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The Relation of Zonulin and Intestinal Fatty Acid-binding Protein with Cognitive Functions in Patients with Bipolar Disorder

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Objective: This study aimed to investigate the relationship between zonulin and intestinal fatty acid-binding protein (I-FABP), biomarkers of intestinal permeability, and cognitive functions in patients with bipolar disorder (BD) compared to healthy controls (HCs).
Methods: The study included 40 individuals diagnosed with BD and HCs. Cognitive functions were evaluated using the Stroop Test, Trail Making Test, Öktem Verbal Memory Processes Test, Wisconsin Card Sorting Test, and Digit Span Test. Serum zonulin and I-FABP levels were measured.
Results: Zonulin and I-FABP levels were significantly higher in the BD group than in HCs ($p < 0.001$). After correction for multiple comparisons, significant associations remained between zonulin levels and cognitive performance measures, including the Stroop effect (r = 0.46, $p < 0.001$), delayed recall (r = −0.41, $p < 0.001$), and recognition performance (r = 0.42, $p < 0.001$). No significant correlations were found between I-FABP levels and cognitive test results.
Conclusion: The observed associations between zonulin levels and cognitive performance suggests a potential relationship of intestinal barrier integrity and gut microbiota with cognitive functions in BD. These findings should be interpreted as associative rather than causal, and future longitudinal studies are required to explore the direction of these relationships.

KEY WORDS: Bipolar disorder; Intestinal permeability; Blood–brain barrier; Cognition; Zonulin; Fatty acid-binding proteins.

INTRODUCTION

Bipolar disorder (BD) is a chronic psychiatric condition characterized by recurrent depressive, manic, or hypomanic episodes. It often leads to significant functional impairment and reduced quality of life. BD's lifetime prevalence is estimated at around 1%, however broader spectrum definitions suggest that the rate is up to 5% [1]. While it is primarily known for episodic mood disturbances, BD has increasingly been recognized as a disorder that involves cognitive dysfunction as well as affective instability. These cognitive impairments persist even during euthymic phases, and thus significantly contribute to long term disability and poor psychosocial outcomes [2].

The etiopathogenesis of BD is complex and includes multiple factors that encompass genetic, neurobiological and immune mechanisms. Recent studies have stressed the effect of systemic inflammation and immune dysregu-

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lation on both cognitive impairment and mood instability. Identification of the peripheral biomarkers that reflect these underlying processes may help improve early diagnosis, treatment monitoring and prognostic assessment [3,4].

The gut microbiota forms the microbial ecosystem that is in bidirectional communication with the central nervous system. This two-way communication, known as gut-brain axis, is through immune, neural and endocrine pathways [5].

Among these mechanisms, the vagus nerve plays a particular role in bidirectional signaling, whereas immune mediators and microbial metabolites have been related with alterations in neuronal activity and behavior [6-9]. Disruption in the microbial composition of the gut microbiota, i.e., dysbiosis, has been associated with increased intestinal permeability and low grade systemic inflammation. It was suggested that increased intestinal permeability allows the movement of microbial metabolites and immune molecules into the bloodstream, resulting in modulation of neuroinflammatory and metabolic pathways associated with cognition and emotion [10,11].

Through the epithelial tight junctions, the intestinal barrier regulates the selective passage of luminal antigens and bacteria, thereby preserving the mucosal integrity [12,13]. Disruption of these epithelial tight junctions, referred to as leaky gut, may cause microbial translocation and activate innate immune responses, particularly via Toll-like receptor 4 signaling [14,15]. By leading to pro-inflammatory cytokine release and systemic inflammation, this chain of reactions further degrades the integrity of the intestinal barrier and contributes to neuroinflammatory pathways [16].

The blood-brain barrier and intestinal barrier share similarities in terms of their structure and function. Both rely on tight junctions regulating paracellular permeability and are highly sensitive to inflammatory stress [17]. Disruptions in these barriers and alterations in the gut-brain axis were reported in several psychiatric conditions, including not only BD but also attention-deficit/hyperactivity disorder, autism spectrum disorder, major depressive disorder and schizophrenia. Each of these conditions is associated with microbial imbalance and increased intestinal permeability, implying that the peripheral barrier dysfunction and systemic inflammation may play a role in the regulation of mood and cognition in BD [18,19].

