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Research report

Characteristics of Bipolar I patients grouped by externalizing disorders

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ABSTRACT

Background: 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 and a subgroup with externalizing symptoms prior to age 15 have different clinical features than those without externalizing disorders and whether these could be attributed to specific genetic variations.
Methods: A large cohort ($N=2505$) of Bipolar I subjects was analyzed. Course of illness parameters were compared between an Externalizing Group, an Early-Onset Subgroup and a Non-Externalizing Group in the Discovery sample ($N=1268$). Findings were validated using an independent set of 1237 BPI subjects (Validation sample). Genetic analyses were carried out.
Results: Subjects in the Externalizing Group (and Early-Onset Subgroup) tended to have a more severe clinical course, even in areas specifically related to mood disorder such as cycling frequency and rapid mood switching. Regression analysis showed that the differences are not completely explainable by substance use. Genetic analyses identified nominally associated SNPs; calcium channel genes were not enriched in the gene variants identified.
Limitations: Validation in independent samples is needed to confirm the genetic findings in the present study.
Conclusions: Our findings support the presence of an externalizing disorder subphenotype within BPI with greater severity of mood disorder and possible specific genetic features.

1. Introduction

Bipolar disorder (BP) includes distinct episodes with altered mood, activity, and thought patterns. The mean age at onset for Bipolar Disorder Type I (BPI) is 18.4 years and the lifetime prevalence is 0.6% (Merikangas et al., 2011). BP may occur in conjunction with a number of other disorders such as substance use and anxiety disorders (Merikangas et al., 2011). Substance use has been shown to be highly prevalent in BP patients (Merikangas et al., 2011; Regier et al., 1990). Prior studies have identified more severe outcomes among BP patients with co-occurring substance use disorders (Cardoso et al., 2008; Elizabeth Sublette et al., 2009; Frye and Salloum, 2006; Grunebaum et al., 2006). The presence of substance abuse also makes it more difficult to treat BP (Swann, 2010). The disorder has a substantial genetic component. Monozygotic twin concordance rates range from 45% to 70% and sibling recurrence risk ranges from 5% to 10% (Craddock and Forty, 2006). Genome-Wide Association Studies (GWAS) have identified 10 common variants with modest effects (Chen et al., 2013; Ferreira et al., 2008; Seifuddin et al., 2012; Sklar, 2013; Sklar et al., 2011; Smith et al., 2009, 2011), and analyses using the entire set of variants tested with common GWAS platforms suggest that many additional vulnerability genes remain to be identified as larger samples become available (Cross-Disorder Group of the Psychiatric Genomics Consortium et al., 2013; Lee et al., 2011). Many course of illness parameters have also been shown to be heritable (Potash et al., 2007). 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. We were particularly interested in whether the characteristics of the mood disorder itself (apart from externalizing symptoms) differentiated the subgroups. Two sets of BPI subj ects ascertained by the National Institute of Mental Health (NIMH) Bipolar Disorder Genetics Initiative and evaluated with the Diagn ostic Instrument for Genetic Studies (Nurnberger et al., 1994) were

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included (a Discovery sample and a Validation sample). We also performed GWAS analyses using the combined sample to identify genetic variations that may help characterize these groups.

2. Methods

2.1. Clinical parameters

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. Subjects were from Indiana University, Johns Hopkins University, the National Institute of Mental Health Intramural (NIMH) Program, Washington University at St. Louis, 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, Rush University, and Howard University.

The study protocol was approved by the Institutional Review Board of the respective universities. After description of the study to the subjects, written informed consent was obtained.

2.1.1. Discovery sample

Subjects totaled 951 unrelated European American (EA) individuals and 317 unrelated African American (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 (Table 1).

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 sample 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

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), attention-deficit hyperactivity disorder (ADHD) and CD (Table 2). Of note, about 70% of subjects in this sample came from multiplex families (additional affected relatives with BPI disorder).

2.1.2. Validation sample

The validation sample consisted of 1237 unrelated EA BPI subjects from the same study. 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 Subgroup-307 subjects (Table 1). Of note, about 20% of subjects in this sample came from multiplex families.

2.1.3. Clinical assessment

All subjects were interviewed with the Diagnostic Interview for Genetic Studies (DIGS) (Nurnberger et al., 1994), 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.

2.2. Genome-wide association analyses

2.2.1. Genotyping and quality control of data available on dbGaP

Genotyping was carried out at 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 50 ng/μl 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 (Purcell et al., 2007). 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

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 sample (*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