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Association between the Arylalkylamine N-Acetyltransferase (*AANAT*) Gene and Seasonality in Patients with Bipolar Disorder

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Objective Bipolar disorder (BD) is complex genetic disorder. Therefore, approaches using clinical phenotypes such as biological rhythm disruption could be an alternative. In this study, we explored the relationship between melatonin pathway genes with circadian and seasonal rhythms of BD.

Methods We recruited clinically stable patients with BD (n=324). We measured the seasonal variation of mood and behavior (seasonality), and circadian preference, on a lifetime basis. We analyzed 34 variants in four genes (*MTNR1a*, *MTNR1b*, *AANAT*, *ASMT*) involved in the melatonin pathway.

Results Four variants were nominally associated with seasonality and circadian preference. After multiple test corrections, the rs116879618 in *AANAT* remained significantly associated with seasonality (corrected p=0.0151). When analyzing additional variants of *AANAT* through imputation, the rs117849139, rs77121614 and rs28936679 (corrected p=0.0086, 0.0154, and 0.0092) also showed a significant association with seasonality.

Conclusion This is the first study reporting the relationship between variants of *AANAT* and seasonality in patients with BD. Since *AANAT* controls the level of melatonin production in accordance with light and darkness, this study suggests that melatonin may be involved in the pathogenesis of BD, which frequently shows a seasonality of behaviors and symptom manifestations.

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Key Words Bipolar disorder, Seasonality, Circadian rhythm, Melatonin pathway, *AANAT*.

INTRODUCTION

Bipolar disorder (BD) is chronic psychiatric disorder characterized by episodic changes in mood and energy level.[1] BD has a high heritability, close to 80%.[2,3] However, because of the heterogeneity of the biological basis and complexity of genetic architecture, there have been limited findings in genetic studies.[4,5] Therefore, alternative approaches adopting specific clinical features and biological markers as phenotypes for genetic studies were suggested.[6-8]

Organisms have evolved innate biological clocks that oscillate with the environmental cycles of day and night (circadian rhythm) and photoperiod change (seasonal rhythm) for adaptation.[9-11] This process is also important for humans to adjust to external cues,[12,13] but abnormally exaggerated or dampened rhythmic behavioral changes have been observed in some mental disorders.[14,15] In BD, the variation of biological rhythms, such as seasonal mood, behavioral changes, or circadian rhythm abnormalities have been suggested as distinctive clinical characteristics.[16-18] Although abnormalities in biological rhythms are more prominent during episodes, they are also identified in euthymic state BD and regarded as lifetime traits. A seasonal

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pattern is common in BD, even among treated patients whose seasonal fluctuations may have been modified by such treatment. In addition, BD who not experienced either seasonal manic or depressive episodes, also reported to have higher scores for seasonal variation in mood and behavior when compared to the general population or patients with depression.[19,20] Climatic conditions may trigger BD symptoms or episodes[21-23] and antimanic drugs have been known to stabilize circadian rhythms.[24,25] Also, BD with seasonal depressive episodes or evening preference have been reported to be associated with other specific clinical profiles, e.g., bipolar II disorder subtype, comorbid eating disorders and premenstrual syndrome, more relapses and rapid cycling.[18,26-29]

Melatonin is a neurohormone secreted from the pineal gland during the hours of darkness. It falls rapidly with light onset. Arylalkylamine N-acetyltransferase (AANAT) is a key regulatory enzyme in the melatonin biosynthesis pathway, which converts serotonin to N-acetylserotonin. N-acetylserotonin is converted to melatonin by acetylserotonin O-methyltransferase (ASMT).[30] Oscillating levels of activated *AANAT* result in the rhythmic synthesis and secretion of melatonin.[31] Seasonal photoperiod-induced changes in melatonin secretion have wide-reaching effects on seasonal animal physiology and behavior, as the MT1 and MT2 melatonin receptors (encoded by *MTNR1a* and *MTNR1b*) are distributed widely throughout the body, including the central nervous system, heart, endocrine system, and immune system.[11,32]

Considering the abnormal biological rhythms and melatonin levels observed in patients with BD[17,18,33] and the role of melatonin in diurnal and seasonal changes,[11,32] melatonin seems to be related to the biological mechanisms that develops the clinical characteristics of the subgroup of BD. Genetic studies for BD focusing on genes in the melatonin pathway reported mixed results.[33-36] A genetic heterogeneity of BD may be one reasons for this discrepancy. There have also been association studies using biological rhythm disruption as an alternative phenotype in BD. Geoffroy et al.[37] reported a negative finding in their investigation of the association between seasonal depressive episodes and the circadian genes in the melatonin pathway. Other study regarding sleep patterns of BD and healthy controls showed an association between the *ASMT* variant and the inter-day stability of sleep.[38]

We hypothesized that patients with BD with abnormal biological rhythms as a lifetime trait are likely to have a specific genetic underpinning, especially related to the melatonin pathway. This study investigated the association between the melatonin pathway genes, the circadian preferences, and seasonal mood and behavior changes in BD.

METHODS

Subjects

We recruited clinically stable patients who met the DSM-IV diagnostic criteria for BD (n=324), including bipolar I disorder (n=182), bipolar II disorder (n=134) and BD not otherwise specified (n=8) between 18 and 60 years of age from the outpatient and inpatient units of the Samsung Medical Center (n=148) and the Seoul National University Bundang Hospital (n=176) in South Korea. Board-certified psychiatrists who had at least one year of research experience examined the participants' psychiatric diagnoses using the DSM-IV criteria with either the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID)[39] or the Korean version of the Diagnostic Interview for Genetic Studies (DIGS).[40] SCID was used at Seoul National University Bundang Hospital and DIGS was used at the Samsung Medical Center. No significant difference in terms of demographic characteristics was detected between participants evaluated using SCID and DIGS. We excluded participants if they could not clearly remember their lifetime traits due to illnesses. Moreover, to prevent the effect of current mood state or medication in evaluating their lifetime characteristics, we included participants after their medication had been stabilized. All participants were under the standard pharmacological treatment, including mood stabilizers (lithium, valproate, lamotrigine, and carbamazepine) or atypical antipsychotics (quetiapine, olanzapine, risperidone, aripiprazole, and ziprasidone). We excluded those with mental retardation, substance abuse, medical illnesses, or long-term use of hormonal agents known to affect mood. Because sustained social rhythm can deeply affect the biological rhythm,[41] we also excluded night-shift workers as participants. We obtained written informed consent from all subjects after a complete explanation of the study. This study was approved by the Institutional Review Boards of the Samsung Medical Center (IRB No. 2012-09-056) and the Seoul National University Bundang Hospital (IRB No. B-1105/128-008).

