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Linkage Disequilibrium Mapping of the Chromosome 6q21–22.31 Bipolar I Disorder Susceptibility Locus

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Abstract

We previously reported genome-wide significant evidence for linkage between chromosome 6q and bipolar I disorder (BPI) by performing a meta-analysis of original genotype data from 11 genome scan linkage studies. We now present follow-up linkage disequilibrium mapping of the linked region utilizing 3,047 single nucleotide polymorphism (SNP) markers in a case–control sample (N = 530 cases, 534 controls) and family-based sample (N = 256 nuclear families, 1,301 individuals). The strongest single SNP result (rs6938431, P=6.72× 10^{-5}) was observed in the case–control sample, near the solute carrier family 22, member 16 gene (*SLC22A16*). In a replication study, we genotyped 151 SNPs in an independent sample (N = 622 cases, 1,181 controls) and observed further evidence of association between variants at *SLC22A16* and BPI. Although consistent evidence of association with any single variant was not seen across samples, SNP-wise and gene-based test results in the three samples provided convergent evidence for association with *SLC22A16*, a carnitine transporter, implicating this gene as a novel candidate for BPI risk. Further studies in larger samples are warranted to clarify which, if any, genes in the 6q region confer risk for bipolar disorder.

Keywords

bipolar disorder; genetic; association; *SLC22A16*; 6q

Introduction

Bipolar disorder (BPD) is a chronic and often disabling disorder that, in broadest conceptualization, affects up to 4% of the US population and, in the most narrow (Type I) classification, consistently affects about 1% of adults worldwide [Kessler et al., 2005]. Despite the availability of effective treatments for mania and depression, the recurrence rate and disability associated with BPD remain substantial even among adequately treated patients [Fagiolini et al., 2005]. Thus, there is intense interest in determining the etiology of BPD, with the goal of developing more effective treatment and prevention strategies. Family and twin studies have consistently documented that BPD is familial and heritable [Smoller and Finn, 2003], but efforts to identify specific susceptibility genes have been complicated by the genetic and phenotypic complexity of the disorder.

For the most part, two parallel approaches have been exploited in the search for BPD susceptibility genes. The first has focused on biologically relevant candidate genes thought to underlie BPD based on prior knowledge of the biological basis or mechanism of action of drug treatments for BPD. For example, variants within the serotonin transporter gene (*SLC6A4*) have been implicated in BPD by meta-analyses of association studies [Cho et al, 2005; Lasky-Su et al., 2005]. However, the identification of such candidates is constrained by the state of knowledge about the biology of the disorder. The second strategy involves "unbiased" genome-wide approaches, including whole genome linkage and association methods that do not require pre-specified hypotheses about genes of interest. Genome-wide linkage scans can localize chromosomal regions harboring susceptibility loci which can then be examined using linkage disequilibrium (LD) methods to identify the relevant genes, a strategy that has proven successful in other complex diseases [e.g., Crohn's disease; Hugot et al., 2001; Rioux et al., 2001]. More recently, genome-wide association studies (GWAS) have become feasible, permitting LD analysis across the entire genome. Initial results have implicated several putative loci in BPD, though none has yet been established [Baum et al., 2007; Wellcome Trust Case Control Consortium, 2007; Sklar et al., 2008].

We have been pursuing both of these strategies, and here we report the primary results of a large-scale effort to combine linkage mapping and LD analyses to identify BPD susceptibility variants. We began by performing a meta-analysis of genome scan linkage studies in which we combined original genotype data from 11 linkage studies, comprising more than 5,000 individuals from 1,067 families [McQueen et al., 2005]. We identified two regions that achieved genome-wide significance: the first, at chromosome 6q, achieved a LOD score of 4.19 for BPI disorder (narrow phenotypic definition); and the second on chromosome 8q yielded a LOD score of 3.4 for a broad phenotypic definition (bipolar I + bipolar II) [McQueen et al., 2005]. Here we report LD analyses aimed at identifying susceptibility variants within the 6q locus.We employed a staged approach beginning with a screening set of 530 BPI cases and 534 unscreened controls and an independent family-based sample comprising 256 nuclear families in which offspring were BPI cases. The strongest results from stage 1 were followed up in an independent sample of 622 BPI cases and 1,181 screened controls. We also examined two sub-phenotypes of BPD (psychotic BP and early-onset BP) that have previously been shown to be familial subtypes of the disorder {Potash et al., 2003 #2102; Saunders et al., 2008 #8782}.

