination, resulting in a final data set of 3469 individuals (cases $n=1461$; controls $n=2008$).

Marker exclusions. After excluding individuals as described above, SNPs were excluded for the following reasons: (1) call rate of less than 95% ($n=23\,673$), (2) minor allele frequency of less than 1% ($n=67\,661$), (3) Hardy–Weinberg equilibrium $P < 1 \times 10^{-6}$ in controls ($n=11\,671$) and (4) differential rates of missing genotypes between cases and controls, using Fisher's exact test, $P < 1 \times 10^{-3}$ ($n=388$) (see Supple-

mentary Table 2). We also tested whether genotypes were nonrandomly missing with respect to genotype (potentially unobserved), as indicated by a local haplotype strongly predicting genotyping failure; we excluded SNPs with a P -value $< 1 \times 10^{-10}$ ($n=17\,021$). Finally, as genotypes were called on a per plate basis, we identified 'bad plates' where SNPs showed grossly different allele frequencies to all other plates ($P < 1 \times 10^{-10}$) and removed those SNPs ($n=2397$). Following the exclusion there remained 372 193 SNPs for further analysis.

Population stratification. As all primary analyses were based on the combined STEP-BD and UCL samples, to control for possible systematic differences reflecting differential ancestry and/or DNA collection conditions, the primary analyses condition on analysis panel (STEP-BD versus UCL). To further control for possible effects of population stratification within panel, we matched cases and controls based on the proportion of alleles IBS, using complete linkage hierarchical clustering. Within panel, individuals were clustered to ensure (a) at least one case and one control in clusters with two or more individuals; (b) that no two individuals in the same cluster fail the population pairwise concordance test with a $P < 1 \times 10^{-3}$; (c) only individuals from the same sample (US or UK) were clustered with each other. For a given pair of individuals, the pairwise concordance test compares the ratio of IBS 0 (AA:BB pairs) versus IBS 2 SNPs (AB:AB pairs) conditional on observing two of each allele; if both individuals belong to the same random mating population, these two classes should be observed in a 1:2 ratio. Finally, parallel to our cluster-based approach, we also applied classical multidimensional scaling based on the matrix of IBS distances to visually represent any stratification (Supplementary Figure 1).

Association analyses

Primary whole-genome association analysis. The primary analysis was of single SNPs using the Cochran–Mantel–Haenszel test to assess allelic association with disease conditional on the strata as defined by the stratification analysis. For each SNP, we also calculated standard, allelic association tests not conditioned on strata, based on a χ^2 test for independence.

Multimarker and haplotype tests. We augmented the single SNP tests with multimarker haplotype tests. We attempted 565 560 (345 288 unique tests) tests, of which 459 414 (340 925 unique) were examined (minor haplotype frequency of greater than 0.01)³⁵ and tested for association (allowing for potential ambiguity in statistically inferred haplotype phase and imputed genotype).

We used additional haplotype-based analyses to validate and refine the signals of the most associated SNPs (labeled 'reference SNPs' here). First, we used

local haplotype information to probabilistically reconstruct missing genotypes for each reference SNP, to further ensure that the associations were not due to biased genotyping failure (labeled the pSNP test). Second, we scanned the local region for haplotypes (not including the reference SNP) which we call 'proxies,' that were in linkage disequilibrium (LD) with the reference SNP ($r^2 > 0.2$). All proxy haplotypes of up to four SNPs out of up to six SNPs on either side and within 250 kb of the reference SNP are then tested for association with disease (the pHAP test): a positive result here is encouraging in that it shows that the original signal is not due to just a single SNP (and, therefore, not due to technical artifact that might influence a single SNP). As well as technical validation, if the reference SNP is in fact an imperfect proxy for some underlying haplotype, this approach could also help to refine the association signal.

In total, we performed 372 193 single SNP tests, 340 925 unique multimarker tests, 200 pSNP tests and 183 513 pHAP tests (which represents approximately 10% of all possible haplotypes, that is, if the constraint of correlation with the reference SNP was ignored), giving a total of 896 831 primary tests of association with BP disorder. Naturally, a high proportion of these tests will be strongly correlated with one or more other tests, particularly the pHAP tests which were selected for being highly correlated with only 200 reference SNPs.