Measurements of phenotypes

We measured circadian preference by using the standardized Korean version of the Composite Scale of Morningness (CSM),[42,43] containing 13 questions concerning what time participants preferred to go to bed, wake up, and perform specific activities. The sum of the answers to these questions yielded a single score to represent the level of circadian preference (range, 13−55), with lower scores indicating a stronger evening preference. We measured seasonality with Seasonal Pattern Assessment Questionnaire (SPAQ)[44] containing 6 items to measure seasonal variations in sleep, social activity, mood, weight, appetite, and energy level. The Korean version of the SPAQ that

was translated into Korean was used with the permission of the original author. The translation and validation process for this scale has been described in our previous study.[45] We used the sum of individual item scores, each on a 5-point scale ranging from 0 (no change) to 4 (extremely marked change) to indicate a global seasonality score (GSS). Based on the criteria of Kasper et al.[46,47] for seasonal affective disorder (SAD) and subsyndromal SAD, and a GSS of 9 or greater, we designated scores with a subjective rating of having at least mild difficulty with seasonal changes [on a 6-point scale from 0 point (no difficulty) to 5 points (a disabling difficulty); 1 points=mild] or a GSS of 11 or higher as having significant seasonality. We assigned both seasonality and circadian preference on a lifetime-basis.

Table 1. Characteristics of SNPs in melatonin pathway genes

Gene	SNP	Genomic location*	Intragenic location	M	m	MAF	HWE
MTNR1a	rs28611030	Chr4:187448441	3'-near	A	G	0.213	0.867
MTNR1a	rs7440284	Chr4:187449434	3'-near	C	T	0.039	1.000
MTNR1a	rs1800884	Chr4:187455426	Exon 3	G	A	0.011	1.000
MTNR1a	rs34532313	Chr4:187460490	Intron 1	T	C	0.475	1.000
MTNR1a	rs116952947	Chr4:187463252	Intron 1	C	T	0.028	1.000
MTNR1a	rs6820205	Chr4:187464867	Intron 1	C	T	0.092	0.315
MTNR1a	rs13140444	Chr4:187465729	Intron 1	C	T	0.406	0.908
MTNR1a	rs149982127	Chr4:187472823	Intron 1	T	C	0.017	1.000
MTNR1a	rs4862706	Chr4:187473694	Intron 1	A	G	0.088	0.292
MTNR1a	rs1800885	Chr4:187476360	Exon 1	G	A	0.039	1.000
MTNR1a	rs76691596	Chr4:187479828	5'-near	G	T	0.056	0.066
MTNR1a	rs6858707	Chr4:187484762	5'-near	G	A	0.386	0.724
MTNR1a	rs13131052	Chr4:187485933	5'-near	T	G	0.196	0.110
MTNR1b	rs75715438	Chr11:92699831	5'-near	T	C	0.025	1.000
MTNR1b	rs75153006	Chr11:92700218	5'-near	G	T	0.037	0.357
MTNR1b	rs4753426	Chr11:92701596	5'-near	C	T	0.313	0.437
MTNR1b	rs10830963	Chr11:92708710	Intron 1	C	G	0.428	0.733
MTNR1b	rs3781637	Chr11:92713770	Intron 2	T	C	0.146	0.826
MTNR1b	rs148736119	Chr11:92714049	Intron 2	G	A	0.020	1.000
MTNR1b	rs76309303	Chr11:92717504	Intron 3	G	A	0.014	1.000
MTNR1b	rs1447350	Chr11:92718127	Exon 4 (3'-UTR)	C	G	0.315	0.607
MTNR1b	rs12225378	Chr11:92718649	Exon 4 (3'-UTR)	T	C	0.079	0.434
MTNR1b	rs1447352	Chr11:92722761	3'-near	A	G	0.315	0.605
AANAT	rs495055	Chr17:74440890	5'-near	A	G	0.071	1.000
AANAT	rs9896887	Chr17:74444059	5'-near	G	A	0.495	0.823
AANAT	rs116879618	Chr17:74448849	5'-near	C	T	0.042	0.434
AANAT	rs77537806	Chr17:74449204	5'-near	G	T	0.022	1.000
AANAT	rs3744044	Chr17:74475014	3'-near	C	T	0.011	1.000
ASMT	rs17149149	ChrX:1734143	Exon 2	C	A	0.020	1.000
ASMT	rs62593301	ChrX:1743087	Intron 3	T	C	0.195	0.374
ASMT	rs28675287	ChrX:1748910	Intron 6	T	C	0.346	0.112
ASMT	rs4521942	ChrX:1752017	Intron 7	G	T	0.059	0.297
ASMT	rs4639690	ChrX:1755236	Intron 8	G	A	0.353	0.542
ASMT	rs4933063	ChrX:1755404	Exon 9	C	T	0.311	1.000

*Genomic Location was released from Human Feb. 2009 (GRCh37/hg19) Assembly. Chr: chromosome, SNP: single nucleotide polymorphism, M: major allele, m: minor allele, MAF: minor allele frequency, HWE: p-value of the Hardy-Weinberg equilibrium test, UTR: untranslated region

SNP selection and genotyping

We isolated a genomic DNA sample from peripheral blood leukocytes by using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA), according to the manufacturer's instructions. We produced the genotype data by using the Korea Biobank Array Chip (K-CHIP), version 1.0 from the K-CHIP consortium. The K-CHIP, consisting of about 833K SNPs for the whole genome, was designed by the Center for Genome Science, Korea National Institute of Health, Korea (4845-301, 3000-3031). According to the Affymetrix Axiom® 2.0 Assay User Guide, the K-CHIP assay was conducted by Axiom® 2.0 Reagent Kit (Affymetrix, Santa Clara, CA, USA), which contains a series of reactions including amplification, fragmentation, hybridization, and ligation. After ligation, we stained the reaction product, imaged and analyzed it for genotype reading by using the Genotyping Console™ Software (Affymetrix, Santa Clara, CA, USA). We produced the genotype data for approximately 790K SNPs after having checked quality control for the samples.

