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Characteristics of Bipolar I Patients Grouped by Externalizing Disorders

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Abstract (244/250 words)

Objective: Bipolar disorder co-occurs with a number of disorders with externalizing features. The aim of this study is to determine whether Bipolar I (BPI) subjects with comorbid externalizing disorders have different clinical features than those without externalizing disorders. We also sought to determine whether the observed differences could be attributed to specific genetic variations (single nucleotide polymorphisms or SNPs). **Method:** 1268 BPI subjects in the Genetic Association Information Network (GAIN) study were divided into two groups: those with comorbid externalizing disorders (N=796) and those without such disorders (N=472); a subgroup of subjects with externalizing symptoms beginning prior to age 15 was also defined. Externalizing disorders were defined as the presence of alcohol abuse/dependence, drug abuse/dependence, pathological gambling, attention-deficit hyperactivity disorder, anti-social personality disorder or conduct disorder. Course of illness parameters were compared between the externalizing (and early-onset subgroup) and non-externalizing groups. The findings were validated using 1237 BPI subjects in an independent study. Genome-wide association studies (GWAS) were carried out. **Results:** Subjects in the externalizing group tended to have poorer clinical outcome with earlier ages at onset of BPI, depression and mania, higher depressive and manic episode frequency, more suicide attempts, rapid switching and rapid cycling, compared to the non-externalizing group. GWAS analyses did not reveal any SNP that reached genome-wide significance, but identified a number of nominally associated SNPs. **Conclusions:** The clinical and genetic findings in the present study support the presence of an externalizing disorder subphenotype within BPI that merits further investigation.

Introduction

Bipolar disorder (BP), also known as manic-depressive illness, is a brain disorder in which people experience distinctive episodes with altered mood, activity, and thought patterns. An episode can be an overly activated excited state (a manic episode), an extremely sad or hopeless state (a depressive episode), or an episode with both manic and depressive symptoms (a mixed episode). The symptoms of BP can be severe, resulting in damaged relationships, poor job or school performance, and even suicide. Bipolar I (BPI) disorder is defined in the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV) by manic or mixed episodes that are present for at least seven days or by manic symptoms so severe that the person requires hospitalization. The person also usually has depressive episodes that last at least two weeks. The mean age at onset for BPI is 18.4 years and the lifetime prevalence of BPI is 0.6% (1).

BP has a substantial genetic component. Monozygotic twin concordance rates range from 45 to 70% and sibling recurrence risk ranges from 5 to 10% (2). Genome Wide Association Studies (GWAS) have tried to identify genetic variants that might increase BP risk, with recent results identifying four common variants with modest effects (3-8).

BP may occur in conjunction with a number of other disorders. It has been shown to be characterized by increased lifetime rates of anxiety disorders and substance use in comparison to controls (1). Increased rates of other psychiatric disorders such as obsessive-compulsive disorders, panic disorder, eating disorders and attention-deficit hyperactivity disorder (ADHD), and medical conditions such as migraine, cardiovascular and endocrine abnormalities have been reported (9, 10). Substance use disorders, especially alcohol abuse/dependence, are highly prevalent (36.6%) (1) in BP patients and are associated with significant mortality and morbidity (11). The co-occurrence of alcohol use disorders and BP is associated with numerous

negative consequences, such as greater risk of medication noncompliance, slower recovery from mood episodes and more frequent hospitalizations (12).

The aim of this study is to determine whether a subphenotype of BPI subjects can be defined based on the presence of externalizing disorders, and whether these subjects are clinically and/or genetically different from those who did not have externalizing disorders. BPI subjects were recruited by the National Institute of Mental Health (NIMH) Bipolar Disorder Genetics Initiative. A subset of subjects genotyped as part of the Genetic Association Information Network (GAIN) (6, 13) was used as the Discovery sample; a different subset genotyped at the Translational Genomics Research Institute (TGEN) (8) was used in the Validation phase. Many course of illness parameters have been shown to be heritable (14) and thus we also performed GWAS analyses using the combined sample to identify genetic variations that may help characterize these groups.