Replication sample association analyses

We analyzed the combined NIMH-GI and Edinburgh replication samples using the DFAM test implemented in PLINK, which effectively combines a standard with a Cochran–Mantel–Haenszel test (for a single stratum of all Edinburgh samples) into a single test statistic. When presenting results separately for the two samples, we used the transmission disequilibrium test (TDT) for the NIMH-GI family data and a standard allelic association test for the Edinburgh sample. All tests were one-sided (given the direction of effect in the screening sample). We report tests where $P < 0.05$ in one of the samples or the combined sample.

We combined P -values obtained online for the Wellcome Trust Case–Control Consortium (WTCCC) with the original STEP-BD/UCL sample using Fisher's rule.³⁶

Results

We analyzed 372 193 SNPs genotyped on the Affymetrix GeneChip Human Mapping Array in 1461 BPI patients and 2008 controls following the data-cleaning procedure described above. The overall genotyping call rate for analyzed SNPs was 99.4%. Including the multimarker predictors, our data set is estimated to capture 78.7% of common variation (SNPs with minor allele frequency $\geq 5\%$) in the CEPH HapMap CEU samples with $r^2 > 0.8$.

Assessment and handling of population stratification

The IBS-based constrained hierarchical clustering resulted in 67 clusters (ranging from size 2 to 126 individuals per cluster, mean 51.8, median 48). The final results were not particularly sensitive to different pairwise concordance test thresholds or clustering schemes, as expected given the initial removal of population outliers and the generally well-matched nature of the remaining samples. Supplementary Figure 1 shows a series of multidimensional scaling plots representing these samples and the HapMap samples. Relative to the entire HapMap, all current samples cluster quite closely with the CEU HapMap panel. There is greater dispersion among the US samples compared to the UK samples, which is to be expected given the ascertainment schemes and prematching for the UK samples, but importantly, within US and UK samples, the cases and controls show similar patterns. In general, we did not observe obviously different patterns of results whether we conditioned on sample site and population strata or not.

Association analysis

The genome-wide association results for BP disorder from the Cochran–Mantel–Haenszel test are shown in Supplementary Figure 2. Our most significant single SNP had a P -value of 1.66×10^{-7} . We did not observe any deviation in the extreme tail of the distribution of single SNP test statistics (see Q–Q plot Supplementary Figure 3).

Table 1 lists the 20 most associated single SNPs ranked according to the Cochran–Mantel–Haenszel test. Five of these results are found for common SNPs (minor allele frequency in cases between 0.042 and 0.39) within the introns of brain-expressed genes (myosin5b (*MYO5B*); tetraspanin-8 (*TSPAN8*); epidermal growth factor receptor (*EGFR*); ornithine transcarbamylase (*OTC*) and raft-linking protein (*RFTN*)) conferring OR up to 0.59 and 1.51, for protective and risk alleles, respectively. Previous association to BP disorder has not been reported for these genes. Four SNPs in strong LD are found within 142 kb on chromosome 9, in a region containing an open reading frame and *ATP6V1G1*. Two independent associations are found on 18q; in addition to *MYO5B* at 45.7 Mb, there are an additional six SNPs approximately 15.6 Mb telomeric. One SNP is found near transmembrane protein 132E (*TMEM132E*) on chromosome 17. Chromosomes 12 and 13 each have a SNP in a region containing no annotated genes or transcripts.

Association results for the top 200 SNPs can be seen in Supplementary Table 3; a complete listing of all single SNP statistics is available at <http://pngu.mgh.harvard.edu/purcell/bpwgas/>. Of the two-marker haplotypes used in multimarker tests of SNPs not directly genotyped, no additional associations were identified at $P < 5 \times 10^{-5}$ except for within the region of *EGFR*, that are already identified by single SNP analysis (Supplementary Table 4). Analyzing