We selected four melatonin related genes (*MTNR1a*, *MTNR1b*, *AANAT*, *ASMT*). We explored single nucleotide polymorphisms (SNPs) within 10 kilobase pairs upstream and downstream from the coding and regulatory region of each of the genes. The former three genes are located in autosomes, and the *ASMT* is located in the pseudoautosomal region 1 (PAR1) of sex chromosomes. It had previously been suggested that these are linked to BD.[48] We included 34 SNPs with minor allele frequencies (MAF) greater than 1%, and genotype call rates >97% with the associated analyses. Table 1 summarizes the localization of the studied SNPs. To confirm the reliability of the genotyping method using the K-CHIP, we analyzed all SNPs with cluster plots. Four selected SNPs (rs34532313 on *MTNR1a*, rs10830963 on *MTNR1b*, rs9896887 on *AANAT*, and rs4639690 on *ASMT*) that showed the highest MAF for each gene were re-genotyped for 48 random samples by sequencing reaction using ABI PRISM® BigDye Terminator v 3.1 Cycle Sequencing Kits (Applied Biosystems, Foster City, CA, USA). The concordance rate of genotype data between sequencing and assay using the array Chip was 99.5%.

Statistical analyses

We checked the Hardy-Weinberg equilibrium with the Fisher's exact test for genotype analysis. We observed no significant deviation in any of these SNPs (Table 1). We evaluated the genotype associations between the selected SNPs and each phenotype by logistic and linear regression analysis, with age and sex as covariates. We considered the additive genetic models based on the minor alleles of each SNP. We controlled for experimental type I errors by using Bonferroni correction, covering all included SNPs (corrected p=0.05*SNP number). We did the statistical analyses with the R program, v.3.2.2 (R Foundation for Statistical Computing, Vienna, Austria) and PLINK, v. 1.9 (www.cog-genomics.org/plink/1.9).[49]

We imputed genes that have a significant variant in the above process by using the reference panel of the EAS data from the 1000 Genomes Project Integrated Phase 3 Release. Likewise, we included SNPs within 10 kilobase pairs upstream and downstream from each gene. The data was phased using SHAPEIT (v2.837)[50] and imputed using IMPUTE2 (2.3.0). We selected variants with a high imputation quality ('info' score >0.5).[51] We subsequently analyzed imputed SNPs with minor allele frequencies (MAF) greater than 1%, and genotype call rates >97% for association by using PLINK v.1.9. We used the Haploview software 4.0[52] to estimate and plot pairwise linkage disequilibrium (LD) measures of SNPs that were included in associated analyses in this study. The LD blocks were defined according to Gabriel criteria.[53]

RESULTS

Demographic and clinical characteristics of participants (n=324) were demonstrated in Table 2. About 38% (n=123) of the participants presented with seasonality and 25% (n=83) presented with evening preference based on the cut-off value derived from the Korean general population.[43] Patient with seasonality demonstrated much younger age (p=0.04), earlier age of onset (p=0.02) and no difference in bipolar I or II subtypes. The earlier onset age in BD with seasonality was consistent with other previous studies (Table 3).[26,37]

Table 2. Demographic and clinical characteristics of subjects (N=324)

Characteristics	
Sex, male, N (%)	111 (34.26)
Age, years, mean (SD)	34.94 (10.49)
Education, college graduate or more, N (%)	214 (66.25)
Marital state, married, N (%)	144 (44.58)
Occupation, present, N (%)	263 (81.17)
GSS, mean (SD)	7.45 (5.29)
Seasonality*, present, N (%)	123 (38.32)
CSM score†, mean (SD)	31.06 (7.76)
Evening preference, present, N (%)	83 (25.61)
Age at onset, years, mean (SD)	23.89 (8.75)
Duration of illness, years, mean (SD)	11.79 (7.53)

*the significant seasonality was defined by having either seasonal affective disorder or subsyndromal seasonal affective disorder according to the definition of Kasper et al.,[46] †the lower CSM score indicates a stronger evening preference. SD: standard deviation, GSS: global seasonality score, CSM: composite scale of morningness

Table 3. Differential clinical characteristics of patients with or without seasonality

	Seasonality*		
	(-) (N=198)	(+) (N=123)	p
Sex, male, N (%)	71 (35.9)	39 (31.7)	0.522
Age, mean (SD)	35.0 (26.0;44.0)	32.0 (24.0;40.5)	0.040
Education, college graduate or more, N (%)	132 (67.0)	80 (65.0)	0.810
Occupation, present, N (%)	161 (81.3)	99 (80.5)	0.971
Marrital state, married (%)	89 (44.9)	54 (44.3)	0.997
Subtype, type I, N (%)	114 (57.6)	66 (53.7)	0.407
Age at onset, mean (SD)	22.0 [18.0;30.0]	20.0 [16.0;27.0]	0.020
Duration of illness, mean (SD)	11.0 [6.0;16.0]	9.0 [6.0;17.0]	0.678
CSM_score, mean (SD)	31.6±7.6	30.2±8.1	0.133

*the significant seasonality was defined by having either seasonal affective disorder or subsyndromal seasonal affective disorder by the definition from Kasper et al.[46] This statistic excluded patients with missing values (N=3). SD: standard deviation

Single marker analysis identified nominal associations (uncorrected p<0.05) between seasonality and three SNPs in melatonin pathway genes. These were rs116879618 located in *AANAT* (p=0.0004), rs1800885 in *MTNR1a* (p=0.031), and rs4639690 in *ASMT* (p=0.0497). After adjusting for multiple testing by using the Bonferroni correction, the association remained significant for rs116879618 in *AANAT* (corrected p=0.0151). GSS showed nominal associations with rs116879618 located in *AANAT* (p=0.0199), but it did not remain significant after multiple test corrections. Circadian preference showed a nominal association (uncorrected p<0.05) with rs75715438 in *MTNR1b* (p=0.0144), but it did not remain significant after adjusting multiple testing (Table 4). All analyses data are shown in Supplementary Table 1 (in the online-only Data Supplement).

