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Review article: intestinal barrier dysfunction and central nervous system disorders – a controversial association

M. Julio-Pieper*, J. A. Bravo*, E. Aliaga† & M. Gotteland‡

*Instituto de Química, Facultad de Ciencias, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile.
†Escuela de Kinesiología, Facultad de Ciencias, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile.
‡Departamento de Nutrición, Facultad de Medicina, Universidad de Chile, Santiago, Chile.

Correspondence to:
Dr M. Julio-Pieper, Instituto de Química, Facultad de Ciencias, Pontificia Universidad Católica de Valparaíso, Avenida Universidad 330, Curauma, Valparaíso, Chile.
E-mail: marcela.julio@ucv.cl

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SUMMARY

Background
Central nervous system (CNS) development and physiopathology are greatly affected by environmental stimuli. The intestinal barrier restricts the entrance of toxins, pathogens, and antigens while modulating the expression of various neuroactive compounds. The existence of a rich gut-to-brain communication raises the possibility that intestinal barrier alterations may take part in the pathophysiology of CNS disorders.

Aim
To review evidence associating intestinal barrier dysfunction with the development of CNS disorders.

Methods
Literature search was conducted on PubMed using the following terms: intestinal barrier, intestinal permeability, central nervous system, mental disorders, schizophrenia, autism, stress, anxiety, depression, and neurodegeneration.

Results
Clinical and animal model studies of the association between intestinal barrier and schizophrenia, autism spectrum disorders, neurodegenerative diseases or depression were reviewed. The majority of reports concentrated on schizophrenia and autism spectrum disorders. About half of these described increased intestinal permeability/mucosal damage in patients compared with healthy controls, with up to 43% of children with autism spectrum disorders and up to 35% of schizophrenia patients displaying abnormally high urinary excretion of the sugars used as permeability markers. However, another substantial group of studies did not find such differences. In autism spectrum disorders, some reports show that the use of diets such as the gluten-free casein-free diet may contribute to the normalisation of lactulose/mannitol ratio, but to date there is no adequately controlled study showing improvement in behavioural symptoms following these dietary interventions.

Conclusions
Evidence of altered intestinal permeability in individuals suffering from CNS disorders is limited and cannot be regarded as proven. Moreover the efficacy of targeting gut barrier in the management of neurological and behavioural aspects of CNS disorders has not yet been established, and needs further investigation.

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INTRODUCTION

For some time clinical experience has supported the existence of a functional relationship between the central nervous system (CNS) and the gastrointestinal tract,[1, 2] a paradigm known as the 'brain-gut axis'. Data gathered in controlled experimental conditions using animal models reveal that brain disturbances, such as those induced by physical or psychological stress, can affect gut function[3, 4] but also alterations of the gastrointestinal micro-environment can induce behavioural and neurochemical changes.[5, 6] The latter evidence is supported by the few human studies currently available in this area.[7, 8] It is therefore possible to define 'brain to gut' as well as a 'gut to brain' modulation.[9]

The 'gut to brain' aspect of the brain-gut axis has recently gained more attention and efforts have been focused in studying CNS function in various animal models, including chronically altered nutrition[10] and acute gastrointestinal pain.[11] Similarly, there is now greater knowledge that the complex interaction of the intestinal mucosa with its luminal content may also be relevant to CNS maturation and function.[12] This review summarises the latest reports that have investigated the following hypotheses: (i) whether intestinal barrier disturbances are associated to or have an impact on CNS disorders and (ii) whether dietary interventions aimed to restore a healthy gut barrier have a therapeutic effect on CNS disorders.

A comprehensive literature search was conducted on PubMed using one or more of the following search terms: intestinal barrier, intestinal permeability, central nervous system, mental disorders, schizophrenia, autism, stress, anxiety, depression, and neurodegeneration. English language articles published between 1953 and 2014 were revised. Bibliographies of selected publications were also checked to identify additional relevant articles.