Methods

Samples

Case–control sample (*STEP1*)—A sample of 530 individuals with BPI were obtained from the genetic repository of the Systematic Treatment Enhancement Program for BPD (STEP-BD), a longitudinal cohort study designed to examine the effectiveness of treatments and their impact on the course of BPD [Sachs et al., 2003]. STEP-BD enrolled 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. Only self-defined Caucasian participants that met consensus diagnosis of BPI on both the Affective Disorders Evaluation

and the Mini-International Neuropsychiatric Interview were included in the current genetic study. Caucasian control subjects (n = 534) were obtained from the NIMH Genetics Initiative through the NIMH Center for Collaborative Studies (http://zork.wustl.edu/nimh/). Control samples were derived from anonymous cord blood samples and thus were phenotypically unscreened [Mansour et al., 2005]. Such samples are normally discarded following normal delivery.

Family-based sample (*NIMH*)—Nuclear families (n = 256 families, 1,301 individuals) were selected from samples previously collected for linkage studies by the National Institute of Mental Health (NIMH) Genetics Collaborative Study of BPD waves 1 through 4 [Edenberg et al., 1997; Stine et al., 1997; Dick et al., 2003; McInnis et al., 2003]. In brief, diagnosis was determined using the Diagnostic Interview for Genetic Studies [Nurnberger et al., 1994] with best-estimate diagnosis assigned by two independent psychiatrists based on the Diagnostic Interview for Genetic Studies result, family informants, and review of medical records. For the present study, we initially identified all complete affected parent-proband trios for whom DNA was available from the Rutgers University repository (http://www.nimhgenetics.org) using abroad definition that included BPI, BPII, or SAB probands; from these, the families which offspring affected with BPI, based on phenotypic data (NIMH release 3.05; http://www.nimhgenetics.org), were selected for primary analyses. The same group of families were included in additional studies by our group [Perlis et al., 2008; Sklar et al., 2008].

Follow-up case–control sample (STEP2)—For follow-up analyses, a second sample of BPI cases (n = 622) were obtained from the STEP-BD genetic repository, in the same way as STEP1. The control subjects (N = 1,181) were obtained from the NIMH Genetics Initiative through the NIMH Center for Collaborative Studies (http://zork.wustl.edu/nimh/). The control sample comprised a sub-sample of a panel of self-reported Caucasian individuals who completed an online self-administered psychiatric screen and were recruited via random-digit dialing by a marketing research company, Knowledge Networks (Menlo Park, CA) [Sanders et al., 2008] 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 BPD using a three-part question: “Have you ever received treatment for, or been diagnosed with, any of the following conditions: (a) Schizophrenia or schizoaffective disorder; (b) Hearing voices others could not hear or believing things that others said were not true (such as that people were trying to harm you); (c) BPD (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 impairment based on their responses to depression items were excluded. The STEP1 and STEP2 samples and the controls were also included in a recent GWAS of BPD [Sklar et al., 2008].

Gene and SNP Selection

Stage I: screening SNP selection and genotyping—Based on the results of our genome-scan meta-analysis [McQueen et al., 2005], single nucleotide polymorphism (SNP) genotyping focused on a ∼20 Mb core linkage peak region of chromosome 6q (spanning 105.34–125.73 Mb, NCBI build 34). The chromosome 6 linkage region boundaries were identified using a meta-analytic approach as described by McQueen et al. [2006]. In particular, the contiguous region on chromosome 6q for which the 95% confidence interval of the summary IBD estimate did not include null allele sharing (IBD = 0.5) defined the fine-mapping region. In addition, 22 genes (data shown in Supplement Table s1) located within 25 Mb flanking the core linkage peak region were selected for genotyping if they met at least one of two criteria: (1) known to be involved in neurotransmitter systems or central nervous system function; and/or (2) evidence of association with psychiatric disorders in prior genetic studies.