Since *AANAT* showed significant association with BD, we performed additional imputation analysis. We generated the genotypes of 20 additional SNPs through imputation following quality control, and we conducted an associated analysis for each SNP and seasonality. Figure 1 presents the locations of the *AANAT* SNPs and their linkage disequilibrium block. An additional five SNPs showed a nominal association, and three SNPs, rs117849139, rs77121614, and rs28936679 (corrected p=0.0086, 0.0154, and 0.0092) showed a significance association following multiple test corrections. Among these SNPs, rs28936679 is a nonsynonymous (missense) variant in exon 1 (Table 5). Supplementary Table 2 (in the online-only Data Supplement) presents detailed information about the imputed SNPs and the statistical data.

DISCUSSION

The aim of this study was to evaluate whether the melatonin pathway genes are associated with biological rhythm changes in patients with BD. Assuming that specific clinical presentations have a more homogeneous genetic underpinning, we evaluated the genetic association of phenotypes reflecting the circadian and seasonal rhythms with the melatonin pathway genes as functional candidate genes.

In a previous study, BD patients demonstrated a hypersensitive pineal response to ocular light exposure when compared to a control, independent of the disease state.[54] Bipolar I disorder showed a lower baseline melatonin level and a lower nadir melatonin level on the light night and a greater amplitude of variation in melatonin secretion than did the controls on the dark night.[55] These studies and the result of the current study support the possibility that the melatonin pathway genes are involved in the exaggerated biological rhythm changes in BD.

Four variants in *AANAT*, *ASMT*, *MTNR1a*, and *MTNR1b* have been nominally associated with seasonality and circadian preference. Following correction for multiple testing, the rs116879618 in the *AANAT* remained significantly associated with the seasonality of BD. In past studies, genomewide linkage analyses with bipolar families have shown that the 17q25 region, including the *AANAT*, is linked to BD,[56-58] and associated analyses with the bipolar population shows polymorphism of *AANAT* being associated with BD.[34] But some studies failed to find a significant result.[35,36] The rs116879618, which appeared to be significantly associated with the seasonality of BD in the current study, has not been reported to be significantly associated with BD or any other psychiatric disorders. Its clinical significance was not well defined, but it is located in 5'-UTR of the *AANAT* and may influence the regulatory function of this region. The *AANAT* has been labeled the "timezyme"[59] because of its role in the timing of melatonin production by the pineal gland along with light and darkness. Activity increases 10 to 100 fold at night, causing an increase in the production and the release of melatonin, and in response to light, it shows rap-

Table 4. Summary of association analysis of SNPs in melatonin pathway genes and seasonality and circadian preference of patients with bipolar disorder

Gene	SNP	Global seasonality score			Seasonality			CSM score		
		Beta	SE	p†	OR	CI	p†	Beta	SE	p†
MTNR1a	rs28611030	-0.183	0.506	0.718	0.933	0.628–1.385	0.729	-0.294	0.709	0.678
MTNR1a	rs7440284	1.310	1.096	0.233	2.161	0.942–4.961	0.069	-0.782	1.529	0.610
MTNR1a	rs1800884	-0.867	2.011	0.667	0.649	0.122–3.444	0.612	1.883	2.754	0.495
MTNR1a	rs34532313	-0.400	0.418	0.340	0.974	0.704–1.346	0.872	0.809	0.578	0.163
MTNR1a	rs116952947	-0.740	1.319	0.575	1.266	0.463–3.463	0.647	1.015	1.803	0.574
MTNR1a	rs6820205	-0.024	0.707	0.973	1.206	0.701–2.075	0.499	1.712	0.960	0.076
MTNR1a	rs13140444	-0.210	0.426	0.623	0.930	0.669–1.293	0.666	0.718	0.588	0.223
MTNR1a	rs149982127	-0.603	1.621	0.710	0.677	0.173–2.647	0.575	0.283	2.305	0.902
MTNR1a	rs4862706	-0.083	0.711	0.907	1.151	0.667–1.986	0.613	1.347	0.973	0.167
MTNR1a	rs1800885	1.631	1.092	0.136	2.522	1.088–5.847	0.031	-0.100	1.534	0.948
MTNR1a	rs76691596	-0.974	0.853	0.255	1.049	0.544–2.025	0.886	2.006	1.161	0.085
MTNR1a	rs6858707	-0.356	0.436	0.414	0.817	0.581–1.149	0.245	0.166	0.605	0.783
MTNR1a	rs13131052	-0.372	0.501	0.458	1.002	0.680–1.477	0.991	0.598	0.691	0.387
MTNR1b	rs75715438	-0.868	1.349	0.520	0.775	0.260–2.313	0.647	4.513	1.834	0.014
MTNR1b	rs75153006	-0.300	1.069	0.779	0.962	0.419–2.210	0.927	-2.809	1.458	0.055
MTNR1b	rs4753426	0.268	0.439	0.542	1.046	0.743–1.473	0.795	0.698	0.611	0.255
MTNR1b	rs10830963	-0.560	0.412	0.175	0.820	0.594–1.134	0.230	0.113	0.573	0.844
MTNR1b	rs3781637	0.011	0.595	0.985	1.150	0.729–1.813	0.548	0.987	0.818	0.228
MTNR1b	rs148736119	-1.685	1.468	0.252	0.446	0.118–1.686	0.234	-2.785	2.118	0.190
MTNR1b	rs76309303	0.373	1.888	0.843	1.714	0.418–7.034	0.454	0.492	2.586	0.849
MTNR1b	rs1447350	0.316	0.441	0.474	1.073	0.764–1.508	0.685	0.927	0.604	0.126
MTNR1b	rs12225378	1.401	0.753	0.064	1.332	0.748–2.370	0.330	1.630	1.044	0.120
MTNR1b	rs1447352	0.330	0.440	0.454	1.069	0.761–1.502	0.700	0.907	0.607	0.136
AANAT	rs495055	-0.906	0.823	0.272	0.682	0.349–1.335	0.264	-1.189	1.130	0.294
AANAT	rs9896887	0.668	0.422	0.115	1.207	0.870–1.677	0.260	-0.843	0.579	0.147
AANAT	rs116879618	2.368	1.012	0.020	5.034	2.043–12.410	<0.001*	-1.040	1.390	0.455
AANAT	rs77537806	1.533	1.444	0.289	2.419	0.801–7.304	0.117	0.463	1.987	0.816
AANAT	rs3744044	1.698	2.017	0.400	2.405	0.521–11.100	0.261	-1.358	2.767	0.624
ASMT	rs17149149	1.191	1.490	0.425	0.962	0.304–3.050	0.948	2.308	2.122	0.278
ASMT	rs62593301	0.635	0.511	0.215	1.169	0.791–1.729	0.434	0.108	0.700	0.877
ASMT	rs28675287	-0.312	0.456	0.495	1.035	0.728–1.470	0.849	0.461	0.629	0.464
ASMT	rs4521942	0.053	0.859	0.951	0.730	0.365–1.457	0.372	-0.138	1.191	0.908
ASMT	rs4639690	0.828	0.446	0.064	1.414	1.000–1.999	0.050	-0.256	0.619	0.679
ASMT	rs4933063	-0.427	0.451	0.344	0.890	0.626–1.266	0.518	-0.613	0.618	0.322

