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Leaky gut biomarkers in depression and suicidal behavior

Ohlsson L, Gustafsson A, Lavant E, Suneson K, Brundin L, Westrin Å, Ljunggren L, Lindqvist D. Leaky gut biomarkers in depression and suicidal behavior.

Objective: Inflammation is associated with major depressive disorder (MDD) and suicidal behavior. According to the 'leaky gut hypothesis', increased intestinal permeability may contribute to this relationship via bacterial translocation across enterocytes. We measured plasma levels of gut permeability markers, in patients with a recent suicide attempt (rSA), MDD subjects with no history of a suicide attempt (nsMDD), and healthy controls (HC), and related these markers to symptom severity and inflammation.
Method: We enrolled rSA (n = 54), nsMDD (n = 13), and HC (n = 17). Zonulin, intestinal fatty acid binding protein (I-FABP), soluble CD14, and interleukin-6 (IL-6) were quantified in plasma. Montgomery–Åsberg Depression Rating Scale (MADRS) and Suicide Assessment Scale (SUAS) were used for symptom assessments.
Results: The rSA group displayed higher I-FABP and lower zonulin levels compared with both the nsMDD and the HC groups (all $P < 0.001$). IL-6 correlated positively with I-FABP ($r = 0.24$, $P < 0.05$) and negatively with zonulin ($r = -0.25$, $P < 0.05$). In all subjects, I-FABP levels correlated positively with MADRS ($r = 0.25$, $P < 0.05$) and SUAS scores ($r = 0.38$, $P < 0.001$), and the latter correlation was significant also in the nsMDD group ($r = 0.60$, $P < 0.05$).
Conclusion: The 'leaky gut hypothesis' may improve our understanding of the link between inflammation and suicidal behavior. These findings should be considered preliminary until replicated in larger cohorts.

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Key words: suicide, attempted; depressive disorder, major; zonulin; intestinal fatty acid binding protein; intestinal permeability

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Significant outcomes

- Gut permeability markers zonulin and intestinal fatty acid binding protein were altered in patients with a recent suicide attempt.
- Gut permeability markers correlated significantly with interleukin-6 – a marker of systemic inflammation.
- The 'leaky gut hypothesis' may help explain part of the association between of the inflammation and suicidal behavior.

Limitations

- The sample size was relatively small; hence, these findings need to be replicated in larger samples.
- It is possible that our results were confounded by unmeasured variable such as smoking, alcohol intake, or dietary habits.

Introduction

Several lines of evidence support an association between inflammation and major depressive disorder (MDD) (1–3). Some reports suggest that this immune activation might be even more pronounced in suicidal individuals (4–10). The underlying pathobiology behind inflammation in suicidal

behavior and depression is not fully understood. The so-called gut–brain axis, linking emotional and cognitive brain centers with gastrointestinal function, has recently received substantial attention in relation to psychiatric disorders (11). This bidirectional crosstalk between the digestive system and the brain could be mediated via changes in gut microbiota resulting in immune activation, potentially generating various types of psychiatric symptoms (12–17). Specifically, a leaking gut allows translocation of lipopolysaccharides (LPS), molecules found on the outer membrane of gram-negative bacteria, from the gut into the circulation. LPS, in turn, activate various immune cells, leading to increased secretion of pro-inflammatory cytokines and systemic low-grade inflammation (18, 19).

Some currently used markers of gut permeability are lactulose/mannitol challenge test, fecal calprotectin, and histological analysis of intestinal biopsies (14). Intestinal permeability can also be determined in blood plasma, for example, by measuring zonulin and intestinal fatty acid binding protein (I-FABP). Zonulin, first described in 2000 by Fasano et al. (20), is a protein involved in modulating the permeability of the small intestine. Zonulin has been shown to induce disassembly of tight junctions between cells of the duodenum and small intestine, resulting in increased permeability (21). Another potential marker of gut integrity is I-FABP, also known as FABP2 (22). This cytoplasmic protein is found in the enterocytes of the small intestine and elevated levels indicate enterocyte damage (23, 24). Although zonulin and I-FABP have not been studied in psychiatric samples, one previous study on individuals with HIV found that these two markers are inversely correlated and that high I-FABP and low zonulin predicted mortality (25). The underlying mechanisms are currently unknown but it has been hypothesized that greater gut epithelial cell death or dysfunction might decrease the expression of zonulin (25), suggesting that low plasma zonulin levels may be indicative of greater gut permeability. Soluble CD14 (sCD14) is a co-receptor for LPS considered to be an activation marker for monocytes and other blood mononuclear cells released after stimulation (26). LPS induce secretion of sCD14 from immune cells (27); hence, high plasma levels of sCD14 are thought to reflect exposure to LPS (28, 29). sCD14 is increased in conditions thought to be characterized by greater gut permeability such as celiac disease (30, 31), potentially as a consequence of bacterial translocation across enterocytes (31). However, given that sCD14 is a non-specific marker of monocyte activation that can be released from immune cells via other, non-LPS dependent, mechanisms (26), any specificity as a biomarker of gut permeability is yet to be determined. To the best of our knowledge, no studies have investigated biomarkers of increased gut permeability in patients with MDD and in patients with recent suicidal behavior, or the relationship between such markers and systemic inflammation and illness severity.