OVERVIEW OF THE INTESTINAL BARRIER

The intestinal mucosa is the largest surface of interaction between the internal milieu and the external environment. It has a dual role because it must regulate the absorption of water, electrolytes, and nutrients from the lumen into the circulation, while preventing the penetration of harmful pathogens and noxious luminal substances, including dietary antigens.[13] This selective, dynamic, and highly regulated barrier depends on many actors, such as: the intestinal microbiota, the epithelial cells apically bound through the tight-junction complex, secreted products of epithelial origin, the intestinal endothelium, the resident immune cells present in the mucosa and the enteric nervous system (ENS).[13–16]

The ENS is important in the modulation of gut permeability. The intestine is innervated by submucosal and myenteric ganglia. These are formed by enteric neurons and glia, both able to release mediators that can affect wound healing, epithelial proliferation/differentiation and paracellular permeability.[14, 17, 18] Not only is the ENS able to regulate gut permeability, it could also represent a path of communication between luminal events and the CNS. For example, mice fed with a strain of *Lactobacillus rhamnosus* displayed reduced anxiety- and depression-related behaviours,[6] however these effects were not found in vagotomised mice. The ENS is anatomically connected to the vagus nerve,[19] which could explain a potential signalling pathway from the gut lumen to the brain.

Although the adequate function of all components of the intestinal barrier is crucial to prevent access of harmful entities to the circulation, it is noteworthy that its establishment depends on the presence of luminal microbiota at key developmental stages.[20, 21] It is well documented that animals reared in a germ-free environment display a poorly developed intestinal barrier, with striking differences in morphological, immune, biochemical, and biophysical parameters, when compared to conventional counterparts. For instance, the intestinal wall of germ-free mice shows very few lymphoid follicles and a less complex vascular network, together with reduced mucus thickness and sIgA concentrations than conventionally reared mice.[21] Germ-free mice also show ENS alterations, with excitability being decreased in a subgroup of myenteric neurons.[22] Therefore, it is possible to suggest that early-life dysbiosis could alter the properties of the gut barrier and potentially affect health later on.

Increasingly, studies are investigating intestinal permeability in patients suffering from disorders of the CNS.[23] Translocation of intestinal luminal contents could affect CNS function, either directly or indirectly, through one or more of the routes shown in Figure 1. Although it is still unknown whether disruption of intestinal barrier may be a cause or an effect in these situations, treatments directed to restore intestinal barrier integrity might be of great interest to counteract inflammation-related symptoms, therefore improving the quality of life of these patients.

IMMUNE REGULATION OF CNS DEVELOPMENT AND PHYSIOLOGY

The nervous and immune systems are intertwined in a complex relationship. Immune activation subsequent to intestinal pathogen permeation[43, 44] affect CNS