*corrected p-value<0.05 (corrected p=0.05*SNP number). †nominal p-value by logistic regression with age and sex covariates. SNP: single nucleotide polymorphism, CSM: composite scale of morningness, SE: standard error, OR: odds ratio, CI: confidence interval

id degradation, thereby reducing the synthesis of melatonin.[60,61] The *AANAT* regulatory regions enabled the cAMP to control rapid activation and degradation switching by phosphorylating the Ser/Thr residues in the PKA/14-3-3 motifs.[59]

Following imputation, the rs28936679 in the exon of *AANAT* also showed an association with the seasonality of BD. The rs28936679 is a nonsynonymous (missense) variant in the *AANAT* exon1, which participates in an amino acid substitution from alanine to threonine. Because of different chemical characteristics, such as the structure and polarity between these amino acids, the activity of *AANAT* might be affected by this substitution.[62] This SNP was also reported to be related to de-

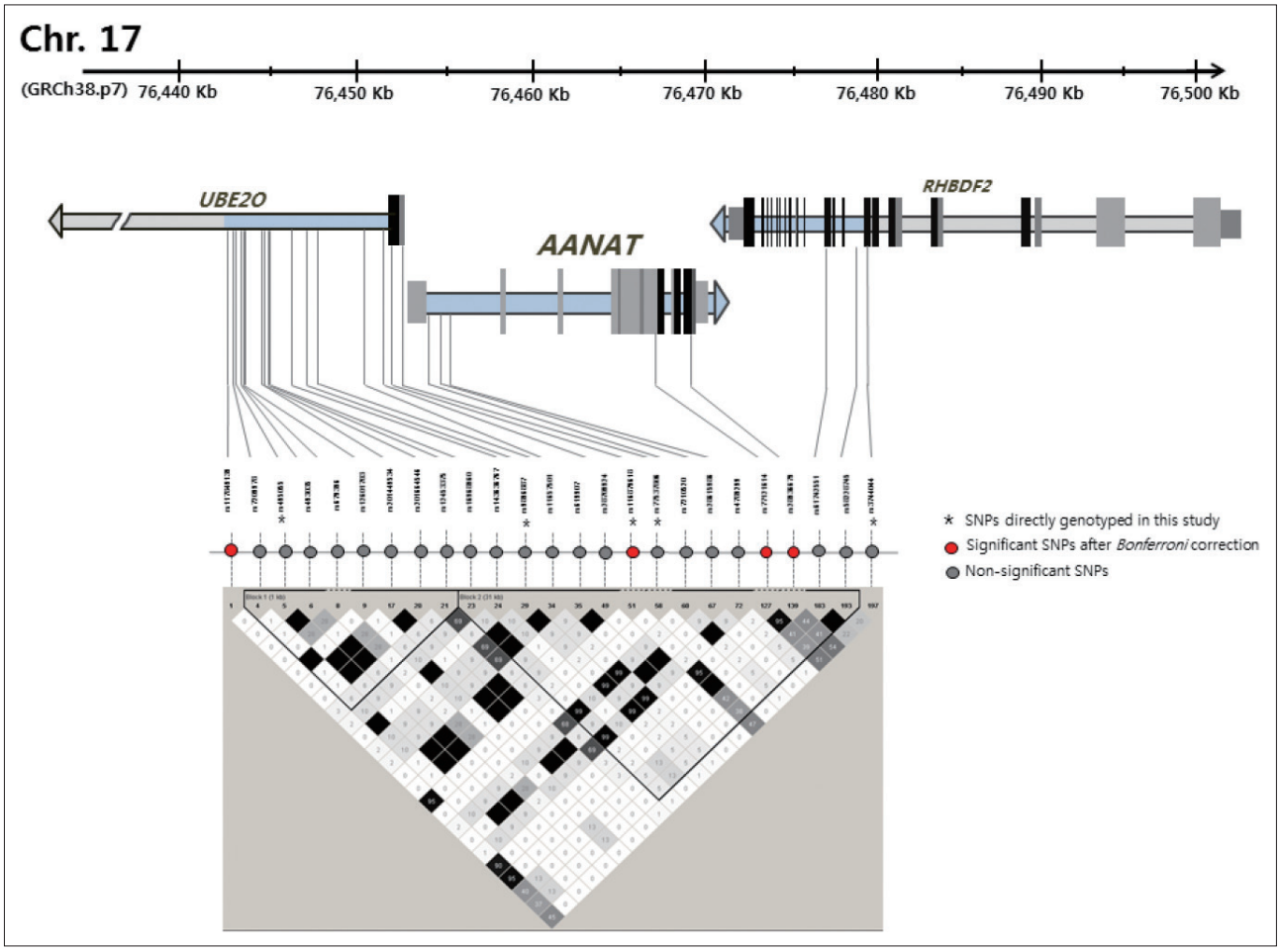

Figure 1. Scaled graphical representation of the 10 Kb genomic region surrounding the AANAT including association analysis results. Relative positions of SNPs directly genotyped in this study and imputed SNPs are presented. Coding exons of the genes in the regions are shown in gray, noncoding exons in black. SNPs that showed significant association with seasonality of BD are highlighted in the red circle. Bottom of diagram, there is the LD pattern of the region determined and visualized using the Haploview, and LD blocks were defined according to Gabriel criteria.[53] SNP: single nucleotide polymorphism, BD: bipolar disorder, LD: linkage disequilibrium.

layed sleep phase syndrome.[62]

Notably in current study, the variant of *AANAT* was related to seasonality rather than circadian preference. A previous study based on the general population suggested that genetic variants may also contribute to the seasonality phenotype that is similar to the current study in some instances.[63] The suprachiasmatic nuclei regulate seasonal rhythmicity by perceiving and encoding changes in day length and transmitting them to the pineal gland, where they regulate melatonin production.[11,64] In animal studies, when entrained to a long photoperiod, the light-induced decrease in *AANAT* is advanced by an earlier dawn, whereas the dark-induced increase in *AANAT* is delayed by a later dusk. As a result, daily melatonin secretion is compressed during the summer and prolonged during the winter.[65,66] Therefore, the variants of *AANAT* can cause change in the normal seasonal rhythm, and could affect the seasonality of BD.

