BMC Psychiatry

RESEARCH Open Access

Mood disorders and 5-HTR2A genetic variants – the moderator effect of inflammation on expression of affective polarity phenotype

Maja Pantovic-Stefanovic[1,2*], Jelena Karanovic[3,4], Vladimir Jurisic[5], Bojana Dunjic-Kostic[2,6], Milica Nesic[1,2], Sara Dodic[1,2], Marta Gostiljac[1], Marija Puric[1,2], Dusanka Savic Pavicevic[3] and Maja Ivkovic[1,2]

Abstract

Background Although repeatedly confirmed, the molecular nature of gene-environment (GxE) interactions has rarely been investigated in the clinical context of mood disorders. This study assesses the relationship between *HTR2A* genetic variants and the modulatory effect of inflammation in a collective cohort of patients with major depressive disorder (MDD) and bipolar disorder (BD), as a unified group with two distinct phenotypes.

Methods The study included 138 patients with acute mood episodes (BD = 83; MDD = 55). *HTR2A* rs6313 and rs6314 genotyping was performed while measuring platelet-derived indicators of inflammation (platelet count (PLT), mean platelet volume (MPV), plateletcrit, and platelet distribution width) and the MPV/PLT ratio.

Results The *HTR2A* rs6313 variant is a significant predictor of the polarity phenotype in mood disorders, with the MPV/PLT ratio moderating this relationship, but only under low-inflammatory conditions. In more pronounced inflammatory states, genetic influences lose their predictive role.

Conclusions To our knowledge, this is the first study to investigate the complex interplay between platelet-derived indicators of inflammation and *HTR2A* variants in the context of mood disorders. Without pro-inflammatory conditions, mood disorders seem to be more genetically determined. Under pro-inflammatory conditions, phenotypic presentation is less dependent on genetic factors. GxE interactions in mood disorders are multifaceted, context-dependent and relevant for assessing their clinical presentation and course.

Keywords *HTR2A* rs6313, Mood disorders, Unipolar depression, Bipolar disorder, Gene-environment interactions

*Correspondence:
Maja Pantovic-Stefanovic
majapantovic@yahoo.it
[1]Department for Bipolar Disorders, Clinic for Psychiatry, University Clinical Centre of Serbia, Pasterova 2, Belgrade 11000, Serbia
[2]Faculty of Medicine, University of Belgrade, Dr Subotica 8, Belgrade 11000, Serbia
[3]Centre for Human Molecular Genetics, Faculty of Biology, University of Belgrade, Studentski trg 16, Belgrade 11000, Serbia
[4]Present address: Laboratory for Molecular Biology, Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Vojvode Stepe 444A, Belgrade 11042, Serbia
[5]Faculty of Medical Scinces, University of Kragujevac, Svetozara Markovica 69, Kragujevac 11000, Serbia
[6]Institute of Mental Health, Milana Kasanina 3, Belgrade 11000, Serbia

Background

Mood disorders affect millions of individuals and cause considerable social, economic, and personal burden [1, 2]. The two most important phenotypic expressions of mood disorders, major depressive disorder (MDD) and bipolar disorder (BD), share a part of their clinical presentation, leaving almost 60% of BD patients misdiagnosed at their first episode, further increasing the burden of BD [3, 4]. Moreover, the conversion rate of MDD to BD is high, and it is likely that more than one in ten patients diagnosed with MDD will convert to BD within a decade [5, 6]. However, the two disorders significantly differ in course, prognosis and treatment, making timely identification of discriminating factors of crucial importance.

Both MDD and BD are believed to stem from a complex interplay of genetic susceptibility and factors mediating that susceptibility [7]. In that sense, there is increasing evidence that inflammation shapes the already present vulnerability of the serotonergic system [4, 7]. Despite advancements in psychiatric research, the precise etiological mechanisms connecting the two and defining phenotypic expressions of mood disorders remain elusive.

Various streams of evidence suggest a connection between the clinical presentation of mood disorders and alterations in serotonin (5-hydroxytryptamine, 5-HT) receptor genetic variants. The serotonin receptor 2 A (5-HTR2A) genetic variants, specifically rs6313 (T102C) and rs6314 (C1354T or His452Tyr/H452Y), have gathered particular attention due to their potential role in affecting receptor function, thus influencing mood regulation. At the same time, they have been implicated in multiple psychiatric disorders or their symptoms, although not consistently. Namely, rs6313 variant is associated with a variety of symptoms in depressed patients, including anxiety and neuroticism, but also suicidality, impulsivity, and seasonal mood variations [8–12]. Furthermore, rs6313 has been linked to antidepressant-induced dysphoria in patients with misdiagnosed bipolar spectrum disorders [13], suggesting its significance in both unipolar and bipolar phenotypes of mood disorders. Although the rs6313 is a silent mutation (the receptor with a T to C substitution on rs6313 remains the same), the presence of mood disorders has almost exclusively been related to the C allele. This suggests that the functional significance of receptor properties is most probably subject to environmentally mediated epigenetic changes [14].

Another genetic variant of the 5-HTR2A receptor, rs6314, has been implicated in altered receptor expression and functionality, leading to decreased working memory and attention. It has also been linked to alterations in the synaptic plasticity mechanisms related to 5-HT2AR signaling and its interplay with the glutamate system, as well as to hippocampal volume and activation [15]. At the same time, it has been associated with MDD, antidepressant treatment response and lack of remission, suggesting its role in the development of unipolar depression [16, 17]. It has also been related to the occurrence of postpartum depression, a condition frequently related to the onset episode of bipolar disorder [18], while other studies have shown it to be part of the vulnerability haplotype of BD [19]. However, the data regarding the association of the polymorphism with specific mood symptoms, or with unipolar or bipolar disorders, have not been consistent [20–22].

The link between affective disorders and the immune system has also been repeatedly demonstrated, so much so that they are thought to be significantly shaped by immune-mediated epigenetic processes [7, 23]. MDD has been frequently related to both pro-inflammatory and regulatory immune mediators, while BD has been associated with more pronounced, mostly pro-inflammatory conditions [4, 24]. In these states, various immune mediators interact with several pathways implicated in mood disorders, including the serotonergic system [25, 26], redirecting tryptophan metabolism towards the kynurenine pathway, thereby decreasing serotonin availability [26]. However, some studies indicate that this effect is not caused by changes in serotonin levels directly but rather by immune-mediated changes in the 5-HTR2A receptor [27].

Platelets are involved in these processes and are considered to be indicators of the inflammatory state and a link connecting mental disorders and inflammation. Interestingly, they exhibit some similarities with neurons, including serotonin trafficking. Human platelets also express functional 5-HTR2A receptors, reflecting their central nervous system (CNS) activation [28–30]. Simultaneously, the rs6314 T allele was found to influence platelet aggregation in response to serotonin [31, 32], while rs6313 has been associated with somatic disorders connected to aberrant platelet functioning [33, 34]. Several platelet-related parameters of inflammation have also been investigated as potential indicators of immune activation in mood disorders. For instance, increased mean platelet volume (MPV), a marker of platelet activation, has been considered to be indicative of the intensity of inflammatory processes but also is subjective to habits and lifestyle. Moreover, it has proven to provide important information on the course and prognosis of many pathological conditions, including psychiatric disorders [35, 36]. Elevated MPV has been associated with MDD [37, 38], while antidepressant treatment has been found to restore MPV levels within physiological ranges [39]. Plateletcrit (PCT), defined as percentage of platelet mass to the whole blood volume and an indicator of immune activation, has also been used to assess the degree of

inflammation. In mood disorders, it has been altered during manic episodes [40], and the same was noted for both absolute platelet count (PLT) and MPV [40, 41]. Moreover, when comparing MDD, BD and healthy subjects, patients with BD show the highest MPV, and the lowest PLT, followed by MDD and healthy individuals, indicating that the state of inflammation gradually elevates according to the severity of psychopathology [36]. The relationship between MPV and PLT, has been proven to be a powerful prognostic marker and indicator of inflammation. It has also proven to be more informative than MPV as a predictor of long-term mortality in many disorders [42]. However, to the best of our knowledge it has not been previously assessed in mood disorders [43]. Also, the studies even less explore the implication of indicators of inflammation on functioning of serotonergic system, namely *HTR2A* variants, in mood disorders.