Method

Clinical parameters

The BPI subjects were selected from those collected and characterized by the National Institute of Mental Health (NIMH) Bipolar Disorder Genetics Initiative over the past 18 years. The subjects were collected in five waves. Waves 1-4 comprise the Bipolar Family Dataset (BFD), which includes 2,936 subjects from 646 pedigrees, each ascertained via a proband with a BPI diagnosis and an additional first degree relative with BPI or Schizoaffective Disorder, Bipolar Type (SABP) diagnosis (15, 16). All subjects were diagnosed with a standard best-estimate procedure (see below). Waves 1 and 2 involved recruitment of primarily large pedigrees through four sites (Indiana University, Johns Hopkins University, the National Institute of Mental Health Intramural (NIMH) Program, and Washington University at St. Louis) (15). In Waves 3 and 4, smaller pedigrees with a minimal ascertainment criterion of a BPI-BPI/SABP affected sibling pair

were recruited through an expanded series of sites (i.e., including those above, as well as University of Pennsylvania, University of California at San Diego, University of California at Irvine, University of California at San Francisco, University of Iowa, University of Chicago, and Rush University). In Wave 5, BPI cases only were recruited through the above sites with the addition of Howard University.

After description of the study to the subjects, written informed consent was obtained.

Discovery Sample. The BPI subjects include 162 unrelated European American (EA) subjects from Waves 1 and 2, and 382 unrelated EA subjects from Waves 3 and 4 (16). In addition, the sample included 724 subjects from the Wave 5 data collection, which included 317 unrelated African American (AA) subjects and 407 unrelated EA subjects. Subjects from Waves 1-5 of the Genetics Initiative totaled 951 EA individuals and 317 AA individuals. EA status was determined based on the subject's self-report that all four grandparents were of EA heritage. AA status was based on self-report of at least one grandparent being of AA heritage.

The 1268 BPI subjects were divided into the following groups: 1) the Non-externalizing group-472 subjects; 2) the Externalizing group-796 subjects who had at least one externalizing disorder and 3) the Early-onset subgroup-329 subjects in the externalizing group who had two or more symptoms of conduct disorder (CD) prior to age 15. Externalizing disorders included one or more of the following DSM-IV diagnoses: alcohol abuse/dependence, drug abuse/dependence, pathological gambling, anti-social personality disorder (ASPD), ADHD and CD (Table 1).

Validation Sample. The validation sample consisted of 1237 BPI subjects from Wave 5 of the NIMH Genetics Initiative (8) who were not included in the discovery sample. Based on the above mentioned criteria, these subjects were also divided into 1) the Non-externalizing group-

436 subjects; 2) the Externalizing group-801 subjects and 3) the Early-onset sub-group-307 subjects (Table 1).

Clinical Assessment. All subjects were interviewed with the Diagnostic Interview for Genetic Studies (DIGS) (17), a diagnostic instrument developed for determining mood disorders and related conditions and shown to have excellent test-retest reliability. Final diagnoses were made by two independent clinicians incorporating all available information using a best-estimate procedure (Best Estimate Final Diagnosis or BEFD). If no agreement could be reached between the two clinicians, a third clinician reviews all information and determines a final diagnosis. Waves 1 and 2 were assessed with the DIGS 2.0, and Waves 3 and 4 were assessed with the DIGS 3.0 (16). The DIGS was revised (DIGS 4.0) between Waves 4 and 5 to allow collection of additional data on posttraumatic stress disorder and ADHD, as well as additional phenotypic information on BP. A change was also made in the BEFD process at the start of Wave 5 to incorporate clinician judgment of multiple phenotypic indicators including diagnosis by DSM-IV, DSM-III-R, the Research Diagnostic Criteria (RDC) (18), age at onset and number of episodes for depression, hypomania and mania, temporal relationship of mood disorder to substance abuse and psychosis, evidence of mixed episodes and rapid cycling, and a summary of family history information. All of these indicators were scored independently by a senior clinician (generally a psychiatrist) based on all available information, including medical records, interviewer observations, the coded DIGS, and the Family Instrument for Genetic Studies (FIGS, developed for the NIMH Genetics Initiative; available at https://www.nimhgenetics.org/). The FIGS incorporates clinician judgment on family patterns of illness, including presence or absence of BP, unipolar disorder, and/or other psychiatric disorders in first- and second-degree relatives.