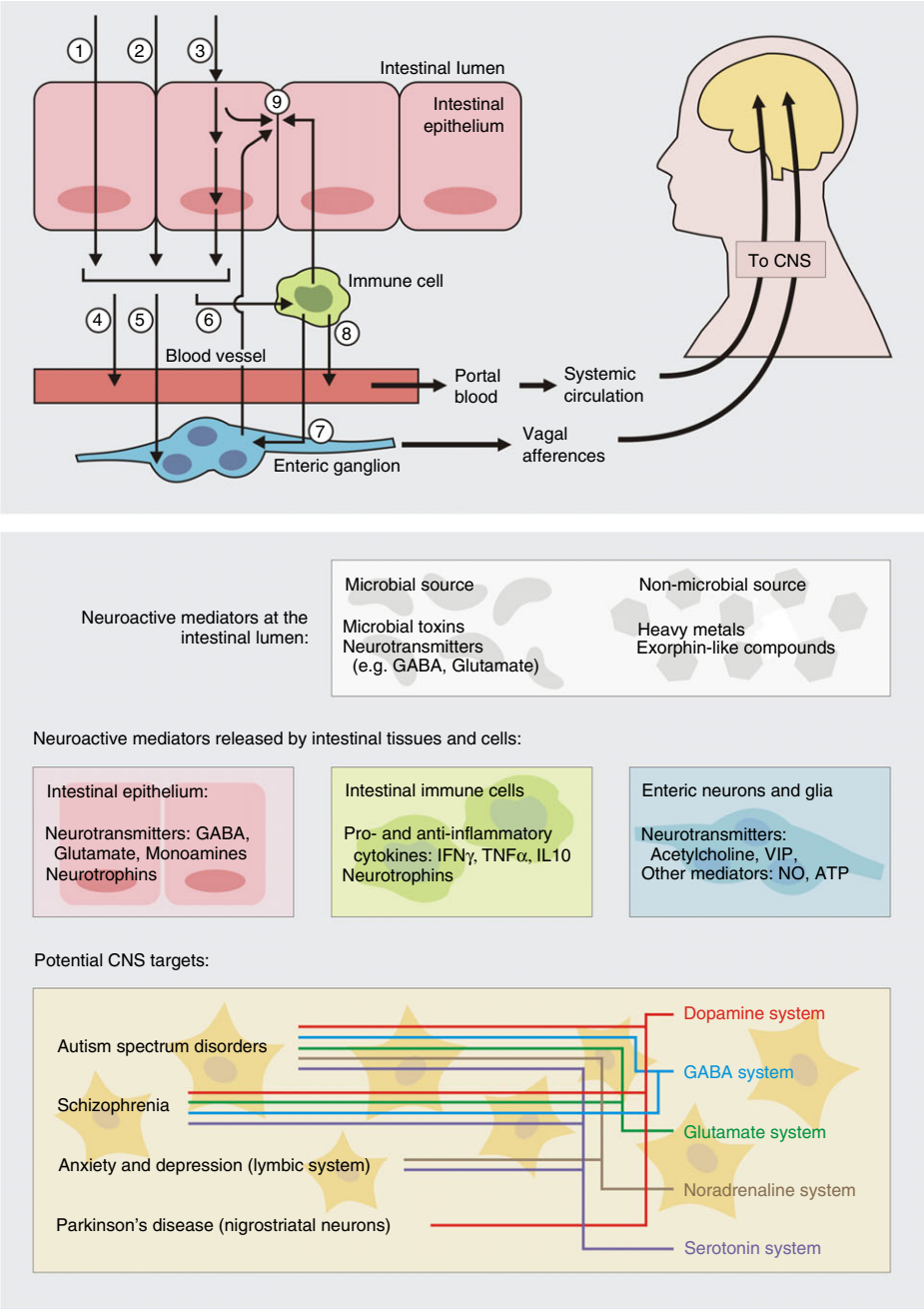

Figure 1 | Potential pathways connecting the intestinal lumen and the CNS. The upper panel shows anatomical and humoral pathways of comunication between the gut and the brain. Intestinal luminal contents may access the submucosal compartment by crossing the epithelium through a transcellular (1) or paracellular (2) pathway. Alternatively, epithelial cells release mediators basolaterally upon luminal stimulation (3). Components in the submucosal area may subsequently access the local (4) and systemic circulation and also reach the enteric neurons (5) and stimulate vagal afferences; these two destinations could facilitate the induction of changes in CNS function. In parallel, immune cells that reside in the epithelial and submucosal compartments can also be activated by intestinal luminal substances (6) and release mediators with the ability to affect enteric neuron function (7) or enter the blood (8). In addition, the properties of junctional complexes in the epithelium can be modulated by immune, neuronal, glial and epithelial cells themselves (9), generating a more or less permeable barrier. The lower panel indicates key intestinal neuroactive mediators at the intestinal lumen and intestinal tissues, as described by others.[98, 105–109] Also potential targets are shown for some CNS disorders, based on the current physiopathology and pharmacology for each condition.[110–115]

Table 1 | Clinical and laboratory tests used for the determination of intestinal permeability