Overall, a ∼23 Mb region (core linkage peak region plus 22 candidate gene spanning 2.6 Mb) was selected for LD mapping. We initially used HapMap Phase I data (www.hapmap.org) to identify tagging SNPs that captured variation at common SNPs (minor allele frequency ≥5%) within the selected regions. The tagging SNPs were selected by H-clust software [Rinaldo et al., 2005] using an r^2 threshold of 0.8 or greater.

A total of 3,047 6q SNPs were selected (1,503 in gene regions), with an average marker density in the core linkage peak region of 1 SNP per ∼7.6 kb (2,697 SNPs/20,393 kb). In total, 3,072 SNPs were genotyped in STEP1 and NIMH samples using two Oligo Pool Arrays (OPAs) by the Illumina BeadArray platform (San Diego, CA) at the Broad Institute. Between the two OPAs, eight SNPs (seven non-chr6 SNPs and one chr6 SNP) were duplicated for internal quality control purposes (data shown in Supplement Table s2). One control sample from the Centre d'E′ tude du Polymorphisme Humain set was also included on each 96-well plate as inter-plate controls.

Stage II: follow-up SNPs selection and genotyping—Based on the results observed in the STEP1 and NIMH samples, follow-up genotyping was performed for six gene regions that showed evidence of association with BPI in analyses of the STEP1 and/or NIMH trio samples (data shown in Supplement Table s3). First, SNPs in these six regions from the original OPAs were selected and genotyped in STEP2 sample (81 SNPs). Second, additional tagging SNPs for these loci were selected using Tagger [de Bakker et al, 2005] based on HapMap Phase II data (www.hapmap.org) and pair-wise tagging parameters, with a minimum r^2 threshold of 0.8 and minimum minor allele frequency of 0.05. These SNPs were supplemented by known coding sequence SNPs in the gene regions derived from dbSNP (http://www.ncbi.nlm.nih.gov/SNP/). In total, 70 additional SNPs were genotyped in all three groups (STEP1, NIMH and STEP2). Third, an additional 56 SNPs were genotyped in NIMH sample only since they were genotyped in STEP1 and STEP2 samples in our GWAS [Sklar et al., 2008].

All SNPs in our follow-up tests were genotyped by an allele-specific primer extension of amplified products with detection by matrix-assisted laser desorption ionization time-of-flight mass spectroscopy using the Sequenom iPLEX genotyping platform (San Diego, CA)

at the Broad Institute. Among them, 12 SNPs were genotyped in duplicate in both STEP1 and NIMH samples by Illumina OPAs and follow-up iPlex pools. The genotype concordance rate between Illumina OPAs and Sequenom iPLEX genotyping platforms was calculated using these duplicated SNPs.

Data Analyses

Quality control (QC) analyses—Genetic quality control analyses were performed using the PLINK software package [Purcell et al., 2007]. The original genetic dataset comprised 530 STEP1 cases, 534 anonymous controls, and 1,301 individuals from the NIMH nuclear families genotyped for 3,072 SNPs. We excluded 34 STEP1 individuals (17 cases and 17 controls), 57 NIMH individuals, and 269 SNPs that failed the OPAs quality control (QC) filters (QC data are shown in Supplement Tables s4 and s5). After QC, the STEP1 and NIMH datasets consisted of 513 STEP cases, 517 controls, and 1,244 individuals from the NIMH nuclear families genotyped for 2,803 SNPs for which genotype call rates exceeded 95%. For the analyzable duplicate individuals and SNPs, the within and between plate concordance exceeded 99.9%, and concordance with published HapMap CEPH genotypes was greater than 99.7%. The inter-OPAs concordance was greater than 99.9% on the basis of seven duplicated SNPs.

To determine the tagging performance of SNPs that passed QC thresholds in the core linkage peak region of chromosome 6q, we employed Tagger [de Bakker et al., 2005] using HapMap phase II data (HapMap Release 21). Pair-wise tagging parameters included a minimum r^2 threshold of 0.8 and minimum minor allele frequency of 0.05. Of a total of 16,805 HapMap phase II SNPs in the core linkage peak region, 74% were captured with $r^2 \geq 0.8$, with mean $r^2 = 0.88$, suggesting that our final set of SNPs adequately captured common variation in this region.