Statistical Analyses. Statistical analyses were performed between the non-externalizing group and the externalizing group of BPI subjects (including both early-onset and later-onset subjects), and between the non-externalizing group and the early-onset subgroup. Categorical variables were analyzed using the Pearson chi-square test (two-sided) and continuous variables were analyzed using an independent samples t-test (two-sided). Levene's Test for Equality of Variances was performed to check the equality of variances assumption. All statistical analyses were performed in PASW Statistics 18.

Genome-wide Association Analyses

Genotyping and Quality Control of Data Available on dbGaP. Genotyping was carried out at the The Broad Institute Center for Genotyping and Analysis. PicoGreen fluorometry was used to check DNA quantity, and sample quality was initially assessed by genotyping a 24-single nucleotide polymorphism (SNP) panel on the Sequenom iPLEX platform containing a sex determining assay. Samples were plated at 50ng/uL in 96 well plates at the Rutgers University Cell and DNA Repository. The Centre d'Etude du Polymorphisme Human (CEPH; http://www.cephb.fr/en/cephdb/) sample NA12144 was placed on each production plate at the Broad Institute. Genotyping was carried out separately for the EA and AA samples using the Affymetrix Genome-Wide Human SNP Array 6.0. Allele calling was performed using the BirdSeed algorithm Affymetrix Power Tools version apt-1.8.6 and cluster models ('priors') file. Concordance between genotypes from the array and those from the initial quality control (QC) panel was evaluated to confirm sample ID. BPI EA Discovery and Validation samples were pooled together for the GWAS.

Samples were not used in the analysis if they had a low call rate (<98.5%) or incompatibility between reported gender and genetically determined gender. Pairwise identity-by-descent estimation was used to check for unexpected familial relationships in PLINK v1.07 (19). SNPs were not analyzed if the minor allele frequency was <0.01, call rate <95%, Hardy

Weinberg Equilibrium was violated ($p<10^{-6}$) in control samples, if there were three or more Mendelian errors, or if there was more than one discrepancy among duplicate samples. 2064 samples and 677,171 SNPs passed all QC tests. Further information on QC can be obtained in (6, 8).

Statistical Analyses. All genetic analyses were conducted using PLINK v1.07 (19). The analyses involved testing the association of each SNP coded additively with externalizing and early-onset status, with subject sex included as a covariate in the logistic model employed. Manhattan plots (Figure SF1) displaying chromosomal position (x-axis) against the $-\log_{10}(p)$ value for association were generated in Haploview version 4.2.

Results

Subject Distribution

1268 BPI subjects (951 EA, 317 AA) were used in the Discovery phase of the analysis and the findings were validated using 1237 BPI subjects in the Validation sample (Table 1). The distribution of BPI subjects among the non-externalizing and externalizing groups, and the early-onset subgroup was similar for the two samples.

Prevalence of Externalizing Disorders

We then examined the distribution of externalizing disorders in the externalizing group of BPI subjects in the two studies (Table 2). A majority of subjects had alcohol abuse/dependence and/or drug abuse/dependence. A higher proportion ($p<0.05$) of Discovery subjects had drug abuse/dependence and ASPD compared to Validation sample subjects. Conversely, a higher proportion ($p<0.05$) of Validation sample subjects had ADHD compared to Discovery subjects, but this may be related to assessment methodology (see Discussion). Further information of the

breakdown of alcohol abuse, alcohol dependence, drug abuse and drug dependence in the externalizing group of BPI subjects in the entire Discovery and Validation sample cohort can be found in Table ST1.