Table 1 Association results for top 20 SNPs in BP disorder

Chromosomes	SNP	Rank	Position (base pairs)	Nearest gene or transcript	Distance from	Frequency affected	Frequency unaffected	Allele1/Allele2	P-value CMH	P-value allelic	Odds ratio	pSNP test P-value	pHAP test P-value	r ²
18	rs4939921	1	45716326	MYO5B	Intron	0.115	0.080	G/A	1.66E-07	5.02E-07	1.51	5.09E-07	2.04E-08	0.42
12	rs1705236	2	69831825	TSPAN8	Intron	0.042	0.070	T/A	6.11E-07	1.47E-06	0.58	1.46E-06	7.57E-07	0.87
9	rs16931058	3	114547107	C9orf91	(-59589)	0.055	0.032	A/G	3.57E-06	2.34E-06	1.76	2.34E-06	9.11E-06	0.88
13	rs1341139	4	78439189	C13orf7	(+307874)	0.025	0.043	C/A	7.23E-06	3.16E-05	0.56	2.15E-05	1.59E-04	0.45
12	rs11050038	5	29149594	MLST1D1	+43806	0.167	0.207	C/T	1.47E-05	2.18E-05	0.77	2.18E-05	2.60E-05	0.98
9	rs2274598	6	114499686	C9orf91	-12168	0.040	0.022	C/T	1.68E-05	1.28E-05	1.87	2.30E-05	7.04E-06	0.83
18	rs1444067	7	61321666	CDH7	(+247471)	0.470	0.419	T/G	1.83E-05	3.41E-05	1.23	7.26E-05	5.56E-06	0.61
18	rs2858046	8	61268622	CDH7	(+300515)	0.473	0.424	T/C	1.93E-05	4.36E-05	1.22	6.02E-05	2.50E-05	0.98
9	rs2900591	9	114524833	C9orf91	-37315	0.048	0.029	T/C	2.06E-05	2.68E-05	1.71	8.05E-06	6.66E-06	0.55
18	rs2658046	10	61273210	CDH7	(+295927)	0.473	0.423	G/A	2.09E-05	4.31E-05	1.22	5.34E-05	2.31E-05	0.94
17	rs10491113	11	30028246	TMEM132E	-37797	0.144	0.185	A/G	2.69E-05	9.30E-06	0.74	1.03E-05	7.36E-02	0.21
18	rs12870791	12	61316783	CDH7	(+252354)	0.467	0.418	A/C	2.73E-05	5.30E-05	1.22	5.55E-05	1.22E-02	0.65
18	rs2850699	13	61268473	CDH7	(+300664)	0.472	0.424	C/A	2.78E-05	6.02E-05	1.22	6.02E-05	4.40E-05	0.95
18	rs4455070	14	61305341	CDH7	(+263796)	0.472	0.423	T/A	3.26E-05	5.60E-05	1.22	4.10E-05	1.34E-05	0.64
7	rs17172438	15	54925746	EGFR	Intron	0.203	0.162	C/T	3.26E-05	1.26E-05	1.32	1.58E-05	9.28E-07	0.69
7	rs729969	16	54902416	EGFR	Intron	0.163	0.125	T/C	3.30E-05	9.29E-06	1.36	8.67E-06	8.36E-08	0.68
9	rs10982292	17	114404845	ATP6V1G1	+24703	0.039	0.022	A/G	3.32E-05	2.86E-05	1.82	2.52E-05	6.81E-06	0.77
12	rs11049998	18	29085712	MLST1D1	(+107688)	0.166	0.206	G/A	3.33E-05	3.93E-05	0.77	2.95E-05	2.05E-04	0.78
X	rs5963419	19	38028410	OTC	Intron	0.386	0.331	C/T	3.44E-05	3.53E-05	1.27	4.22E-05	4.71E-05	0.99
3	rs9817739	20	16455831	RFTN1	Intron	0.184	0.223	T/C	3.47E-05	7.26E-05	0.79	7.30E-05	7.24E-03	0.28

Abbreviations: BP, bipolar; CMH, Cochran–Mantel–Haenszel; SNP, single nucleotide polymorphism.

STEP-BD and UCL individuals separately, no single SNP from either site exceeds those results listed in Table 1 (Supplementary Table 5). There was no heterogeneity of ORs between study sites for the top 200 markers (Breslow–Day test for homogeneity of ORs, $P > 0.01$).

Reconstruction of missing genotypes for these top 200 SNPs, based on local haplotype information, indicated that none of these tests were substantially biased by missing genotype data (pSNP test; see Materials and methods section). Next, we established proxy alleles and haplotypes that were highly associated with the reference SNP, but did not contain the SNP (pHAP test). In general, this confirmed that the original signals were not driven by solely a single SNP. In particular, rs1705236 in *TSPAN8* (ranked second) does not have any flanking SNPs showing association, but does show a strong association with a number of proxy haplotypes (see Supplementary Figure 4). For *MYO5B*, *EGFR* and *TSPAN8* (single SNP results shown in Figure 1) the pHAP tests showed haplotypic P -values of 2.04×10^{-8} , 8.4×10^{-8} and 7.5×10^{-7} , respectively (Table 1). The *MYO5B* result withstands conservative correction for 1 million tests ($P = 5 \times 10^{-8}$) and has an OR of 1.51 (frequency in cases = 0.115 and controls = 0.08). Plots of genes and regions surrounding additional SNPs from Table 1 can be seen in Supplementary Figure 5.