Zonulin, a pre-haptoglobin 2 protein, is one of the most physiologically active modulators of epithelial tight junctions, and elevated zonulin serum levels in the blood have been associated with increased intestinal permeability and systemic inflammation [20]. Recent clinical studies provide evidence that individuals with BD may have higher levels of zonulin in their bloodstream than healthy controls (HCs), indicating a possible relationship between disrupted intestinal barrier regulations and BD [21,22]. Nevertheless, the data specific to BD is limited. Intestinal fatty acid-binding protein (I-FABP or FABP2), another biomarker, signals enterocyte impairment and serves as a peripheral indicator of epithelial integrity [23,24]. Similarly, it has been reported that populations with mood disorders have increased I-FABP levels, and this suggests mild epithelial injury being possibly related to affective pathology, without implying a causal relationship [25]. These markers, collectively, represent complementary aspects of intestinal barrier function and may help identify the biological relationships between gut permeability and cognitive function in BD.

Cognitive impairment in BD occurs in all phases of the illness and often continues during remission [26]. Meta-analysis studies consistently demonstrate significant impairments in executive functioning, verbal learning and attention in euthymic patients [26]. While the relationship between BD and cognitive dysfunction is widely recognized, factors such as medication exposure, residual symptoms, and illness chronicity may influence these outcomes. Furthermore, recent research suggested that systemic inflammation and altered intestinal permeability may contribute to the cognitive deficits [27]. Nevertheless, the possibility of reverse causality should also be considered as cognitive impairment can influence behavioral or lifestyle factors that indirectly affect gut microbiota and barrier function.

Recent evidence supports the view that BD is characterized by systemic immune activation and intestinal barrier alterations; and both of these may impact gut-brain communication [28]. Increased intestinal permeability was suggested as a peripheral biological pathway that links immune dysregulation and cognitive function in psychiatric disorders [29]. In this context, the purpose of the present study is to examine the associations between the biomarkers of intestinal permeability, which are serum zonulin and I-FABP levels, and cognitive perform-

ance in patients with BD, compared to HCs. In the existing body of research, evidence that directly relates intestinal permeability markers, such as zonulin and I-FABP, to cognitive outcomes in BD is limited. This study aims to address this by examining whether alterations in these peripheral biomarkers are associated with cognitive performance in BD.

Based on the existing literature and biological rationale, the following hypotheses were formulated:

1. Serum levels of zonulin and I-FABP would differ between patients with BD and HCs, reflecting increased intestinal permeability in BD.

2. Patients with BD would perform worse than HCs across multiple cognitive domains, particularly executive functioning, attention, and memory.

3. Higher zonulin and I-FABP levels would be associated with poorer cognitive performance among patients with BD.

METHODS

Subjects

The study sample consisted of 40 patients diagnosed with BD in remission and 40 age- and sex-matched HCs. All patients met the criteria for BD type I. Participants were recruited from the outpatient clinic of the Department of Psychiatry at Bezmialem Vakif University Faculty of Medicine between May 2019 and January 2020. The age range of participants was between 18 and 65 years. The BD and HC groups were comparable in terms of age, gender, body mass index (BMI), and smoking status.

Diagnoses were confirmed using the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I), administered by a trained psychiatrist. Remission status of patients was confirmed by clinical interview and standardized rating scales: Hamilton Depression Rating Scale (HDRS) scores below 7 and Young Mania Rating Scale (YMRS) scores below 5 were required for inclusion, ensuring the absence of significant residual depressive or manic symptoms at the time of assessment. The requirement for HCs was having no current or past psychiatric diagnosis according to the SCID-I and no first-degree relatives with a history of major psychiatric disorders. All participants in the study had sufficient intellectual capacity to complete the neurocognitive assessments. The exclusion criteria applied to both groups as follows: (a) age under 18 or over 65 years; (b) current or past alcohol or substance use disorder; (c) diagnosis of any systemic illness, including but not limited to coronary artery disease, diabetes mellitus, hypertension, malignancy, thyroid disorder, or acute infections; (d) intellectual disability; (e) presence of neurodegenerative or other neurological disorders; (f) presence of any major psychiatric disorder as identified by the SCID-I in the non-BD group; (g) refusal to provide informed consent; (h) illiteracy; (i) BMI < 18.5 kg/m^2 or > 30 kg/m^2.

Cognitive functioning was assessed using a neuropsychological battery consisting of the Stroop Test, Trail Making Test, Öktem Verbal Memory Processes Test (VMPT), Wisconsin Card Sorting Test (WCST), and Digit Span Test (DST).