Thus, this study aimed to assess the nature of the relationship between genetic variants in *HTR2A*—specifically, rs6313 and rs6314—and the modulatory effect of platelet-related indicators of immune activation in a collective cohort of patients diagnosed with MDD and BD, as a unified group with two distinct phenotypes.

Methods

Patients and study design

The required sample size to obtain a power of $1-\beta=0.80$ at $\alpha=0.05$ was calculated to be fifty-one subjects per group for determining the intergroup differences. One hundred-seventy-nine patients hospitalized at the Clinic for Psychiatry, University Clinical Centre of Serbia, Belgrade, for the treatment of acute mood episode (MDD=78 and BD=101) provided written informed consent to participate in the study. Subsequent screening for inclusion/exclusion criteria derived a final sample of 138 patients with mood disorders (bipolar affective phenotype/bipolar disorder (BD=83 (39 in manic episode and 44 in depressive episode) or unipolar affective phenotype/major depressive disorder (MDD=55)). The study was approved by the Ethics Committee of the Clinical Centre of Serbia (3672/10 and 4072/5) and conducted in accordance with the Helsinki Declaration of 1989 and the recommendations of the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement. In the patient's group, psychiatric diagnosis was confirmed on the basis of the ICD-10 and the Mini-International Neuropsychiatric Interview (MINI) [44, 45].

The severity of depressive symptoms was assessed with the Hamilton Depression Rating Scale (HDRS) [46]. Manic symptoms were evaluated by Young Mania Rating Scale (YMRS) [47]. The Brief Psychiatric Rating Scale (BPRS) was used to assess the overall level of psychiatric symptoms, additionally using 5 subscales to assess the severity of particular groups of symptoms: Affect; Positive Symptoms; Negative Symptoms; Resistance; and Activation [48]. Patients were enrolled in the study if they met the following criteria: (a) their baseline HDRS was ≥18 points (in a depressive episode) or their baseline YMRS score was ≥20 points (in a manic episode), and (b) complete data regarding socio-demographic information, course of illness information, immune indicators and genetic variants. Remission was defined as HDRS score≤7 and YMRS<8 [46, 49, 50]. All psychometric testing was done at two time points. The initial assessment was done within three days upon the admission. The second assessment was done on the day of the discharge and if, upon psychometric testing, the patients reached study remission criteria they were included in the final sample.

Diagnosis and psychometric evaluations were made by two blinded, licensed psychiatrists through complete semi-structured interviews in combination with all other available data from medical records. The exclusion criteria were as follows: (a) the history or current diagnosis of any other ICD-10 disorder excluding personality disorders, (b)>8 mood episodes within the previous 12 months, (c) the history of treatment resistance, (d) medical conditions associated with changes in inflammatory response [51], and (e) sociodemographic characteristics previously associated with alterations of immune mediators [51]: obesity [body mass index (BMI)≥30 kg/m^2] or undernutrition with recent weight loss, and smoking≥20 cigarettes/day. In addition, all patients were screened for acute infection by a complete physical examination, body temperature quantification, erythrocyte sedimentation rate, complete blood count, hepatic enzymes, and electrolytes. All biochemical measurements were conducted by investigators who were blinded to the diagnostic status of the patients.

Biochemical analysis

Blood samples were taken from the antecubital veins of patients between 7 a.m. and 8 a.m. after an overnight fast. The first blood samples were taken within three days upon the admission, while the second blood samples were drawn on the day of the discharge, if upon psychometric testing, the patients reached remission in accordance with the study criteria.

For the measurement of complete blood cell characteristics (PLT, MPV, PCT and platelet distribution width - PDW), blood was collected using Vacutainer plastic blood collection tubes containing EDTA as an anticoagulant and was analyzed in an automated blood cell counter (Beckman Coulter LH 750, Fullerton, CA, USA). The MPV/PLT ratio was calculated by dividing the MPV with the absolute PLT.

Genetic analysis

DNA was isolated from blood samples of all subjects using the QIA amp DNA Blood Mini Kit (Qiagen, Hilden, Germany). Genotyping of the *HTR2A* rs6313 and rs6314 genetic variants was performed as previously described [52].

Statistical analysis

The data were analyzed using the Software Package for Social Sciences for Windows v.26.0 (SPSS Inc. Chicago, IL), PROCESS macro for SPSS. The Kolmogorov–Smirnov test was used to determine the normality of the distribution of the numerical values.

There is a lack of confidence about the functional effects of the T and C alleles of the *HTR2A* rs6313 and rs6314 genetic variants. In order to study the effect of the T and C alleles in a systematic and unbiased way and to minimize the chance of a type II error three models were considered for both variants (a) recessive: TT homozygote *versus* C allele carriers, (b) dominant: T allele carriers *versus* CC homozygote, and (c) additive: TT *versus* CT *versus* CC genotype. As there was no rs6314 TT homozygote among studied patients only heterozygous T allele carriers (CT) *versus* CC homozygote were tested. Bearing in mind the number of different genetic models we included, in order to avoid risk of type I error in test including genetic variants, results were considered to be significant only if p-value was <0.023 [53].

The data are described using standard descriptive statistics (i.e., frequencies and percentages for attributive variables and mean value with standard deviation for numeric variables). For discrete variables, χ^2, with continuity correction according to Yates or Fisher's exact probability test were used. To compare the variables between the groups t-test between two groups and one-way analysis of variance (ANOVA) among multiple groups were used.

Multivariable analysis, after adjusting for potential confounding factors, was performed by ANCOVA to assess the differences in the levels of immune mediators between the study groups. Adjustment for multiple testing was carried out by post-hoc LSD test. To estimate the association between immune mediators' levels and clinical variables, the partial correlation coefficients controlling for age and sex were calculated.

Confounding factors mutual for all analyses were age and sex. Other confounding factors were added in accordance to the phase of the disorder (acute or remission). Namely, for all multivariable assessments (ANCOVA, partial correlation) involving immune mediators, the analyses of the total patient sample were additionally covaried for type of affective episode (mania/depression), in the acute phase. In remission, for all assessments involving immune mediators, the analyses of the total patient sample were additionally covaried for type of the disorder (unipolar depression/bipolar disorder). For the multivariable assessments (ANCOVA) including genetic variants and clinical characteristics, all analyses were also covaried for type of the disorder. Finally, moderation analyses were performed using PROCESS (macro for SPSS) [54], based on ordinary least square regression within path analytical framework. Given that 5-HTR2A genetic variants, indicators of inflammation and affective phenotype expression were interrelated, we investigated the association between the biological markers and affective phenotype of the mood disorders, controlling for potential confounding factors. Using two separate models, we examined the moderating effects of inflammation on the relationship between HTR2A variants as predictors and affective phenotype expression as dependent variable. Age, sex, and type of the current affective episode were included as covariates.

To increase the precision of the data bootstrapping method was used in all testing. Adjusted odds ratios with 95% confidence interval (95% CI) were computed. The results were regarded as statistically significant only if both p-value was <0.05 and confidence intervals (CI) were adequate.

Results

HTR2A variants and indicators of inflammation in patients with mood disorders

Socio-demographic and clinical characteristics of the study groups as well as *HTR2A* variants and indicators of inflammation of subjects in our study are shown in Table 1.

HTR2A variants and clinical characteristics

We explored *HTR2A* rs6313 and rs6314 variants in regard to phenotypic expression of the mood disorder, severity of the psychopathology and characteristics of the course of illness.

For rs6313, C allele carriers more frequently presented with bipolar phenotype of mood disorder ($p=0.012$). On the other hand, compared to CC homozygotes, rs6314 T allele carriers had an earlier age of onset (CC homozygotes: 34.13 ± 11.23, T allele carriers: 33.40 ± 14.18, $F=25.636$, $p=0.000$), longer duration of untreated disorder (CC homozygotes: 17.26 ± 28.21, T allele carriers: 66.85 ± 75.00, $F=9.852$, $p=0.000$), more affective episodes (CC homozygotes: 7.39 ± 4.43, T allele carriers: 9.07 ± 9.81, $F=16.698$, $p=0.000$) and more inpatient treatments (CC homozygotes: 5.49 ± 4.02, T allele carriers: 8.55 ± 11.61, $F=11.705$, $p=0.000$).