***Functional tests:* One or more probes are administered orally. Urine is collected and probe detected**	
Type of probe	Interpretation
Sucrose	Increased excretion indicates an elevation in gastric permeability[25, 26]
Cellobiose + mannitol	Increased C/M ratio of excretion indicates an elevation in small intestinal permeability[27]
Lactulose + mannitol	Increased L/M ratio of excretion indicates an elevation in small intestinal permeability[28]
Sucralose + mannitol	Increased S/M ratio of excretion indicates an elevation in whole gut permeability[29]
^{51}Cr-labelled EDTA	Increased excretion indicates an elevation in whole gut permeability[30, 31]
Polyethylene glycol (PEG)	Excretion is decreased in coeliac disease, Crohn's disease and rheumatoid arthritis[32, 33] but increased in eczema[34]
Serological markers of intestinal mucosa damage	
Food antigen antibodies	
Antibodies to *Saccharomyces cerevisiae* (ASCA)[35, 36]	
Antibodies to bovine milk casein[37]	
Antibodies to gluten derived gliadin (AGA)[38]	
Antibodies to deamidated gliadin peptide (DGP-AGA)[39]	
Auto antibodies	
Antibodies to tissue transglutaminase (tTGA)/endomysium (AEA)[40, 41]	
Other markers	
Zonulin (pre-haptoglobulin 2)[42]	

Various compounds have been used as probes for gastric and intestinal permeability; it is important to note that excretion of marker probes can be influenced by diet, concomitant medication and health status (for a review see Mishra *et al.*[24]). Serological markers have also been used to measure the presence and/or extent of intestinal mucosal damage.

physiology and behaviour, typically not tipically inducing fatigue and social withdrawal.[45] Increases in early proinflammatory cytokines such as TNF-α and IL-1β can alter behaviour potentially through modifications on neuron ceramide synthesis, whereas interferons and IL-6 appear to act later to amplify the responses initiated by TNFα or IL-1β.[45] Conversely, stressful stimuli can activate the hypothalamus–pituitary–adrenal (HPA) axis, promoting the secretion of glucocorticoids from the adrenal cortex. These hormones (cortisol in humans and corticosterone in rodents) are strong immune-suppressants, however alterations in the HPA axis can lead to inadequate immune suppression, which may be linked to the appearance of diseases that share as a common feature a low degree of inflammation, such as irritable bowel syndrome (IBS)[46] and mood disorders.[47]

In addition, the nervous system can rapidly exert an anti-inflammatory response when sensing the presence of pathogenic antigens in the periphery. Such effect is mediated through the 'cholinergic anti-inflammatory pathway'[48], where vagal afferent sensory fibres signalling from the gastrointestinal system to the brain are stimulated by cytokines produced by immune cells.[49–51] These fibres terminate within the dorsal vagal complex, which connects with the PVN of the hypothalamus,[52] initiating a rapid neural activation of early anti-inflammatory responses, including HPA activation. Moreover, acetylcholine released from vagal efferent fibres have anti-inflammatory effects on immune cells,[51] which may be mediated by the α_7 subunit of the nicotinic receptor.[51] The secretion of IgG1, IFN-γ and IL-6 also depend on muscarinic receptor activation.[53] To summarise, alterations of this cross-talk between the physiological response to physical or psychological stimuli and the defence against pathogens could potentially lead to the appearance of disease.

Such alterations can appear as a consequence of aberrant immune-to-brain communication during development. Evidence shows that immune activation during pregnancy can lead to several behavioural abnormalities in nonhuman primates and rodents.[54, 55] In Rhesus monkeys (*Macaca mulatta*) exposure of pregnant dams with A/Sydney/5/97 (H3N2) virus at week 17 of the 24-week gestation period, affects foetal brain development, as shown by a reduction in cortical grey matter volume in the offspring, but without affecting behaviour.[55] In mice, injection of poly (I:C) (a synthetic analogue to dsRNA) on a key day during pregnancy (i.e. embryonic day 12.5) leads to behavioural alterations in the offspring, which are reminiscent of schizophrenia and autism.[54, 56–58] Such effects can be mimicked by IL-6 administration and are prevented by the co-administration of anti-IL-6 antibody.[57] Furthermore, the