Aims of the study

The aim of the present study was to measure plasma levels of zonulin and I-FABP in three groups: patients with a recent suicide attempt (rSA), MDD subjects with no history of a suicide attempt (nsMDD), and healthy controls (HC), and to relate these markers to interleukin-6 (IL-6) (a cytokine previously found to be elevated in suicide attempters (7, 32)), sCD14, and symptom severity. Based on previous studies linking inflammation to suicidal behavior *per se,* we hypothesized that any evidence of increased gut permeability would be most pronounced among rSA, followed by nsMDD, and HC.

Material and methods

Subjects

All included subjects gave written informed consent to participate, and the study was approved by Lund University Medical Ethics Committee. nsMDD subjects (n = 13) were recruited from the psychiatric clinic at Lund University Hospital between 2001 and 2003. They all fulfilled the Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV criteria for moderate to severe MDD. None of these subjects had a history of a previous suicide attempt. Additional exclusion criteria were pregnancy, cardiovascular disease, and treatment with antidepressants, neuroleptics, or mood stabilizers during the last month. One subject had ongoing alcohol abuse, and one subject had previous alcohol abuse. HCs (n = 17) were recruited between 2001 and 2003. They were randomly selected from the municipal population register in Lund, Sweden. They were somatically healthy and had no history of mental disorders, as determined by medical history, routine blood screening, and physical examination. The rSA group (n = 54) was enrolled following admission to Lund University Hospital after a suicide attempt between 2006 and 2009. Psychiatric diagnoses were determined according to the DSM-IV. Depressive symptoms were rated using the Montgomery–Åsberg Depression Rating Scale (MADRS) (33). Suicidality was

assessed by means of the Suicide Assessment Scale (SUAS) (34). Characteristics of the study population are presented in Table 1.

Sample handling

Blood samples were collected in EDTA vacuum tubes. The samples were immediately placed on ice and centrifuged at 4°C and 2000× *g* for 10 min within 1 h of collection and stored at −80°C until analysis (mean 12 ± 2 years after sample collection).

Assays

Plasma zonulin (P-zonulin) was measured using a competitive enzyme immunoassay (Immundiagnostik AG, Bensheim, Germany) according to the manufacturer's instructions. The assay only detects the active (uncleaved) form of zonulin. Plasma I-FABP and sCD14 concentrations were measured using a sandwich enzyme immunoassay (RnD Systems, Abingdon, UK) according to the manufacturer's instructions. Detection limits: zonulin = 0.22 ng/ml, I-FABP = 6.2 pg/ml, and sCD14 = 0.125 ng/ml. All samples were above detection limits. Intra-assay coefficients of variation (CV) were 3.5% for I-FABP, 4.3% for Zonulin, and 5.4% for sCD14. Interassay CVs were 8.4% for I-FABP, 13.4% for Zonulin, and 6.3% for sCD14.

IL-6 was assayed on three 96-well plates with samples from HCs, nsMDD, and rSA subjects distributed on all three plates. To avoid batch-to-batch variation, all the reagents used for the cytokine analysis were from the same kit. IL-6 was measured in the plasma using ultra-sensitive electrochemiluminescence immunoassays according to the manufacturer's recommendations (Meso Scale Discovery, UK). Standards and samples were analyzed in duplicate. The detection limit was 0.050 pg/ml IL-6. IL-6 data from this sample have been published previously (7).

