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FKBP5 Gene Polymorphisms and Insomnia Symptoms During Depressive Episodes in Stress-Related Bipolar Disorder

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Abstract

Stress-related bipolar disorder (BP) is shaped by intricate interactions among genetic, environmental, and clinical factors. Although the FKBP5 gene functions as a central modulator of the stress response and has been linked to several mood disorders, its potential involvement in insomnia during depressive episodes of BP remains insufficiently explored. This study aimed to examine the association between FKBP5 gene variants and insomnia symptoms emerging during depressive episodes in BP. The study enrolled 347 individuals diagnosed with BP (42% male, 58% female), of whom 78% experienced insomnia symptoms. Diagnostic assessments were conducted using the SCID and OPCRIT instruments, while eight FKBP5 single nucleotide polymorphisms (SNPs) were genotyped through the TaqMan method. Participants were divided according to the presence or absence of a significant stressor preceding their first mood episode. Statistical analyses, including ANCOVA and Chi-square tests with pairwise post hoc comparisons, were performed using Statistica 13.3 and R software. Functional characterization of variants with significant associations was carried out via Ensembl VEP, RegulomeDB, HaploReg, and SNPnexus. The FKBP5 rs755658 variant showed a potential link with insomnia symptoms among participants with prior stress exposure, where CT/CC genotypes were more frequently associated with insomnia ($p = 0.03$; BH-adjusted $p = 0.22$, below the 0.25 threshold) compared to the TT genotype. Additionally, seven other FKBP5 polymorphisms displayed significant associations with BP subtypes in participants without identifiable stressors, suggesting a genetic component independent of environmental triggers. Functional prediction analysis indicated that rs755658 may influence transcriptional activity, transcription factor binding, and post-transcriptional gene regulation. The results suggest that FKBP5 genetic variants could modulate vulnerability to insomnia in stress-affected individuals during depressive episodes of BP, underscoring their potential contribution to stress regulation pathways. Given the exploratory scope of this research, replication in larger, independent samples is necessary. Future investigations should focus on the molecular mechanisms and potential clinical implications for personalized treatment approaches.

Keywords: Stress, Depressive episode, Bipolar disorder, Insomnia, FKBP5 gene variants

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Introduction

FKBP prolyl isomerase 5 (FKBP5) is a 51-kDa immunophilin protein that regulates glucocorticoid receptor (GR) sensitivity through its interaction with the steroid receptor complex. Its activity is influenced by genetic polymorphisms, environmental stressors, and epigenetic modifications [1-3]. Variations in the FKBP5 gene affect stress reactivity by modulating the hypothalamic–pituitary–adrenal (HPA) axis, altering the GR's responsiveness to cortisol—the principal stress hormone—and thereby shaping individual susceptibility to stress-related and mood disorders [4-13]. Numerous studies have reported associations between FKBP5 polymorphisms and affective disorders, particularly in the context of maladaptive stress regulation [14-16].

Insomnia frequently accompanies depressive, anxiety, and adjustment disorders [17-19]. However, findings regarding the relationship between FKBP5 variants and bipolar disorder (BP) remain inconsistent. These discrepancies may arise from differences in allele frequencies across populations, ethnic diversity, and gene–environment interactions, all of which contribute to BP heterogeneity and complicate genetic interpretations. The diverse clinical manifestations of BP—spanning depressive and manic episodes—further obscure the genetic contribution of FKBP5 to the disorder. For example, Willour *et al.* [20] proposed that FKBP5 variants may influence the number of depressive episodes in BP, whereas Szczepankiewicz *et al.* [21] found no significant relationship between FKBP5 polymorphisms and BP but did observe associations with major depressive disorder. Similarly, another study linked the FKBP5 rs3800373 polymorphism to the depressed subtype of BP [22]. Since depressive episodes tend to impair daily functioning more severely than manic phases, they substantially increase the overall burden of the illness [23, 24].

Insomnia, a prevalent sleep disturbance, is characterized by difficulty initiating or maintaining sleep—manifesting as broken sleep, early morning awakening (often seen in melancholic depression), or non-restorative sleep—and can present either as an independent condition or as a symptom secondary to psychiatric disorders [25, 26]. It may be classified as a symptom, a normal variant, or a clinical disorder (acute, chronic, or comorbid with medical, psychiatric, or substance-related conditions) [27]. According to the *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition* (DSM-5), insomnia is defined as a disorder only when it warrants separate clinical attention and cannot be fully explained by another underlying condition [28]. Poon *et al.* [29] describe insomnia symptoms as difficulty falling asleep, frequent awakenings, early rising, daytime fatigue, and impaired functioning, though diagnostic criteria vary slightly across classification systems. Sleep disturbance has been shown to exacerbate the course of BP, contributing to mood instability, psychotic symptoms, and increased treatment resistance, while also intensifying emotional distress and stress reactivity [30, 31].

Genetic factors have been implicated in sleep regulation within BP. For instance, in a Polish cohort, associations were identified between three FKBP5 polymorphisms (rs1360780, rs7748266, and rs9296158), one ACP1 variant (rs300774), and one glucocorticoid-induced transcript 1 (GLCCI1) variant (rs37972) with lithium treatment response [4]. Moreover, FKBP5 polymorphisms have been linked to disrupted human sleep architecture and may contribute to sleep disturbance vulnerability [32]. Experimental evidence supports this role, as FKBP5-deficient mice exhibit enhanced stress resilience and improved sleep, emphasizing the gene's involvement in both stress regulation and sleep homeostasis [33].

Despite extensive research connecting FKBP5 polymorphisms with stress regulation, mood disorders, and sleep disturbances, the potential relationship between FKBP5 variants and insomnia symptoms specifically within stress-related BP remains unexplored. The present study addresses this gap by investigating whether FKBP5 polymorphisms are associated with insomnia symptoms during depressive episodes of BP, and whether these associations differ depending on exposure to stressors. Understanding this relationship may clarify the contribution of FKBP5 to sleep pathology in BP and the moderating role of stress.