Fasting blood samples were collected from all participants between 8:00 AM and 10:00 AM to minimize circadian variation and analyzed at the Biochemistry Laboratory of Bezmialem Vakif University Hospital. Serum levels of zonulin and I-FABP were measured to assess intestinal permeability. In addition, glucose and triglyceride levels were measured to evaluate the general metabolic status of the participants.

Ethical approval for the study was obtained from the Clinical Research Ethics Committee of Bezmialem Vakif University (Approval No.: 31.01.2019-1925). All procedures were conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants prior to their participation in the study.

Measurements

Cognitive tests

The Stroop Test is a commonly used measure of executive function which assesses the ability to flexibly directing attention in the presence of distraction, inhibiting habitual behavioral patterns, and demonstrating adaptive behavior. For instance, the Stroop effect (SE) occurs when the color of the word displayed is different from the color that the word represents. This interference known as the SE, results from the increased time required to name the ink color compared to reading the word itself [30].

The Trail Making Test (Parts A and B) is a neuropsychological assessment tool is used to evaluate attention, mental flexibility, visual tracking, and motor skills of

individuals. In Part A of the test, participants are required to connect numbers from 1 to 25 in sequential order. In Part B, numbers and letters are alternated sequentially (e.g., 1-A-2-B, etc.). The duration required to complete each part was recorded in the present study [31].

The Öktem VMPT provides data regarding immediate memory, attention, learning, recall, and recognition processes. This test is the Turkish adaptation of the Rey Auditory Verbal Learning Test. It includes scores for immediate memory (number of responses remembered after the first presentation of words), total learning (total number of words remembered across trials), and maximum learning (the highest number of words remembered in a single trial). After 45 minutes, participants are asked to recall the same 15 words (delayed free recall) and to recognize the words from a provided list (recognition) [32].

The WCST is used to measure the ability to identify a classification rule based on feedback, and to abandon that rule when it no longer results in correct responses. WCST is also used in evaluating selective attention and cognitive flexibility. A computerized version of the test was applied in this study [33].

The DST a tool that is applied in measuring aspects of working memory and attention. It has two parts, namely digits forward and digits backward spans. In the forward part, participants are required to repeat a sequence of numbers in the presented order. In the backward part, they repeat the numbers in reverse order [34].

Biochemical analysis: determination of serum zonulin and I-FABP

Approximately 10 ml of venous blood was collected from each participant between 08:00 and 10:00 AM, following an overnight fast. Blood samples were drawn into gel-containing biochemical tubes. Serum was separated by centrifugation at 3,000 rpm for 10 minutes and stored at −80°C until the day of analysis.

Serum zonulin and I-FABP levels were measured using the enzyme-linked immunosorbent assay (ELISA) method in accordance with the manufacturer's instructions (Zonulin: BT Lab –E3704Hu; I-FABP: BT Lab – E7143Hu). Readings were taken at a wavelength of 450 nm using an ELISA microplate reader (Thermo Scientific™ Varioskan™ Flash Multimode Reader). Results were expressed in ng/ml and ng/L.

Statistical analyses

The distribution of the data was assessed using the Shapiro-Wilk test. For the comparison of two independent groups with normally distributed data, independent samples t tests were applied. In cases where the data were not normally distributed, the Mann-Whitney U test was used. Differences among categorical variables were evaluated using Pearson's chi-square test, Fisher's exact chi-square test, and the Fisher-Freeman-Halton test, as appropriate. Multiple comparison bias was controlled using the false discovery rate procedure, with Bonferroni correction applied in supplementary tables.

Spearman's correlation analysis was conducted to examine relationships between numerical variables. Descriptive statistics were expressed as mean ± standard deviation (SD) for normally distributed variables, and as median (minimum−maximum) for non-normally distributed variables. Categorical variables were summarized using frequencies and percentages.

All statistical analyses applied in this study were conducted using IBM SPSS Statistics version 26.0 (IBM Co.), with a significance level set at $\alpha = 0.05$.

To verify the adequacy of the sample size, a preliminary power analysis was conducted using G*Power 3.1. Based on previous studies evaluating intestinal permeability markers in psychiatric populations, a medium effect size (Cohen's d = 0.6) was assumed for between-group comparisons [35,36]. With a significance level of $\alpha = 0.05$ and power of 0.80, the minimum required sample size was 36 participants per group. Therefore, 40 participants were included in each group within this study to ensure meaningful statistical analysis.

