

Zonulin as a Biomarker in Mental Illness - Review

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Abstract

Mental health conditions are on the rise worldwide. This has determined an acceleration in the last two years in research focusing on mental health and brain-gut microbiome axis, highlighting the relevance of bidirectional communication. This systematic review aimed to gather evidence from studies of intestinal permeability biomarkers, in adults diagnosed with mental disorders, by following the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA). The selected articles provided evidence of intestinal permeability in adults diagnosed with neurocognitive impairment in Alzheimer's disease, schizophrenia, depression and suicide risk, bipolar affective disorder and alcohol consumption. Research shows that increased permeability and changes in the intestinal microbiota contribute to the pathogenesis of mental disorders. These findings support that this intestinal permeability biomarker had a role in mental disorders, but more information is needed for shedding light on this topic.

Key words: biomarkers, mental disorders, Zonulin.

J.E.L. classification: I130, I180

1. Introduction

Mental health problems are a major cause of disability (GBD 2017 Disease and Injury Incidence and Prevalence Collaborators, 2018), thus there is of a great necessity to identify new therapeutic approaches based on the clarification of pathophysiological processes.

Multiple studies investigating intestinal permeability in patients suffering from mental disorders are published. Asbjornsdottir et al. (2020) selected five papers focusing on children for their systematic review, where the intestinal permeability was determined by altered levels of zonulin. While in some particular studies zonulin level is correlated with the severity of the condition (i.e., correlated with Childhood Autism Rating Scale; Esnafoglu et al., 2017) or subtypes (i.e., in ADHD patients; Özyurt et al., 2018), other studies come in a certain contradiction when testing with the relevance of the age. While Esnafoglu et al. (2017) reported that zonulin level does not seem to be correlated with age, but rather with body mass index in children with autism spectrum disorder (ASD), Józefczuk et al. (2018) showed a positive correlation between zonulin level and age, in children of 5 years old the zonulin level being higher than in older children. Thus, more studies are needed for better understanding the correlations between zonulin level and specific parameters or mental disorders.

This systematic review aimed to gather evidence from studies of intestinal permeability biomarkers in adults diagnosed with mental disorders by following the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta Analysis (PRISMA).

2. Theoretical background

Alterations in the composition of gut microbiota determine increased permeability of the gut barrier and immune activation, leading to systemic inflammation, which in turn may impair the blood-brain barrier and promote neuroinflammation, neural injury, and ultimately neurodegeneration (Mulak et al, 2019).

Research focusing on mental health and the brain-gut-microbiome axis has accelerated in the last decade (Foster et al, 2017; Grenham et al, 2011; Iannone et al, 2019) highlighting the relevance of the bidirectional communication on the brain-gut- microbiome axis in mental disorders. The gut and the central nervous system (CNS) have communication network which includes the enteric nervous system (ENS), sympathetic and parasympathetic branches of the autonomic nervous system (ANS), neuroendocrine signaling pathways, and neuro-immune systems (Grenham et al, 2011; Foster et al, 2017). Dysbiosis increases the permeability of the gut-blood barrier, translocating gut microbiota. It may cause immune system activation, with local and systemic proinflammatory state and more permissive intestinal barrier (Iannone et al, 2019). Chronic inflammation and activated immune could be a pathogenesis for mental disorders (Mulak et al, 2021).

Zonulin has been identified as a pre-haptoglobin, a molecule that has been initially considered the inactive precursor of haptoglobin 2. However, data suggest pre-HP2 is a multifunctional molecule that, in a single-chain intact form, regulates intestinal permeability and in a split form, with two chains, is a precursor for hemoglobin (Fasano, 2012). Zonulin is probably the most important biomarker of intestinal permeability, an increased concentration of this protein being correlated with increased intestinal permeability (Fasano, 2012). This intracellular protein of the tight junction complex has an atomic mass of 47 kilodaltons (kDa) and binds the cell cytoskeleton to tight transmembrane junction proteins. In the epithelial cells, it acts as a modulator of intracellular tight junctions and paracellular permeability in the intestine-blood and brain-blood barriers (Lu et al, 2000).

3. Research methodology

This systematic review followed items according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines (Moher et al, 2010). The database includes records found in libraries available on PubMed, Cochrane Library, Web of Science, Springer Link, Science Direct, and Scopus, using as inclusion criterion the analysis of intestinal permeability biomarkers in studies to adults with mental disorders. The keywords used for the search were biomarkers, mental disorders, and zonulin. Data collected included general information, characteristics, statistical methods, number of participants involved, and conclusions.