Table 2 | Clinical studies investigating intestinal barrier alterations in patients suffering from CNS disorders

Source	Nature of patients studied (*n*)	Control/ comparison group (*n*)	Biochemical tests performed and markers evaluated in the study	Main outcome (e.g. prevalence of intestinal barrier dysfunction)	*P* value	Comments
Wood *et al.*[62]	Schizophrenic patients (17)	None	Functional gut permeability test	Cellobiose/mannitol ratio was normal in 11 (65%) and was abnormally high (range 0.032–0.46) in six (35%) patients	Not available	There is no control group; data were compared to a control population recovery ratio ranging from 0.002% to 0.03%. Cellobiose/mannitol ratio was also abnormally high in 33% of nonschizophrenic long-stay psychiatric patients
Lambert *et al.*[63]	Schizophrenic patients (24)	Healthy volunteers (43)	Functional gut permeability test	There was no significant difference in percentage excretion of ^{51}Cr-EDTA	Nonsignificant	Among schizophrenic patients, no difference in percentage excretion was found between the ones in remission and those in relapse
Cascella *et al.*[64]	Schizophrenic patients (1401)	Attendees at primary health-care units who answered a questionnaire indicating elevated risk for coeliac disease (900)	Markers for dietary antigen exposure Autoantibodies	23.1% of schizophrenic patients and 3.1% of the control group had moderate to high levels of AGA Moderate to high levels of tTGA were present in 5.4% of schizophrenic patients vs. 0.8% of the control group	<0.001 <0.001	No screening for schizophrenia was performed in the control group
Severance *et al.*[65]	Nonrecent onset (143) and recent onset schizophrenic patients (67)	Individuals with no history of psychiatric illness (207)	Markers for yeast antigen exposure Markers for dietary antigen exposure	Levels of ASCA antibodies were significantly elevated in nonrecent onset schizophrenics compared to controls (1.45 vs. 1.00) ASCA were also higher in recent onset schizophrenics compared to controls (2.03 vs. 1.00) Not reported as independent data	≤0.004 ≤0.00001 Not available	Data for dietary antigen exposure were only reported in correlation with ASCA
D'Eufemia *et al.*[66]	Children with autism (21)	Age-matched children (40)	Functional gut permeability test	L/M recovery ratio ranged from 0.010 to 0.034 in the control group. 57% of autistic patients had normal L/M recovery ratio (range 0.017–0.032), and 43% had an abnormally high L/M recovery ratio (range 0.036–0.298)	Not available	Participants showing clinical evidence of gastrointestinal disease, individual or family history of allergy or positive serum anti-gliadin and anti-endomysium antibodies were excluded from the study
Robertson *et al.*[67]	Children with autism (14). Other types of ASD were excluded from the study	Controls were siblings or friends of the subjects, or were related to the investigators (15)	Autoantibodies	No differences in mean urinary lactulose/mannitol ratio between autistic children and controls	Nonsignificant	In this study all children had normal IgA levels and were negative for anti-endomysium antibodies

Table 2 | (Continued)