RESULTS

Participant Characteristics

The demographic and clinical characteristics of the participants are presented in Table 1. As shown in Table 1, the BD and HCs did not differ significantly in demographic or metabolic parameters (all $p > 0.05$). However, c-reactive protein (CRP) levels were significantly higher in the BD group ($p < 0.001$).

The mean age of onset for patients with BD was 25.55 ± 8.62 years, and the mean duration of illness was 11.98 ± 7.74 years. The mean HDRS score was 1.18 ± 1.24, and the mean YMRS score was 0.06 ± 0.018. The mean num-

Table 1-1. Group characteristics and metabolic parameters

Group comparisons	BD (n = 40)	HCs (n = 40)	p value / test statistic
Sex (female, %)	28 (70.0)	24 (60.0)	0.34 / χ^2 = 0.879
Age (yr)	37 (19−60)	33.5 (23−56)	0.37 / Z = −0.881
Education, n (%)	Elem: 10 (25.0) High: 11 (27.5) Univ: 19 (47.5)	Elem: 13 (32.5) High: 8 (20.0) Univ: 19 (47.5)	0.65 / χ^2 = 0.865
Smoking status (%)	9 (22.5)	12 (30.0)	0.40 / χ^2 = 0.201
BMI (kg/m^2)	26.55 (19.8−30)	25.30 (18−37)	0.67 / Z = −0.424
Glucose (mg/dl)	91.43 ± 7.55	90.57 ± 7.41	0.62 / Z = 0.493
Triglyceride (mg/dl)	102 (43−310)	81 (26−284)	0.58 / Z = −0.542
HDL (mg/dl)	54 (34−98)	56 (32−76)	0.96 / Z = −0.043
CRP (mg/L)	0.57 (0.02−5.26)	0.20 (0.02−3.22)	< 0.001 / Z = −3.287

Data are presented as mean ± standard deviation, n (%), or median (interquartile range, Q1−Q3), as appropriate. Mean rank Z values were obtained using the Mann–Whitney U test; χ^2 indicates the chi-square test.
BD, bipolar disorder patients; HCs, healthy controls; BMI, body mass index; HDL, high-density lipoprotein; CRP, C-reactive protein; Elem, elementary school; High, high school; Univ, university.

Table 1-2. Clinical characteristics of BD

	BD (n = 40)
Age of onset (yr)	25.55 ± 8.62
Disease duration (yr)	11.98 ± 7.74
HDRS score	1.18 ± 1.24
YMRS score	0.06 ± 0.018
Number of depressive episodes	4.73 ± 4.80
Number of manic episodes	2.58 ± 2.45

Data are presented as mean ± standard deviation.
BD, bipolar disorder patients; HDRS, Hamilton Depression Rating Scale; YMRS, Young Mania Rating Scale.

ber of lifetime depressive episodes was 4.73 ± 4.80, and the mean number of lifetime manic episodes was 2.58 ± 2.45.

Zonulin and I-FABP Levels

As shown in Figure 1 and Table 2, patients with BD exhibited significantly higher serum levels of zonulin and I-FABP compared with HCs (both $p < 0.001$), indicating increased intestinal permeability in the BD group.

Neurocognitive Assessment

Table 3 presents the mean scores and SDs for the neurocognitive assessments. The analysis revealed that the SE was significantly prolonged in the patient group compared with HCs ($p < 0.001$). Similarly, patients demonstrated significantly longer completion times on both the Trail Making Test Part A (TMT-A) and Part B (TMT-B) ($p < 0.001$ for both).

In the Öktem VMPT, the BD group showed significantly lower scores in immediate memory, learning, highest learning, and delayed free recall compared with HCs ($p < 0.001$ for all). Recognition scores were higher in the BD group, but this difference is not statistically significant ($p = 0.50$).

The BD group made a greater number of total and perseverative errors and responses, while achieving fewer correct responses and completed categories ($p = 0.011$, $p = 0.002$, $p < 0.001$, and $p = 0.051$, respectively). In contrast, the number of total correct and completed categories were significantly lower in the BD group ($p = 0.008$ and $p = 0.51$, respectively).

Finally, performance on the DST revealed significantly lower scores for both forward and backward spans in the BD ($p < 0.001$ for both).