Statistical analyses

SPSS was used for statistical analysis of data. Correlations were tested using Pearson's *r*. Pearson's chi-square was used to compare proportions between groups. Non-normally distributed variables were log-transformed to achieve normality. In cases when log-transformation was insufficient (viz., IL-6 and I-FABP levels), we used Blom transformation (35), a statistical procedure replacing each raw score with its rank value and adjusting the scale distances between the ranks to achieve a normal distribution. One-way ANOVA with Bonferroni correction was used to test between-group differences adjusting for covariates when appropriate (ANCOVA). We adjusted group-wise comparisons for age, gender, body mass index (BMI), and substance use. We conducted a series of sensitivity analyses in order to take into account the potentially confounding effects of concurrent medications and somatic comorbidity.

Results

Demographics

There were no significant between-group differences in sex distribution, age, or BMI (Table 1). The nsMDD group had the highest MADRS score, followed by rSA and HCs. The rSA had the highest SUAS score, followed by nsMDD and HCs. Psychiatric/somatic diagnoses and medications are summarized in Table 2.

Group differences

The rSA group had significantly higher I-FABP and lower zonulin levels compared to both HCs

Table 1. Demographic and clinical characteristics for the three groups

	rSA (*n* = 54)	nsMDD (*n* = 13)	HC (*n* = 17)	*P*-value
Sex (f/m)	30/24	7/6	8/9	0.83*
Age (years; mean ± SD)	38.5 ± 14.5	34.5 ± 11.5	34.4 ± 11.4	0.42†
Body mass index (kg/m^2; mean ± SD)	25.7 ± 4.4	25.9 ± 8.7	23.1 ± 3.1	0.16†
MADRS score (mean ± SD)	21.0 ± 11.7	28.7 ± 7.6	0.8 ± 1.5	<0.001†
SUAS score (mean ± SD)	38.8 ± 16.9	28.3 ± 6.3	0.8 ± 2.2	<0.001†
Zonulin, ng/ml (median, IQR)	5.8, 3.7–7.3	26.4, 22.8–34.2	22.4, 20.3–29.5	<0.001†
Intestinal fatty acid binding protein, pg/ml (median, IQR)	2027.5, 1277.8–2723.8	559.8, 431.4–976.5	667.8, 474.6–1378.0	<0.001†
Soluble CD14, ng/ml (median, IQR)	1012.5, 769.5–1222.5	917.5, 325.5–1082.7	704.9, 345.3–1090.7	0.13†

rSA, patients with a recent suicide attempt; nsMDD, MDD subjects with no history of a suicide attempt; HC, healthy controls. Non-normally distributed biomarkers were log- or Blom-transformed prior to analyses; IQR, interquartile range; MADRS, Montgomery–Åsberg Depression Rating Scale; SUAS, Suicide Assessment Scale.

*Pearson's chi-square.

†One-way ANOVA.

Table 2. Principal psychiatric diagnoses, somatic comorbidities that could potentially interfere with biomarkers, and medications in all subjects

	rSA (n = 54)	nsMDD (n = 13)	HC (n = 17)
Principal DSM diagnosis (n)	MDD = 12 Depressive disorder NOS = 3 Schizoaffective disorder = 2 Psychotic disorder NOS = 1 Bipolar disorder 1 = 3 Bipolar disorder II = 12 GAD = 1 Anxiety disorder NOS = 4 Dysthymic disorder = 3 Alcohol dependence = 6 Substance dependence = 3 Adjustment disorder = 3 Adjustment disorder with Depressed mood = 1	MDD = 13	N/A
Somatic comorbidities (n)*	Asthma/allergy = 2 Diabetes = 2 Psoriasis = 1	Asthma/allergy = 1 Ulcerative colitis = 1	Asthma/allergy = 2
Psychiatric medications (n)	Antidepressants = 25 Mood stabilizers only = 4 Mood stabilizers + antidepressants = 6 Neuroleptics + antidepressants = 8 Other combinations = 3 No psychotropics = 8	N/A	N/A
Somatic medications	Regular NSAID = 3 Antibiotics = 3	N/A	N/A

rSA, patients with a recent suicide attempt; nsMDD, MDD subjects with no history of a suicide attempt; HC, healthy controls; DSM, Diagnostic and Statistical Manual of Mental Disorders; NOS, not otherwise specified; GAD, generalized anxiety disorder; NSAID, non-steroid anti-inflammatory drug.
*One individual had both asthma and diabetes.