Testing in independent samples

We selected a total of 304 SNPs for genotyping in two independent samples: the family-based NIMH-GI sample and population-based Edinburgh sample. As well as genotyping of the 200 top hits (both samples), we report here additional SNPs that were genotyped earlier in the study only in the NIMH-GI sample, based on the results of interim analyses with less stringent QC metrics. We observed evidence for association, defined as same allele, same direction of effect, one-sided $P < 0.05$, in the combined NIMH-GI and Edinburgh samples for 13 SNPs (2 SNPs at $P < 0.01$) (Supplementary Table 6). Of these, 9 were from our top 200 hits, whereas 4 (rs12967023, rs9268853, rs749044 and rs6424785) were selected based on interim analyses (ranked 566th, 1220th, 662nd and 2405th out of the 372 193 SNPs in the final analysis, with Cochran–Mantel–Haenszel P -values of 0.001165, 0.002575, 0.00137 and 0.005191, respectively).

Four of these 13 SNPs are in strong LD in a region of chromosome 18q22 with no annotated genes, approximately 246 kb from cadherin 7 (*CDH7*); they were ranked 7th, 8th, 10th and 13th most significant in the whole-genome scan and are marginally significant at $P < 0.05$ (one-sided) in this follow-up. The next highest ranking SNP from the original scan that is also significant in the follow-up is rs10491113 (ranked 11th in the original scan), which is 38 kb downstream of *TMEM132E* on chromosome 17. The strongest follow-up results are for rs12967023 ($P = 0.00091$), which is in myelin basic protein, and