Clinical Characteristics

A number of clinical parameters were found to be significantly (p<0.05) different when comparing the externalizing group to the non-externalizing group of BPI subjects in the Discovery sample (Tables 3 and 4). Subjects in the externalizing group were more likely to be male (χ^2=9.012, df=1, p=0.003), to be disabled (χ^2=6.932, df=1, p=0.008) and to have fewer years of schooling (t=6.694, df=1217, p<0.001). They had an earlier age at onset of BPI (t=7.250, df=776.393, p<0.001) (including earlier onset ages for both depression (t=5.533, df=796.274, p<0.001) and mania (t=5.807, df=837.128, p<0.001)), a higher number of clean depressive (χ^2=20.902, df=1, p<0.001) and manic episodes (χ^2=12.613, df=1, p<0.001) and a higher frequency of episodes (χ^2=9.763, df=1, p=0.002; χ^2=4.179, df=1, p=0.041). They had an increased frequency of incidents of (non-suicidal) self-harm (χ^2=25.797, df=1, p<0.001) and more suicide attempts (t=-2.680, df=478.609, p=0.008). They were rated as more impaired on the interepisode Global Assessment Scale (GAS) (t=3.771, df=474, p<0.001) and were more likely to report a history of rapid switching (χ^2=21.696, df=1, p<0.001) and rapid cycling (χ^2=25.516, df=1, p<0.001). They also tended to start drinking (t=6.392, df=617, p<0.001) and use tobacco at an earlier age (t=2.855, df=821, p=0.004). These findings were also replicated in BPI subjects in the Validation sample (Tables ST2 and ST3).

Examination of the clinical variables in the early-onset subgroup shows the same pattern as in the externalizing group as a whole, but in general the differences are greater. Non-suicidal self-harm was seen in 30.2% of early-onset subjects as compared to 10.0% of non-externalizing subjects (and 26.0% of externalizing subjects as a whole). Rapid switching was seen in 70.6% of early-onset subjects as compared to 48.6% of non-externalizing subjects (and 62.8% of

externalizing subjects). Although they are younger, 56.7% of early-onset externalizing subjects have had >8 clean depressive episodes in their lifetime compared with 35.5% of non-externalizing subjects (and 49.5% of externalizing subjects); 55.1% have had >4 clean manic episodes in their lifetime compared with 42.8% of non-externalizing subjects (and 53.5% of externalizing subjects). They have an earlier age of onset of bipolar disorder by more than 5 years (at 16.0 years compared to 20.4 years) and these ages are similar to the ages of first depressive episode; age of first mania is also advanced by more than 5 years (21.3 years compared to 26.6 years). Number of suicide attempts is greater (3.6 vs 2.6) and age of first attempt is >4 years earlier (20.3 years vs 24.5 years). Patterns are very similar in the Validation sample.

Genetic Association Analyses

Two GWAS analyses were performed comparing: 1) 784 subjects in the non-externalizing group vs. 1280 subjects in the externalizing group; 2) 784 subjects in the non-externalizing group vs. 502 subjects in the early-onset subgroup. QC was performed on all subjects and all SNPs, as summarized above. Although no SNP reached genome-wide significance ($p<5 \times 10^{-8}$) from the two analyses, a number of SNPs were found to be associated at a p-value of 10^{-5} or lower. Manhattan plots and details of 20 SNPs with the strongest association signals from each of the two analyses are shown in Figure SF1 and Table 5. Two SNPs (rs17099448 and rs17622252) were among the top 20 SNPs in both analyses. We also performed GWAS analyses comparing each of the three groups with the control group, but did not identify any genome-wide significant SNPs in any of these comparisons (data not shown).

Discussion

A majority of Discovery and Validation sample BPI subjects had externalizing disorders. Previous studies have shown that BP co-occurs with a number of medical conditions such as substance use disorders, diabetes, obesity, migraine and anxiety (9, 10). Two factors: internalizing and externalizing, have been shown to substantially account for psychiatric comorbidity among BPI subjects (Monahan et al., submitted). In that report, four subject clusters of comorbidity in BPI subjects were identified: pure BPI, BPI plus one or more internalizing disorders, BPI plus one or more externalizing disorders, and BPI plus internalizing and externalizing disorders.