Source	Nature of patients studied (*n*)	Control/ comparison group (*n*)	Biochemical tests performed and markers evaluated in the study	Main outcome (e.g. prevalence of intestinal barrier dysfunction)	*P* value	Comments
Kemperman *et al.*[68]	Children with pervasive developmental disorder (31)	None	Functional gut permeability test	Lactulose/mannitol ratio had a median value of 0.017 and a range 0.008–0.035, indicating that none of the patients had increased intestinal permeability	Not available	There is no control group; a urinary lactulose/mannitol ratio above 0.090 was considered to be indicative of abnormal GI integrity/ increased intestinal permeability. The study only included children, who; were negative for anti-endomysium antibodies
Reichelt *et al.*[69]	Girls and women diagnosed with Rett syndrome (23)	Healthy girls and women (53)	Markers for dietary antigen exposure Autoantibodies	Rett syndrome patients had higher levels of IgA against: Gluten Gliadin Casein No changes were found in anti-endomysium/tTGA	 0.001 0.005 0.005 Nonsignificant	
Maes *et al.* [70]	Patients with depression (28)	Staff or their family members (33)	Markers for bacterial antigen exposure	Abnormally high levels of IgM and IGA against bacterial LPS were more frequent in patients than in controls IgM: 12/28 vs. 0/23 IgA: 11/28 vs. 2/23	 0.0002 0.01	
Maes *et al.*[23]	Patients with depression (112), 35 of whom had chronic depression (>2 years)	Healthy volunteers (28)	Markers for bacterial antigen exposure	Confirms results by Maes *et al.*[70] IgM responses of patients with chronic depression were higher than those of normal controls No difference was found between depressed patients with a duration of illness <2 year and controls	0.0001 Nonsignificant	
Severance *et al.*[71]	Patients with bipolar disorder, without (226) and with (38) a recent onset of psychosis	Individuals with no history of psychiatric illness (207)	Markers for yeast antigen exposure Markers for dietary antigen exposure	Both bipolar disorder groups had higher ASCA levels than controls Not reported as independent data	≤0.00001 Not available	Data for dietary antigen exposure were only reported in correlation with ASCA
Forsyth *et al.*[72]	Subjects with Parkinson's disease (9)	Age-matched healthy volunteers (10)	Functional gut permeability test Markers for bacterial antigen exposure	24 h urinary sucralose excretion was significantly higher in patients compared to controls (1.12 vs. 0.58%) Score for intensity of *Escherichia coli* staining in the lamina propia of sigmoid colon was also higher in patients than controls (medians 2 vs. 0.5)	<0.05 <0.05	

behavioural effects of poly (I:C) exposure *in utero* are absent in IL-6 knock-out mice offspring.[57] Together these observations strongly suggest that IL-6 is highly relevant for the development of brain disturbances.[57]

The brain-to-immune pathway has been investigated using maternal separation in rats. This is a model of early-life stress that is frequently used for studying mood disorders and more recently, IBS.[59] Once the rats reach adulthood, they display impaired behaviour towards stressful situations,[59, 60] increased corticosterone secretion and altered gut microbiota diversity. Also, these rats display an altered immune response as shown by an exaggerated release of TNF-α in LPS-stimulated whole blood in comparison to controls.[3] It is interesting to note that in this model, behaviour and biological markers are affected by early post-natal stress, which suggests that the development of a balanced brain function and immune interaction may be affected not only during pregnancy but also during infancy. Overall, these data indicate that activation of the immune system, stressful situations during infancy, and post-natal therapeutic interventions (as suggested by Blaser[61]) can affect the way brain and immune system interact, and may in part explain an enhanced susceptibility of the CNS to immune alterations induced by intestinal barrier dysfunction.

CNS DISORDERS ASSOCIATED WITH INTESTINAL BARRIER DYSFUNCTION

Schizophrenia

Already in the early 1950s, there were data showing higher prevalence of coeliac disease among schizophrenics.[76] 20 years later, it was shown in a subgroup of patients, that dietary gluten withdrawal led to the improvement of the psychiatric symptoms of the disease.[77] One of the earliest studies aimed to associate schizophrenia with intestinal barrier alterations was done by Wood.[62] (For a summary of intestinal permeability tests see Table 1) In this study, the cellobiose/mannitol test was applied to 32 long-stay psychiatric patients, including 17 diagnosed with schizophrenia and 15 classified with nonschizophrenic psychiatric disease. As shown in Table 2, the cellobiose/mannitol ratio was abnormally high in 35% of the schizophrenic patients and in 33% of the rest of psychiatric patients. It is important to state that no control group was included in this study, meaning that the authors compared their data obtained in patients to control values reported by others. The small intestinal mucosal morphology was normal in all patients tested, with no signs of coeliac disease.[62] A few years later, a different research group assessed GI permeability in 43 healthy controls and 24 patients with schizophrenia (12 in remission and 12 in relapse), by using the ^{51}Cr-EDTA test. No difference was found in the urinary excretion of ^{51}Cr-EDTA in patients vs. controls, nor between schizophrenic patients in remission and those in relapse.[63]