Indicators of inflammation and clinical characteristics

The patients with mood disorders did not differ in the MPV, PLT, PCT, PDW or MPV/PLT in regard to the

Table 1 Socio-demographic, clinical, genetic and blood cells characteristics of the study groups

Socio-demographic variables	BD group ($n=83$)		MDD ($n=55$)		BD vs. MDD (p-value)	
	Acute phase	Remission phase	Acute phase	Remission phase	Acute phase	Remission phase
Sex (female, %)	63.60		65.5		[a]0.084	
Age (years)	45.61 ± 11.05		52.50 ± 9.64		[b]0.000	
BMI (kg/m^2)	25.33 ± 4.01		23.12 ± 1.37		[b]0.004	
Smoking (cigarettes/day)	14.28 ± 5.26		10.47 ± 10.52		[b]0.002	
Marital state (with partner, %)	42.70		76.40		[a]0.000	
Education (years)	12.78 ± 2.15		11.28 ± 2.25		[b]0.004	
Clinical variables						
Age of onset (years)	27.43 ± 8.90		39.98 ± 11.22		[b]0.001	
Duration of illness (years)	18.18 ± 10.24		11.86 ± 8.52		[b]0.002	
Number of previous episodes	10.39 ± 7.19		5.39 ± 3.09		[b]0.002	
Number of inpatient treatments	7.77 ± 7.21		4.28 ± 3.45		[b]0.007	
Number of suicide attempts	0.85 ± 1.44		0.72 ± 1.09		[b]0.795	
Family history (n, %)						
Psychosis	12.04		9.10		[a]0.531	
Mood disorders	55.42		42.30		[a]0.083	
Suicide attempts/suicide	28.92		25.50		[a]0.474	
Psychotropic treatment (n, %)						
Antidepressants	25.60		100.00		[a]0.000	
Mood stabilizers	84.61		37.70		[a]0.000	
Antipsychotics	69.50		26.90		[a]0.000	
Time to remission		49.80 ± 17.73		48.00 ± 9.27	[b]0.361	
Psychometric properties						
BPRS	30.57 ± 10.95	9.37 ± 7.71	36.36 ± 7.37	3.53 ± 2.79	[b]0.003	[b]0.001
BPRS Positive Symptoms	5.71 ± 5.17	1.78 ± 2.72	5.08 ± 2.00	0.58 ± 0.75	[b]0.307	[b]0.001
BPRS Affect	9.65 ± 3.87	2.98 ± 2.50	12.66 ± 2.57	1.02 ± 0.97	[b]0.000	[b]0.000
BPRS Negative Symptoms	4.65 ± 3.88	1.51 ± 1.77	8.57 ± 2.72	0.47 ± 0.79	[b]0.000	[b]0.000
BPRS Resistance	4.56 ± 4.17	1.70 ± 2.65	4.00 ± 1.44	0.51 ± 0.77	[b]0.269	[b]0.001
BPRS Activation	5.38 ± 2.03	1.55 ± 1.18	6.06 ± 1.62	0.94 ± 1.15	[b]0.605	[b]0.007
HAM-D	20.51 ± 11.29	3.94 ± 2.74	26.08 ± 5.34	9 ± 2.45	[b]0.001	[b]0.736
YMRS	15.89 ± 12.45	2.89 ± 2.69	0.55 ± 0.91	0.56 ± 0.93	[b]0.001	[b]0.001
***HTR2A*variants**						
rs 6313 TT vs. CT vs. CC	3.3/ 55.7/ 41.0		18.9/ 41.5/ 39.6		[a]0.000	
rs 6314 (TT vs. CT) vs. CC	13.1/ 86.9		22.6/ 77.4		[a]0.139	
Blood cell characteristics						
White blood cell count (x10^3)	7.15 ± 2.51	7.69 ± 2.41	6.93 ± 2.13	6.94 ± 1.55	[c]0.457	[c]0.734
Platelets (N)	235.16 ± 67.39	224.49 ± 68.84	229.28 ± 61.91	259.01 ± 92.98	[c]0.829	[c]0.141
Mean platelet volume	8.59 ± 0.97	8.67 ± 0.96	8.59 ± 0.67	8.31 ± 0.65	[c]0.146	[c]0.852
Plateletcrit	0.20 ± 0.04	0.18 ± 0.04	0.19 ± 0.05	0.78 ± 2.27	[c]0.690	[c]0.534
Platelet distribution width	16.39 ± 0.42	16.66 ± 0.57	16.61 ± 0.66	15.70 ± 4.20	[c]0.379	[c]0.616
MPV / PLT ratio	0.40 ± 0.11	0.38 ± 0.01	0.41 ± 0.12	0.50 ± 0.50	[c]0.703	[c]0.667

Values are means ± standard deviations unless otherwise stated

[a]Chi-squared test; [b]Independent samples t-test; [c]Analysis of covariance (ANCOVA)

Abbreviations: BMI, body mass index; BD, bipolar disorder; MDD, major depressive disorder; BPRS, Brief Psychiatric Rating Scale; HDRS, Hamilton Depression Rating Scale; YMRS, Young Mania Rating Scale; PLT, absolute platelet count; MPV, mean platelet volume; PCT, plateletcrit; PDW, platelet distribution width

different affective phenotypes (unipolar vs. bipolar), in acute phase and subsequent remission (Table 1).

During the exacerbation of the illness, patients with mood disorders and higher MPV/PLT had more severe overall pathology, as measured by the BPRS score ($r=0.303$, $p=0.017$). More specifically, the MPV/PLT was significantly related to the BPRS thinking subscore ($r=0.230$, $p=0.033$) and exhibited a trend towards significance in regard to the severity of depressive symptoms measured by the HAMD ($r=0.204$, $p=0.056$). Likewise, a lower PLT and lower PCT correlated with overall severity of the pathology (PLT: r=-0.354, $p=0.005$; PCT: r=-0.396, $p=0.003$), as well as disturbances in thinking (PLT: r=-0.244, $p=0.024$; PCT: r=-0.221, $p=0.048$) and activation (PLT: r=-0.275, $p=0.011$; PCT: r=-0.293, $p=0.008$), as measured by the BPRS.

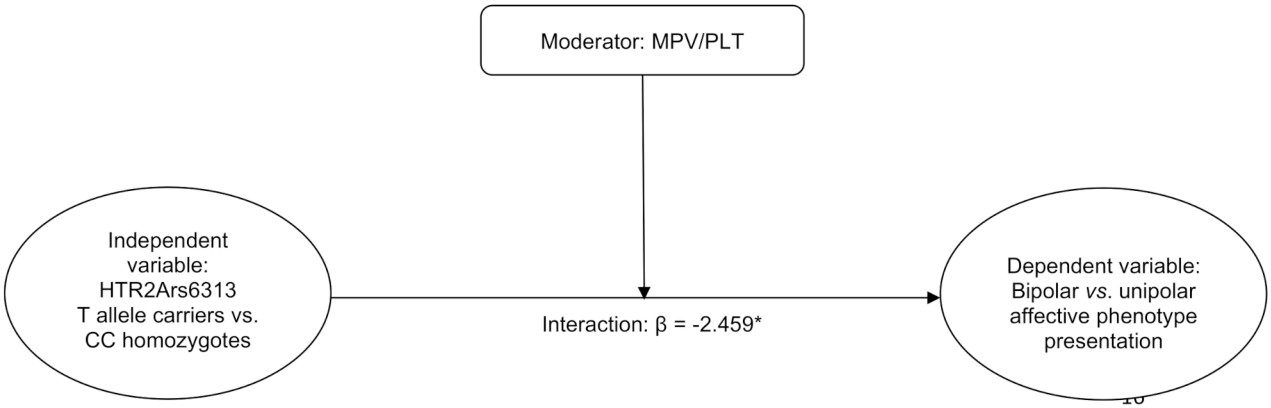


Fig. 1 Model of moderation effect of MPV/PLT on relationship between *HTR2A* rs6313 and affective phenotype expression