This research offers a novel perspective by focusing on FKBP5 single-nucleotide polymorphisms (SNPs) within a gene–environment interaction framework. Unlike recent large-scale genome-wide association studies (GWAS) such as that by Watanabe *et al.* [34], which identified 554 insomnia-related loci across more than 2.3 million individuals without implicating FKBP5, this targeted approach centers on a biologically plausible candidate gene with established relevance to stress pathways. By stratifying participants based on stress exposure and clinically defined insomnia symptoms, this study captures phenotype-specific associations that broad population-based GWAS may overlook. Such an approach enhances understanding of subtype-specific mechanisms underlying insomnia in BP and may inform personalized therapeutic strategies.

In this context, the term *stress-related BP* refers to cases in which patients reported a stressor (type unspecified) preceding the onset of illness. A stressor, as defined by Halbreich [35], is "any event, situation, or environmental condition subjectively perceived as having a negative impact on the individual." Stressors may be biological (e.g., infection, microbiota imbalance, nutritional deficiencies), physical (e.g., temperature fluctuations,

disrupted light–dark cycles), or psychosocial (e.g., aggression, job loss, social isolation). The interaction between such environmental factors and individual genetic predisposition ultimately influences the manifestation and course of BP.

Materials and Methods

Participants

The study comprised 347 individuals diagnosed with bipolar disorder (BP) according to the *International Classification of Diseases, 10th Revision* (ICD-10, code F31: Bipolar affective disorder) and the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (DSM-IV) [36]. The sample included 146 males (42%) and 201 females (58%). Data collection was conducted at the Department of Psychiatric Genetics, Poznan University of Medical Sciences (Poland). All participants were of Polish origin and Caucasian ethnicity.

Measures

Comprehensive clinical information was obtained using the *Structured Clinical Interview for DSM Disorders* (SCID-I) [37]. This included documentation of insomnia and hypersomnia symptoms observed during depressive episodes of BP, with additional assessment of melancholic features. Sleep-related variables were further characterized using the *Operational Criteria Checklist* (OPCRIT) [38], which records specific sleep symptoms—initial insomnia, middle insomnia (fragmented sleep), early morning awakening, and hypersomnia. The OPCRIT also includes a variable identifying the presence or absence of a stressor prior to the onset of the first mood episode. Information regarding stressors was collected retrospectively and was based on patients' self-reports.

Genotyping

Genotyping was performed using TaqMan assays, as described in previous research from our group [13, 21]. Eight FKBP5 single-nucleotide polymorphisms (SNPs)—rs1360780, rs755658, rs9470080, rs4713916, rs7748266, rs9296158, rs9394309, and rs3800373—were selected for analysis. These variants were chosen based on earlier findings by Szczepankiewicz *et al.* [21], which demonstrated significant associations between these FKBP5 loci and major depressive disorder (MDD). Although those findings were specific to MDD, the shared pathophysiological mechanisms between MDD and BP, particularly the dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis and the stress response, justified their inclusion in the present study. Considering the known role of stress and sleep disturbances in bipolar depression [39], the present investigation aimed to determine whether these FKBP5 variants are similarly linked to insomnia symptoms during depressive episodes of BP. Thus, this work extends previous findings to a related clinical population with overlapping biological mechanisms.

Statistical analysis

All statistical analyses were performed using *Statistica* software version 13.3 (StatSoft, Krakow, Poland) and *R* programming language (version 4.4.2) [40], employing the following packages: *dplyr*, *readxl*, *ggplot2*, *tidyr*, and *scales* [41-45]. Analyses focused on identifying associations between FKBP5 polymorphisms and insomnia symptoms. Categorical variables—including sex, BP subtype (coded as 1 for type I and 2 for type II), stressor presence, and genotype categories—were analyzed as factors in all models.

*G*Power* version 3.1 [46, 47] was used for post hoc power analysis, employing a two-tailed, two-sample *t*-test to evaluate the effect size between BP participants with and without reported stressors. In studies involving multiple comparisons, such as those examining genetic polymorphisms, maintaining a higher statistical power (90%) is recommended to account for correction procedures such as Bonferroni or false discovery rate (FDR) adjustments, which may otherwise obscure true associations [48-51]. Accordingly, a power level of 0.9 [52] was applied for effect size estimation.

The Hardy–Weinberg equilibrium (HWE) for genotype distributions was tested using chi-square analyses in *R* (see Supplementary Material S1). Associations between FKBP5 polymorphisms and categorical variables (e.g., insomnia symptoms, sex, BP subtype) were assessed using chi-square tests. Consistent with previous research by Stramecki *et al.* [53], analysis of covariance (ANCOVA) was employed to examine relationships between FKBP5 variants and the continuous variable of age at onset. Following significant chi-square results, the Benjamini–Hochberg (BH) procedure was applied with an FDR threshold of 25%, following the methodological precedent of Stramecki *et al.* [54]. Results were considered significant when the BH-adjusted *p*-value was below the 0.25 threshold (detailed *R* script available in Supplementary Material S1).

The use of a 25% FDR threshold aligns with practices in exploratory genetic and psychiatric research, balancing the need to detect meaningful associations against the control of false positives [55]. This approach is particularly appropriate for screening analyses aimed at identifying potential candidate variants for further investigation and is widely accepted in exploratory biological and psychiatric studies [54, 56–62].

Post hoc pairwise comparisons of proportions, adjusted using the BH correction and evaluated against the 25% FDR threshold, were conducted in cases of significant two-way interactions among participants reporting stress exposure (see Supplementary Material S1 for *R*

methodology). Pairwise comparisons are appropriate following omnibus tests such as chi-square, as they allow identification of specific group differences within significant overall effects [63].