In agreement to earlier reports, it has been proposed that a history of coeliac disease is a risk factor for schizophrenia.[78] Recently, larger studies have tested schizophrenia patients for several markers of intestinal mucosa inflammation. Significant levels of anti-gliadin and anti-transglutaminase (tTGA) antibodies, considered as markers of coeliac disease and gluten sensitivity, have been detected in schizophrenia patients: a study by Cascella reported that 23.1% of patients had medium to high levels of anti-gliadin antibodies vs. 3.1% of controls and 5.6% of patients had medium to high levels of tTGA vs. 0.8% of controls.[64] Another study was performed in two cohorts of patients. In the first group, the levels of anti-*Saccharomyces cerevisiae* antibodies (ASCA) were significantly elevated in both nonrecent and recent onset of schizophrenia (controls: 1.00 ± 0.08; nonrecent onset: 1.45 ± 0.14, $P \leq 0.004$; recent onset: 2.03 ± 0.35, $P \leq 0.00001$).[65] The effect of therapeutic treatment was evaluated in a second cohort of patients (See Table 3 for a summary on pharmacological and dietary treatments). Those who were anti-psychotic-naïve had significantly higher ASCA levels (1.46 ± 0.20, $P \leq 0.05$) than the patients who received anti-psychotic medications (1.0 ± 0.13).[65] These data reinforce the notion that intestinal inflammation associated to either dietary antigen sensitivity or microbial infection may be a relevant component in schizophrenia, which may also be sensitive to anti-psychotics.

Autism spectrum disorders

Gastrointestinal dysfunctions including abdominal pain, diarrhoea, constipation, and bloating are frequently reported in autism spectrum disorder (ASD) patients.[79] Moreover, gastrointestinal symptoms appear to be correlated with autism severity,[80] although part of the expert medical community feels that the prevalence of these conditions is not yet completely understood.[81] It has been proposed that GI symptoms in ASD children may be originated by inadequate nutrition due to restrictive and repetitive dietary patterns, which are commonly present in these patients.[81, 82] On the other hand, the leaky gut hypothesis suggests that food compounds which are able to cross through a hyper-permeable

Table 3 | Clinical studies investigating the impact of pharmacological or dietary interventions on intestinal barrier and/or behavioural symptoms in patients suffering from CNS disorders