Relationship of I-FABP and Zonulin Levels with Neurocognitive Assessment in Patients with Bipolar Disorder and Healthy Controls

Table 4 shows the correlation of zonulin and I-FABP levels with cognitive test scores in patients with bipolar disorder. Among the BD group, a statistically significant positive correlation was found between serum zonulin levels and the duration of the SE (r = 0.46, $p < 0.001$).

Similarly, zonulin levels were inversely correlated with VMPT learning scores (r = −0.305, $p = 0.05$), highest learning scores (r = −0.381, $p = 0.01$), and delayed free recall scores (r = −0.416, $p < 0.001$). After Bonferroni adjustment, only the correlation with delayed recall remained statistically significant, suggesting that elevated

Table 1-3. Current treatments in bipolar disorder patients

Drug / combination	Number	Daily dose (mg/day, mean ± SD)	Distribution (n, %)	Approx. CPZ-eq (mg/day)
Li	11	955 ± 181	600 mg: 1 (9.1); 900 mg: 7 (63.6); 1,200 mg: 3 (27.3)	-
VPA	7	1,429 ± 214	1,000 mg: 2 (28.6); 1,500 mg: 5 (71.4)	-
Antipsychotic monotherapy	2	-	Ari 15 mg (n = 1); Ari 20 mg (n = 1)	300−400
Li + VPA	4	-	2× (Li 900 + VPA 1,000); 2× (Li 600 + VPA 1,500)	-
Li + antipsychotic	6	Li 600−1,200; APs: Olz 10−15, Que 300−800, Ari 15−20	-	200−800
VPA + antipsychotic	9	VPA 1,000; APs: Olz 10−20, Que 300−400, Ari 15−20	-	300−700
Lam + antipsychotic	1	Lam 200 + Que 400	-	~400

Li, lithium; VPA, valproic acid; Ari, aripiprazole; Olz, olanzapine; Que, quetiapine; Lam, lamotrigine; CPZ-eq, chlorpromazine-equivalent dose; -, not available.

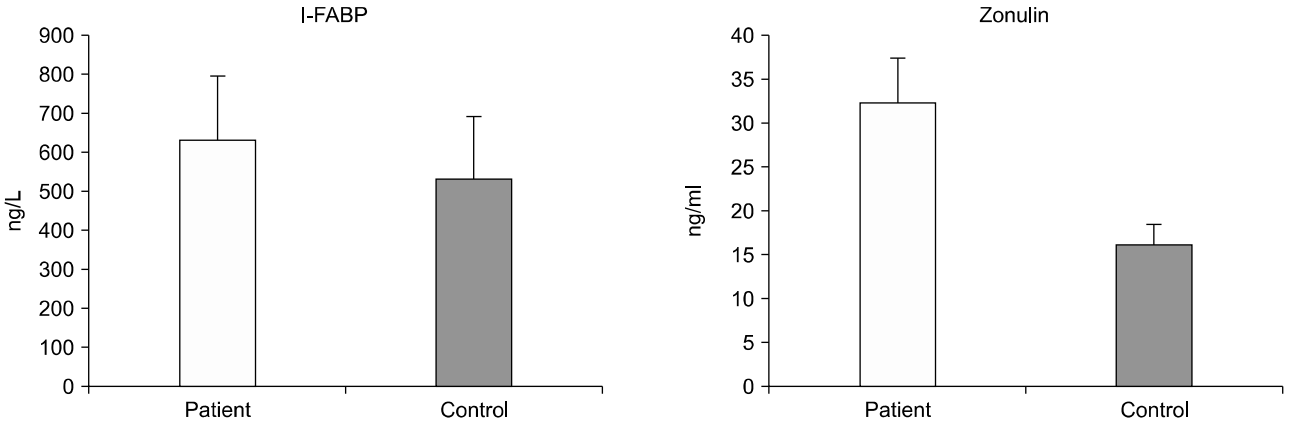

Fig. 1. Levels of I-FABP and zonulin in patients with bipolar disorder and healthy controls.
I-FABP, intestinal fatty acid-binding protein.

Table 2. Serum zonulin and I-FABP levels in patients with BD and HCs

Parameter	BD (n = 40)	HCs (n = 40)	p value	Test statistic (Z)
Zonulin (ng/ml)	26.7 (12.25−45.81)	15.13 (5.34−20.79)	< 0.001	−6.842
I-FABP (ng/L)	659.44 (268.34−987.15)	379.61 (128.90−915.83)	< 0.001	−3.560

Data are presented as median (min−max). Z values were obtained using the Mann-Whitney U test.
BD, bipolar disorder; HCs, healthy controls; I-FABP, intestinal fatty acid-binding protein.

zonulin may be particularly associated with impaired memory consolidation. Conversely, recognition scores showed a positive correlation with zonulin levels (r = 0.423, $p < 0.001$), which remained significant after correction, suggesting a compensatory reliance on recognition-based retrieval in the context of impaired recall.