Table 2 Moderation effect of MPV/PLT values on HTR2A rs6313 variant and affective phenotype relationship

	B	SE	z	95% CI	*p*
Constant	-23.485	19665.659	−0.001	-38567.469–38520.498	0.999
MPV / PLT ratio	-10.285	27.021	−0.380	-63.246–42.6759	0.703
rs 6313 variant	-0.748	0.719	-1.040	-2.159–0.662	0.298
Age	-0.032	0.028	-1.140	-0.0871–0.023	0.254
Sex	1.442	0.800	1.802	-0.126–3.011	0.072
Type of the episode	22.956	19665.658	0.001	-38521.027 - 38566.940	0.999
rs 6313 variant X MPV / PLT ratio	140.199	59.086	2.373	24.392–256.006	**0.017**

Abbreviations: PLT, absolute platelet count; MPV, mean platelet volume

In the euthymic state, the PDW was higher in patients with more previous episodes requiring hospital treatment (r=0.380, p=0.035). It also exhibited a trend towards significance in regard to more lifetime episodes (r=0.351, p=0.053). Other indicators of inflammation showed no relationship with clinical characteristics in remission.

HTR2A variants and indicators of inflammation interplay

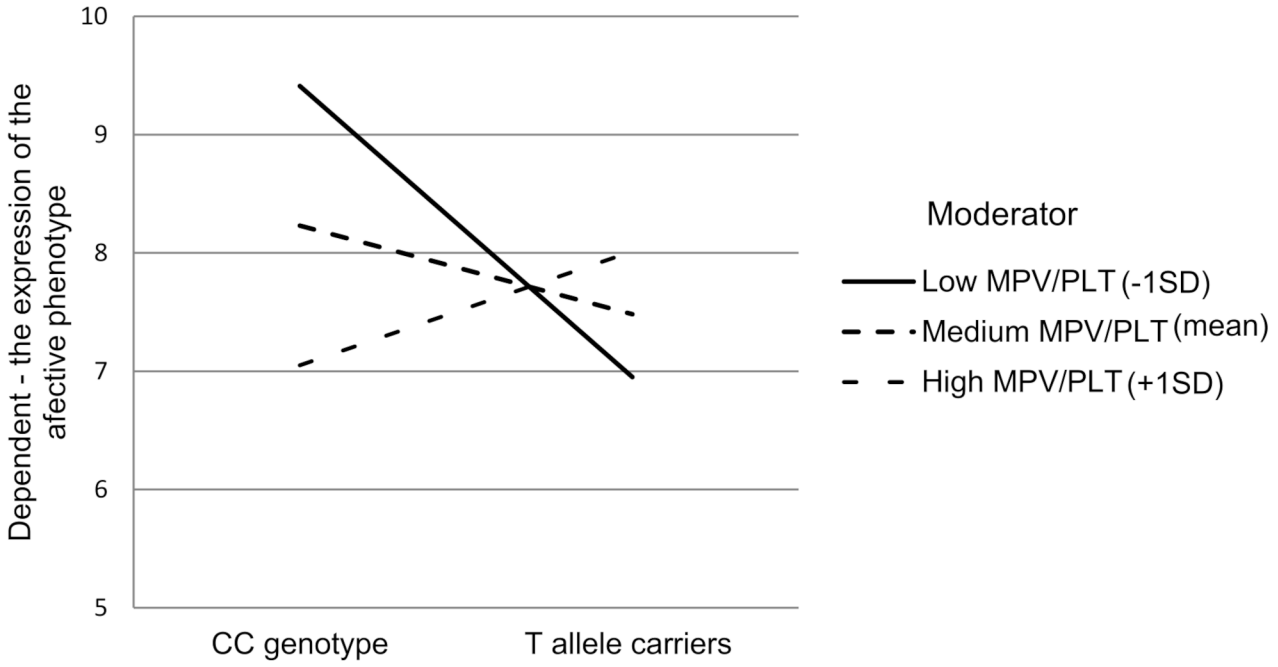
During the acute exacerbation of the disorder, the MPV/PLT exhibited a trend toward being significantly higher in patients with CC genotype (0.043±0.012) vs. T allele carriers (0.038±0.013) of *HTR2A* rs6313 (F=3.781, p=0.055). Patients with the CC genotype had lower absolute PLT (CC homozygotes: 210.73±9.97, T allele carriers: 242.91±7.86, F=6.113, p=0.016) and PCT (CC homozygotes: 0.177±0.008, T allele carriers: 0.201±0.007, F=6.070, p=0.016), but higher PDW (CC homozygotes: 16.617±0.088, T allele carriers: 16.350±0.065, F=6.070, p=0.016). With regard to being C allele carriers vs. TT homozygote of the rs6313, the patients did not differ in MPV/PLT, MPV, PLT, PCT, or PDW. Additionally, the MPV/PLT, MPV, PLT, PCT, and PDW did not differ among the rs6314 genotypes, combined or individually.

In remission, patients with the rs6313 CC genotype maintained a lower absolute PLT (CC homozygotes: 209.48±10.40, T allele carriers: 241.78±8.26, F=5.916, p=0.018). Other indicators of inflammation did not differ among the other genotype combinations of the *HTR2A* rs6313 and rs6314 variants.

The value of *HTR2A* variants and indicators of inflammation in predicting affective phenotype expression

In the first model, we found a significant moderation effect of the MPV/PLT values in the acute phase of the disorder on *HTR2A* rs6313 variant and affective phenotype relationship (Cox and Snell R^2=0.352, Nagelkerke R^2=0.495, p=0.000). In the model, T allele presence of rs6313 was a significant predictor of the unipolar phenotype, while the CC genotype was a significant predictor of bipolar phenotype of mood disorders, but only in patients with below median values of MPV/PLT (β=-2.459, p=0.035, CI: -4.747 – -0.172). Other variables in the model showed no independent predictive effect. Moreover, the presence of median (β=-2.459, p=0.298, CI: -2.159 – 0.661) to high (β=0.9624, p=0.254, CI: -0.693 – 2.618) values of MPV/PLT had no potentiating effect on the relationship between genotype and affective phenotype expression, although with higher MPV/PLT values the change in the direction of the effect was observed (Figure 1) (Table 2) (Graph 1).

No moderation effect of acute MPV/PLT on *HTR2A* rs6314 variant and affective phenotype relationship was found (β=44.363, p=0.450, CI: -70.848–159.574).



Graph 1 Expression of the affective phenotype as a function of multiple risk factors

Discussion

The relationship and the direction of the relationship between hereditary and environmental factors, such as inflammation has rarely been investigated in clinical context. In our study, *HTR2A* rs6313 genetic variant has been associated with increased risk of more severe presentation of mood disorders, affecting mood polarity. Indirect indicators of inflammation, such as the MPV/PLT were also related to more severe pathology in mood disorders as reported previously [55]. Furthermore, rs6313 remains a significant predictor of polarity phenotype of mood disorders. However, this is observed only under low inflammatory conditions, with the T allele predicting unipolar, and the CC genotype predicting the bipolar phenotype of the disorder. In more pronounced inflammatory states, genetic influences lose their significance, giving rise to other more complex factors that seem to contribute to the phenotypic presentation of mood disorders.

Several *HTR2A* variants have been frequently associated with a range of psychiatric disorders including BD and MDD [56]. Genetic variants, particularly rs6313, are related to an increased risk of developing mood disorders [56, 57]. Studies have found that rs6313 C allele may be associated with an increased risk of developing MDD and BD, while individuals with the T allele may be at a decreased risk [58, 59]. In addition, the C allele has also been linked to depression following seasonal patterns [8], a clinical condition possibly part of bipolar spectrum disorders [60]. However, a recent meta-analysis suggests the absence of a relationship between *HTR2A* rs6313 and both MDD [61] and BD [62].

A different stream of evidence showed that the symptoms rather than the disorders are more determined by the genetic variants. The rs6313 C allele increased the risk of suicidal behavior [8, 11, 12], a lack of empathy, greater anxiety, and communication problems [10–12] and even attention and cognitive impairments [63]. On the other hand, TT genotype was associated with positive personality features such as novelty seeking, in a mixed sample of patients with both unipolar and bipolar affective phenotypes [57].