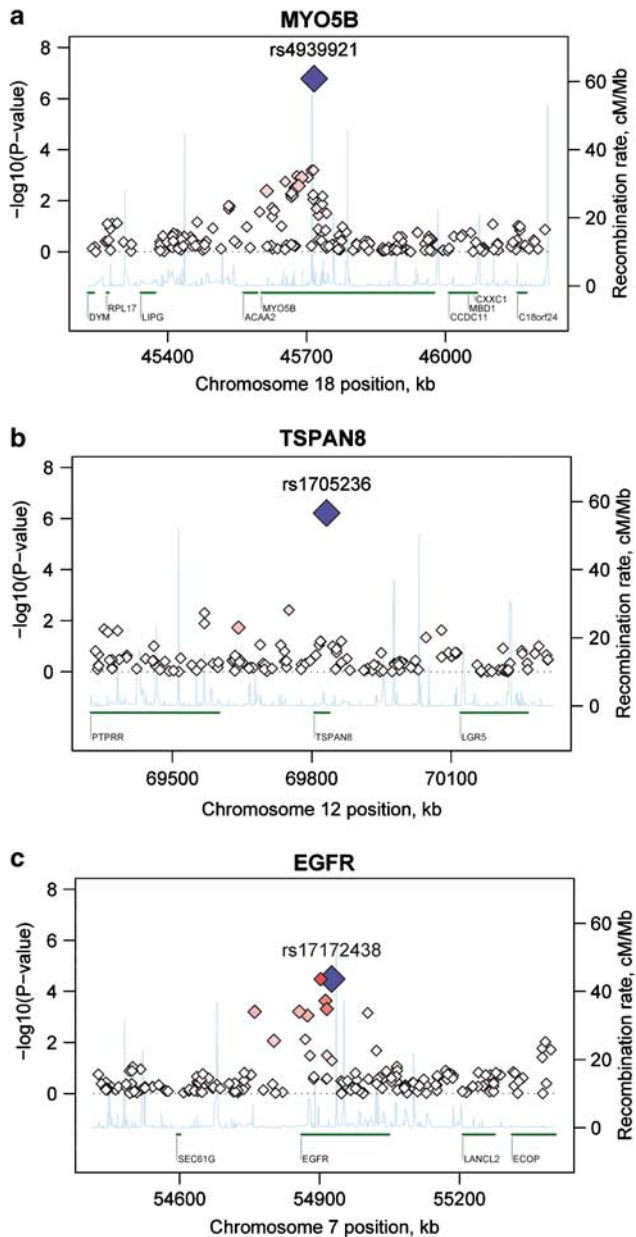


Figure 1 Plot of *MYO5B*, *TSPAN8* and *EGFR* regions of association. Association results ($-\log_{10} p$) are plotted for all single nucleotide polymorphisms (SNPs) passing quality control in the combined STEP-BD and UCL sample. (a) *MYO5B*, (b) *TSPAN8* and (c) *EGFR*. Chromosome position is plotted with reference to the NCBI build 35 and gene names are plotted with reference to the University of California at Santa Cruz Genome Browser. Recombination rate as estimated from the HapMap is plotted in light blue. Blue diamonds: SNP with strongest evidence for association. Red diamonds: strength of linkage disequilibrium (LD) with blue diamond SNP (darker red indicates stronger LD).

rs9268853 ($P=0.0011$) on chromosome 6 near HLA-DRA; neither SNP was in the top 200 in the original scan, however. We see modest evidence for association with rs4979416 in the gene calcium/calmodulin-dependent serine protein kinase-interacting protein (*DFNB31*) (ranked 172nd in the original screen,

$P=0.013$ in follow-up) and an association for rs2237554, in the metabotropic glutamate receptor 3 gene (*GRM3*) (ranked 121st in the original screen, $P=0.035$ in follow-up). Importantly, though, given that 304 follow-up tests were performed, this number of significant results is consistent with that expected by chance.

Comparison to previous whole-genome scan data

Two groups have recently published whole-genome association studies of BP disorder. The WTCCC genotyped 1900 primarily BPI patients from the UK and 3000 controls using the same platform as described in this paper.¹ The strongest signal observed in that study was at rs420259 near the partner and localizer of *BRCA2* (*PALB2*) under a recessive genetic model. We do not observe evidence for association at this SNP under allelic, dominant or recessive models ($P>0.05$).

Looking at the WTCCC allelic P -values (as available online), we do not observe any evidence for association with the 20 SNPs in Table 1 noted (all $P>0.05$). For our top 200 SNPs, we calculated combined P -values for our study and the WTCCC (Supplementary Table 7). Of our top 200 SNPs, the most significant result in the WTCCC consistent with our data was rs1006737 (OR = 1.21 this study, OR = 1.16 WTCCC) that is located in the third intron of the alpha subunit of the L-type, voltage-gated calcium channel (*CACNA1C*). Within *CACNA1C*, there are numerous other associated SNPs in both our sample and the WTCCC in an area of strong LD as shown in Figure 2. However, we did not observe statistically significant association with this SNP in our replication samples (although the Edinburgh case-control sample displays broadly similar allele frequencies as well as an OR of 1.15).

Baum *et al.*¹⁶ recently reported the results of a whole-genome scan using a pooled DNA strategy on the Illumina HumanHap550 followed by individual genotyping of 37 SNPs. They identified SNPs in diacylglycerol kinase eta as associated with BP disorder in US and German samples. As we expect some degree of overlap between our control sample and those used by Baum *et al.*, we have excluded 1044 controls from the following analyses, to create a data set of 1461 cases and 964 controls that are guaranteed to be independent of Baum *et al.* We do not see evidence for association with the 41 SNPs present on our arrays in diacylglycerol kinase eta (minimum $P=0.03$). Furthermore, imputing the missing genotypes for the three SNPs reported as associated in diacylglycerol kinase eta (using the CEU HapMap samples as a reference panel and the pSNP test, as these SNPs were not directly genotyped in this study) we do not see association for these SNPs, with pSNP tests imputing the missing genotype data showing $P=0.257$, 0.744 and 0.381, respectively; the strongest haplotype-based proxies in our data (from the pHAP test) gave $P=0.066$, 0.289 and 0.247 for r^2 with the Baum *et al.* SNPs of 0.40, 0.68 and 0.91, respectively).

