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Research report

# A genome-wide association study of seasonal pattern mania identifies *NF1A* as a possible susceptibility gene for bipolar disorder

Heon-Jeong Lee [a,1], Hyun Goo Woo [b,1], Tiffany A. Greenwood [c], Daniel F. Kripke [c], John R. Kelsoe [c,d,*]

[a] Department of Psychiatry, Korea University College of Medicine, Seoul, South Korea
[b] Department of Physiology, Ajou University School of Medicine, Suwon, South Korea
[c] Department of Psychiatry and Institute for Genomic Medicine, University of San Diego, La Jolla, CA, USA
[d] San Diego VA Healthcare System, San Diego, CA, USA

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ABSTRACT

*Objective:* The use of subphenotypes may be an effective approach for genetic studies of complex diseases. Manic episodes with a seasonal pattern may distinguish phenotypic subgroups of bipolar subjects that may also differ genetically.
*Method:* We have performed a genome-wide association study using GAIN genotype data from the Bipolar Genome Study (BiGS) and bipolar subjects that were categorized as having either seasonal or non-seasonal patterned manic episodes.
*Results:* A bipolar case-only analysis identified three genomic regions that differed between seasonal and non-seasonal patterned manic episodes of bipolar subjects. The most significant association was for rs41350144, which lies within an intron of *NF1A* gene on 1p31 ($P=3.08\times10^{-7}$, OR=2.27). Haplotype construction using flanking three SNPs (rs41453448, rs1125777, and rs12568010) spanning 7549 bp showed a more significant association ($P=2.12\times10^{-7}$, OR=0.4).
*Conclusions:* These data suggest that genetic variants in the *NF1A* gene region may predispose to seasonal patterned of mania in bipolar disorder.



## 1. Introduction

Bipolar disorder (BD), also known as manic-depressive illness, has a lifetime prevalence of approximately 5–6.4% in the general population (Akiskal et al., 2000; Judd and Akiskal, 2003), although the lifetime prevalence of bipolar I disorder (BPI) is around 1%. Family, twin, and adoption studies suggest heritability estimates of 60–80% for BDI (Tsuang and Faraone, 1990). Although BD is highly heritable, the identification of specific genetic variations has yielded limited findings (Baum et al., 2008a, 2008b, Sklar et al., 2008, 2011, Wellcome Trust Case Control Consortium, 2007, Smith et al., 2011). Creating subgroups of patients with BD according to clinical subphenotypes has been suggested as a possible approach for further genetic studies in BD (McQueen et al., 2005).

Circadian rhythm dysfunction is hypothesized to play a role in the pathophysiology of BD (Kripke et al., 2009; Jones, 2001; Mansour et al., 2005; McClung, 2007; Wehr et al., 1983; McCarthy et al., 2012). BD patients usually demonstrate circadian rhythm-related symptoms, including a periodicity of manic-depressive episodes, diurnal variation of mood, and sleep disturbance, such as sleeplessness during mania and insomnia or hypersomnia during depression. Sleep disturbances may be caused by circadian dysfunctions in BD and may promote emotion dysregulation (Harvey et al., 2006). However, the causal relationship between sleep disturbance and emotional problems may be bidirectional (Dahl, 2004). Furthermore, sleep disturbances are very common symptoms in psychiatric illness, and most mood episodes heighten sleep problems. While it is difficult to distinguish core circadian disturbance as a subphenotype from the complex mood disorder symptomatology, a seasonal pattern of manic episodes is a more clearly recognizable sub-phenotype of circadian dysfunction in BD.

Seasonal pattern in mood disorders has been well recognized since ancient times when Hippocrates described the correlation between season and the precipitation of manic episodes in BD. Many studies have since revealed that BD patients have more manic episodes during the spring and summer (Barbini et al., 1995; Parker and Walter, 1982; Takei et al., 1992; Volpe and Del Porto, 2006; Lee et al., 2007; Mulder et al., 1990; Sayer et al., 1991). Although a positive association between the photoperiod and BD mania has been reported in some studies BDI patients

* Corresponding author at: University of California, Department of Psychiatry, San Diego, 9500 Gilman Drive, Mail Code 0603, La Jolla, CA 92093, United States Tel.: +1 858 534 5927; fax: +1 858 534 5527.
*E-mail address:* jkelsoe@ucsd.edu (J.R. Kelsoe).
[1] HJL and HGW equally contributed to the study.

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(Lee et al., 2002), others have not found an association (Silverstone et al., 1995; Whitney et al., 1999). Despite these contradictory findings, there seems to be some evidence for a higher prevalence of manic BD episodes in the spring and summer months. Seasonality in mood disorder was reported associated with a family history for mood disorders (Brambilla et al., 2012) and self-reported seasonal mood changes were reported to be heritable in a twin study (Jang et al., 1997).

These observations suggest that seasonal pattern subtypes of mania may represent genetically distinct subtypes of BD. We explored this hypothesis in a genomewide association (GWA) analysis of seasonal pattern mania *vs.* non-seasonal mania in BDI subjects and controls of European ancestry genotyped as part of Genetic Association Information Network (GAIN) by the Bipolar Genome Study (BiGS).