No statistically significant correlations were observed between zonulin or I-FABP levels and WCST parameters

Table 3. Comparison of neurocognitive test performance between patients with BD and HCs

Cognitive measure	BD (n = 40)	HCs (n = 40)	*p* value	Test statistic (*t*/Z)
SE (sec)	31 (3−58)	12 (0−37)	< 0.001	−6.032
TMT-A (sec)	43 (23−103)	34.5 (10−74)	< 0.001	−3.601
TMT-B (sec)	100 (55−200)	69 (25−194)	< 0.001	−3.977
VMPT-IM	4 (2−7)	5 (0−10)	< 0.001	−3.330
VMPT-LS	88 (44−136)	114 (68−145)	< 0.001	−4.68
VMPT-HVLS	10.79 ± 3.07	13.20 ± 2.01	< 0.001	−4.090
VMPT-DR	5 (0−10)	10 (4−15)	< 0.001	−5.590
VMPT-R	7 (5−11)	4 (4−8)	0.050	−2.530
WCST-TC	64 (26−112)	91 (32−112)	0.008	−2.652
WCST-TE	64 (16−98)	37 (16−96)	0.011	−2.556
WCST-PR	36 (8−122)	20 (0−109)	0.002	−3.134
WCST-PE	32.5 (8−92)	17 (0−74)	< 0.001	−3.486
WCST-C	2 (0−10)	6 (0−10)	0.051	−1.947
DST-DF	3 (1−7)	5 (3−7)	< 0.001	−3.972
DST-DB	2 (0−5)	3 (2−6)	< 0.001	−2.880

Data are presented as median (min−max) or mean ± standard deviation, as appropriate. *t* and Z values were obtained using independent samples *t* test and Mann–Whitney *U* test.
BD, bipolar disorder; HCs, healthy controls; SE, Stroop effect; TMT, Trail Making Test; VMPT, Verbal Memory Process Test; IM, immediate memory; LS, learning score; HVLS, high verbal learning score; DR, delayed recall; R, recognition; WCST, Wisconsin Card Sorting Test; TC, total correct; TE, total error; PR, perseverative response; PE, perseverative error; C, categories; DST, Digit Span Test; DF, digit forward; DB, digit backward.

Table 4. Correlation of zonulin and I-FABP levels with cognitive test scores in the bipolar disorder patients

Cognitive test	Zonulin (r)	Zonulin (*p*)	Zonulin (*p*, Bonf.)	I-FABP (r)	I-FABP (*p*)	I-FABP (*p*, Bonf.)
SE	0.469	< 0.001	< 0.001	0.055	0.73	9.49
TMT-A	0.199	0.21	2.73	−0.180	0.26	3.38
TMT-B	0.782	0.01	0.13	0.267	0.90	11.70
VMPT-IM	−0.060	0.71	9.23	−0.010	0.95	12.35
VMPT-LS	−0.305	0.05	0.65	0.092	0.57	7.41
VMPT-HVLS	−0.381	0.01	0.13	0.134	0.41	5.33
VMPT-DR	−0.416	< 0.001	< 0.001	0.133	0.41	5.33
VMPT-R	0.423	< 0.001	< 0.001	−0.193	0.23	2.99
WCST-TC	0.182	0.26	3.38	0.140	0.38	4.94
WCST-TE	−0.167	0.30	3.90	−0.173	0.28	3.64
WCST-PR	−0.118	0.46	5.98	−0.132	0.41	5.33
WCST-PE	−0.127	0.43	5.59	−0.133	0.41	5.33
WCST-C	0.159	0.32	4.16	0.255	0.16	2.08
DST-DF	0.297	0.06	0.78	−0.016	0.92	11.96
DST-DB	0.133	0.41	5.33	−0.006	0.97	12.61

r = Spearman correlation coefficient; Bonferroni-corrected *p* values are reported.
I-FABP, intestinal fatty acid-binding protein; SE, Stroop effect; TMT, Trail Making Test; VMPT, Verbal Memory Process Test; IM, immediate memory; LS, learning score; HVLS, high verbal learning score; DR, delayed recall; R, recognition; WCST, Wisconsin Card Sorting Test; TC, total correct; TE, total error; PR, perseverative response; PE, perseverative error; C, categories; DST, Digit Span Test; DF digit forward; DB, digit backward.

or DST scores in the patient group. Similarly, I-FABP levels were not significantly associated with any of the cognitive test performances either before or after Bonferroni correction.