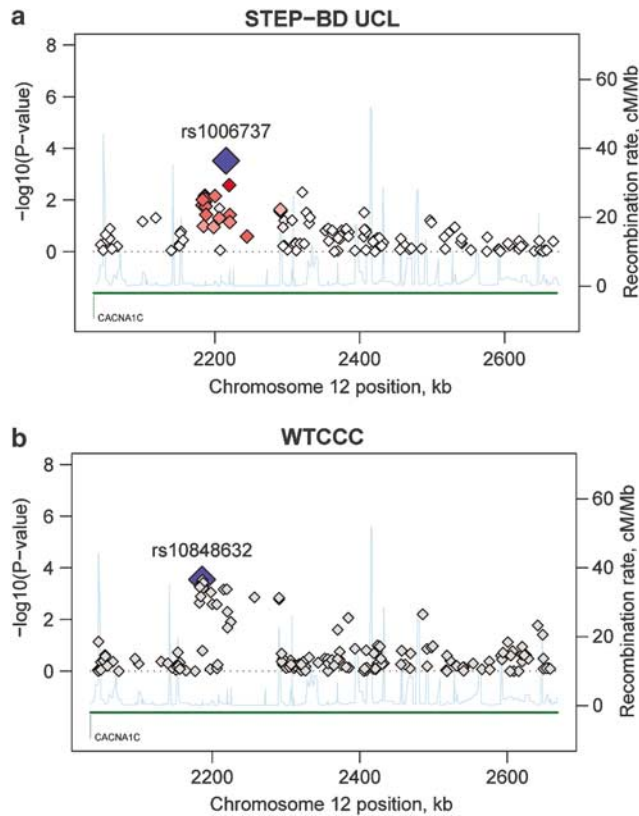


Figure 2 Plot of *CACNA1C* region of association. Association results ($-\log_{10} p$) are plotted for all single nucleotide polymorphisms (SNPs) passing quality control. **(a)** Combined STEP-BD and UCL samples, and **(b)** WTCCC. See Figure 1 for description of plots.

The fifth most associated SNP in Baum *et al.* is rs942518 ($P=0.0001$, not present in our genotyping platform) that is located near *DFNB31*. In our full sample including all controls, we observed an association with rs4979416 (located in *DFNB31* approximately 10 kb from rs942518), which was ranked 172nd ($P=0.0003$, OR 1.45, MAF 7% in cases, 5% in controls; $P=0.0027$ in the nonoverlapping subsample) and showed replication in our follow-up ($P=0.013$). Although, the NIMH-GI sample overlaps with the case samples used in Baum *et al.*, we observe a marginal association in the Edinburgh sample ($P=0.06$, OR 1.4, 7.4% in cases, 5.4% in cases) as well as the NIMH-GI trios ($P=0.06$, OR 1.4). This SNP is in moderate LD with rs942518, based on HapMap data ($r^2=0.35$). The WTCCC also shows a number of SNPs (rs11787667, rs10982246, rs10982256, rs1535964 and rs998548) flanking and within ~20 kb of rs4979416 that show association with BP (all $P < 5 \times 10^{-4}$ Supplementary Figure 6). However, as shown in Supplementary Figure 7, the association in this region appears complex and there is no obvious haplotype spanning the region that can account for the entire signal. Although in this study, the WTCCC and Baum *et al.* all identify associations physically near each other in *DFNB31*, the WTCCC association would appear to be independent of the signal seen

in our study and Baum *et al.*; further, the association in *DFNB31* from our study appears to be at least partially representing indirect association with a stronger signal ~60 kb downstream, to a region containing multiple SNPs that were ranked in our top 10 associations. Further work is warranted to confirm and refine the nature of association in this region.

Discussion

We report results from a large whole-genome association study of BP disorder. For our strongest signals, we also report replication efforts using a combined family-based and case-control sample. Furthermore, we compared our top results with that of other whole-genome association studies of BP disorder. Although we did not detect single SNP signals that meet stringent criteria for genome-wide significance, a haplotype in the gene *MYO5B* achieved genome-wide significance and we identified several interesting loci that will require examination in much larger samples. In addition to its size and the genotyping of individual rather than pooled samples, specific strengths of this study include the rigor of the diagnostic assessments for both the STEP-BD and UCL samples, the use of psychiatrically screened controls for the majority of the control sample and the methodology implemented to limit the effects of population stratification.