The original iPLEX genotype datasets (Stage 2) comprised 1,064 STEP1 individuals (530 cases, 534 controls), 1,301 members of NIMH nuclear families and 1,803 STEP2 individuals (622 cases, 1,181 controls) genotyped for 70, 126, and 151 SNPs, respectively. We excluded 92 individuals and 8 SNPs from STEP1 dataset, 57 individuals and 12 SNPs from NIMH dataset, 221 individuals and 18 SNPs from STEP2 dataset that failed the iPLEX QC filters (data shown in Supplement Table s5). After QC, all remaining individuals and SNPs had genotype call rates greater than 95%. The concordance between OPA and iPLEX genotyping platforms was greater than 99.9% on the basis of seven duplicated SNPs that passed both QC filters.

Single-marker analyses—Single-marker association analyses were conducted using PLINK [Purcell et al., 2007] for the case–control samples (STEP1 and STEP2) and FBAT for the family-based sample (NIMH) [Laird et al., 2000]. We report P-values under an additive model using the Cochran–Armitage trend test implemented in PLINK, and from the family-based test in FBAT. We also report *P*-values for *Z*-scores based on combined results from the three independent samples. The lambda value [Devlin and Roeder, 1999] for the STEP1 sample was 1.06, suggesting that adjustment for population substructure was not necessary.

Gene-based analyses—Gene regions were defined by all geno-typed SNPs within 10 kb up- and down-stream of gene transcription sequences based on NCBI build 36.1 (March 2006). Overall, there are 117 RefSeq genes in our 6q region with at least one SNP genotyped by Illumina OPAs. The set-based test implemented in the PLINK software package was performed in case–control samples (STEP1 and STEP2). The *min-p* test implemented in the FBAT software (which computes the *P*-value for the minimum observed marker *P*-value in a gene using Monte Carlo simulations) was performed in family-based samples (NIMH). We also report the Fisher's combined *P*-value, as a combined measure of results from the case–control and family-based samples.

The sequence of analyses are summarized in Figure 1.

Results

Primary Association Analyses

First, we performed single-SNP analyses for 2,803 SNPs genotyped in the Stage 1 samples (STEP1 and NIMH). Single-marker *P*-values from the Cochran–Armitage trend test in the STEP1 sample and from the FBAT test in the NIMH trio sample are shown in Figure 2, respectively. Supplementary Table s6 and s7 show the top 10 SNPs from these analyses, for STEP1 and NIMH, respectively, along with corresponding *P*-value, direction of effect (+ for OR > 1 and – for OR < 1), physical position and, where applicable, the gene region the SNP sits in.

As shown in Supplementary Table s6, no single marker achieved statistical significance after Bonferroni correction for the full set of markers tested (Bonferroni-corrected threshold $0.05/2{,}803 = 1.8 \times 10^{-5}$). The strongest evidence for association was seen for rs6938431 ($P = 6.7 \times 10^{-5}$), which is located 8.9 kb 5′ of D-aspartate oxidase gene (*DDO*) and 304 bp 3′ of Solute carrier family 22 (organic cation/carnitine transporter), member 16 (*SLC22A16*). Of note, another SNP in the top 10 lists (rs9400393) is also in the *SLC22A16* region (the r^2 is 0.094 between these two SNPs).

Supplementary Table s7 shows the top 10 SNP results for the NIMH family-based sample. While none of the SNPs that showed the strongest association in STEP1 were associated with BPI in the NIMH sample, another SNP in the *SLC22A16* region, rs17071722, showed evidence of association in the NIMH sample. This SNP is not in LD with the *SLC22A16* SNPs in Supplementary Table s6 (rs17071722 has an r^2 of 0.019 with rs6938431, and 0.045 with rs9400393). The strongest association in the NIMH sample was observed for rs6931341 ($P = 0.0003$), located ∼28 kb upstream of the transcription start point of alpha chain isoform 4 of laminin gene (*LAMA4*), In addition, two SNPs (r^2 between them is 0.431) in the gene encoding prolyl endopeptidase (*PREP*), a gene implicated in the action of mood stabilizers [Williams et al., 2002], were among the top 10 results for the NIMH sample.