In silico prediction of variant functionality

The functional impact of significant FKBP5 polymorphisms was evaluated *in silico* using multiple bioinformatics tools, including Ensembl Variant Effect Predictor (VEP) Cache version 113.0 (https://www.ensembl.org/Homo_sapiens/Tools/VEP), RegulomeDB version 2.2 (https://regulomedb.org), HaploReg version 4.2 (https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php), and SNPnexus version 4 (https://www.snp-nexus.org/v4/).

The Ensembl VEP tool annotates and predicts the potential functional consequences of genomic variants on genes, transcripts, and protein products [64]. Variants were analyzed based on the GRCh38.p14 human genome assembly using Ensembl VEP v113.0, which integrates data from several major databases, including GENCODE v47, dbSNP 156, ClinVar (version 202404), and gnomAD v4.1. VEP evaluates variant consequences in relation to transcript biotypes, regulatory elements, and co-located known variants, while functional impact predictions were derived from SIFT, PolyPhen, CADD, SpliceAI, and ClinPred.

RegulomeDB was used to assess the potential regulatory functions of single-nucleotide variants (SNVs) located in non-coding regions. This tool integrates data from multiple functional genomic assays such as transcription factor chromatin immunoprecipitation sequencing (TF ChIP-seq) and DNase-seq from the ENCODE project, along with quantitative trait locus (QTL) analyses [65, 66]. Each queried SNV is assigned a regulatory score ranging from 1 to 7 (with 1 denoting the strongest evidence of regulatory function) and a model score between 0 and 1 (higher values indicating greater regulatory potential). RegulomeDB facilitates the interpretation of intronic and non-coding regulatory variants by mapping them to transcription factor binding sites, promoters, enhancers, and methylation regions, thereby providing insight into their biological relevance [66–68]. The role of non-coding RNA (ncRNA) was also considered, given its conserved structure and significant contribution to cellular signaling and disease regulation [69].

HaploReg was employed to explore the regulatory potential of non-coding genetic variants by integrating linkage disequilibrium data with epigenomic profiles, transcription factor binding, and expression QTL annotations [70]. In parallel, SNPnexus provided comprehensive annotation of known and novel genetic variants, enabling the identification of functionally relevant SNPs and small insertions/deletions across multiple human genome assemblies [71–75].

Ethical considerations

This research was conducted in compliance with the ethical standards of the 1964 Declaration of Helsinki and its subsequent amendments. Ethical approval was obtained from the Bioethics Committee of Poznan University of Medical Sciences, Poland (Approval No. 1194/16). All procedures adhered to institutional and international guidelines for human research. Written informed consent was obtained from all participants prior to their inclusion in the study.

Results

Effect size determination

Post hoc power analysis revealed a small-to-moderate effect size (d = 0.4). According to Cohen's criteria [76], this indicates a moderate difference between BP patients who experienced stressors prior to disease onset and those without such exposure.

Study population and data presentation

A detailed demographic and clinical characterization of the study cohort (N = 347) is provided in **Table 1**.

Table 1. Description of the analysed population

Empty Cell	**Count (%) or Mean (SD)**	Empty Cell
Empty Cell	**Total, n = 347**	Empty Cell
Stressor present prior the onset of BP	204 (59 %)	
Stressor absent prior the onset of BP	143 (41 %)	
Insomnia present in depressive episode	271 (78 %)	
Insomnia absent in depressive episode	76 (22 %)	
Male	146 (42 %)	
Female	201 (58 %)	
BP 1	263 (76 %)	
BP 2	84 (24 %)	
		Min, Max
Age of onset, mean (SD)	31 (11.3)	10, 59
***FKBP5* genotypes distribution in all BP patients**		
rs1360780		
CC	192 (55 %)	
CT	134 (39 %)	
TT	21 (6 %)	
rs755658		
CC	284 (82 %)	
CT	60 (17 %)	
TT	3 (1 %)	
rs9470080		

CC	172 (50 %)
CT	150 (43 %)
TT	25 (7 %)
rs4713916	
AA	20 (6 %)
AG	138 (40 %)
GG	189 (54 %)
rs7748266	
CC	265 (76 %)
CT	79 (23 %)
TT	3 (1 %)
rs9296158	
AA	23 (7 %)
AG	130 (37 %)
GG	194 (56 %)
rs9394309	
AA	184 (53 %)
AG	140 (40 %)
GG	23 (7 %)
rs3800373	
AA	203 (59 %)
AC	124 (36 %)
CC	20 (6 %)

Abbreviations: SD - standard deviation; BP – bipolar disorder.

The genotype distributions for the polymorphisms adhered to Hardy-Weinberg Equilibrium.

A chi-squared test revealed a statistically significant association between the *FKBP5* rs755658 variant and insomnia symptoms ($\chi^2 = 7.17$, df = 2, $p = 0.03$) for BP individuals with stressors **(Table 2)**.

Table 2. Interactions between the *FKBP5* gene polymorphisms and insomnia symptoms