and the nsMDD group (all $P < 0.001$; Fig. 1). There were no other significant between-group differences. Adjusting for age, sex, BMI, and substance use did not significantly alter these findings (all $P < 0.001$). rSA continued to have significantly higher I-FABP and lower zonulin levels compared to both nsMDD and HCs after (i) including only those rSA free of psychotropic medications (n = 8) (all $P < 0.01$), (ii) excluding all subjects taking anti-inflammatory medications or antibiotics (n = 6) (all $P < 0.001$), and (iii) excluding all subjects with somatic conditions that could have an impact on the biomarkers (asthma/allergies, inflammatory bowel disease, psoriasis, diabetes; n = 8) (all $P < 0.001$). In exploratory analyses, we compared biomarker levels between the three largest diagnostic groups within the rSA group (bipolar mood disorders, n = 15; unipolar mood disorders, n = 18; and alcohol/substance dependence, n = 9), but there were no significant between-group differences in zonulin, I-FABP, or sCD14 (one-way ANOVA, all $P > 0.29$).

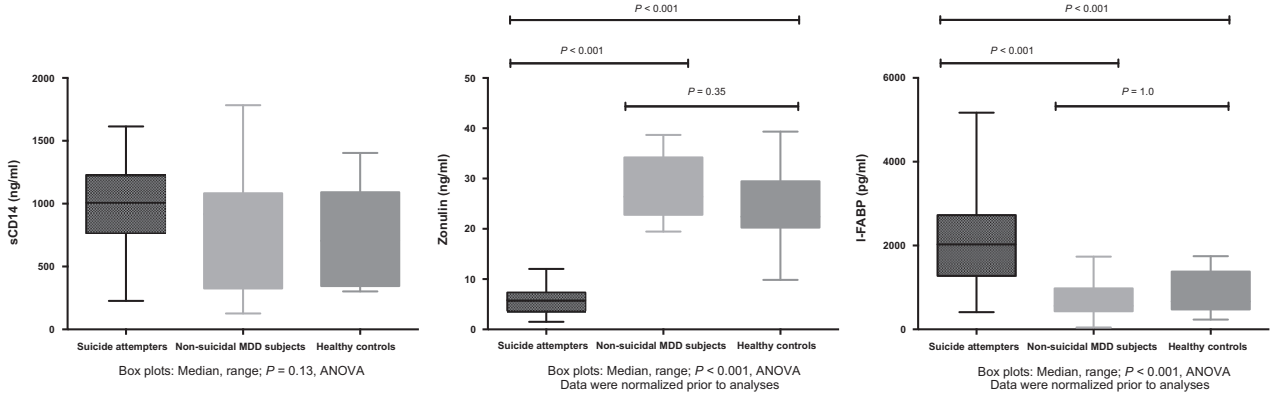

Fig. 1. Zonulin, intestinal fatty acid binding protein (I-FABP), and soluble CD14 levels in patients with a recent suicide attempt, MDD subjects with no history of a suicide attempt, and healthy controls. One-way ANOVA with Bonferroni correction on normalized data. Box plots indicate median and interquartile range (IQR), and whiskers indicate range.

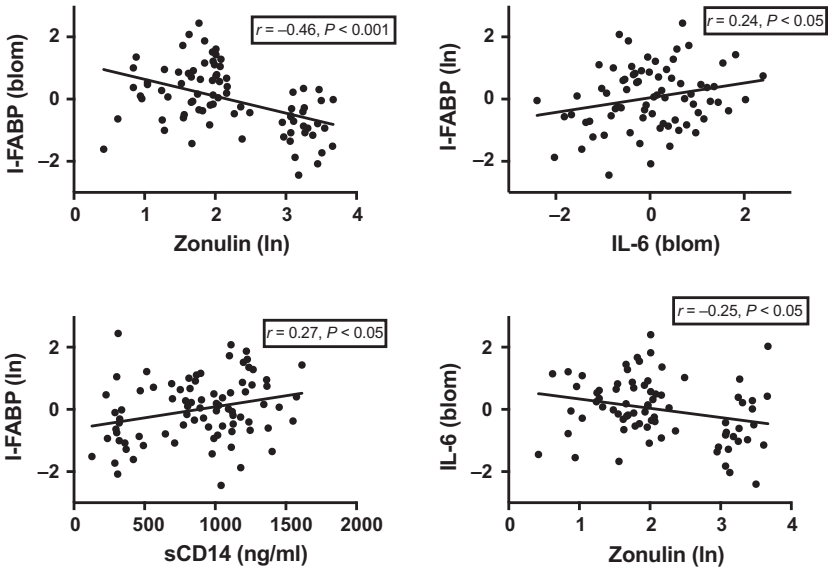

Fig. 2. Intercorrelations between biomarkers in all subjects (Pearson's *r*). Non-normally distributed variables were log- or Blom-transformed prior to analyses. I-FABP, intestinal fatty acid binding protein; sCD14, soluble CD14; IL-6, interleukin-6.