In Supplementary Table s8, we show the top 10 results from the pooled analysis of both the NIMH and STEP1 samples, combined according to *Z*-scores. There are two SNPs in this list that are in the *DDO/SLC22A16* region, and two SNPs in this list that are in the 5′-nucleotidase domain containing one gene region (*NT5DC1*).

Gene-Based Tests

As an additional analysis, we performed gene-based tests for 117 genes. In Supplementary Tables s9 and s10 we show the top 10 genes in the STEP1 and NIMH samples, respectively.

As expected from the findings reported above, *SLC22A16* was in the top 10 lists for both samples. In Supplementary Table s11, we show the combined results from both samples, combining *P*-values using Fisher's method. The leading gene-based results were observed for *SLC22A16* (*P*=0.00048), *DDO* (*P*=0.0033), and *PREP* (*P*=0.0148), although none survive Bonferroni correction for all the marker tests we performed.

Follow-Up and Replication Analyses

Based on the pattern of findings from the NIMH and STEP1 samples, we performed follow-up analyses focusing on six loci, including five gene regions—*SLC22A16*, *DDO*, *PREP*, *NT5DC1*, *GPR6*, and the region around rs794854 (the top SNP in the combined analysis: Supplementary Table s8).

None of the additional SNPs in our follow-up study achieved lower *P*-values than the original set of SNPs within the NIMH and STEP1 samples. However, genotyping in the STEP2 sample added new, independent evidence for *SLC22A16*. Notably, 5 of the top 10 SNPs in STEP2 (Supplementary Table s12) are in the *SLC22A16* region. In addition, one of these SNPs (rs17071722) was nominally associated with BPI in all three (STEP1, NIMH, and STEP2) samples (*P*=0.016, 0.002, and 0.02, respectively), although the associated allele is flipped between STEP1 (major allele) and the NIMH (minor allele)/STEP2 (minor allele) samples. Results for single marker tests in the *SLC22A16* region are summarized in Figure 3.

The gene-based test for STEP2 resulted in nominally significant *P*-values for two genes: *SLC22A16* (*P*=0.03) and *C6orf204* (*P*=0.036). It is interesting that *SLC22A16* is nominally significant in all three samples (with *P*-values 0.001, 0.03, and 0.03 in STEP1, NIMH, and STEP2), although the top ranking SNP from STEP1, rs6938431 (Supplementary Tables s6), has an opposite direction of effect in STEP2 (Supplementary Tables s12).

Phenotypic Subtype Analyses (Psychosis and Early-Onset BPI)

In light of the suggestive evidence of association observed for *SLC22A16*, we explored whether a stronger association might be observed within genetically relevant subtypes of BPD. In particular, psychotic BPD and early-onset BPD have been shown to be familial and heritable subtypes that may reflect more genetically homogeneous influences [Smoller and Gardner-Schuster, 2007]. Early-onset BPD was defined as having a first manic or depressive episode before age 18, and psychosis was defined as having had psychotic symptoms or a psychotic diagnosis on any of the STEP-BD instruments that assessed psychosis. For the NIMH sample, psychosis was identified in individuals reporting lifetime history of either delusions or hallucinations, as assessed by the DIGS [Nurnberger et al., 1994]. The resulting sample sizes for the three datasets and the two sub-phenotypes are shown in Supplementary Table s13.

For the subtype of psychotic BPI, nominally significant association for the gene-based test of *SLC22A16* was observed only for the NIMH sample (*P*=0.0073) sample. For the early-onset BPD sub-phenotype, the STEP1 and STEP2 both showed nominally significant *P*-values (STEP1 *P*=0.0008, STEP2 *P*=0.027) (data shown in Supplementary Table s13).

Discussion

We report results from a large-scale LD mapping study of BPI disorder using independent case–control and family-based samples. Although we did not detect single marker or gene signals that meet stringent criteria for study-wide significance, we identified several interesting loci that warrant further examination in much larger samples.