Stress factor	Effect	rs1360780	rs755658	rs9470080	rs4713916	rs7748266	rs9296158	rs9394309	rs3800373
Stressor present	**Sex**	$\chi^2 =$ 1.17; $p =$ 0.558	$\chi^2 =$ 1.05; $p =$ 0.592	$\chi^2 =$ 0.18; $p =$ 0.913	$\chi^2 =$ 0.51; $p =$ 0.773	$\chi^2 =$ 3.59; $p =$ 0.166	$\chi^2 =$ 0.84; $p =$ 0.656	$\chi^2 =$ 0.65; $p =$ 0.722	$\chi^2 =$ 0.05; $p =$ 0.974
	Age of onset	F = 0.63; $p =$ 0.533	F = 0.65; $p =$ 0.526	F = 1.12; $p =$ 0.328	F = 0.85; $p =$ 0.428	F = 0.08; $p =$ 0.921	F = 0.70; $p =$ 0.496	F = 0.55; $p =$ 0.576	F = 1.39; $p =$ 0.254
	BP type 1 and 2	$\chi^2 =$ 0.40; $p =$ 0.820	$\chi^2 =$ 1.28; $p =$ 0.528	$\chi^2 =$ 0.16; $p =$ 0.923	$\chi^2 =$ 0.19; $p =$ 0.908	$\chi^2 =$ 0.74; $p =$ 0.690	$\chi^2 =$ 0.69; $p =$ 0.707	$\chi^2 =$ 0.89; $p =$ 0.640	$\chi^2 =$ 1.33; $p =$ 0.513
	***FKBP5* x insomnia symptoms**	$\chi^2 =$ 1.01; $p =$ 0.605	**$\chi^2 =$ 7.17; $p =$ 0.028**	$\chi^2 =$ 1.60; $p =$ 0.449	$\chi^2 =$ 0.88; $p =$ 0.644	$\chi^2 =$ 0.73; $p =$ 0.694	$\chi^2 =$ 0.12; $p =$ 0.942	$\chi^2 =$ 1.36; $p =$ 0.507	$\chi^2 =$ 0.79; $p =$ 0.673
Stressor absent	**Sex**	$\chi^2 =$ 0.86; $p =$ 0.652	$\chi^2 =$ 1.05; $p =$ 0.591	$\chi^2 =$ 1.99; $p =$ 0.37	$\chi^2 =$ 0.87; $p =$ 0.649	$\chi^2 =$ 2.18; $p =$ 0.336	$\chi^2 =$ 0.78; $p =$ 0.678	$\chi^2 =$ 0.63; $p =$ 0.731	$\chi^2 =$ 1.69; $p =$ 0.429
	Age of onset	F = 0.08; $p =$ 0.927	F = 0.30; $p =$ 0.742	F = 0.14; $p =$ 0.870	F = < 0.01; $p =$ 0.996	F = 0.37; $p =$ 0.689	F = 0.18; $p =$ 0.838	F = 0.14; $p =$ 0.868	F = 0.08; $p =$ 0.927
	BP type 1 and 2	$\chi^2 =$ **9.06; $p =$ 0.011**	$\chi^2 =$ 1.93; $p =$ 0.380	$\chi^2 =$ **9.86; $p =$ 0.007**	$\chi^2 =$ **10.81; $p =$ 0.004**	$\chi^2 =$ **7.14; $p =$ 0.028**	$\chi^2 =$ **9.90; $p =$ 0.007**	$\chi^2 =$ **10.18; $p =$ 0.006**	$\chi^2 =$ **6.56; $p =$ 0.038**
	***FKBP5* x insomnia symptoms**	$\chi^2 =$ 1.36; $p =$ 0.506	$\chi^2 =$ 0.68; $p =$ 0.710	$\chi^2 =$ 1.28; $p =$ 0.526	$\chi^2 =$ 3.04; $p =$ 0.219	$\chi^2 =$ 2.90; $p =$ 0.234	$\chi^2 =$ 1.87; $p =$ 0.392	$\chi^2 =$ 3.77; $p =$ 0.152	$\chi^2 =$ 1.42; $p =$ 0.491

Bolded values indicate statistically significant results.

After the BH correction, the *p* value of 0.03 remained statistically significant, as shown in **Table 3** (R methodology is available in Supplementary Material S1).

Table 3. Multiple testing correction results for *FKBP5* rs755658 interaction with insomnia symptoms for participants with stressors.

Raw *p* values	BH adjusted *p* values	Significance
0.60	0.79	FALSE
0.03	**0.22**	**TRUE**
0.45	0.79	FALSE
0.64	0.79	FALSE
0.69	0.79	FALSE
0.94	0.94	FALSE
0.51	0.79	FALSE
0.67	0.79	FALSE

Abbreviations: BH - Benjamini-Hochberg.

Following the detection of a statistically significant association between the FKBP5 rs755658 genotype and insomnia symptoms using the chi-square test ($p = 0.03$), a pairwise comparison of proportions was subsequently performed. The Benjamini–Hochberg (BH) correction method was applied with a false discovery rate (FDR)

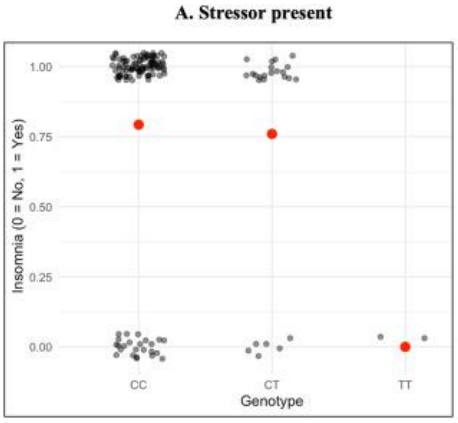
threshold of 25% to account for multiple testing **(Table 4)**. Details of the *R* analytical procedure are provided in Supplementary Material S1**.** The pairwise analysis revealed statistically significant differences between the TT and CC genotypes, as well as between the TT and CT genotypes.

Table 4. Post-hoc pairwise comparison of interaction between *FKBP5* rs755658 polymorphism and insomnia symptoms in participants with stressors

Empty Cell	Genotype	CC	CT
1	CT	0.92	–
3	TT	**0.21**	**0.22**

Bolded values indicate statistically significant results.

To illustrate the relationship between the FKBP5 rs755658 variant and insomnia symptoms, a jitter (dot) plot was generated **(Figure 1)** to visually complement the statistical findings by highlighting potential genotype-specific patterns in the occurrence of insomnia. This visualization displayed individual-level data, allowing for a clear depiction of the proportion of participants reporting insomnia symptoms (coded as 1) across different genotypes. The TT genotype appeared to be less frequent among participants with insomnia, particularly within the stress-exposed subgroup, suggesting a possible inverse relationship. This trend indicates that individuals carrying the TT genotype may be less susceptible to developing insomnia symptoms under stress conditions or following exposure to a stressor.