Our study supports the majority of previous findings connecting *HTR2A* rs6313 and the clinical presentation of mood disorders [8, 10, 12, 57–59]. The results we obtained show that the C allele/CC genotype could be associated not only with more severe forms of mood disorders, but also with bipolar phenotype. However, the overall findings so far are still inconclusive, as some of the earlier studies explored patients with various diagnoses [57, 63], or included only single allele carriers without homozygotes being separately evaluated [12]. Alternatively, the studies propose the effect could be masked by confounders such as epistasis, epigenetic regulation, or RNA regulation [58, 61]. It is still unclear how and which additional factors lead to clinical expression of particular mood disorder.

The heterogeneity in the findings could be attributed to the complex and multifaceted functioning of the serotonergic system [64]. Previous studies linked the rs6313 C

allele with changes in platelet 5-HTR2A receptor density, binding potential, and sensitivity and expression, all of which were previously associated with mood disorders [13, 65, 66]. Although both the increased receptor density and binding related to the *HTR2A* variants might contribute to the development of mood disorders, research suggests that increased receptor density and hypersensitivity, conditions previously related to the C allele of rs6313, are more likely tied to the development of bipolar disorder [13, 65].

Abovementioned supports our study but also other research showing that individuals with higher 5-HTR2A receptor density and hyperactive receptors are more prone to experiencing symptoms of depression and anxiety, but more importantly to impulsivity, dysphoria, irritability and mood swings, all of which are features of the bipolar phenotype of mood disorders [13, 66–68]. It is worth mentioning that the studies exploring the connection used strict selection criteria and predominantly focused on drug-naïve or drug-free patients and those without significant comorbidities [66, 68]. Some of them also accounted for the effect of age [68] but other confounding factors have not been evaluated. While it has been discussed that C allele of rs6313 presents with increased levels and binding of the 5-HTR2A receptor, but also with significantly higher mRNA expression [58], some find contradictory results [14, 61, 69]. This is most likely related to environmental factors and epigenetic mechanisms, primarily C allele methylation, which has been known to affect the regulation of *HTR2A* expression [10, 14, 70].

It has been suggested that inflammation can influence methylation, leading to changes in gene expression [71]. Studies exploring the clinical significance of this effect are very important but still scarce. Animal studies are more frequent and find that inflammatory conditions can lead to changes in 5-HTR2A receptor expression and density in the brain, with a sustained effect even after acute inflammation [27, 72, 73]. In addition, other research suggests that inflammation may be able to modify the signaling pathways that regulate the expression of the 5-HTR2A receptor [27, 74–76], while the direction of these changes may depend on the level of inflammation [27, 72–74]. It is likely that inflammation upregulates 5-HTR2A receptor expression, rather than reduces availability of serotonin, which can be responsible for consequent depressive like symptoms [27]. Bearing in mind that the activation of serotonin 5-HTR2A receptors may lead to systemic anti-inflammatory effects [75, 76], the enhanced receptor expression in inflammatory conditions could be compensatory, but at the same time pose a vulnerability to individuals with already constitutively enhanced 5-HTR2A expression. When applied to our study, patients with the more vulnerable rs6313 CC genotype could be particularly sensitive to inflammation, creating a higher risk of the pathogenic shift towards the bipolar phenotype.

Patients showing inflammatory states with elevated PLTs have an impaired course of illness [77] or express more severe forms of depression [78]. In acute inflammation, mean platelet volume (MPV) tends to be lower and to decrease due to increased inflammation and cytokine release affecting platelet size. Conversely, in chronic inflammatory disorders, MPV is higher, indicating a role of platelets in chronic inflammation and suggesting that increased MPV values may be associated with chronic inflammatory conditions [79, 80]. Similarly, in acute inflammation, the number of PLT is often low, as seen in conditions like acute infectious diseases where platelet count decreases during the active inflammatory period before treatment and increases significantly after treatment. On the other hand, in chronic inflammatory disorders PLT is higher indicating a role for platelets in chronic inflammation [79, 81]. Also, the MPV/PLT ratio, has particularly been determined to be indicative of inflammatory processes [42]. The previous studies highlight the potential clinical significance of the MPV/PLT ratio in various medical conditions and populations. The research shows that MPV/PLT is an independent predictor of poor prognosis and mortality in acute exacerbation of chronic obstructive pulmonary disease [82]. It has also been shown to be indicative of cardiovascular risk and cancer prognosis as well as an indicator of liver function in pediatric and adult populations [83–87].

While some previous studies provide valuable insights into the independent relationship between mental disorders and PLT and mental disorders and MPV [38, 41], direct investigations specifically focusing on the MPV/PLT ratio in mental health contexts are limited. In our patients, it moderated the course of the disorders, derived more likely from lower PLT than higher MPV, pointing to the potentially compensatory response in pro-inflammatory conditions [88] and the importance of immune processes in shaping not only somatic, but also mental disorders.

The recent and scarce clinical research indicates that immune mediators and variations in the *HTR2A* gene are associated with distinct impacts on mood symptoms, but with a cumulative effect [89]. Namely, the presence of the *HTR2A* gene variant can elevate the likelihood of experiencing psychiatric symptoms, but similar to animal studies [27, 72–74], the effect is variable and dependent on the degree of inflammation [89]. Our results also indicate that inflammation could play an important role in mood disorders by directly moderating the effect of genes on the affective phenotype. Although the 5-HTR2A receptor itself was not the subject of our research, we did explore the variants that affects its expression [10, 70, 90]. It

seems that the genetic underpinning could more clearly affect the clinical presentation only in patients in certain inflammatory conditions, similar to recent findings of Wang et al. [89] and Snijders et al. [91].

In our study, the T allele of rs6313 was a significant predictor of the unipolar phenotype, whereas the CC genotype was a significant predictor of the bipolar phenotype, but only in low-inflammatory states. In the presence of median to high inflammation, other factors seem more likely to moderate the clinical expression of mood disorders. Clinically, this means more severe form of the disorders could be pronounced in the presence of factors causing median to high inflammation [10, 92, 93] even if the patient is not genetically susceptible to it. This confirms the previous substantial research on gene-by-environment interactions (GxE), indicating that inflammation invariably influences gene expression, though the exact sequence of events remains unclear [94–96].

The findings should be interpreted considering both their limitations and strengths. We did not measure participants' 5-HT levels. This would have enhanced the reliability of the results and further explore the role of 5-HT in the relationship between immune mediators and 5-HTR2A receptors in defining the clinical presentation of mood disorders. The rare studies on expression or onset of mood disorder so far predominantly explored the relationship between serotonin-related genes and the stress-diathesis model [7] without including more delicate biological mechanisms underpinning the relationship. It also not to undermine the potential effect of 5HT2A polymorphisms on the PLT number and function, independent from a specific psychiatric condition. The inclusion of other, platelet-non-related, indicators of inflammation might provide additional information and the precision of the results. Another constraint of the study is that it does not account for the possible association between platelet-related indicators of inflammation and type of treatment. Also, the results observed could stem not solely from the type of the treatment but also from the duration and dosage-dependent administration of the therapy. Also, we did not evaluate the potential effect of psychotherapy and counseling interventions that could have had a protective effect in regard to the inflammatory status and clinical presentation of the disorder. However, we have tried to include other covariates, such as type of the current episode, that we believe could at least be partially helpful to account for the choice of treatment. The number of study participants is modest, but in line with majority of similar previous studies [61]. However, our study is a clinical one and provides support to the findings of previous experimental studies. It is also important to acknowledge that it addresses and improves upon certain limitations identified in prior research [13, 61]. The studies discuss rs6313 in bipolar spectrum disorders [13] on a single case basis, or offer the assessment of the relationship between rs6313 and susceptibility to MDD [61]. Interestingly, the meta-analysis of White et al. shows multiple studies with samples sizes similar to ours but without further engaging into the nature of the relationship between the polymorphism and different presentations of mood disorders. Notably, our study encompassed patients with different phenotypic expression of affective disorders, investigated multiple inflammation indicators, and controlled for variety of potential confounders. The decision to analyze patients collectively underscores our commitment to unraveling shared biological pathways that may contribute to the heterogeneity of the clinical presentations within mood disorders. Our approach enabled us to explore the direction of the relationships among 5-HTR2A genetic variants, inflammation and clinical presentation of mood disorders.