This study has several limitations. First, since it did not include normal control subjects, we are not sure if the current study's findings are confined to BD or if they could be generalized in the normal population. Second, we included only four genes that directly participate in melatonin synthesis and action. Some other genes that may indirectly affect melatonergic function were not evaluated. Further studies that include more related genes and that cover genetic interactions are needed. Third, although night-shift workers were excluded from these subjects, we could not completely adjust for the effect of other social environments that could affect the individual's lifestyle and sleep–wake cycle. Finally, current study showed an association between subgroup of BD patient and a melatonin pathway gene, but it is unclear whether this gene causes BD. In order to reveal this causality, future studies using methods such as Mendelian randomization are needed.

This study demonstrated an association between a genetic variation in *AANAT* and seasonality in BD. Further studies on the biological mechanisms of melatonin on the seasonality of BD are needed.

Table 5. Association analysis of seasonality of BD with imputed SNPs in AANAT†

SNP	Genomic location‡	Intragenic location	M	m	MAF	HWE	OR	CI	p§
rs117849139	74440536	5'-near	T	C	0.030	0.173	5.639	2.187–14.540	<0.001*
rs7209978	74440875	5'-near	A	G	0.094	0.227	0.963	0.549–1.689	0.895
rs495055	74440890	5'-near	A	G	0.082	1.000	0.674	0.345–1.318	0.249
rs493035	74441157	5'-near	C	T	0.082	1.000	0.674	0.345–1.318	0.249
rs679396	74441310	5'-near	T	C	0.021	0.310	0.478	0.125–1.829	0.281
rs12601703	74441316	5'-near	T	C	0.094	0.227	0.963	0.549–1.689	0.895
rs201449534	74442545	5'-near	-	C	0.082	1.000	0.674	0.345–1.318	0.249
rs201664546	74442551	5'-near	A	C	0.082	1.000	0.674	0.345–1.318	0.249
rs12453375	74442650	5'-near	G	A	0.397	0.480	1.256	0.905–1.744	0.172
rs16968960	74442942	5'-near	A	G	0.491	0.200	1.242	0.894–1.726	0.196
rs143636767	74442979	5'-near	TGTGTT TTTGAC	-	0.094	0.228	0.958	0.546–1.681	0.882
rs9896887	74444059	5'-near	G	A	0.492	0.201	1.242	0.894–1.726	0.196
rs11657501	74445051	5'-near	A	G	0.489	0.176	1.242	0.894–1.726	0.196
rs619907	74445776	5'-near	G	A	0.082	1.000	0.674	0.345–1.318	0.249
rs28709924	74448395	5'-near	C	G	0.082	1.000	0.674	0.345–1.318	0.249
rs116879618	74448849	5'-near	C	T	0.031	0.193	4.918	2.001–12.090	<0.001*
rs77537806	74449204	5'-near	G	T	0.015	0.171	2.331	0.772–7.035	0.133
rs7210520	74450040	Intron	C	T	0.491	0.248	1.232	0.886–1.713	0.215
rs28615986	74451270	Intron	G	C	0.082	0.824	0.690	0.352–1.353	0.280
rs4789299	74452010	Intron	G	A	0.488	0.247	1.250	0.898–1.741	0.187
rs77121614	74464146	Intron	C	T	0.028	0.489	5.282	2.037–13.700	<0.001*
rs28936679	74465813	Exon (n-syn)	G	A	0.027	0.458	6.411	2.306–17.830	<0.001*
rs61742551	74472998	Exon (syn)	G	A	0.060	0.535	2.588	1.370–4.887	0.003
rs58228745	74474330	Intron	C	T	0.061	0.554	2.568	1.377–4.788	0.003
rs3744044	74475014	3'-near	C	T	0.012	0.114	2.819	0.736–10.800	0.131

*corrected p-value<0.05 (corrected p=0.05*SNP number), †imputation using the reference panel of the EAS data from the 1000 Genomes Project Integrated Phase 3 Release, ‡Genomic Location was released from Human Feb. 2009 (GRCh37/hg19) Assembly, §nominal p-value by logistic regression with age and sex covariates. SNP: single nucleotide polymorphism, M: major allele, m: minor allele, MAF: minor allele frequency, HWE: p-value of the Hardy–Weinberg equilibrium test, UTR: untranslated region, OR: odds ratio, CI: confidence interval, n-syn: nonsynonymous, syn: synonymous

Supplementary Materials

The online-only Data Supplement is available with this article at https://doi.org/10.30773/pi.2020.0436.

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Conflicts of Interest

The authors have no potential conflicts of interest to disclose.

Author Contributions

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Supplementary Table 1. Results of association test of SNPs in melatonin pathway genes with bipolar patients' seasonality and circadian preference