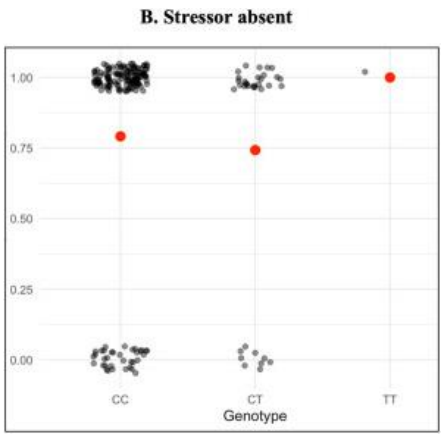


Figure 1. Jitter/dot plot showing rs755658 genotype and insomnia symptom interaction. Red dots represent the mean. Insomnia 0 = absence of insomnia symptoms, 1 = presence of insomnia symptoms

A notable association was also found between BP types 1 and 2 and seven FKBP5 polymorphisms (rs1360780, rs9470080, rs4713916, rs7748266, rs9296158, rs9394309, rs3800373) among participants without stress exposure, all of which remained significant after BH correction, as presented in **Table 5** (details of the R methodology are provided in Supplementary Material S1).

Table 5. Multiple testing correction results for *FKBP5* rs755658 interaction with BP types for participants without stressors

Raw *p* values	BH adjusted *p* values	Significance
0.01	**0.02**	**TRUE**
0.38	0.38	FALSE
0.01	**0.01**	**TRUE**
< 0.01	**0.01**	**TRUE**
0.03	**0.04**	**TRUE**
0.01	**0.01**	**TRUE**
0.01	**0.01**	**TRUE**
0.04	**0.04**	**TRUE**

Abbreviations: BH - Benjamini-Hochberg.
Bolded values indicate statistically significant results.

To illustrate the relationship between FKBP5 variant genotypes and BP types, as well as the genotype distribution among participants with stressors, a facet-wrapped stacked proportional bar plot was employed **(Figure 2)**. This visualization highlights the proportion of each BP type across genotypes and facilitates comparison of BP type distribution among various SNPs.

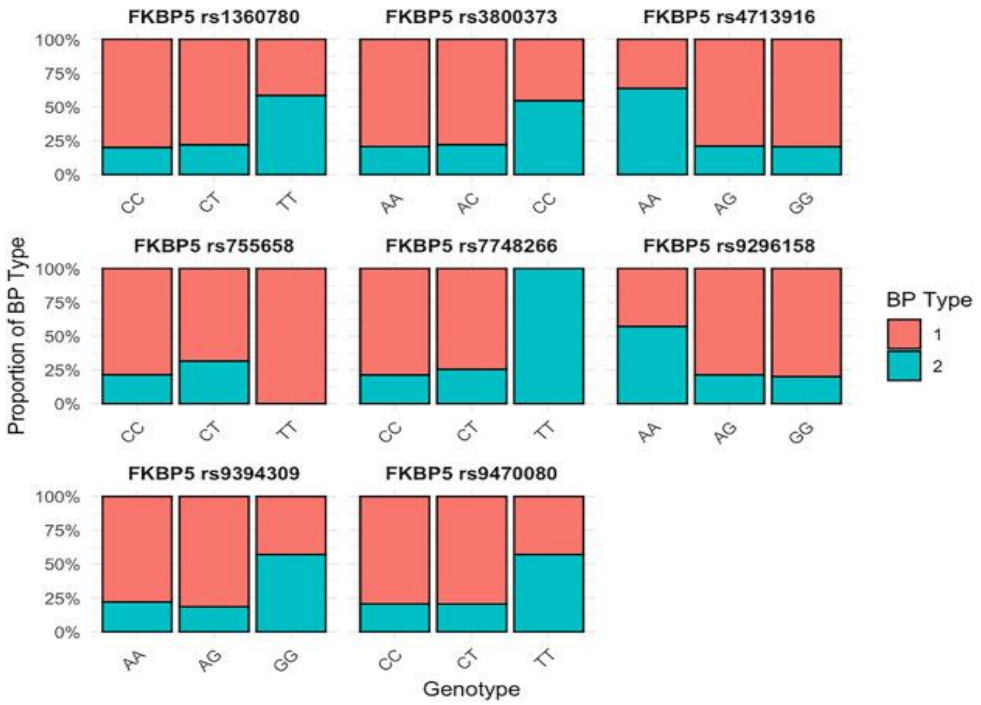


Figure 2. Distribution of bipolar disorder (BP) types across *FKBP5* genotypes when stressors are absent (R methodology is available in Supplementary Material S1)

Figure 3 presents a facet-wrapped proportional bar plot illustrating the association between FKBP5 genotypes and BP types, along with genotype distribution in participants without stressors. In most genotypes, Type 1 BP predominates, accounting for approximately 60–80% of cases. However, rs7748266 TT exhibits complete dominance of Type 2 BP (with the exception of rs755658, which is expected since it lacks statistical significance), while rs9296158 AA shows roughly 60% representation of Type 2 BP.

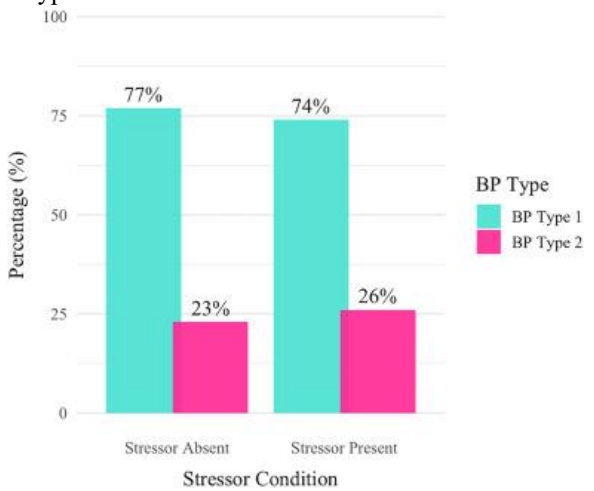


Figure 3. Bipolar disorder (BP) type 1 and 2 distribution in participants with and without stressors. (R methodology is available in Supplementary Material S1)

Figure 3 shows the distribution of BP types in the overall population.