## 2. Methods

### 2.1. Subject ascertainment

For genotyping as part of the Bipolar Genome Study (BiGS), BDI subjects were selected from those collected by the NIMH Genetics Initiative for bipolar disorder in five waves at 11 sites across the United States as described elsewhere in detail (Smith et al., 2009). Recruitment for waves 1–2 consisted of extended multiplex families with a BDI proband, waves 3–4 included families with a BDI proband and at least one other sibling with BDI or schizoaffective disorder, bipolar type, whereas Wave 5 consisted of unrelated BDI cases. All subjects provided written informed consent according to the local IRB protocols and were interviewed using the Diagnostic Interview for Genetic Studies (DIGS) (Nurnberger et al., 1994). Information was obtained from other family informants and medical records and reviewed along with the interview by a panel of experienced clinicians to obtain a final best-estimate diagnosis. The complete DIGS interview, which includes over 2000 questions with detailed information regarding manic and depressive episodes, is available for these subjects.

Control subjects were selected from those ascertained through a NIMH-supported contract mechanism between Dr. Pablo Gejman and Knowledge Networks, Inc. All subjects donated a blood sample and were given a medical questionnaire. The selected controls were matched for gender and ethnicity with the BD cases, and all control subjects who endorsed a history of BD, psychosis, or recurrent major depression were excluded from our study.

### 2.2. Genotyping and cleaning

The initial sample was genotyped at the Broad Institute as part of the Genetic Association Information Network (GAIN) using the Affymetrix 6.0 (1M SNP) array. A total of 1001 BD cases, 1033 controls, and 724,067 SNPs were available for analysis following an extensive QC process (Smith et al., 2009) to eliminate individuals with $>10\%$ missing data and SNPs with poor allele clustering, $>10\%$ missing data, duplicate errors, minor allele frequencies (MAFs) $<0.05$, and Hardy–Weinberg equilibrium $p<10^{-6}$. The second sample was similarly genotyped at the Translational Genomics Institute (TGEN) and underwent a comparable QC process that resulted in 1190 BD cases, 401 controls, and 728,187 SNPs available for analysis. An additional round of QC performed on the merged GAIN and TGEN samples resulted in 703,012 passing SNPs.

### 2.3. Phenotypes

Phenotypes were derived from the Phenome Database, which compiles data across the DIGS 2, 3, and 4 to arrive at a common set of variables for each subject in the sample (Potash et al., 2007). As a part of the DIGS interview, BD subjects were queried, "*Do your episodes (mania/hypomania) tend to begin in any particular season?*" Subjects who answered 'Spring' or 'Summer' were categorized as BD with seasonal patterned mania and subjects who answered 'no pattern' were categorized as BD with non-seasonal patterned mania. Those who answered 'Fall', 'Winter', or 'Unknown' or who endorsed more than one season were categorized as missing because their patterns may be not related to circadian response to increased light exposure during Spring or Summer. It may be an instead related to other social factors such as anniversary reactions during other seasons. There were 392 BD subjects in the seasonal mania (SM) group and 930 subjects in the non-seasonal mania (NSM) group.

### 2.4. Association analyses

To assess genetic factors contributing to seasonal patterned mania, we first performed a case-only analysis of SM *vs.* NSM. In order to differentiate those genetic factors that are unique to seasonal patterned mania, as opposed to those that may modify the expression of mania in BD, the SM group was compared to controls in a secondary analysis. A similar analysis was performed for NSM. All association analyses were performed using logistic regression in PLINK (Purcell et al., 2007) with covariate adjustment for sex. Label-switching permutations were performed to assess the empirical significance of the results, since spurious results may result from a sampling bias through the selection of a small subset of individuals.

### 2.5. Linkage disequilibrium analysis

Linkage disequilibrium (LD) among SNPs were obtained by $D'$ and r2 using Haploview with default parameters and HapMap CEU+TSI (R2). (Barrett et al., 2005) The proxy SNPs with regional recombination rates were assessed by using SNAP and 1000 Genomes Pilot 1 data (Johnson et al., 2008).

### 2.6. SNP imputation

The genotypes for missing markers in a dataset can be confidently inferred by LD and the correlation between genotypes in a reference data set. Association tests of genotyped markers should show similar levels of association compared with imputed markers. Missing SNPs were imputed using the expectation–maximization (E–M) algorithm in PLINK and the HapMap CEU r23a reference panel. The imputed SNPs were used for the functional enrichment analysis.

### 2.7. Functional enrichment analysis

Gene ontology analysis was performed on the gene sets harboring the identified SNPs ($P<0.005$) using DAVID software (Huang et al., 2009a, 2009b). The enriched gene functions with more than five genes were identified from the SM *vs.* NSM, SM *vs.* control, and NSM *vs.* control analyses, respectively.

## 3. Results

We performed a primary GWA of SM *vs.* NSM to identify SNPs associated with seasonal patterns of manic episodes. As shown in