Conclusion

To our knowledge, this is the first study to investigate the complex interplay between platelet-derived indicators of inflammation and *HTR2A* variants on the expression of mood disorders. In the absence of pro-inflammatory conditions, mood disorders seem to be more genetically determined, while in the presence of adverse environmental factors such as inflammation, the phenotypic presentation is susceptible to change and less dependent on genetic factors. The nature of the GxE interaction in mood disorders is multifaceted, context-dependent and important for the clinical assessment of the course of mood disorders and calls for more studies in the future.

Abbreviations

ANCOVA	Analysis of Covariance
ANOVA	One-way Analysis of Variance
BD	Bipolar Disorder
BMI	Body Mass Index
BPRS	Brief Psychiatric Rating Scale
GxE	Gene-Environment Interactions
HDRS	Hamilton Depression Rating Scale
MDD	Major Depressive Disorder
MPV	Mean Platelet Volume
5-HTR2A	Serotonin Receptor 2 A
PCT	Plateletcrit
PDW	Platelet Distribution Width
PLT	Absolute Platelet Count
YMRS	Young Mania Rating Scale

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12888-024-06207-y.

Supplementary Material 1

Acknowledgements

Not applicable.

Author contributions
M.P.S: Conceptualization, Methodology, Writing- Original draft preparation, Data curation. J.K: Writing- Original draft preparation, Data curation. V.J, B.D.K, M.N: Writing- Reviewing and Editing. S.D, M.G, M.P: Visualization, Investigation, Data curation, Software. D.S.P and M.I: Data curation, Supervision.

Funding
None to declare.

Data availability
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical approval and consent to participate
The study was approved by the Ethics Committee (EC) of Clinical Centre of Serbia (3672/10 and 4072/5) and conducted in accordance with the Helsinki Declaration of 1989.

Consent to participate
All participants provided written informed consent to participate in the study, according to EC approval.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 25 June 2024 / Accepted: 22 October 2024
Published online: 29 October 2024

References

1. Ferrari AJ, Stockings E, Khoo J-P, Erskine HE, Degenhardt L, Vos T, et al. The prevalence and burden of bipolar disorder: findings from the global burden of Disease Study 2013. Bipolar Disord. 2016;18:440–50.
2. Li S, Zhang X, Cai Y, Zheng L, Pang H, Lou L. Sex difference in incidence of major depressive disorder: an analysis from the global burden of Disease Study 2019. Ann Gen Psychiatry. 2023;22:53.
3. Goodwin GM. Bipolar depression and treatment with antidepressants. Br J Psychiatry J Ment Sci. 2012;200:5–6.
4. Poletti S, Vai B, Mazza MG, Zanardi R, Lorenzi C, Calesella F, et al. A peripheral inflammatory signature discriminates bipolar from unipolar depression: a machine learning approach. Prog Neuropsychopharmacol Biol Psychiatry. 2021;105:110136.
5. Baryshnikov I, Sund R, Marttunen M, Svirskis T, Partonen T, Pirkola S, et al. Diagnostic conversion from unipolar depression to bipolar disorder, schizophrenia, or schizoaffective disorder: a nationwide prospective 15-year register study on 43 495 inpatients. Bipolar Disord. 2020;22:582–92.
6. Kessing LV, Willer I, Andersen PK, Bukh JD. Rate and predictors of conversion from unipolar to bipolar disorder: a systematic review and meta-analysis. Bipolar Disord. 2017;19:324–35.
7. Musci RJ, Augustinavicius JL, Volk H. Gene-environment interactions in Psychiatry: recent evidence and clinical implications. Curr Psychiatry Rep. 2019;21:81.
8. Arias B, Gastó C, Catalán R, Gutiérrez B, Pintor L, Fañanás L. The 5-HT(2A) receptor gene 102T/C polymorphism is associated with suicidal behavior in depressed patients. Am J Med Genet. 2001;105:801–4.
9. Comings DE, MacMurray JP. Molecular heterosis: a review. Mol Genet Metab. 2000;71:19–31.
10. Ghasemi A, Seifi M, Baybordi F, Danaei N, Samadi Rad B. Association between serotonin 2A receptor genetic variations, stressful life events and suicide. Gene. 2018;658:191–7.
11. Gong P, Liu J, Blue PR, Li S, Zhou X. Serotonin receptor gene (HTR2A) T102C polymorphism modulates individuals' perspective taking ability and autistic-like traits. Front Hum Neurosci. 2015;9:575.
12. González-Castro TB, Tovilla-Zárate C, Juárez-Rojop I, Pool García S, Velázquez-Sánchez MP, Genis A, et al. Association of the 5HTR2A gene with suicidal behavior: case-control study and updated meta-analysis. BMC Psychiatry. 2013;13:25.
13. Fortinguerra S, Sorrenti V, Giusti P, Zusso M, Buriani A. Pharmacogenomic characterization in Bipolar Spectrum disorders. Pharmaceutics. 2019;12:13.
14. Polesskaya OO, Sokolov BP. Differential expression of the C and T alleles of the 5-HT2A receptor gene in the temporal cortex of normal individuals and schizophrenics. J Neurosci Res. 2002;67:812–22.
15. Blasi G, De Virgilio C, Papazacharias A, Taurisano P, Gelao B, Fazio L, et al. Converging evidence for the association of functional genetic variation in the serotonin receptor 2a gene with prefrontal function and olanzapine treatment. JAMA Psychiatry. 2013;70:921–30.
16. Guiard BP, Giovanni GD. Central serotonin-2A (5-HT2A) receptor dysfunction in depression and epilepsy: the missing link? Front Pharmacol. 2015;6:46.
17. Wilkie MJV, Smith G, Day RK, Matthews K, Smith D, Blackwood D, et al. Polymorphisms in the SLC6A4 and HTR2A genes influence treatment outcome following antidepressant therapy. Pharmacogenomics J. 2009;9:61–70.
18. Pinsonneault JK, Sullivan D, Sadee W, Soares CN, Hampson E, Steiner M. Association study of the estrogen receptor gene ESR1 with postpartum depression–a pilot study. Arch Womens Ment Health. 2013;16:499–509.
19. McAuley EZ, Fullerton JM, Blair IP, Donald JA, Mitchell PB, Schofield PR. Association between the serotonin 2A receptor gene and bipolar affective disorder in an Australian cohort. Psychiatr Genet. 2009;19:244–52.
20. Atan Y, Akbaba M, Kul S, Tataroğlu Z, Temiz E, Nacarkahya G, et al. Does the serotonin receptor gene (rs6313 and rs6314) polymorphism have a role in suicidal attempts? J Forensic Leg Med. 2018;56:32–6.
21. Gadow KD, Smith RM, Pinsonneault JK. Serotonin 2A receptor gene (HTR2A) regulatory variants: possible association with severity of depression symptoms in children with autism spectrum disorder. Cogn Behav Neurol off J Soc Behav Cogn Neurol. 2014;27:107–16.
22. Gu L, Tan J, Su L, Qin Y, Long J, Chen Q, et al. Meta-analysis of the HTR2A–1354 C/T polymorphism in schizophrenia and bipolar disorder. Acta Neuropsychiatr. 2013;25:311–9.
23. Leonard BE. The concept of depression as a dysfunction of the immune system. Curr Immunol Rev. 2010;6:205–12.
24. Guglielmo R, Miskowiak KW, Hasler G. Evaluating endophenotypes for bipolar disorder. Int J Bipolar Disord. 2021;9:17.
25. Miller AH, Maletic V, Raison CL. Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. Biol Psychiatry. 2009;65:732–41.
26. Miller AH, Haroon E, Raison CL, Felger JC. Cytokine targets in the brain: impact on neurotransmitters and neurocircuits. Depress Anxiety. 2013;30:297–306.
27. Couch Y, Xie Q, Lundberg L, Sharp T, Anthony DC. A model of post-infection fatigue is Associated with increased TNF and 5-HT2A receptor expression in mice. PLoS ONE. 2015;10:e0130643.
28. Canobbio I. Blood platelets: circulating mirrors of neurons? Res Pract Thromb Haemost. 2019;3:564–5.
29. Goubau C, Buyse GM, Di Michele M, Van Geet C, Freson K. Regulated granule trafficking in platelets and neurons: a common molecular machinery. Eur J Paediatr Neurol EJPN off J Eur Paediatr Neurol Soc. 2013;17:117–25.
30. Izzi B, Tirozzi A, Cerletti C, Donati MB, de Gaetano G, Hoylaerts MF, et al. Beyond Haemostasis and thrombosis: platelets in Depression and its co-morbidities. Int J Mol Sci. 2020;21:8817.
31. Beretta L, Cossu M, Marchini M, Cappiello F, Artoni A, Motta G, et al. A polymorphism in the human serotonin 5-HT2A receptor gene may protect against systemic sclerosis by reducing platelet aggregation. Arthritis Res Ther. 2008;10:R103.
32. Cerrito F, Lazzaro MP, Gaudio E, Arminio P, Aloisi G. 5HT2-receptors and serotonin release: their role in human platelet aggregation. Life Sci. 1993;53:209–15.
33. Silva TO, Jung I, Trott A, Bica CG, Casarin JN, Fortuna PC et al. Association between T102C 5-HT2A receptor gene polymorphism and 5-year mortality risk among Brazilian Amazon riparian elderly population. Am J Hum Biol off J Hum Biol Counc. 2017;29.
34. Yamada S, Akita H, Kanazawa K, Ishida T, Hirata K, Ito K, et al. T102C polymorphism of the serotonin (5-HT) 2A receptor gene in patients with non-fatal acute myocardial infarction. Atherosclerosis. 2000;150:143–8.
35. Korniluk A, Koper-Lenkiewicz OM, Kamińska J, Kemona H, Dymicka-Piekarska V. Mean platelet volume (MPV): New perspectives for an old marker in the course and prognosis of inflammatory conditions. Mediators Inflamm. 2019;2019:9213074.