Correlation analyses

In all subjects, I-FABP correlated negatively with zonulin ($r = -0.46$, $P < 0.001$) and positively with IL-6 ($r = 0.24$, $P < 0.05$) and sCD14 ($r = 0.27$, $P < 0.05$). Zonulin correlated negatively and significantly with IL-6 ($r = -0.25$, $P < 0.05$) but not with sCD14 ($r = -0.16$, $P = 0.16$) (Fig. 2).

In all subjects, MADRS scores correlated significantly and positively with I-FABP ($r = 0.25$, $P < 0.05$) and negatively at trend level with zonulin ($r = -0.21$, $P = 0.07$). When rSA and nsMDD were analyzed separately, these correlations did not reach significance (all $P > 0.2$).

In all subjects, SUAS scores correlated significantly and positively with I-FABP ($r = 0.38$, $P < 0.001$) and negatively with zonulin ($r = -0.51$, $P < 0.001$). When rSA and nsMDD were analyzed separately, the positive correlation between SUAS and I-FABP remained significant in the nsMDD group ($r = 0.60$, $P < 0.05$) (Fig. 3), but none of the other correlations were significant in any of the groups (all $P > 0.51$).

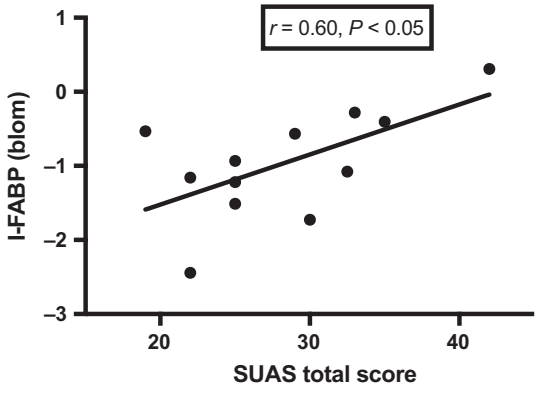

Fig. 3. Correlation between Suicide Assessment Scale (SUAS) total score and intestinal fatty acid binding protein (I-FABP) in subjects with major depressive disorder without a history of a suicide attempt (Pearson's *r*). I-FABP levels were Blom-transformed prior to analysis.

Discussion

This is, to the best of our knowledge, the first study to investigate biomarkers of gut permeability in patients with suicidal behavior and depressed patients without a history of a suicide attempt. Consistent with our hypothesis of an association between suicidal behavior and increased gut permeability, I-FABP, a marker of enterocyte damage, was significantly elevated in rSA and directly correlated with severity of depressive symptoms. Interestingly, high I-FABP levels were directly correlated with severity of suicidal symptoms also among those depressed patients who had not attempted suicide, supporting a link also between suicidal ideation and increased gut permeability and/or enterocyte damage. Plasma zonulin levels, however, were significantly decreased in rSA and negatively associated with I-FABP, suggesting that these two blood markers may represent different aspects of gut integrity in this sample. Finally, the observed group differences did not seem to be confounded by the effects of sex, age, BMI, medication use, substance abuse, or somatic comorbidities.

Several studies have reported a link between various psychiatric disorders, gut integrity, and microbiota (14, 36–39), although any causal relationships have not yet been determined. In support of the notion that gastrointestinal alterations may actually cause depressive symptoms, Kelly et al.

(40) demonstrated that oral transplantation of fecal microbiota from depressed patients to microbiota-depleted rats induces depressive-like symptoms. Moreover, some (41, 42), but not all (43), clinical trials report that probiotics may alleviate symptoms of depression and anxiety, further supporting a mechanistic relationship between dysbiosis and psychiatric symptoms. These biological mechanisms are, however, not restricted to psychiatric disorders. Increased gut permeability and/or dysbiosis have been suggested as pathophysiological mechanisms also in metabolic disorders (18, 44, 45), rheumatoid disorders (46, 47), and HIV infection (25, 48), all conditions with a persistent inflammatory component and a heightened risk of depression (49–52). Although a direct causal link between gut permeability and some of these conditions has not yet been fully established, we hypothesize that this could represent a common pathophysiological mechanism in some cases, explaining part of the comorbidity between certain psychiatric and somatic disorders. Despite some evidence suggesting a biological link between gut integrity and psychiatric symptoms, it is also possible that this association can be partly explained by a psychological reaction to a debilitating somatic condition. For instance, individuals with a gastrointestinal disorder may be more prone to develop depressive symptoms due to the psychological burden of their somatic illness. Future studies are needed to tease apart these effects.