We noted a higher proportion of subjects in the Validation sample with ADHD compared to the Discovery sample. A section for adult ADHD was included in DIGS 4.0 used for Wave 5 subjects, but not in earlier versions. The earlier versions had only a retrospective report of childhood ADHD. Inclusion of the adult ADHD section may have increased the frequency of ADHD reports (including retrospective childhood ADHD). It is not clear to us how to explain the decrease in ASPD diagnoses and drug abuse/dependence diagnoses in the Validation Sample compared to the Discovery sample. Site effects cannot be ruled out (as the four original sites contributed to a larger proportion of the Discovery sample in comparison to the Validation sample), and such effects have been demonstrated in other studies of bipolar subphenotypes (20). However it is reassuring to note the similarity of the proportions of externalizing and early-onset subjects in the two samples, as well as the very similar results with respect to clinical variables (Tables 3 and 4; Supplementary Tables 2 and 3).

A significant difference in a majority of clinical parameters were found when we compared subjects in the non-externalizing and externalizing groups as well as subjects in the non-externalizing group and the early-onset subgroup (Tables 3, 4, ST2 and ST3). A possible explanation for the differences seen between these groups is the high proportion of subjects with an alcohol abuse/dependence and/or drug abuse/dependence (Table 2). Substance use has been shown to be highly prevalent in BP patients (1, 21). BP patients with co-occurring

substance use disorders have an earlier age at onset of first mood episodes, higher rates of mixed or dysphoric mania, rapid cycling, increased manic and depressed symptom severity and higher levels of novelty seeking, suicidality, aggression and impulsivity when compared to BP patients without co-occurring alcohol use disorders (12, 22-24). The presence of substance abuse also makes it more difficult to treat BP (25).

To determine whether the differences in the clinical parameters between subjects in the non-externalizing and externalizing groups can be accounted by externalizing disorders, separate linear regression analyses were performed using frequency of clean depressive episodes/clean manic episodes as the dependent variable, and externalizing disorder status, presence of alcohol abuse/dependence, presence of drug abuse/dependence, ethnicity, presence/absence of first degree relatives with BPI and cohort (ascertainment Wave) as independent variables controlling for age and gender in the Discovery sample. The frequency of clean depressive episodes was found to be a function of externalizing disorder status (standardized β=0.119, p=0.021), but was not completely explained by the presence of alcohol abuse/dependence (p>0.05) or drug abuse/dependence (p>0.05). The frequency of clean manic episodes was not found to be a function of externalizing disorder status (p>0.05), but appeared to be related to cohort (Waves 1 and 2: standardized β=0.069, p=0.047; Waves 3 and 4: standardized β=0.084, p=0.025, compared to Wave 5). This may be related to the preponderance of heavily loaded families in Waves 1-4 in comparison to Wave 5. We further examined the effect of externalizing disorder status controlling for alcohol abuse/dependence on age at onset of BPI, one of the clinical parameters observed to be significantly (p<0.05) different between the non-externalizing and externalizing groups. A significant (p<0.05) effect of the externalizing disorder status was still observed (data not shown). This suggests that other externalizing disorders such as ASPD (26), pathological gambling (27), ADHD and CD (28) may explain a substantial part of the differences seen in the clinical parameters between the externalizing and non-externalizing groups warranting further investigation.

The most remarkable result of this study was the increased severity of affective illness in the externalizing group and particularly in the early-onset externalizing subgroup. In every parameter tested, subjects with externalizing disorders show evidence of greater symptomatology, earlier onset, and more impairment. This is true even when care is taken to exclude the direct effects of substances (e.g. the exclusion of substance-related depressive or manic episodes). In general the chronology suggests that substance use precedes episodes of major mood disorder but it is not clear whether minor mood problems may precede substance use or abuse. In any case, the divisions highlighted in this report appear to be clinically salient. Also, the analyses noted above suggest that the group differences are not entirely explainable by substance use.

Although no SNP reached genome-wide significance ($p<5 \times 10^{-8}$) in the two GWAS analyses, a number of potential candidate SNPs were found to be nominally associated ($p<10^{-5}$) (Table 5 and Figure SF1). Two intronic SNPs (rs9359856 and rs6934804) and one coding SNP (rs2273238) were identified in the *ANKRD6* (ankyrin repeat domain 6) gene on chromosome 6. Also known as Diversin, the gene has been shown to be an essential component of the Wnt-signaling pathway, controlling fusion of heart precursors and gastrulation movements in zebrafish embryogenesis (29, 30). Variants in other identified genes identified have been associated with fasting glucose-related traits (*GLIS3*, *TMEM195* and *ZMAT4*) (31, 32) and Alzheimer's disease (*MTHFD1L*) (33). Thus, a number of SNPs associated with other diseases/complex traits may play a role in predisposition to externalizing disorders in the presence of bipolar illness.