Gene	CHR	SNP	Global seasonality score						
			BETA	SE	L95	U95	STAT	p	Corr_p
MTNR1a	4	rs28611030	-0.1829	0.5059	-1.175	0.8087	-0.3616	0.7179	1
MTNR1a	4	rs7440284	1.31	1.096	-0.8379	3.458	1.195	0.2328	1
MTNR1a	4	rs1800884	-0.8667	2.011	-4.807	3.074	-0.4311	0.6667	1
MTNR1a	4	rs34532313	-0.3995	0.4184	-1.22	0.4205	-0.9549	0.3404	1
MTNR1a	4	rs116952947	-0.7398	1.319	-3.326	1.846	-0.5608	0.5754	1
MTNR1a	4	rs6820205	-0.02435	0.7066	-1.409	1.361	-0.03445	0.9725	1
MTNR1a	4	rs13140444	-0.2099	0.4264	-1.046	0.6259	-0.4921	0.623	1
MTNR1a	4	rs149982127	-0.6026	1.621	-3.779	2.574	-0.3718	0.7103	1
MTNR1a	4	rs4862706	-0.08323	0.7111	-1.477	1.311	-0.117	0.9069	1
MTNR1a	4	rs1800885	1.631	1.092	-0.5098	3.772	1.493	0.1364	1
MTNR1a	4	rs76691596	-0.9735	0.8534	-2.646	0.6991	-1.141	0.2548	1
MTNR1a	4	rs6858707	-0.3562	0.4357	-1.21	0.4979	-0.8174	0.4143	1
MTNR1a	4	rs13131052	-0.372	0.5011	-1.354	0.6101	-0.7424	0.4584	1
MTNR1b	11	rs75715438	-0.868	1.349	-3.512	1.776	-0.6435	0.5204	1
MTNR1b	11	rs75153006	-0.2996	1.069	-2.394	1.795	-0.2803	0.7794	1
MTNR1b	11	rs4753426	0.268	0.4385	-0.5915	1.127	0.6111	0.5416	1
MTNR1b	11	rs10830963	-0.5603	0.4121	-1.368	0.2473	-1.36	0.1749	1
MTNR1b	11	rs3781637	0.01138	0.5948	-1.154	1.177	0.01914	0.9847	1
MTNR1b	11	rs148736119	-1.685	1.468	-4.563	1.193	-1.147	0.2521	1
MTNR1b	11	rs76309303	0.3733	1.888	-3.327	4.074	0.1977	0.8434	1
MTNR1b	11	rs1447350	0.3156	0.4405	-0.5478	1.179	0.7164	0.4743	1
MTNR1b	11	rs12225378	1.401	0.7534	-0.07611	2.877	1.859	0.06397	1
MTNR1b	11	rs1447352	0.3297	0.4396	-0.5319	1.191	0.7501	0.4538	1
AANAT	17	rs495055	-0.9056	0.823	-2.519	0.7074	-1.1	0.272	1
AANAT	17	rs9896887	0.6683	0.4223	-0.1595	1.496	1.582	0.1146	1
AANAT	17	rs116879618	2.368	1.012	0.3842	4.352	2.34	0.01993	0.6777
AANAT	17	rs77537806	1.533	1.444	-1.297	4.363	1.062	0.2891	1
AANAT	17	rs3744044	1.698	2.017	-2.254	5.651	0.8422	0.4003	1
ASMT	25	rs17149149	1.191	1.49	-1.729	4.112	0.7994	0.4246	1
ASMT	25	rs62593301	0.6348	0.5105	-0.3656	1.635	1.244	0.2145	1
ASMT	25	rs28675287	-0.3121	0.4563	-1.206	0.5823	-0.6839	0.4946	1
ASMT	25	rs4521942	0.05253	0.8588	-1.631	1.736	0.06117	0.9513	1
ASMT	25	rs4639690	0.8284	0.4455	-0.0447	1.702	1.86	0.06388	1
ASMT	25	rs4933063	-0.4273	0.451	-1.311	0.4567	-0.9473	0.3442	1

	CHR	SNP	Seasonality					
			OR	L95	U95	STAT	p	Corr_p
MTNR1a	4	rs28611030	0.9325	0.6278	1.385	-0.3465	0.729	1
MTNR1a	4	rs7440284	2.161	0.9415	4.961	1.818	0.06912	1
MTNR1a	4	rs1800884	0.6488	0.1222	3.444	-0.5079	0.6115	1
MTNR1a	4	rs34532313	0.9737	0.7041	1.346	-0.1614	0.8718	1
MTNR1a	4	rs116952947	1.266	0.4625	3.463	0.4585	0.6466	1
MTNR1a	4	rs6820205	1.206	0.7007	2.075	0.6756	0.4993	1
MTNR1a	4	rs13140444	0.9299	0.6688	1.293	-0.4321	0.6657	1
MTNR1a	4	rs149982127	0.6773	0.1733	2.647	-0.5602	0.5753	1
MTNR1a	4	rs4862706	1.151	0.6671	1.986	0.5053	0.6133	1
MTNR1a	4	rs1800885	2.522	1.088	5.847	2.157	0.03104	1
MTNR1a	4	rs76691596	1.049	0.5437	2.025	0.1435	0.8859	1
MTNR1a	4	rs6858707	0.8168	0.5808	1.149	-1.163	0.2446	1
MTNR1a	4	rs13131052	1.002	0.6799	1.477	0.01137	0.9909	1
MTNR1b	11	rs75715438	0.7747	0.2595	2.313	-0.4575	0.6473	1
MTNR1b	11	rs75153006	0.9617	0.4186	2.21	-0.09201	0.9267	1
MTNR1b	11	rs4753426	1.046	0.7433	1.473	0.2603	0.7946	1
MTNR1b	11	rs10830963	0.8204	0.5936	1.134	-1.199	0.2304	1
MTNR1b	11	rs3781637	1.15	0.7292	1.813	0.6005	0.5481	1
MTNR1b	11	rs148736119	0.4459	0.1179	1.686	-1.19	0.234	1
MTNR1b	11	rs76309303	1.714	0.4179	7.034	0.7484	0.4542	1
MTNR1b	11	rs1447350	1.073	0.7636	1.508	0.4056	0.685	1
MTNR1b	11	rs12225378	1.332	0.7484	2.37	0.9745	0.3298	1
MTNR1b	11	rs1447352	1.069	0.7609	1.502	0.3855	0.6999	1
AANAT	17	rs495055	0.6822	0.3487	1.335	-1.117	0.2641	1
AANAT	17	rs9896887	1.207	0.8696	1.677	1.126	0.2602	1
AANAT	17	rs116879618	5.034	2.043	12.41	3.512	0.0004442	0.0151
AANAT	17	rs77537806	2.419	0.8014	7.304	1.567	0.1171	1
AANAT	17	rs3744044	2.405	0.521	11.1	1.125	0.2608	1
ASMT	25	rs17149149	0.9623	0.3036	3.05	-0.06523	0.948	1
ASMT	25	rs62593301	1.169	0.7905	1.729	0.7821	0.4342	1
ASMT	25	rs28675287	1.035	0.7283	1.47	0.1907	0.8488	1
ASMT	25	rs4521942	0.7296	0.3653	1.457	-0.8935	0.3716	1
ASMT	25	rs4639690	1.414	1	1.999	1.962	0.04972	1
ASMT	25	rs4933063	0.8903	0.6259	1.266	-0.6465	0.518	1

