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Letter to the editor

Elevated zonulin, a measure of tight-junction permeability, may be implicated in schizophrenia

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Dear Editors:

Increasing evidence suggests that the pathophysiology of schizophrenia may be related to peripheral and neuroinflammation (Fineberg and Ellman, 2013; Fraguas et al., 2017; Jiang et al., 2018; Kroken et al., 2018; Muller, 2018). Additionally, increased leakiness of the epithelial tight junctions of the intestine and the blood brain barrier (BBB) may also play a role in neuroinflammation (Kealy et al., 2018; Najjar et al., 2017). We propose a potential role for zonulin in the mechanism of neuroinflammation. Zonulin, an endothelial growth factor receptor stimulator, has been shown to regulate tight junction permeability of the gut and the BBB. It has previously been implicated in a loss of barrier function of gastrointestinal tract, allowing the passage of macromolecules, including endotoxins, into the body and resulting in an immune response (Fasano, 2011). This process leads to the establishment of chronic inflammation as suggested from many other chronic inflammatory diseases, including Celiac Disease (Sturgeon and Fasano, 2016). This Letter to the Editor suggests a similar role for zonulin in the pathogenesis of schizophrenia.

1. Methods

We collected blood samples and symptom data from 98 male and female patients with DSM-IV-TR diagnosis of schizophrenia. Other inclusion and exclusion criteria for the schizophrenia participants included between the ages of 18 to 75 years and able to give informed consent. All participants were seen for a one time visit for clinical assessments and a laboratory draw. The primary blood sample drawn was for serum levels for zonulin. Zonulin was measured at the laboratory of Dr. Alessio Fasano at Massachusetts General Hospital (Guerrant et al., 2016), as commercial assays are known to be insufficient in detecting the actual protein (Ajamian et al., 2019). Human sera (70 μg per well) was run under nondenaturing conditions on 4–20% Tris-Glycine gels (Invitrogen). Protein was transferred onto a PVDF membrane (Millipore) and probed with 1.5 μg/mL mouse monoclonal zonulin antibody (Bio-Rad). Bands were detected with Alexa Flor 680 conjugated goat anti-mouse IgG antibodies (ThermoFisher). Bands were visualized using the LI-COR system. Densitometry was measured using Image Studio software (LI-COR). All samples were normalized to a reference sample run separately on each gel. Cut off scores for zonulin positivity were determined as 2.33 mg/dL, Cytokines were analyzed by the Cytokine Core Laboratory at the University of Maryland using multiplex (Luminex®) technology (IL-1β and TNF-α).

The clinical assessments included the demographic information and the Brief Psychiatric Rating Scale (BPRS), including five symptom domains of the BPRS (activation, psychosis, hostility, negative symptoms and anxiety/depression). This study was approved by the Institutional review Board at the University of Maryland School of Medicine.

Statistical analysis was performed using SPSS statistical software. The analysis was pilot in nature, examining one of the first reports of serum zonulin in people with schizophrenia. We explored associations between zonulin levels and factors related to symptoms of schizophrenia, demographic information, and inflammatory markers. The analysis included a variety of statistical tools to evaluate the relationship between zonulin levels, IL-1β, TNF-α, and BRPS scores, including Spearman's correlation and multivariable analyses.

2. Results

In this population the mean age was 32.5 ± 9.7 years, 68% were male, 58% were Caucasian and the mean BPRS total score was 31.6 ± 6.6. The mean zonulin level of this schizophrenia cohort was 2.38 mg/dL (SD 1.23 mg/dL) with 42/98 (42.9%) having levels higher than the cut off for elevated levels (>2.33 mg/dL). There was no difference in mean values for total BPRS or any subfactors between those with elevated and not elevated zonulin levels ($p > 0.05$). We find no relationship between either zonulin or cytokines alone with psychiatric symptoms ($p > 0.05$) but we did find an interaction (higher zonulin X higher cytokine) for IL-1b (Beta = 2.31, $p = 0.024$) and a trend for TNF-α (Beta = 0.342, $p = 0.06$) with both associated with higher total BPRS scores. Anxiety/depression and hostility subfactors were also significant for the interactions while the model was not predictive for positive and negative symptoms (See Table 1). These findings hint that the combination of gut permeability and chronic inflammation may have some relationship to brain function as they are associated with higher global psychiatric symptoms.