The present study includes >2500 unrelated BPI subjects in two samples with clinical and genetic data. Subjects from two different ethnic populations (EA and AA) were analyzed for clinical parameters. However, this is a modest sample size for GWAS analyses and possible significant associations are likely to have been missed. Validation at genome-wide significance levels would be needed to confirm the suggestive associations identified in the present study.

In sum, we have demonstrated that BPI subjects with externalizing disorders tend to have a poorer clinical outcome then those without externalizing disorders, and this is particularly true of those subjects with early onset of conduct disorder symptoms. Although no SNP reached genome-wide significance in the GWAS analyses, a number of SNPs were nominally associated with the externalizing disorder group of subjects and a number were associated with the early onset subgroup. These results suggest the presence of an externalizing disorder subphenotype within BPI warranting further investigation.

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TABLE 1. BPI Subject Distribution

	Non-externalizing group		Externalizing group		Total N	Early-onset subgroup	
	N	% of Total N	N	% of Total N		N	% of Externalizing group N
Discovery subjects	472	37.2	796	62.8	1268	329	41.3
European Americans	385	40.5	566	59.5	951	235	41.5
African Americans	87	27.4	230	72.6	317	93	40.4
Validation sample subjects	436	35.2	801	64.8	1237	307	38.3

TABLE 2. Breakdown of Externalizing Disorders in the Externalizing Group of Discovery and Validation Sample BPI Subjects

Discovery and Validation sample subjects							
Externalizing disorder	Discovery (N=796)		Validation sample (N=801)		χ^2	df	*p* (two-sided)
	N	%	N	%			
Alcohol abuse/dependence	591	74.2	607	75.8	0.501	1	0.479
Drug abuse/dependence	528	66.3	475	59.3	8.448	1	0.004
Pathological gambling	43	5.4	44	5.5	0.006	1	0.936
Anti-social personality disorder	110	13.8	58	7.2	18.353	1	<0.001
Attention-deficit hyperactivity disorder	153	19.2	263	32.8	38.407	1	<0.001
Conduct disorder	29	3.6	28	3.5	0.025	1	0.874

TABLE 3. Clinical Features in Discovery BPI Subjects Grouped by Externalizing Disorders (Categorical Parameters)

Parameter	NE[a]		E[a]		EO[a]		χ^2 (1 df)		*p* (two-sided)		OR: Value		OR: 95% Confidence Interval			
													Lower		Upper	
	N	%	N	%	N	%	NE,E	NE,EO	NE,E	NE,EO	NE,E	NE,EO	NE,E	NE,EO	NE,E	NE,EO
Gender:																
Male	185	39.2	381	47.9	172	52.3	9.012	13.435	0.003	<0.001	0.702	0.588	0.557	0.443	0.885	0.782
Female	287	60.8	415	52.1	157	47.7										
Disabled:																
Yes	92	20.4	208	27.2	97	30.0	6.932	9.355	0.008	0.002	0.688	0.599	0.521	0.430	0.910	0.833
No	358	79.6	557	60.9	226	70.0										
Self harm:																
Yes	25	10.0	122	26.0	73	32.0	25.797	35.719	<0.001	<0.001	0.316	0.235	0.199	0.143	0.501	0.386
No	226	90.0	348	74.0	155	68.0										
Rapid switching:																
Yes	205	48.6	434	62.8	218	70.6	21.696	35.321	<0.001	<0.001	0.559	0.394	0.438	0.289	0.715	0.538
No	217	51.4	257	37.2	91	29.4										