Our strongest association signals were observed in three genes, *MYO5B*, *TSPAN8* and *EGFR* that were supported by consideration of local haplotype information. *MYO5B* is located on chromosome 18 in a region initially reported as linked to BP disorder,^{37,38} although a subsequent meta-analysis of linkage studies does not support this observation.⁶ *MYO5B* is a large gene (372 kb, 40 exons) encoding a multi-functional protein expressed in a variety of brain structures, including dendritic spines, at high levels.^{39,40} Myosins are known to transport proteins in neurons through binding to their C-terminal globular domain,⁴¹ and *MYO5B* specifically regulates vesicle trafficking.⁴² Strikingly, *MYO5B* has been shown to regulate EGFR cycling in both canine (MDCK) and human cells (A431).⁴³ In light of the current associations with both *MYO5B* and *EGFR*, the functional relationship of these two genes may point to a possible molecular pathway (that is, vesicle trafficking at the plasma membrane) in which converging susceptibility alleles of small effect size, in distinct component genes, may underlie the biological etiology of BP disorder. EGFR, also known as ErbB1/HER1, belongs to the ErbB family of receptor tyrosine kinases that includes ErbB4, the Neuregulin 1 (NRG1) receptor and heterodimeric complexes of EGFR, ErbB2, ErbB3. ErbB4 have been shown to modulate downstream signaling of NRG1 and NRG2.⁴⁴

Abnormalities in circadian rhythms have been hypothesized to lead to episodes of mania and depression in what has been termed the social

zeitgeber theory.^{45,46} The biology that connects mood symptoms and circadian rhythms is unknown. However, Kramer *et al.*⁴⁷ have demonstrated that the actions of transforming growth factor- α , transduced through the EGFR, inhibit locomotor activity on the running wheel in mice and disrupt circadian cycles when infused into the third ventricle. Waved-2 mice are a naturally occurring strain with an EGFR point mutation that reduces kinase activity by more than 80%.⁴⁸ In fact, this loss of function EGFR mutation, which would be predicted to result in excessive activity, demonstrates excessive daytime activity on the running wheel, phenotypically reminiscent of the prominent hyperactivity observed in manic patients. Conversely, *Drosophila* gain-of-function mutants that activate EGFR signaling demonstrate an opposite phenotype, excessive sleep.⁴⁹ From this we would hypothesize that the SNPs and haplotypes associated with increased risk of BP disorder observed with *EGFR* would result in decreased tyrosine kinase activity. Through downstream interactions with known interacting proteins such as GAB1 and ErbB3, decreased PI3K activity could result in decreased phosphorylation (increased activity) of GSK3 β . This is consistent with the effects of mood stabilizers that function as GSK3 β inhibitors. However, these observations remain speculative in the absence of independent replication or knowledge of a causal polymorphism.

TSPAN8, located on chromosome 12, is a member of a large family of tetraspanin proteins that are involved in diverse cellular processes.⁵⁰ Other tetraspanins are known to form tetraspanin-enriched microdomains and may be involved in the clustering of receptors or cell signaling molecules.⁵¹

We did not see evidence for replication of our strongest findings. We did, however, observe replication *P*-values below 0.05 in a 53 kb region of 18q22 with no annotated genes, approximately 246 kb from *CDH7*, a brain-expressed calcium-dependent cell-cell adhesion molecule. Similarly, rs10491113 is found 38 kb downstream of *TMEM132E*, an uncharacterized transmembrane protein. Two SNPs, one in myelin basic protein (*MBP*) and one in *GRM3* followed based on results of interim analyses show stronger results. Myelination and oligodendrocyte functions have been reported as abnormal in BP disorder patients.⁵² We have previously demonstrated upregulation in the full-length 'golli' *MBP* mRNA transcription in mice following treatment of lithium.⁵³ Finally, two groups have tested SNPs in the *GRM3* gene for association with BP disorder with varying results.^{54,55} While we judge the evidence for association with *GRM3* modest, we have previously reported lithium-induced changes in this gene.⁵³

Consistent association findings between our study and the WTCCC are observed within intron 3 of the *CACNA1C*. L-type calcium channels mediate a variety of calcium-dependent processes in neurons and are sensitive to dihydropyridine derivatives, such as verapamil. Treatment of mania with verapamil initially

showed promise, but its efficacy remains ambiguous.^{56,57} Mutations in *CACNA1C* have been shown to cause Timothy's syndrome with severe prolongation of the QT interval on electrocardiogram, syndactyly, cognitive abnormalities and autism spectrum symptoms.⁵⁸

In summary, we have generated a list of genes and regions that warrant follow-up in more samples. These associations can be classified according to two categories: those with strong statistical significance in the primary scan but no supporting evidence, and those that show only moderate association in the primary scan but that also show replication in the follow-up, or some level of consistency with the WTCCC or Baum *et al.* study. In the first category are *MYO5B*, *TSPAN8* and *EGFR*. In the second category are *DFNB31*, *CACNA1C*, *MBP*, *GRM3* and *HLA-DRA*. This study, by itself, cannot unambiguously conclude that any of these genes influence risk for BPI.