4. Findings

Of the 56 records identified in the systematic search, 8 articles were selected for this review (Table 1), encompassing a total of 537 participants who provided evidence of intestinal permeability in addition to the diagnosis of mental disorders. From the total of selected articles, 1 neurocognitive impairment in Alzheimer's diseases, 1 investigated the connection between zonulin level and schizophrenia, 2 depression and suicide risk, 3 bipolar affective disorder, 1 alcohol.

Table no. 1 Summary of the main findings and conclusions

First author, year, journal	Participants (n)	Main findings	Conclusions
Wang X, 2020, <i>Acta Neurologica Scandinavica</i>	AD, n=110 MCI, n=110 Control, n=110	The zonulin and C type lectin like receptor-2 (CLEC-2) levels were higher in Alzheimer disease (AD) patients compared with mild cognitive impairment (MCI) patients. Increased zonulin and CLEC-2 levels were significantly associated with reduced Mini-Mental State Examination (MMSE) score.	Increased zonulin and CLEC-2 are the significant factors for reduced MMSE score in MCI and AD.

<p>Usta A, 2021, <i>European Archives of Psychiatry and Clinical Neuroscience</i></p>	<p>Schizophrenia, n=50 control, n=50</p>	<p>The mean serum zonulin levels were significantly increased in patients with schizophrenia when compared to the control group. Also, in patients with schizophrenia serum claudin-5 levels were decreased when compared to the control group.</p>	<p>The study assumes that zonulin and claudin-5 may be involved in the etiopathogenesis of schizophrenia.</p>
<p>Ohlsson L, 2019, <i>Acta Psychiatrica Scandinavica</i></p>	<p>rSA, n=54 no history of suicide attempt, n=13 control, n=17</p>	<p>The recent suicide attempt (rSA) group displayed lower zonulin levels and higher I-FABP compared with both the nsMDD and the control group. Zonulin was negatively correlated with IL-6, but for I-FABP a positive correlation was found with IL-6.</p>	<p>Patients with suicidal behavior have alterations in gut permeability markers. The 'leaky gut hypotheses' may help explain part of the immune activation in individuals with suicidal behavior.</p>
<p>Liśkiewicz P, 2021, <i>Progress in Neuro-Psychopharmacology & Biological Psychiatry</i></p>	<p>MDD, n=16</p>	<p>Fecal zonulin levels was not correlated with the clinical status Hamilton Depression Rating Scale (HDRS24) in patients with major depressive disorder (MDD). There was a positive correlation with changes in fecal calprotectin during hospitalization, i.e., calprotectin decreases during the study with better clinical outcome.</p>	<p>The severity of depressive symptoms was associated with gut microbiota. These findings do not serve as predictors of symptomatic improvement during antidepressant treatment in inpatient treatment for MDD.</p>
<p>Maget A, 2021, <i>Journal of Affective Disorders</i></p>	<p>Depression, n=76 Euthymia, n=45</p>	<p>Zonulin level was significantly higher in women than in men, independent from affective status. Also, zonulin level was increased in euthymic women comparing to euthymic men. Specific psychopharmacotherapy did not induce any difference in zonulin levels between groups</p>	<p>Epithelial permeability poses an interesting potential factor in neuropsychiatric entities. The severity of the depression was not related to the zonulin level.</p>
<p>Aydın O, 2020, <i>Journal of Neural Transmission</i></p>	<p>BD type I, n=30 control, n=29</p>	<p>In bipolar disorders (BD) type I no significant correlations were found between plasma zonulin and duration of illness, total count of manic and depressive attacks, treatment duration, and hospital admission counts.</p>	<p>Plasma zonulin levels did not oscillate in patients with BD type I between symptom exacerbation and treatment response. Furthermore, plasma zonulin levels did not vary between control and BD.</p>

Kılıç F, 2020, <i>Journal of Affective Disorders</i>	Manic, n=20 In remission, n=21 control, n=41	In patients with manic episodes the mean serum zonulin and claudin-5 levels were significantly higher compared to healthy controls. No difference was found between patients with manic episodes and patients in remission in zonulin and claudin-5 levels.	The findings may contribute to the role of intestinal permeability or brain-blood-barrier in the pathogenesis of bipolar disorder
Jung F, 2021, <i>Nutrients</i>	ALD, n= 37 control, n=17	Zonulin protein concentrations and I-FABP were lower in Alcohol-Related Liver Disease (ALD) patients before alcohol withdrawal, compared to healthy controls, but without reaching the significance level. The concentrations of both zonulin and I-FABP increased almost to the level of controls after the alcohol withdrawal.	Increased bacterial toxin levels are directly related to alcohol intake, the functioning of intestinal barrier markers being altered. After one week of abstinence these alterations are remarkably improved, in association with an improvement of liver healthiness.