In the HC group, a positive correlation was identified between zonulin levels and the completion time for Trail Making Test–Form A (r = 0.360, $p = 0.02$), as well as the number of WCST perseverative errors (r = 0.347, $p = 0.03$) (Supplementary Table 1; available online). However, these findings should be interpreted with caution the absence of multiple-comparison correction in this group.

DISCUSSION

There is existing research in the literature that examines the markers of intestinal permeability in BD. However, to

the best of our knowledge, the present study is the only one directly investigating the association between these biomarkers and cognitive functioning. In line with the previous research, this study found higher zonulin and I-FABP levels in the BD group, which indicates a disruption in intestinal barrier integrity. Stevens *et al.* [37] reported such elevations among patients with depression, anxiety and they argued that these may reflect microbial dysbiosis and low-grade systemic inflammation. Similarly, Kılıç *et al.* [21] showcased persistently increased zonulin and claudin-5 levels in euthymic BD, suggesting that barrier alterations may persist during euthymic states. In other study, Zengil and Laloğlu [22] found elevated serum zonulin and occludin levels in BD, with no significant associations with HDRS scores, and all patients had YMRS scores of 0. Their findings support the idea that increased marker levels are trait-related and observed regardless of the BD patients' mood state.

Arnone [25] also confirmed higher I-FABP levels in patients with mood disorders, reinforcing the view that I-FABP indicates epithelial injury. On the contrary, Ohlsson *et al.* [36] reported an interesting dissociation between I-FABP and zonulin levels among the psychiatric inpatients following suicide attempts. They observed increased I-FABP but reduced zonulin levels, and attributed this to advanced mucosal damage resulting in down-regulated zonulin release.

It is likely that variability among these findings reflect differences in clinical populations, stage of illness, pharmacological exposure, and factors related to methodology such as sampling and assay procedures. Notably, Ioannou *et al.* [28] emphasized that biomarkers like zonulin, LBP, and sCD14 represent distinct physiological pathways within gut-immune signaling; treating them interchangeably may obscure their specific biological significance.

The results of this study, along with the emerging evidence in the literature, support the hypothesis that intestinal barrier disruption may be a consistent biological characteristic of BD, rather than a temporary effect of mood episodes.

Cognitive impairments in BD are well-documented and persist beyond mood episodes into remission. Deficits in psychomotor speed, attention, verbal memory, and executive function have been consistently reported [26]. A landmark meta-analysis revealed that executive dysfunction and verbal memory impairments are more prominent than attention and immediate memory deficits in BD patients during euthymic state [38].

In this context, the Stroop Test was used in this study to evaluate focused attention, interference control, and processing speed, A significantly increased SE was observed in patients ($p < 0.001$), indicating of impaired inhibitory control. Similarly, patients required significantly longer times to complete Trail Making Tests A and B ($p < 0.001$), suggesting deficits in mental flexibility and visuomotor scanning. On the Öktem Verbal Memory Test, patients exhibited reduced immediate recall, learning capacity, maximum learning, and spontaneous recall, however recognition scores were paradoxically elevated, potentially indicating compensatory reliance on recognition memory due to underlying retrieval deficits. Executive dysfunction was further supported by the WCST, where patients displayed increased perseverative errors and fewer completed categories. Lower forward and backward Digit Span scores also pointed to attentional and working memory impairments. Matching educational levels between BD and HCs groups reinforced the validity of these findings by minimizing potential confounding effects. Most patients were receiving mood stabilizers and/or atypical antipsychotics. Daily doses of the medication are presented in Table 1, with antipsychotic doses converted and expressed as chlorpromazine equivalents. However, serum drug concentrations were not assessed at the time of blood sampling, and this limits direct pharmacokinetic interpretation. Because the control group did not receive psychotropic medication, potential confounding effects of treatment cannot be ruled out. Previous studies indicate that mood stabilizers and antipsychotics can influence cognitive performance through both protective and adverse mechanisms depending on treatment duration and dose [39,40]. Therefore, medication exposure should be considered an important contextual factor when interpreting the present results. All participants were in a clinically verified euthymic state at the time of assessment, with YMRS scores of 0 and very low HDRS values. Including HDRS scores as a covariate did not alter the main results, indicating that residual depressive symptoms did not significantly affect either intestinal permeability markers or cognitive outcomes.