To our knowledge, this is one of the first studies to investigate zonulin in relation to inflammatory markers in schizophrenia. A recent paper recently reported higher zonulin levels in schizophrenia compared to healthy controls but measured by an IgM response. They find that deficit schizophrenia may be more related to immune response and zonulin as 57% of the variance in psychiatric symptoms measured by the Schedule for the Deficit Syndrome (SDS) was explained by a variety of immune and tight junction measures (Maes et al., 2019). We did not see any relationship of zonulin and immune function to negative symptoms in our study. No other schizophrenia reports reporting zonulin levesl are available, to our knowledge, however interestingly a recent report found that during a psychosocial stress paradigm zonulin levels increased and were partly associated with changes in inflammatory markers (Linninge et al., 2018). Also, increased zonulin levels have been associated with other behaviors and psychiatric disorders such as hyperactivity and social dysfunction in children with attention deficit hyperactivity disorder (Ozyurt et al., 2018) and anxiety/depression (Stevens et al., 2018).

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Table 1
Effects of inflammatory cytokines vs. interaction of zonulin with inflammatory cytokines on psychiatric symptoms.

BPRS symptom category	Cytokine or interaction coefficient	Standardized coefficients (beta)	p-Value
Total score	IL-1β	−0.165	0.282
	Zon * IL-1β	2.306	0.024*
	TNF-α	−0.893	0.375
	Zon * TNF-α	0.342	0.067
Activation	IL-1β	0.380	0.014
	Zon * IL-1β	−0.129	0.430
	TNF-α	0.410	0.008
	Zon * TNF-α	0.042	0.811
Hostility	IL-1β	−0.213	0.131
	Zon * IL-1β	0.590	0.000*
	TNF-α	−0.229	0.113
	Zon * TNF-α	0.668	0.000*
Negative symptoms	IL-1β	−0.074	0.642
	Zon * IL-1β	0.064	0.709
	TNF-α	−0.064	0.705
	Zon * TNF-α	−0.014	0.944
Positive symptoms	IL-1β	−0.238	0.131
	Zon * IL-1β	0.267	0.113
	TNF-α	−0.232	0.163
	Zon * TNF-α	0.228	0.232
Anxiety/depression	IL-1β	−0.105	0.490
	Zon * IL-1β	0.365	0.027*
	TNF-α	−0.084	0.603
	Zon * TNF-α	0.367	0.050*

* $p < 0.05$.

This pilot study is limited by lack of a normative control group; however, the assay and cutoff scores were determined by an in house assay and defined by healthy control data in Dr. Fasano's laboratory. Future studies should examine the relationship of these apparently elevated zonulin levels and delineate a subgroup for which these may be associated with psychiatric symptoms or the underlying pathophysiology of schizophrenia. We also are limited by not having comprehensive ratings of neuropsychological function (i.e., MATRICS Consensus Cognitive Battery (MCCB)) and specific negative symptom scales (i.e., Scale for the Assessment of Negative Symptoms). It is our hope that zonulin and its role in schizophrenia will stimulate the research community to devote more resources to investigating this topic but also cautiously understanding the challenges with commercially available zonulin assays. This pilot study contributes to the idea that gut permeability and inflammation continue to play an emerging role in the pathogenesis of schizophrenia. Thus, future studies are needed to better understand the complex role of barrier function and inflammation to the pathophysiology of schizophrenia.

Roles on project

Gregory Barber, Robert McMahon, and Deanna Kelly undertook the statistical analysis. Gregory Barber and Deanna Kelly managed the literature search and analysis. Gregory Barber wrote the first draft of the manuscript. Deanna Kelly designed the study and wrote the protocol. Craig Sturgeon collected the data and edited the manuscript. Alessio Fasano oversaw the collection of data and edited the manuscript. Nicola Cascella and William Eaton edited and revised the manuscript.

Declaration of Competing Interest

Dr. Alessio Fasano is a consultant for INOVA Diagnostics and Innovate Biopharmaceuticals, Inc. He is a stockholder for Alba Therapeutics and serves as a speaker for Mead Johnson Nutrition. Dr. Deanna Kelly has served as consultant for Alkermes, Lundbeck and HLS Therapeutics.

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