Source: Authors' own contribution

Neurocognitive disease. In a study conducted on patients suffering from Alzheimer's dementia (AD, n = 110), mild cognitive impairment (MCI, n = 110) and healthy controls (n = 110, mean age over 75 years for all groups), increased zonulin levels, measured using ELISA assay, were observed (Wang et al, 2019). In AD patients serum zonulin levels were significantly higher (5.37 ± 2.18 ng/mL) than those in MCI patients (3.20 ± 0.18 ng/mL) or healthy patients (2.68 ± 0.27 ng/mL). In addition, in this study, a high level of zonulin was positively correlated with a high level of C-type lectin-like receptor 2.

Schizophrenia. Usta et al (2021) showed that zonulin levels were high in patients diagnosed with schizophrenia. In this study, serum zonulin levels in schizophrenia patients (n = 50) were higher than in the healthy controls (n = 50; $t = 3107$, $p = 0.002$). In addition, zonulin was positively correlated with BMI ($r = 0.413$, $p = 0.003$), but not with age or disease duration. No significant correlation was found between serum zonulin concentration and the total Scale for the Assessment of Negative Symptoms (SANS) score ($r = -0.259$, $p = 0.070$), but a negative correlation was identified between serum zonulin concentration and the positive schizophrenia symptoms evaluated through the Scale for the Assessment of Positive Symptoms (SAPS) ($r = -0.306$, $p = 0.031$).

Depression and suicidal behavior. In a study held by Ohlsson et al, (2019), investigating biomarkers of gut permeability in patients with suicidal behavior (n = 54 with recent suicid attempt), depressed patient without a history of suicid attempt (n = 13 with major depressive disorder) and healthy controls (n = 17), zonulin was negatively correlated with the intestinal fatty acid binding protein (I-FABP) ($r = 0.46$, $p < 0.001$), with IL-6 ($r = 0.25$, $p < 0.05$) and with MADRAS Scores ($r = -0.21$, $p = 0.07$).

Another study (Liśkiewicz et al, 2021) investigated patients with mild depressive episode (n = 1), moderate depression (n = 8) and severe depressive episode (n = 7) in treatment with escitalopram, during 6 weeks and showed median results: In week 0, median result of zonulin was 70.97 (ng/ml) (60.69–99.84), in week 6 median results of zonulin was 55.0 (ng/ml), (43.69–102.82). In week 0, median result of calprotectin was 21.76 ($\mu\text{g/ml}$), (12.96–42.24), in week 6 median results of calprotectin was 14.35 ($\mu\text{g/ml}$), W6 (9.52–33.83). The authors found the severity of depressive symptom correlated with gut microbiota and no correlation between severity of depressive symptom and fecal zonulin level (Liśkiewicz et al, 2021).

There was no significant correlation between fecal zonulin levels and the clinical status Hamilton Depression Rating Scale (HDRS24). There was a positive correlation with changes in fecal calprotectin during hospitalization (i.e., calprotectin decreases during the study with better clinical outcome).

Bipolar affective disorder. Kılıç et al (2020) observed 41 patients diagnosed with bipolar affective disorder (21 in remission and 20 with manic episodes) and 41 healthy controls and showed an increase level of serum zonulin claudin-5 levels than healthy controls, with no difference zonulin and claudin-5 levels between patients with manic episodes and patients in remission.

Marget et al (2021) conducted a study on patients with depression (n = 76) and patients considered euthymic (n = 45), showing that serum zonulin level is significantly higher in women compared to men, independent from affective status. ($z = -2.412$, $p = 0.016$). Women showed higher zonulin levels than men in the euthymic subgroup ($z = -2.114$, $p = 0.035$). No difference in zonulin serum levels was found in individuals taking psychopharmacotherapy and no correlation was found with depression severity.

In another study, focussing on patients suffering from bipolar disorder type I (Aydın et al, , 2020), diagnosed patients (n = 30) and healthy controls (n = 29) the plasma zonulin levels did not significantly differed between groups (Wilks' Lambda; $F(1,56) = 0.57$; $p = 0.45$; $\eta^2 = 0.01$) or treatment response period (Wilks' Lambda; $F(1,56) = 0.67$; $p = 0.51$; $\eta^2 = 0.03$) when analyzed using enzyme-linked immunoassay (ELISA) method. Also, there were no associations between plasma zonulin-level and disease symptoms and intestinal barrier integrity was not altered among patients.