Functional prediction result for **FKBP5** *rs755658*

The rs755658 variant was located within the FKBP5 gene (ENSG00000096060) and an additional non-coding transcript (ENSG00000285599) on chromosome 6 at position 35581893-35581893, GRCh38 (Supplementary Material S2 Table S1). Functional annotation indicated that this variant predominantly appears as an intronic variant in three FKBP5 transcripts and as a 3′ UTR variant in one FKBP5 transcript **(Figure 4)**. Moreover, it was identified as two intron/non-coding transcript variants within ENSG00000285599 (lncRNA).

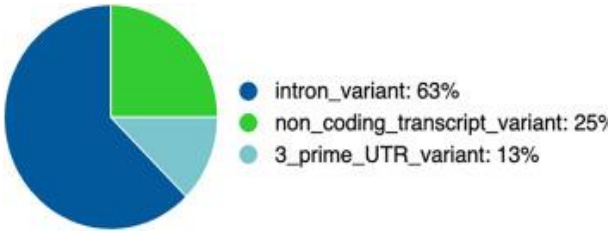


Figure 4. Percentage distribution of the consequence of *FKBP5* rs755658 variant from Variant Effect Predictor (VEP) result

SpliceAI analysis showed no evidence of splicing alteration, as all prediction scores were 0.00, indicating that rs755658 likely does not influence RNA splicing (Supplementary Material S2 Table S1). Although this variant is located in an intronic region, it demonstrated an association with MDD, suggesting it may exert regulatory effects.

According to RegulomeDB, rs755658 possesses potential regulatory activity, receiving a score of 0.55 and a rank of

1f (eQTL/caQTL + TF binding/chromatin accessibility peak), implying possible involvement in transcriptional control. In this system, lower scores denote stronger functional evidence [77]. The variant overlapped with 299 chromatin accessibility peaks, further supporting its regulatory capacity.

ChIP-seq profiling revealed that rs755658 lies within transcriptionally active regions across several tissues. Noteworthy transcription factor binding events were observed in the brain (ZNF70, CTCF), bone marrow (CEBPA), tibial nerve (EP300), and colon (POLR2A), among others (Supplementary Material S2 Table S2). The most prominent signal (114.04) occurred in SK-N-SH neuroblastoma cells for ZNF70 binding **(Figure 5)**, suggesting strong transcriptional regulation within neuronal cells—consistent with the established role of FKBP5 in stress response and psychiatric disorders.

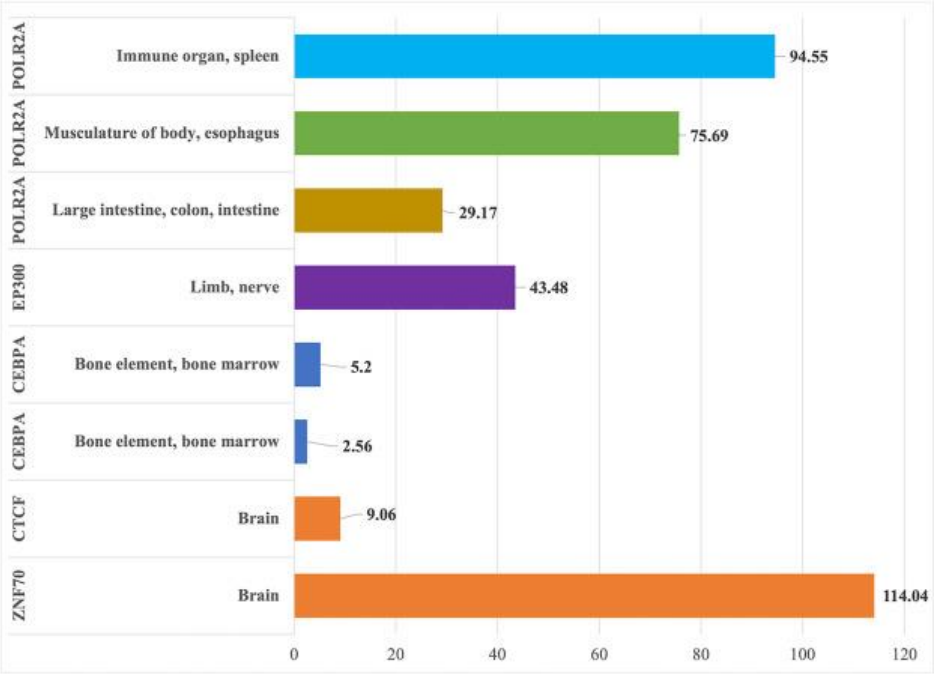


Figure 5. displays a bar chart representing transcription factor binding intensities across various tissues and chromatin peak regions. Abbreviations: ZNF70 – Zinc Finger Protein 70; CTCF – CCCTC-Binding Factor; CEBPA – CCAAT/Enhancer Binding Protein Alpha; EP300 – E1A Binding Protein p300; POLR2A – RNA Polymerase II Subunit A

The rs755658 site overlaps several enhancer elements and chromatin regions characterized by transcriptional activity, including both strong and weak enhancer states (Supplementary Material S2). Expression QTL results indicated that rs755658 affects the expression of *TULP1*, *MAPK13*, *TEAD3*, and *RP3-340B19.3*, rather than *FKBP5*, implying it may act through a trans-regulatory process. These associations were found in several human tissues, particularly within brain areas such as the hypothalamus, frontal cortex, putamen, nucleus accumbens, anterior cingulate cortex, and Ammon's horn (Supplementary Material S2 Table S3).