Our strongest association signal was observed in the STEP1 sample at rs6938431 ($P=6.72 \times 10^{-5}$), which is ∼300 bp 3′ of *SLC22A16*. Another SNP in intron 1 of *SLC22A16* showed a nominally significant association with BPI in all three samples (STEP1, NIMH, and STEP2; *P*=0.016, 0.002, and 0.02, respectively). Although no single SNP showed consistent (in terms of directionality of effect) evidence of association across samples, *SLC22A16* appears in the top 10 results from single-marker and gene-based tests in all three samples. Further studies of the role of *SLC22A16* polymorphisms using large well-characterized clinical samples are warranted. The estimated odds ratios for the seven nominally significant SLC22A16 SNPs in either STEP1 or STEP2 range from 1.35 to 1.63.

The association found between variants in *SLC22A16* and BPI is intriguing since it is located directly under the linkage peak identified by our previous meta-analysis of genome scan linkage studies [McQueen et al., 2005]. The *SLC22A16* gene, which encodes a high affinity carnitine transporter that also transports various organic cations, has not previously been highlighted as a candidate gene for BPD. It is one of a family of organic ion transporters (referred to as amphiphilic solute facilitators, ASFs), that transport various medically and physiologically important compounds, including pharmaceuticals, toxins, hormones, neurotransmitters, and cellular metabolites [Koepsell et al., 2007]. It is expressed in a variety of tissues including brain [Eraly and Nigam, 2002], although its role in the central nervous system has not been characterized. Carnitine itself is essential for the transport of fatty acids into mitochondria for β oxidation; mitochondrial dysfunction, and abnormal bioenergetics have been implicated in the pathophysiology of BPD [Stork and Renshaw, 2005], and preliminary evidence suggests that carnitine may have neuroprotective effects [Soczynska et al., 2008].

The strategy pursued in this study—dense LD mapping of genomic regions implicated by linkage mapping—has been successful in other areas of complex diseases including Crohn's disease, alcoholism, and Alzheimer's disease [Corder et al., 1993; Hugot et al., 2001; Rioux et al., 2001; Dick et al., 2006]. However, the recent advent of genome-wide association studies (GWAS) and their success in identifying susceptibility genes for a variety of complex disorders have highlighted the advantages of the GWAS approach. Nevertheless, there may still be a role for positional mapping given that current GWAS platforms do not comprehensively assay variation throughout the genome. At the same time, one lesson of recent GWAS studies has been that common susceptibility variants confer modest effects

(odds ratios in the range of 1.1–1.5) and thus require very large sample sizes to be successful. Thus, one reason that we may not have detected consistent evidence of association with variants in the 6q region may have been insufficient power. The samples included in the present study also contributed to a GWAS of BPI that we recently reported [Sklar et al., 2008]. In that study (which was undertaken after the analyses reported here), no evidence of association was observed in the 6q region examined in the current study. Data from the Wellcome Trust GWAS of BPD also did not support association with the region encompassing *SLC22A16* [Wellcome Trust Case Control Consortium, 2007]. Of note, only 407 of the 3,047 SNPs genotyped in the current study were included on the Affymetrix GeneChip 500K array used in the GWAS. The most strongly associated SNP at *SLC22A16* in the present study (rs6938431) was not on the 500K Array. Using the same tagging settings described in the Methods Section, the LD coverage of the 500K array for *SLC22A16* is only 64%. The LD coverage of the Illumina OPAs (Stage I of the current study) for *SLC22A16* is 76%, and the LD coverage of Chr6 OPAs plus the *SLC22A16* SNPs genotyped in Stage II is 90%.

In addition to the power constraints of our sample, several additional limitations that may have increased the risk of Type II error in this study should be noted. First, SNP selection focused on common variants (minor allele frequency ≥5%). It is possible that that the genome-wide significant linkage that we observed in our meta-analysis for the 6q region was driven by rare variants that were not well captured by our tagging SNPs. This scenario would be consistent with the strong linkage signal we detected previously, and the weaker association signals we report in the current article. In this scenario, further examination of the region, including re-sequencing for detection of rare variants, may lead to the discovery of additional susceptibility variants. In any case, there is no reason to believe that the common variants we report in the current study account for all of the genetic variance at this locus. Second, differences in ascertainment criteria and diagnostic assessments for the STEP-BD and NIMH Genetics Initiative sample may have resulted in heterogeneity that obscured positive findings. Third, the inclusion of unscreened controls in the STEP1 case–control analysis may have reduced power if occult cases of BPI were present, though the impact of this should be negligible given the low-population prevalence of BPI [Moskvina et al., 2005]. The risk of confounding and spurious association due to population stratification is another concern in case–control samples; however this is unlikely to play a role in our analyses due the small genomic inflation factor (based on mean Chi-squared values) of 1.06 in the STEP1 sample, and the use of family-based samples.