	CHR	SNP	CSM score						
			BETA	SE	L95	U95	STAT	p	Corr_p
MTNR1a	4	rs28611030	-0.2944	0.7092	-1.684	1.096	-0.4151	0.6784	1
MTNR1a	4	rs7440284	-0.7819	1.529	-3.779	2.215	-0.5113	0.6095	1
MTNR1a	4	rs1800884	1.883	2.754	-3.515	7.281	0.6839	0.4946	1
MTNR1a	4	rs34532313	0.8086	0.5777	-0.3236	1.941	1.4	0.1626	1
MTNR1a	4	rs116952947	1.015	1.803	-2.519	4.549	0.563	0.5738	1
MTNR1a	4	rs6820205	1.712	0.9601	-0.1702	3.593	1.783	0.07564	1
MTNR1a	4	rs13140444	0.7178	0.5884	-0.4354	1.871	1.22	0.2234	1
MTNR1a	4	rs149982127	0.2831	2.305	-4.234	4.801	0.1228	0.9023	1
MTNR1a	4	rs4862706	1.347	0.9733	-0.5608	3.254	1.384	0.1674	1
MTNR1a	4	rs1800885	-0.09966	1.534	-3.106	2.907	-0.06496	0.9482	1
MTNR1a	4	rs76691596	2.006	1.161	-0.2701	4.282	1.727	0.08509	1
MTNR1a	4	rs6858707	0.1663	0.6045	-1.018	1.351	0.2752	0.7834	1
MTNR1a	4	rs13131052	0.5981	0.6907	-0.7557	1.952	0.8659	0.3872	1
MTNR1b	11	rs75715438	4.513	1.834	0.9186	8.108	2.461	0.0144	0.4897
MTNR1b	11	rs75153006	-2.809	1.458	-5.668	0.04895	-1.926	0.05496	1
MTNR1b	11	rs4753426	0.698	0.6114	-0.5003	1.896	1.142	0.2545	1
MTNR1b	11	rs10830963	0.1129	0.5731	-1.01	1.236	0.197	0.8439	1
MTNR1b	11	rs3781637	0.9874	0.8182	-0.6162	2.591	1.207	0.2284	1
MTNR1b	11	rs148736119	-2.785	2.118	-6.936	1.366	-1.315	0.1895	1
MTNR1b	11	rs76309303	0.4917	2.586	-4.576	5.56	0.1902	0.8493	1
MTNR1b	11	rs1447350	0.9266	0.6041	-0.2573	2.111	1.534	0.126	1
MTNR1b	11	rs12225378	1.63	1.044	-0.417	3.676	1.561	0.1196	1
MTNR1b	11	rs1447352	0.9073	0.6067	-0.2819	2.096	1.495	0.1358	1
AANAT	17	rs495055	-1.189	1.13	-3.405	1.026	-1.052	0.2936	1
AANAT	17	rs9896887	-0.8427	0.579	-1.977	0.2921	-1.455	0.1466	1
AANAT	17	rs116879618	-1.04	1.39	-3.765	1.685	-0.748	0.455	1
AANAT	17	rs77537806	0.4633	1.987	-3.43	4.357	0.2332	0.8157	1
AANAT	17	rs3744044	-1.358	2.767	-6.783	4.066	-0.4909	0.6238	1
ASMT	25	rs17149149	2.308	2.122	-1.852	6.468	1.087	0.2777	1
ASMT	25	rs62593301	0.1082	0.6999	-1.264	1.48	0.1546	0.8773	1
ASMT	25	rs28675287	0.4612	0.6286	-0.7708	1.693	0.7337	0.4637	1
ASMT	25	rs4521942	-0.138	1.191	-2.472	2.196	-0.1159	0.9078	1
ASMT	25	rs4639690	-0.2563	0.6189	-1.469	0.9567	-0.4142	0.6791	1
ASMT	25	rs4933063	-0.6132	0.6175	-1.823	0.5971	-0.993	0.3215	1

CHR: chromosome, SNP: single nucleotide polymorphism, SE: standard error, L95: lower limit of 95% confidence interval, U95: upper limit of 95% confidence interval STAT: statistica value, Corr_p: corrected p-value, CSM: Composite Scale of Morningness

Supplementary Table 2. Result of association test of seasonality of BD with imputed SNPs in AANAT

SNP	Additive			
	OR	CI	p	Corr_p
rs117849139	5.639	2.187−14.54	0.000345	0.008634
rs7209978	0.9629	0.549−1.689	0.8952	1
rs495055	0.6739	0.3445−1.318	0.249	1
rs493035	0.6739	0.3445−1.318	0.249	1
rs679396	0.4779	0.1249−1.829	0.2808	1
rs12601703	0.9629	0.549−1.689	0.8952	1
rs201449534	0.6739	0.3445−1.318	0.249	1
rs201664546	0.6739	0.3445−1.318	0.249	1
rs12453375	1.256	0.9054−1.744	0.1721	1
rs16968960	1.242	0.8941−1.726	0.196	1
rs143636767	0.9583	0.5464−1.681	0.8819	1
rs9896887	1.242	0.8941−1.726	0.196	1
rs11657501	1.242	0.8944−1.726	0.1955	1
rs619907	0.6739	0.3445−1.318	0.249	1
rs28709924	0.6739	0.3445−1.318	0.249	1
rs116879618	4.918	2.001−12.09	0.000517	0.01292
rs77537806	2.331	0.7721−7.035	0.1333	1
rs7210520	1.232	0.886−1.713	0.2148	1
rs28615986	0.6901	0.352−1.353	0.2803	1
rs4789299	1.25	0.8975−1.741	0.1867	1
rs77121614	5.282	2.037−13.7	0.000619	0.01547
rs28936679	6.411	2.306−17.83	0.00037	0.009236
rs61742551	2.588	1.37−4.887	0.003377	0.08443
rs58228745	2.568	1.377−4.788	0.003017	0.07542
rs3744044	2.819	0.7357−10.8	0.1305	1

BD: bipolar disorder, SNP: single nucleotide polymorphism, OR: odds ratio, CI: confidence interval, Corr_p: corrected p-value