Alcohol. Jung et al (2021) conducted research following patients suffering from alcohol related liver disease (ALD, n = 37) and healthy controls (n = 17), where zonulin and I-FAB levels in plasma were found to be lower before alcohol withdrawal, but not significantly ($p=0.006$ for zonulin and $p=0.27$ for I-FAB). After one week of alcohol withdrawal, zonulin and I-FAB levels increased significantly, almost to the level of controls ($p<0.05$ for both). This effect is a primary result of a restored intestinal mucosa, the patients showing also an improvement of liver healthiness. As a direct consequence of alcohol withdrawal, markers of intestinal permeability are rapidly improved in patients with ALD.

5. Conclusions

The intestines serve as an important barrier between the external environment and the body and play an important role in the transfer of certain antigens to the body and in the development of an immune response (Wei et al, 2005). The study of Usta et al (2021) assumes that zonulin may be involved in the etiopathogenesis of schizophrenia.

The study of (Wang et al, 2019) reveals increased zonulin and C type lectin like receptor-2 as significant factors for reduced Mini-Mental State Examination score in mild cognitive impairment and Alzheimer disease. Some studies reveal the importance of the gut-brain axis in the etiology of schizophrenia. The study of Usta et al (2021) shows that zonulin is negatively correlated with the symptoms evaluated on the SAPS scale. Research findings show patients diagnosed with depression and other mental conditions have demonstrated gut microbiome dysbiosis (Sherwin et al, 2016). Ohlsson et al (2019) suggest that „leaky gut hypothesis” may induce part of immune responses reported in humans with suicidal attempt, because of the importance of the alterations in gut permeability markers. Zonulin and I-FAB have different aspects in gut permeability, in depression lower levels of zonulin could indicate in fact compromise gut integrity and higher levels showing gut epithelial cell death or dysfunction, and I-FAB indicating enterocyte damage. Liśkiewicz et al (2021) find associations between the severity of depressive symptoms and gut microbiota but no significant correlation between zonulin levels and the clinical status. These findings do not serve as predictors of symptomatic improvement during antidepressant treatment in inpatient treatment for MDD. Zonulin as a marker of intestinal permeability in patients with depression need more research also could be focus on the comparison of zonulin with other promising biomarkers like I FABP (Liśkiewicz et al, 2021). Kılıç et al (2020) found that zonulin and claudin-5 levels are higher in patients with manic phase of bipolar disorder. The authors highlight the potential of the findings to contribute to the role of intestinal permeability or brain-blood-barrier in the pathogenesis of bipolar

disorder (Marget et al, 2021). At patients with bipolar disorders no correlation was found between zonulin levels and the severity of the depression, neither between BD type I symptom exacerbation nor treatment response (Aydın et al, 2020). Mentioned, zonulin level was significantly higher in women than in men, independent from affective status. (Marget et al, 2021). Alcohol related liver disease (ALD) included conditions from steatosis to hepatitis, fibrosis and cirrhosis (Mueller et al, 2014). The results of the study (Jung et al, 2021) support the hypothesis that increased bacterial toxin levels are directly related to alcohol intake in ALD patients and that markers of intestinal barrier function are altered. The fact that only one week of abstinence improved the state of patients is a primary result of a restored intestinal mucosa, the patients showing also an improvement of liver healthiness. As a direct consequence of alcohol withdrawal, markers of intestinal permeability are rapidly improved in patients with ALD.

The limiting factors identified within the reviewed papers, (i) small sample size, (ii) intestinal microbiota composition not assessed, (iii) unmeasured sociodemographic and lifestyle variables (i.e., smoking, alcohol intake and dietary habits), (iv) the influence of medications (e.g., cholinergic drugs), constitute important confounding factor which should be taken under consideration in further studies.

All in all, research shows that increased permeability and changes in the intestinal microbiota contribute to the pathogenesis of mental disorders. An increased level of zonulin was found in (i) neurocognitive disorders in Alzheimer's disease and were correlated with low results on Mini-Mental State Exam (MMSE); and in (ii) patients with bipolar disorder or schizophrenia. On the other hand, low level of zonulin was found along with an elevated level of intestinal fatty acid-binding protein (I-FABP) in patients with recent suicide attempts, I-FABP being positively correlated with major depression and Montgomery-Asberg-Depression Rating Scale (MADRAS) results.

Further research should aim to evaluate the therapeutic potential of epithelial barrier amelioration and focus on the comparison of zonulin with other promising biomarkers like FABP. To assess time-dependent dynamics in the interaction of those biomarkers and the course of disease, longitudinal study designs should be preferred.

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