Interestingly, zonulin but not I-FABP levels were significantly associated with cognitive outcomes in this study.

After correction for multiple comparisons, significant associations persisted between zonulin levels and SE, delayed recall performance, and recognition scores. This suggest that increased zonulin may selectively influence executive control, memory consolidation, and recognition-based retrieval processes. After adjusting for CRP levels, the associations between serum zonulin and cognitive performance remained statistically significant, Similarly, including BMI as a covariate did not materially alter the observed associations (Supplementary Table 2; available online), suggesting that these relationships were not primarily explained by BMI either. This suggests that the permeability–cognition link in BD may not simply reflect secondary systemic changes but rather a more direct neurobiological process. This discrepancy likely reflects their involvement in different biological pathways. Zonulin is an active modulator of tight junction permeability in both intestinal and cerebral endothelial barriers, directly regulates paracellular transport and inflammatory signaling within the gut-brain axis. Differently, I-FABP mainly indicates enterocyte injury rather than change in functional permeability, and this may explain the weaker associations found in this study [24-41]. Supporting this interpretation, recent evidence has linked zonulin dysregulation to cognitive and affective disturbances across psychiatric and metabolic disorders. For instance, Maes *et al.* [42] demonstrated that elevated zonulin contributes to paracellular barrier breakdown and neuroinflammation in schizophrenia, while Lee *et al.* [29] reported that gut leakage markers, including zonulin, correlated with attentional performance in ADHD. Building on these observations, several research groups have explored whether probiotics that influence gut permeability might also affect cognitive or emotional symptoms. The outcomes have been mixed. Some trials noted parallel improvements in mood or cognitive measures, while others showed no measurable benefit [43-45]. Given these discrepancies, the connection between gut-barrier modulation and neurocognitive functioning remains uncertain and should be interpreted carefully. Altogether, the evidence points to zonulin as a more active regulator of gut-brain communication compared with I-FABP, which may simply indicate epithelial damage rather than a functional change in barrier permeability.

The findings of this study suggest a meaningful association between intestinal permeability and cognitive dysfunction in BD. However, several points should be considered when interpreting these results. First, the cross-sectional nature of this study limits the ability to identify the direction of cause-and-effect relationship. Future longitudinal research is needed to determine whether changes in gut permeability lead to cognitive decline or occur as a consequence of it.

Second, all BD patients who participated in the study were in a euthymic state, with zero YMRS scores and minimal HDRS values. This reduces, but does not fully eliminate, the possibility that residual symptoms affected cognitive outcomes. Moreover, while medication regimens were stable and dosages were carefully recorded (Table 1), serum concentrations of the medications were not assessed on the day blood sample collection. For this reason, potential pharmacological effects on cognitive performance cannot be entirely ruled out. Previous work suggests that psychotropic medications, especially lithium and atypical antipsychotics, may have mixed influences on cognition, depending on the duration and dosage of treatment. Therefore, future research could include serum-level monitoring to address this issue.

Although CRP and BMI were included as covariates and did not materially change the observed associations, these findings should be interpreted with caution given the relatively modest sample size, the cross-sectional design that precludes causal inference, and possible residual confounding by unmeasured inflammatory or metabolic factors. These constitute the main limitations of the present study.

Overall, these factors highlight the need for future longitudinal studies with larger samples and multimodal approaches combining microbiome, neurocognitive, and neuroimaging data. Such research could explore whether targeting the gut-brain axis through dietary changes, probiotics, or anti-inflammatory treatments could help protect cognitive function in BD.

In conclusion, patients with BD showed increased zonulin and I-FABP levels, indicating impaired intestinal barrier integrity. Only zonulin was consistently associated to poorer performance in executive and memory domains. This association suggests that zonulin-related barrier dysfunction may play a specific role in the cognitive difficulties observed in BD. The findings of this study support the growing evidence that gut-brain interactions contribute to the neurobiology of BD, and represent a promising area for future therapeutic research.

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■ **Conflicts of Interest**

No potential conflict of interest relevant to this article was reported.

■ **Author Contributions**

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