36. Wei Y, Feng J, Ma J, Chen D, Xu H, Yin L, et al. Characteristics of platelet-associated parameters and their predictive values in Chinese patients with affective disorders. BMC Psychiatry. 2022;22:150.
37. Canan F, Dikici S, Kutlucan A, Celbek G, Coskun H, Gungor A, et al. Association of mean platelet volume with DSM-IV major depression in a large community-based population: the MELEN study. J Psychiatr Res. 2012;46:298–302.
38. Chen Z, Wang J, Carru C, Sedda S, Nivoli AM, Li Z. Meta-analysis of peripheral mean platelet volume in patients with mental disorders: comparisons in depression, anxiety, bipolar disorder, and schizophrenia. Brain Behav. 2023;13:e3240.
39. Ataoglu A, Canan F. Mean platelet volume in patients with major depression: effect of escitalopram treatment. J Clin Psychopharmacol. 2009;29:368–71.
40. Mert DG, Terzi H. Mean platelet volume in bipolar disorder: the search for an ideal biomarker. Neuropsychiatr Dis Treat. 2016;12:2057–62.
41. Wysokiński A, Szczepocka E. Platelet parameters (PLT, MPV, P-LCR) in patients with schizophrenia, unipolar depression and bipolar disorder. Psychiatry Res. 2016;237:238–45.
42. Tekin YK, Tekin G. Mean platelet volume-to-platelet count ratio, Mean platelet volume-to-lymphocyte ratio, and red blood cell distribution width-platelet count ratio as markers of inflammation in patients with ascending thoracic aortic aneurysm. Braz J Cardiovasc Surg. 2020;35:175–80.
43. Wu Y-Y, Zhang X, Qin Y-Y, Qin J-Q, Lin F-Q. Mean platelet volume/platelet count ratio in colorectal cancer: a retrospective clinical study. BMC Cancer. 2019;19:314.
44. Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, et al. The mini-international neuropsychiatric interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J Clin Psychiatry. 1998;59(Suppl 20):22–33. quiz 34–57.
45. World Health Organization, editor. The ICD-10 classification of mental and behavioural disorders: clinical descriptions and diagnostic guidelines. Geneva: World Health Organization; 1992.
46. Hamilton M. A rating scale for depression. J Neurol Neurosurg Psychiatry. 1960;23:56–62.
47. Young RC, Biggs JT, Ziegler VE, Meyer DA. A rating scale for mania: reliability, validity and sensitivity. Br J Psychiatry J Ment Sci. 1978;133:429–35.
48. Shafer A. Meta-analysis of the brief psychiatric rating scale factor structure. Psychol Assess. 2005;17:324–35.
49. Durgam S, Earley W, Lu K, Németh G, Laszlovszky I, Volk S, et al. Global improvement with cariprazine in the treatment of bipolar I disorder and schizophrenia: a pooled post hoc analysis. Int J Clin Pract. 2017;71:e13037.
50. Tohen M, Frank E, Bowden CL, Colom F, Ghaemi SN, Yatham LN, et al. The International Society for Bipolar Disorders (ISBD) Task Force report on the nomenclature of course and outcome in bipolar disorders. Bipolar Disord. 2009;11:453–73.
51. Witkowska AM, Borawska MH. Soluble intercellular adhesion molecule-1 (sICAM-1): an overview. Eur Cytokine Netw. 2004;15:91–8.
52. Quist JF, Barr CL, Schachar R, Roberts W, Malone M, Tannock R, et al. Evidence for the serotonin HTR2A receptor gene as a susceptibility factor in attention deficit hyperactivity disorder (ADHD). Mol Psychiatry. 2000;5:537–41.
53. González JR, Carrasco JL, Dudbridge F, Armengol L, Estivill X, Moreno V. Maximizing association statistics over genetic models. Genet Epidemiol. 2008;32:246–54.
54. Hayes AF. Introduction to mediation, moderation, and conditional process analysis: a regression-based approach. Guilford; 2017.
55. Ivković M, Pantović-Stefanović M, Dunjić-Kostić B, Jurišić V, Lačković M, Totić-Poznanović S, et al. Neutrophil-to-lymphocyte ratio predicting suicide risk in euthymic patients with bipolar disorder: moderatory effect of family history. Compr Psychiatry. 2016;66:87–95.
56. Terayama H, Takimoto T, Fukunishi I, Itoh M, Iwahashi K. The serotonin-2A receptor polymorphism and clinical symptoms in mood disorders, schizophrenia and alcohol dependence in Japan. Acta Neuropsychiatr. 2003;15:129–32.
57. Serretti A, Drago A, De Ronchi D. HTR2A gene variants and psychiatric disorders: a review of current literature and selection of SNPs for future studies. Curr Med Chem. 2007;14:2053–69.
58. Abdolmaleky HM, Yaqubi S, Papageorgis P, Lambert AW, Ozturk S, Sivaraman V, et al. Epigenetic dysregulation of HTR2A in the brain of patients with schizophrenia and bipolar disorder. Schizophr Res. 2011;129:183–90.
59. Du L, Faludi G, Palkovits M, Bakish D, Hrdina PD. Serotonergic genes and suicidality. Crisis. 2001;22:54–60.
60. Roecklein KA, Rohan KJ, Postolache TT. Is seasonal affective disorder a bipolar variant? Curr Psychiatry. 2010;9:42–54.
61. White KC, McDonald AK, Compton DM. 5-HTR2A polymorphisms rs6311 and rs6313 and major depressive disorder: a Meta-analysis. J Behav Brain Sci. 2022;12:499–513.
62. Zain MA, Zainal NZ, Kanagasundram S, Mohamed Z. Genetic association study of PDLIM5 and HTR2A variants in Malaysian subjects diagnosed with bipolar disorder; a genetic modelling approach. Neurosci Res Notes. 2018;1:11–20.
63. Sun L, Xu P, Zhou Y-G, Zuo S-R, Liu Y-P. Meta-analysis of polymorphism rs6311 and rs6313 in the 5-HT2AR gene and schizophrenia. Nord J Psychiatry. 2017;71:1–11.
64. Herr N, Bode C, Duerschmied D. The effects of Serotonin in Immune cells. Front Cardiovasc Med. 2017;4:48.
65. Benedetti F, Barbini B, Bernasconi A, Fulgosi MC, Colombo C, Dallaspezia S, et al. Serotonin 5-HT2A receptor gene variants influence antidepressant response to repeated total sleep deprivation in bipolar depression. Prog Neuropsychopharmacol Biol Psychiatry. 2008;32:1863–6.
66. Pandey GN, Pandey SC, Ren X, Dwivedi Y, Janicak PG. Serotonin receptors in platelets of bipolar and schizoaffective patients: effect of lithium treatment. Psychopharmacology. 2003;170:115–23.
67. Popova NK, Tsybko AS, Naumenko VS. The implication of 5-HT receptor family members in aggression, depression and suicide: similarity and difference. Int J Mol Sci. 2022;23:8814.
68. Rosell DR, Thompson JL, Slifstein M, Xu X, Frankle WG, New AS, et al. Increased serotonin 2A receptor availability in the orbitofrontal cortex of physically aggressive personality disordered patients. Biol Psychiatry. 2010;67:1154–62.
69. Kling A, Danell-Boman M, Stenlund H, Dahlqvist R. Association between the use of serotonin receptor 2A-blocking antidepressants and joint disorders. Arthritis Rheum. 2009;61:1322–7.
70. Bani-Fatemi A, Howe AS, Matmari M, Koga A, Zai C, Strauss J, et al. Interaction between methylation and CpG single-nucleotide polymorphisms in the HTR2A Gene: Association Analysis with suicide attempt in Schizophrenia. Neuropsychobiology. 2016;73:10–5.
71. Zhao J, Xu L, Chang C, Jiang P, Wei K, Shi Y, et al. Circulating methylation level of HTR2A is associated with inflammation and disease activity in rheumatoid arthritis. Front Immunol. 2022;13:1054451.
72. Couch Y, Martin CJ, Howarth C, Raley J, Khrapitchev AA, Stratford M, et al. Systemic inflammation alters central 5-HT function as determined by pharmacological MRI. NeuroImage. 2013;75:177–86.
73. Savignac HM, Couch Y, Stratford M, Bannerman DM, Tzortzis G, Anthony DC, et al. Prebiotic administration normalizes lipopolysaccharide (LPS)-induced anxiety and cortical 5-HT2A receptor and IL1-β levels in male mice. Brain Behav Immun. 2016;52:120–31.
74. Atmaca HT. Expression of serotonin 2A, 2 C, 6 and 7 receptor and IL-6 mRNA in experimental toxoplasmic encephalitis in mice. Heliyon. 2019;5:e02890.
75. Nau F, Yu B, Martin D, Nichols CD. Serotonin 5-HT2A receptor activation blocks TNF-α mediated inflammation in vivo. PLoS ONE. 2013;8:e75426.
76. Yu B, Becnel J, Zerfaoui M, Rohatgi R, Boulares AH, Nichols CD. Serotonin 5-hydroxytryptamine(2A) receptor activation suppresses tumor necrosis factor-alpha-induced inflammation with extraordinary potency. J Pharmacol Exp Ther. 2008;327:316–23.
77. Eswarappa M, Neylan TC, Whooley MA, Metzler TJ, Cohen BE. Inflammation as a predictor of disease course in posttraumatic stress disorder and depression: a prospective analysis from the mind your heart study. Brain Behav Immun. 2019;75:220–7.
78. Zainal NH, Newman MG. Increased inflammation predicts nine-year change in major depressive disorder diagnostic status. J Abnorm Psychol. 2021;130:829–40.
79. Gunluoglu G, Yazar EE, Veske NS, Seyhan EC, Altin S. Mean platelet volume as an inflammation marker in active pulmonary tuberculosis. Multidiscip Respir Med. 2014;9:11.
80. Ulasli SS, Ozyurek BA, Yilmaz EB, Ulubay G. Mean platelet volume as an inflammatory marker in acute exacerbation of chronic obstructive pulmonary disease. Pol Arch Med Wewn. 2012;122:284–90.
81. Stokes KY, Granger DN. Platelets: a critical link between inflammation and microvascular dysfunction. J Physiol. 2012;590:1023–34.82.
82. Zhang H, Wu D, Ma D, Deng X, Li L, Wang J. Clinical value of mean platelet volume/platelet count ratio in predicting short-term prognosis of elderly patients with acute exacerbation of chronic obstructive pulmonary. Chinese Journal of Geriatrics 2020;(12):627-631.
83. Wach J, Apallas S, Schneider M, Weller J, Schuss P, Vatter H, Herrlinger U, Güresir E. Mean platelet Volume/Platelet count ratio and risk of Progression