In summary, we performed LD mapping in three independent samples of a region of chromosome 6q that has been strongly linked to BPI disorder. Although consistent evidence of association with any single variant was not seen across samples, SNP-wise and gene-based tests provided convergent evidence for association with *SLC22A16*, implicating this gene as a novel candidate for BPI risk. Further studies in larger samples are warranted to replicate or refute an association of this gene with BPD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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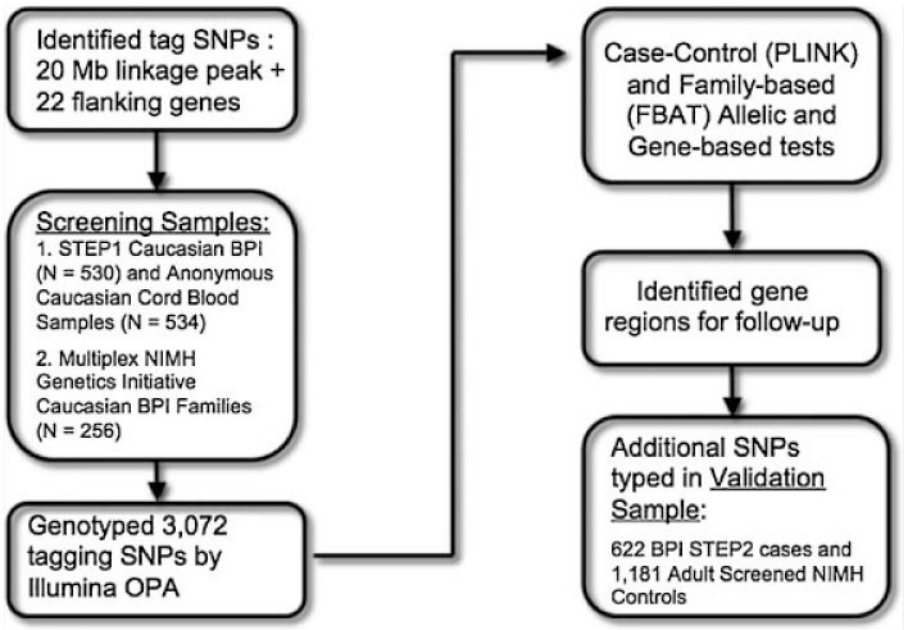

Fig. 1.
Flow chart of analytic approach.