In the present study, we found increased I-FABP in rSA, while zonulin was significantly lower in the same group. Moreover, these two biomarkers were inversely correlated, suggesting that they represent different aspects of gut permeability. I-FABP was also directly correlated with IL-6, a cytokine previously implicated in suicidality (32, 53). We have previously shown, in the same cohort as the present study, that suicide attempters have elevated levels of plasma IL-6 compared to MDD subjects and controls (7). Although the current study is the first psychiatric study relating gut permeability markers to markers of systemic inflammation, the correlation between I-FABP and IL-6 is in line with a previous study on HIV patients (25), a group characterized by increased gut permeability (54). High I-FABP levels indicate greater enterocyte damage, while lower zonulin levels could in fact also indicate that gut integrity is compromised. In support of this, Hunt et al. (25) also reported a negative correlation between zonulin and I-FABP in subjects with HIV infection. Additionally, low zonulin in combination with increased I-FABP predicted mortality in this group (25). Viable gut epithelial cells express zonulin to disassemble intercellular tight junctions, thereby increasing permeability (21). Although speculative and in need of replication, we hypothesize that the lower zonulin levels observed among rSA in our study reflect greater gut epithelial cell death or dysfunction.

Although the cross-sectional design of the current study precludes any causal inferences, there are several different hypotheses that could explain increased gut permeability in patients with suicidal behavior. Increased gut permeability may be partly genetically determined as has been shown in studies on inflammatory bowel disease (55). Moreover, several lifestyle factors, most notably dietary habits, influence gut permeability. Specifically, diets consisting of fast food and processed food have been linked to both increased gut permeability as well as symptoms of depression and suicidality (14, 56). Additionally, gut permeability alterations in the rSA group may be secondary to changes in microbiota composition induced by psychotropics, which has been demonstrated in animal studies (57). This hypothesis is, however, not supported by our sensitivity analysis showing that also psychotropic-free rSA displays low zonulin and high I-FABP. Moreover, stress may be a common upstream cause of increased gut permeability, systemic inflammation, and suicidal behavior. Animal and human studies have shown that both early-life and acute stress may influence gut permeability (14). The gut microbiotia may be a mediator of the well-established link between stress, hypothalamic–pituitary–adrenal axis activity, and the immune system (58)—biological processes also thought to be involved in suicidal behavior (32, 59).

We did not find any significant between-group differences in sCD14 levels. sCD14 is considered a non-specific monocyte activation marker (26), not necessarily indicative of increased gut permeability, which could explain why this marker did not show similar alterations as zonulin or I-FABP.

The current study comes with some limitations including a relatively small sample size. Thus, future studies with larger sample sizes could yield refined methods to possibly identify those with MDD and leaky gut-induced low-grade inflammation. Also, since this was a cross-sectional study based on a single time-point blood and behavioral measurements, we cannot infer any causal relationship between gut permeability markers and psychiatric symptoms. Even though we adjusted for several potential confounders, there is a possibility that yet other, unmeasured variables, such as smoking, alcohol intake, and dietary habits, may have had an impact on the results. Moreover, the

diagnostic heterogeneity within the rSA group complicates the interpretation of our results. Although we did not find evidence that any of the biomarkers differed significantly between the main diagnostic categories within this group, these subgroup analyses may have been underpowered and should therefore be interpreted with some caution. Finally, while there are a large number of potential biomarkers of gut permeability (including fecal markers and lactulose/mannitol ratio tests), we decided to quantify zonulin and I-FABP in blood plasma. The rationale for assaying these specific gut permeability biomarkers was i) plasma samples were available in our cohort, and ii) zonulin and I-FABP are two commonly used plasma biomarkers for gut permeability and they correlate with other indicators of increased gut permeability such as lactulose/mannitol ratio and morphologic epithelial intestinal damage (60, 61).

To conclude, we here show alterations in gut permeability markers in patients with a history of suicidal behavior. Although preliminary and in need of replication, our findings suggest that the 'leaky gut hypothesis' may help explain part of the immune activation frequently reported in individuals with suicidal ideation or behavior.

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Conflict of interest

The authors declare no conflict of interest.

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