According to HaploReg analyses, rs755658 lies within chromosomal regions enriched with histone modifications that mark regulatory activity in the brain **(Figure 6)**. Such epigenetic signatures are typical of enhancer or promoter regions, suggesting that rs755658 could modulate *FKBP5* expression in a brain-region-dependent manner. This regulatory pattern may influence neural functions associated with emotion, cognition, and stress responses, potentially linking it to sleep disturbances observed in bipolar disorder [78-83].

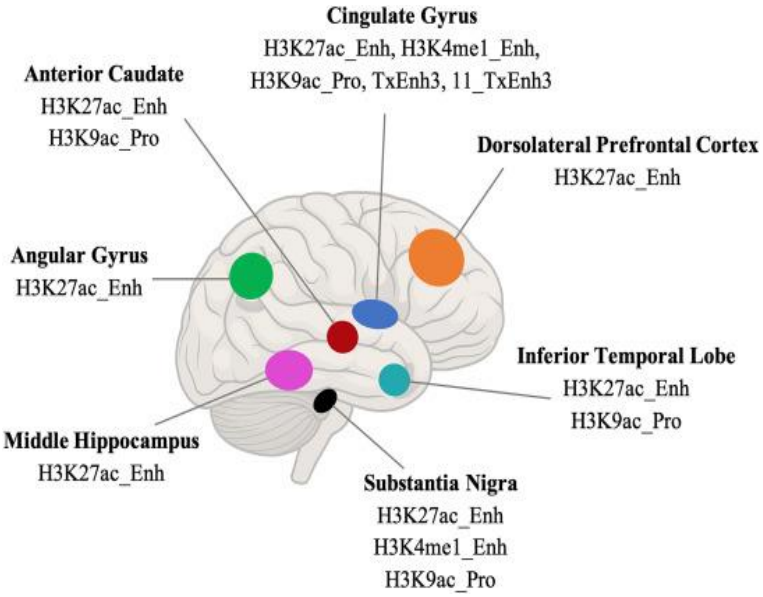


Figure 6. depicts brain regions where the rs755658 SNP is linked to active regulatory chromatin marks. These include H3K27ac, which indicates active enhancers; H3K4me1, marking poised or active enhancers; H3K9ac, signifying active promoters; and the 11_TxEnh3 state, representing transcriptionally active enhancer regions

Reactome pathway enrichment analysis via SNPnexus indicates that rs755658 may play a role in cellular stress responses, MECP2-mediated transcriptional regulation, and nuclear receptor signaling **(Figure 7)**. These pathways are closely connected to mood regulation, neuroplasticity, and psychiatric conditions, supporting the idea that rs755658 could have functional relevance in stress-related disorders [84–86].