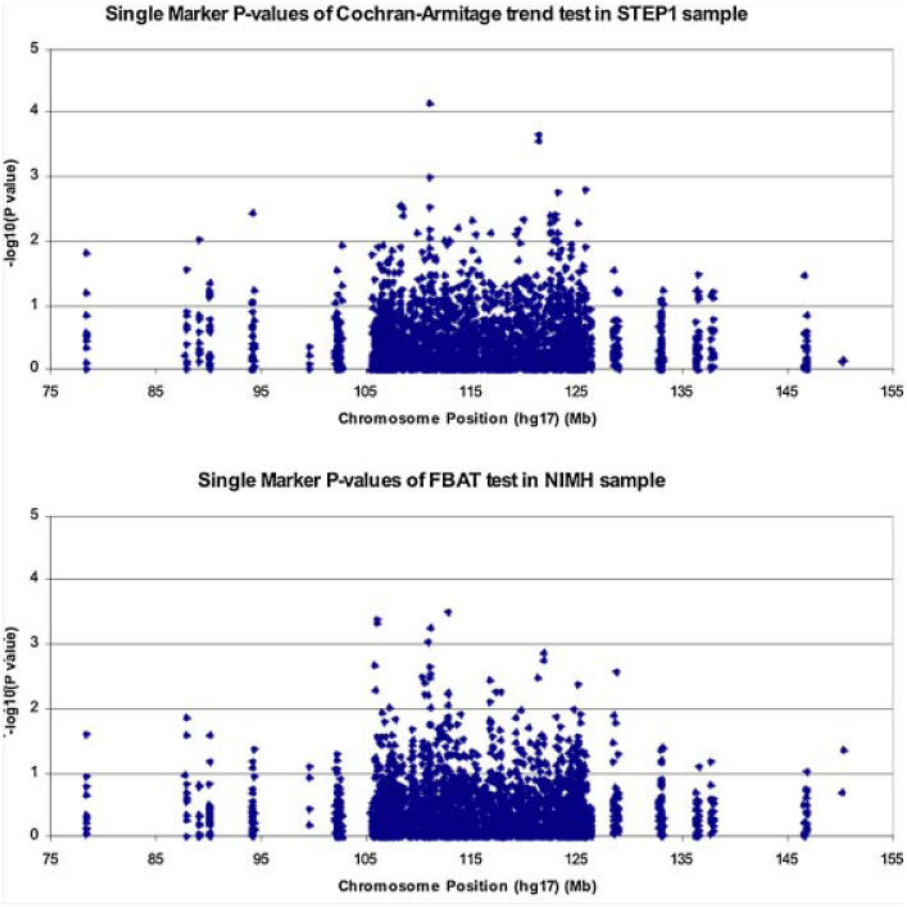

Fig. 2.

Single-marker *P*-values from FBAT test in NIMH sample.

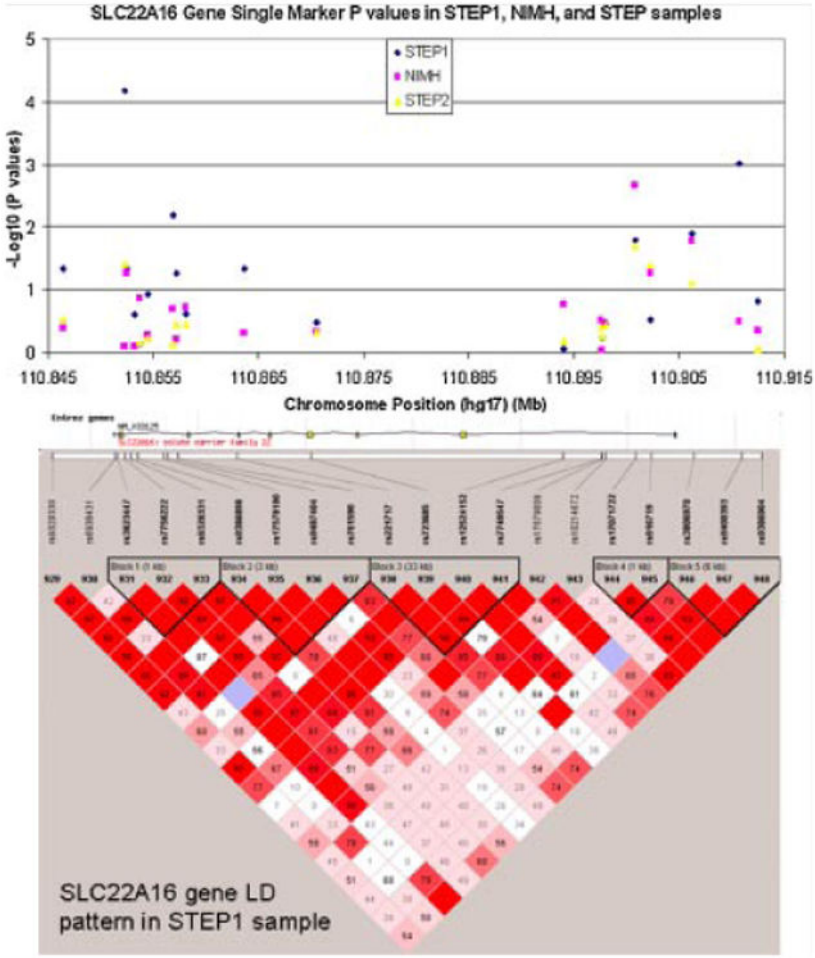

Fig. 3.

Single-marker *P*-values in the SLC22A16 gene region for the STEP1, NIMH, and STEP2 samples. Also shown: LD pattern in STEP1 sample.