in Glioblastoma. Front Oncol. 2021;11:695316. https://doi.org/10.3389/fonc.2021.695316
84. Han X, Xu P, Duan X, Liu Y, Zhang J, Xu H. High mean platelet volume to platelet count ratio as a diagnostic maker for increased risk of liver function damage in pediatric patients with infectious Mononucleosis in China. Exp Ther Med. 2019;18:4523–7.
85. Iida H, Kaibori M, Matsui K, Ishizaki M, Kon M. Ratio of mean platelet volume to platelet count is a potential surrogate marker predicting liver cirrhosis. World J Hepatol. 2018;10(1):82–7. https://doi.org/10.4254/wjh.v10.i1.82
86. Zhu N, Shu H, Jiang W, Wang Y, Zhang S. Mean platelet volume and mean platelet volume/platelet count ratio in nonvalvular atrial fibrillation stroke and large artery atherosclerosis stroke. Med (Baltim). 2020;99(28):e21044. https://doi.org/10.1097/MD.0000000000021044.P
87. Asoğlu R, Asoğlu E. Relationship between Mean platelet volume-to-platelet count ratio and the Presence and Severity of Coronary Artery Ectasia. Int Arch Cardiovasc Dis. 2019;3:024. https://doi.org/10.23937/2643-3966/1710024
88. Gros A, Ollivier V, Ho-Tin-Noé B. Platelets in inflammation: regulation of leukocyte activities and vascular repair. Front Immunol. 2014;5:678.
89. Wang J, Gao X, Gao P, Liu J. A cross-sectional study on the Relationship among cytokines, 5-HT2A receptor polymorphisms, and Sleep Quality of non-manual workers in Xinjiang, China. Front Psychiatry. 2022;13:777566.
90. Polesskaya OO, Aston C, Sokolov BP. Allele C-specific methylation of the 5-HT2A receptor gene: evidence for correlation with its expression and expression of DNA methylase DNMT1. J Neurosci Res. 2006;83:362–73.
91. Snijders G, Brouwer R, Kemner S, Bootsman F, Drexhage HA, Hillegers MHJ. Genetic and environmental influences on circulating NK and T cells and their relation to bipolar disorder. Int J Bipolar Disord. 2019;7:4.
92. Nugent NR, Tyrka AR, Carpenter LL, Price LH. Gene-environment interactions: early life stress and risk for depressive and anxiety disorders. Psychopharmacology. 2011;214:175–96.
93. Parade SH, Novick AM, Parent J, Seifer R, Klaver SJ, Marsit CJ, et al. Stress exposure and psychopathology alter methylation of the serotonin receptor 2A (HTR2A) gene in preschoolers. Dev Psychopathol. 2017;29:1619–26.
94. Barnes J, Mondelli V, Pariante CM. Genetic contributions of inflammation to Depression. Neuropsychopharmacology. 2017;42:81–98.
95. Ben-Efraim YJ, Wasserman D, Wasserman J, Sokolowski M. Family-based study of HTR2A in suicide attempts: observed gene, gene × environment and parent-of-origin associations. Mol Psychiatry. 2013;18:758–66.
96. Beurel E, Toups M, Nemeroff CB. The bidirectional relationship of depression and inflammation: double trouble. Neuron. 2020;107:234–56.

Publisher's note