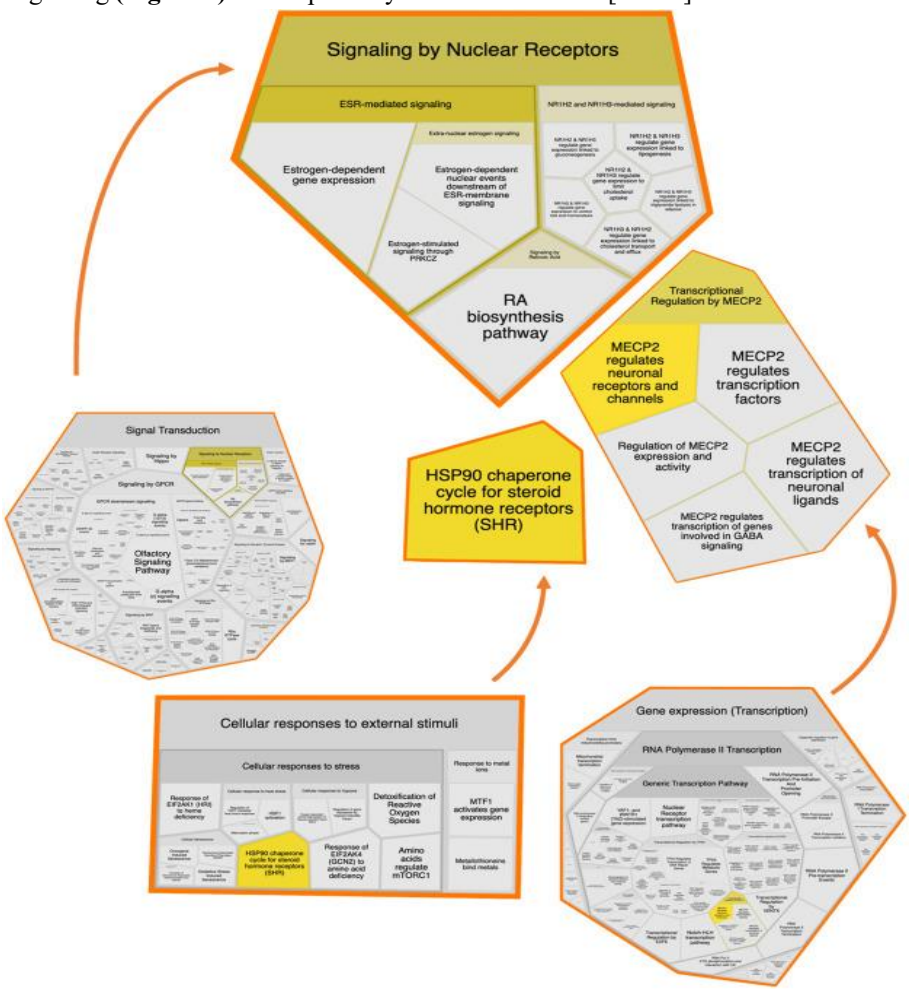


Figure 7. Illustration of the Reactome pathway enrichment of rs755658

Discussion

This study examined the relationship between FKBP5 polymorphisms and insomnia during depressive episodes in bipolar disorder (BP). Notably, FKBP5 rs755658 showed a significant association with insomnia symptoms even after BH correction, suggesting that variations in FKBP5 may exacerbate sleep disturbances, particularly under stress. Interestingly, our previous research did not detect a link between these FKBP5 polymorphisms and BP but did find associations with major depressive disorder (MDD) [21]. Given that MDD has lower heritability (~40%) compared to BP (~80%) [87, 88], environmental factors are likely more influential in its onset. This may explain why Szczepankiewicz *et al.* [21] reported associations with MDD but not BP, indicating that stress-responsive genetic variants such as FKBP5 may play a more prominent role in conditions where environmental triggers are critical. In line with this, the current analysis revealed an association between FKBP5 rs755658 and insomnia specifically in BP patients who experienced stressors prior to disease onset.

Our findings suggest that individuals carrying the CT or CC genotypes of FKBP5 rs755658 may be more susceptible to insomnia during depressive episodes when preceded by stress. This aligns with the study by Li *et al.* [89], which identified the CC genotype of FKBP5 rs3800373 and the CT genotype of FKBP5 rs1360780 as risk factors for sleep disturbances under occupational stress. These results imply that the C allele may act as a risk variant, potentially heightening vulnerability to stress-related insomnia, likely through FKBP5's regulatory role in stress response. Future research should investigate interactions between FKBP5 rs755658 genotypes, environmental stressors, and other biological factors to clarify these mechanisms.

Among BP patients without preceding stressors, our analysis indicates that FKBP5 polymorphisms may be linked to BP subtype **(Figure 2 and 3)**, which could, in turn, influence the likelihood of developing insomnia symptoms during depressive episodes.

To our knowledge, this is the first study to associate FKBP5 rs755658 with insomnia symptoms and, more broadly, with psychiatric manifestations. Functional studies of this variant are scarce. While rs755658 does not appear to directly affect RNA splicing, its genomic context implicates roles in transcription factor binding and post-transcriptional regulation. Though primarily intronic, annotations suggest it may carry regulatory potential. Previous research indicates that over 88% of trait- or disease-associated variants identified by GWAS are located in non-coding regions, with 45% in introns, yet the functional impact of intronic SNVs remains underexplored [68, 90]. Liao *et al.* [77] also highlighted that genetic variants often influence disease through regulatory mechanisms rather than coding changes, affecting elements such as TF binding sites, histone modifications, DNA methylation, and DNase hypersensitivity sites.

In silico predictions additionally identified 3′ UTR variants, which can disrupt miRNA binding, alter mRNA stability, affect polyadenylation, and influence translation efficiency, potentially modulating gene expression and contributing to disease risk [91-96]. Two intron/non-coding variants in lncRNA were also observed. LncRNAs regulate diverse cellular processes, including cell cycle progression, apoptosis, and gene stability [97-100], and dysregulated or mutated lncRNAs are increasingly recognized as critical for understanding transcriptional regulation in brain function [100]. Chromatin state analysis further positions rs755658 within enhancer-associated regions, suggesting it may influence FKBP5 transcription in a tissue-specific manner. Considering FKBP5's role in stress response and psychiatric disorders, this variant could contribute to individual differences in stress-related phenotypes, including insomnia in BP.

Future work should examine the biological pathways linking FKBP5 rs755658 to insomnia, particularly its involvement in stress regulation. At present, the NCBI database indicates that the rs755658 polymorphism occurs predominantly in European populations (~81.72%) (https://www.ncbi.nlm.nih.gov/snp/rs755658), highlighting potential implications for disease susceptibility, drug response, and personalized healthcare strategies. This high prevalence underscores the need for further research to clarify its functional consequences. Additionally, the observed association between seven FKBP5 polymorphisms and bipolar subtype in patients without prior stress exposure is intriguing, but additional studies are required to understand the implications of this finding fully.

Limitations

Several factors may limit the interpretation of our findings. First, stress exposure was based on self-reports, and no detailed data on the type, duration, or intensity of stress were collected, making the results vulnerable to recall or reporting bias. Second, the study examined only eight FKBP5 polymorphisms, which may not reflect the full genetic variability of the gene, potentially restricting the scope of our conclusions. Third, the modest sample size may reduce statistical power and limit generalizability. Additionally, insomnia was assessed as a symptom rather than through formal clinical diagnosis, and standardized sleep assessment tools, such as the Pittsburgh Sleep Quality Index (PSQI) [101] or the Insomnia Severity Index (ISI) [102], were not used. Finally, conclusions regarding the TT genotype are based on only three individuals, making these results preliminary and in need of

replication. Despite these constraints, this work provides valuable insight into a relatively unexplored area, as few studies have addressed the connection between FKBP5 polymorphisms and insomnia during depressive episodes in BP, emphasizing the novelty of our findings.

Conclusion

This study highlights a potential link between FKBP5 genetic variation and insomnia during depressive episodes in stress-sensitive bipolar disorder. The rs755658 polymorphism emerged as significantly associated with insomnia, suggesting it may influence vulnerability to sleep disturbances under stress. Individuals carrying the CT or CC genotypes who experienced stress prior to disease onset were at higher risk, whereas the TT genotype appeared to be less frequently associated with insomnia. Computational predictions indicate that rs755658 may have regulatory effects on transcription factor binding, enhancer activity, and gene expression, although experimental confirmation is needed. These results underscore the interplay between genetic predisposition, environmental stressors, and psychiatric symptoms. Further research is required to clarify the mechanisms through which FKBP5 rs755658 contributes to insomnia and to explore its role in depressive subtypes of BP. A better understanding of this relationship could help guide future interventions aimed at alleviating stress-related sleep disturbances in bipolar disorder.

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