Rapid cycling																
Yes	182	44.6	403	60.4	201	68.1	25.516	38.216	<0.001	<0.001	0.528	0.377	0.411	0.275	0.677	0.515
No	226	55.4	264	39.6	94	31.9										
Number of clean depressive episodes:																
1 to 8	267	64.5	364	50.5	130	43.3	20.902	31.548	<0.001	<0.001	1.781	2.375	1.389	1.752	2.284	3.220
>8	147	35.5	357	49.5	170	56.7										
Number of clean depressive episodes per year:																
0 to 0.4	224	55.3	317	45.5	130	44.5	9.763	7.901	0.002	0.005	1.480	1.542	1.157	1.139	1.893	2.087
>0.4	181	44.7	379	54.5	162	55.5										
Number of																

clean manic episodes:																
1 to 4	254	57.2	343	46.5	136	44.9	12.613	10.960	<0.001	0.001	1.536	1.642	1.211	1.223	1.947	2.203
>4	190	42.8	394	53.5	167	55.1										
Number of clean manic episodes per year:																
0 to 0.4	240	55.7	345	49.4	144	49.5	4.179	2.682	0.041	0.101	1.286	1.283	1.010	0.952	1.636	1.728
>0.4	191	44.3	353	50.6	147	50.5										

[a] NE-Non-externalizing group, E-Externalizing group, EO-Early-onset sub-group.

TABLE 4. Clinical Features in Discovery BPI Subjects Grouped by Externalizing Disorders (Continuous Parameters)

Parameter	NE[b]		E[b]		EO[b]		t		df		*p* (two-sided)[c]	
	Mean	SD	Mean	SD	Mean	SD	NE,E	NE,EO	NE,E	NE,EO	NE,E	NE,EO
Age	44.01	13.49	41.06	11.52	39.80	11.00	3.954	4.825	862.231	770.462	<0.001	<0.001
	(N=467)		(N=782)		(N=325)							
Years of school	15.18	2.81	14.06	2.84	13.45	2.82	6.694	8.464	1217	775	<0.001	<0.001
	(N=452)		(N=767)		(N=325)							
Age at onset of illness	21.36	9.92	17.38	7.94	16.03	7.55	7.250	8.460	776.393	764.647	<0.001	<0.001
	(N=447)		(N=774)		(N=322)							
Age at onset of depression	21.93	10.70	18.48	9.46	16.93	9.10	5.533	6.840	796.274	719.155	<0.001	<0.001
	(N=425)		(N=746)		(N=312)							
Age at onset of mania	26.61	11.52	22.83	9.84	21.29	9.46	5.807	6.962	837.128	733.888	<0.001	<0.001
	(N=452)		(N=750)		(N=309)							
Age of first drink	16.72	4.68	14.06	4.88	12.98	4.32	6.392	8.345	617	401	<0.001	<0.001

	(N=197)		(N=422)		(N=206)							
Age of first tobacco use	16.03	5.35	14.89	5.23	14.02	5.47	2.855	4.208	821	511	0.004	<0.001
	(N=244)		(N=579)		(N=269)							
Number of suicide attempts	2.62	2.55	3.51	4.99	3.64	4.27	-2.680	-2.661	478.609	301.360	0.008	0.008
	(N=144)		(N=380)		(N=181)							
Age of first suicide attempt	24.46	10.32	21.26	8.44	20.30	8.54	2.676	3.012	301	187	0.008	0.003
	(N=74)		(N=229)		(N=115)							
Interepisode GAS[a]	75.30	14.94	70.14	14.64	69.29	14.89	3.771	3.389	474	312	<0.001	0.001
	(N=206)		(N=270)		(N=108)							

[a] GAS-Global Assessment Scale.

[b] NE-Non-externalizing group, E-Externalizing group, EO-Early-onset subgroup.

[c] *p*-values were determined after performing Levene's Test for Equality of Variances to check equality of variances assumption.