In contrast to a number of recent whole genome studies of other common complex disorders, the major findings from each BP disorder study are not obviously supported by any of the other studies. Possible explanations for this apparent non-replication, include inadequate power to detect alleles of modest effect sizes, population-specific disease alleles, phenotypic heterogeneity (or misspecification), epistatic interactions of multiple modest effect genes and effects of copy number variants or other genetic variation not well captured by the panels of common SNPs, including multiple rare disease alleles. Although this study is well powered to detect large effects (ORs > 1.5 for common alleles), most rarer and more modest effects will have a substantial chance of not being detected, in both the whole-genome screen and the replication stage. For example, if we define 5×10^{-4} as the significance threshold for declaring a 'promising SNP' (that is, that we would expect to rank within the top 200 or so results), we can calculate joint power to detect a SNP in both our and the WTCCC samples. For common variants (40% minor allele frequency) the joint power is ~96 and ~48% for SNPs with multiplicative ORs of 1.3 and 1.2, respectively. However, for less common variants (10% minor allele frequency) joint power to detect the SNP in both studies is much lower, now at ~34 and ~3% for ORs of 1.3 and 1.2, respectively. And even if we were able to detect a rarer SNP in our study, for example, one with a minor allele frequency of 5% and ORs of 1.2, then power to replicate in the WTCCC even at very modest significance thresholds ($P < 0.05$ rather than $P < 5 \times 10^{-4}$) is still only approximately 50%. Given these estimates, and the modest effect sizes of replicated variants emerging in other disease areas, the lack of agreement between studies is perhaps less surprising. Joint analysis is expected to be more powerful than replication,⁵⁹ and we are planning to embark on a combined analysis of the WTCCC BP data and our sample with the WTCCC investigators.

In addition to lack of power, phenotypic and genetic heterogeneity are another explanation for the lack of clear findings. It is unlikely, however, that

population-specific alleles account for the observed difference between our study and the WTCCC: both samples are of European background and approximately one-third of the current sample was from the UK (as are the WTCCC samples). Although phenotypic heterogeneity is always a possibility, the STEP-BD and UCL samples are selected from patients with BPI disorder and approximately 70% of the WTCCC sample fit DSM-IV criteria for BPI disorder. Ultimately, meta-analysis of diagnostic subphenotypes, ascertainment strategies and diagnostic conventions will be useful to investigate possible phenotypic heterogeneity between and within studies. In particular, the Affective Disorders Evaluation, Schizophrenia and Affective Disorders Schedule—Life Time version and Operational Criteria checklist used in the diagnostic assessment of the STEP-BD and UCL samples also collects data on age of onset, prior episode frequency, prognosis, clinical features, medical history, family history and mental status. One possibility to be explored is that the genetic influences on BP disorder act on these more specific aspects of the disease or in subtypes of the disorder characterized by age-of-onset or certain clinical symptoms. Future analyses will focus in particular on those aspects of the diagnosis that have been demonstrated to be inherited including psychosis and age-at-onset. The diagnostic instruments used in this study and also by the WTCCC include additional phenotypic information.

Alternatively, the common variant hypothesis may not fit for BP disorder. Whole genome studies are not adequately powered to investigate the hypothesis that there are many rare private alleles (either in a small number of genes or in many genes) leading to disease. Testing of this hypothesis may be possible in the near future as whole genome sequencing technologies become less costly. We are also pursuing alternate analytic approaches to the SNP data presented here, focusing on epistasis, copy number variation and rare variation as indexed by patterns of extended segmental sharing.

We were also unable to provide additional support for the most significant associations reported in the WTCCC and Baum *et al.* studies. Despite this, the high heritability of BP suggests it should be possible to identify risk variants. Given the power and coverage of this study, if common variants exist for BP disorder, they may be of very small effect and thus require very large samples to be reliably detected. This highlights the future need for meta-analysis of all whole-genome BP disorder data already collected as well as for ongoing and larger sample collections.

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Data release policy: Genotypes for the NIMH control samples have been submitted to the NIMH Genetics Repository and are available under the usual data release policies. Genotypes for the STEP-BD case samples will be submitted to the NIMH repository and will be available for release using the same mechanism.

Author contribution statement

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