TABLE 5. SNPs with the Strongest Association Signals from the GWAS Analyses of BPI Subjects

SNP	Chromosome	Base pair position[a]	Closest gene[a,b]	SNP location relative to gene[a]	Minor Allele Frequency	*p*	Odds Ratio
Externalizing group vs. non-externalizing group							
rs17099448	1	76921007	*ST6GALNAC3*	51.79kb downstream	0.025	2.56E-06	0.4633
rs17622252	6	21439886	*CDKAL1*	100.15kb downstream	0.1333	4.39E-06	1.658
rs2764655	1	76942171	*ST6GALNAC3*	72.96kb downstream	0.025	6.62E-06	0.4774
rs10090419	8	124097812	*DERL1*	Intron	0.175	1.94E-05	0.7077
rs2071842	22	41313726	*POLDIP3*	Intron	0.1667	2.16E-05	0.661
rs2958709	8	106838246	*ZFPM2*	Intron	0.1417	2.31E-05	0.6872
rs2273238	6	90383081	*ANKRD6*	Coding region	0.0417	2.33E-05	0.5065
rs11015506	10	27409689	*ANKRD26*	Intron	0.025	2.65E-05	3.33
rs2997227	10	122575842	*WDR11*	24.85kb upstream	0.0778	2.98E-05	0.7426
rs6461161	7	15239240	*TMEM195*	Intron	0.3083	3.00E-05	1.327
rs4869963	6	151341417	*MTHFD1L*	Intron	0.2833	3.48E-05	0.7507
rs7913464	10	23551309	*PTF1A*	28.13kb downstream	0.0583	3.76E-05	0.5527
rs16851722	2	166741590	*SCN9A*	18.36kb downstream	0.0583	4.40E-05	1.655
rs333846	2	117695664	-	-	0.025	4.44E-05	4.72

rs4341952	2	166728262	*SCN9A*	31.68kb downstream	0.1917	4.47E-05	1.393
rs1779518	14	40797200	-	-	0.3222	4.61E-05	1.424
rs7739248	6	151323361	*MTHFD1L*	Intron	0.15	4.85E-05	0.7254
rs11997383	8	40574573	*ZMAT4*	Intron	0.1889	4.85E-05	0.618
rs16831019	2	135259447	*ACMSD*	53.21kb upstream	0.025	4.91E-05	0.4319
rs11013397	10	23660815	*C10orf67*	Intron	0.0667	5.10E-05	0.5823
Early-onset subgroup vs. non-externalizing group							
rs9359856	6	90364704	*ANKRD6*	Intron	0.2	4.52E-06	0.6091
rs4761053	12	127072154	-	-	0.2583	6.95E-06	1.525
rs6934804	6	90372739	*ANKRD6*	Intron	0.1833	1.18E-05	0.6201
rs1438108	20	1792858	*SIRPA*	29.96kb upstream	0.0417	1.45E-05	0.4555
rs1526303	2	174151593	-	-	0.3778	1.93E-05	0.7042
rs1871946	2	213587652	*IKZF2*	Intron	0.4407	2.24E-05	1.413
rs2665452	7	97285019	*ASNS*	34.36kb downstream	0.2333	2.39E-05	0.6398
rs17622252	6	21439886	*CDKAL1*	100.15kb downstream	0.1333	2.71E-05	1.746
rs13080973	3	140078740	*FOXL2*	67.02kb downstream	0.1583	2.89E-05	1.553
rs12538214	7	154969302	*CNPY1*	17.42kb downstream	0.2167	2.91E-05	1.484
rs2289439	15	87486790	*ABHD2*	Intron	0.1083	3.15E-05	0.5478

rs4726457	7	139269639	*TBXAS1*	Intron	0.275	3.45E-05	0.6856
rs2242400	12	24989341	*BCAT1*	Intron	0.1167	3.48E-05	1.733
rs742002	22	25765086	-	-	0.2917	4.14E-05	0.683
rs4339696	9	4285880	*GLIS3*	Intron	0.4333	4.27E-05	1.403
rs2216316	7	53451934	-	-	0.1083	4.31E-05	1.539
rs2530132	7	97286662	*ASNS*	32.72kb downstream	0.2333	4.37E-05	0.6502
rs10868082	9	85828929	*RMI1*	20.12kb downstream	0.1833	4.38E-05	0.6671
rs17099448	1	76921007	*ST6GALNAC3*	51.79kb downstream	0.025	4.51E-05	0.3724
rs1560651	5	167530850	*ODZ2*	Intron	0.4833	4.59E-05	1.392

[a] Genomic locations are based on NCBI Build 36.3.

[b] Closest genes were genes in which the SNP was present or genes located within 200kb (kilobases) of the SNP.