Source	Nature of patients studied	*N*	Type of nutritional/ pharmacological intervention	Tests performed and markers evaluated in the study	Main outcomes	*P* value	Comments
Severance *et al.*[65]	Patients with a first episode schizophrenia, unmedicated	40	Anti-psychotic medication	Markers for yeast antigen exposure	ASCA levels elevated in the untreated group (1.46 vs. 1.0)	≤0.05	Data for dietary antigen exposure were only reported in correlation with ASCA
	Patients with a first episode schizophrenia, medicated	63		Markers for dietary antigen exposure	Not reported as independent data	Not available	
Elder *et al.*[73]	Children with ASD	13	Participants were randomly assigned a to either the GFCF or a placebo diet for 6 weeks, followed by the opposite diet for 6 weeks	Tests for autism symptoms	No differences	Nonsignificant	
				Markers for dietary antigen exposure	No differences in urinary peptide levels of gluten and casein	Nonsignificant	
de Magistris *et al.*[74]	Children with ASD	90	GFCF diet	Functional gut permeability test	Children on GFCF diet had a smaller lactulose/ mannitol ratio than children on a regular diet (0.017 vs. 0.055)	0.034	A placebo group was not included. The ratio between urinary lactulose and mannitol was the only parameter that was reported separately for children under GFCF and regular diet. Behavioural changes as result of treatment were not studied
	Children with ASD on a Gluten-free casein-free (GFCF) diet	23		Markers for dietary antigen exposure	Not reported	Not available	
				Autoantibodies	Not reported	Not available	
Whiteley *et al.*[75]	Children with ASD under regular diet	29	Participants were randomly assigned a to the GFCF or nondiet and tested at baseline, 8, 12 and 24 months	Tests for autism symptoms	A subgroup of children receiving the GFCF diet improved in:		The efficacy of GCFC diet was tested by urine analysis for compounds co-eluting with exogenous opioid peptide standards and/or trans-indolyl-3-acryloylglycine. No placebo group was included
	Children with ASD under GFCF diet	26			social interaction	0.0001	
					communication	0.0022	
					hyperactivity	0.0188	
					degree of inattention	0.0007	
				Markers for dietary antigen exposure	Not reported	Not available	
Maes *et al.*[23]	Patients with depression with anti-depressants	40	Anti-depressant medication	Markers for bacterial antigen exposure	No differences	Nonsignificant	
	Patients with depression without anti-depressants	72					
Severance *et al.*[71]	Patients with bipolar disorder, recent onset of psychosis and anti-psychotics	38	Anti-psychotic medication	Markers for yeast antigen exposure	No differences in ASCA levels between patients who were under medication and those who were drug free	Nonsignificant	

Table 3 | (Continued)

Source	Nature of patients studied	*N*	Type of nutritional/ pharmacological intervention	Tests performed and markers evaluated in the study	Main outcomes	*P* value	Comments
	Patients with bipolar disorder without recent onset of psychosis and with anti-psychotics	158					
	Patients with bipolar disorder without recent onset of psychosis and without anti-psychotics	68					

intestinal mucosa could induce the behavioural symptomatology of autism, although the evidence for abnormal intestinal permeability in individuals with ASDs is still limited, and many reports show methodological caveats including inadequate controls and small subject populations.

An interesting study provided intestinal permeability data from 90 ASD patients as well as 146 relatives. Moreover, healthy child and adult controls were also included (64 and 146 respectively). Of the patients with ASD, 36.7% displayed an abnormal intestinal permeability (lactulose/mannitol ratio), compared with 21.2% of their first-degree relatives, 4.8% of the adult controls and none of the healthy child controls.[74] In another study, girls and women with Rett syndrome (a neurodevelopmental disorder that also falls within the ASD classification) displayed higher IgA antibody levels in serum against gluten, gliadin and casein proteins compared to controls.[69] However, a study performed in children diagnosed with pervasive developmental disorder (which is now considered equivalent to ASD), did not find elevated lactulose/mannitol ratios indicating that intestinal permeability was not altered.[68]

Evidence of intestinal permeability alterations in animal models of ASD is still very limited. A recent report by Hsiao *et al.* shows that the maternal immune activation (MIA) mouse model displays behavioural features of ASD such as decreased sociability and social preference, deficient sensorimotor gating and increased anxiety and stereotyped behaviours.[58] MIA offspring also exhibits gastrointestinal barrier defects and abnormal expression of tight-junction proteins in the colon. In addition, the gut microbiota of the MIA offspring was altered (dysbiosis), being driven primarily by alterations in *Clostridium* and *Bacteroides*. Interestingly, treatment with *Bacteroides fragilis* in order to partially reverse the dysbiosis not only improved gut barrier integrity in the MIA offspring but also improved many of the behavioural alterations. However, deficits in sociability and social preference persisted after this treatment.[58] Circulating levels of the microbially modulated metabolite 4-ethylphenylsulfate (4EPS) were elevated in MIA offspring and restored by *B. fragilis* treatment. In addition, the administration of 4EPS alone increased anxiety-like behaviour in naive mice.[58]

Psychological stress, anxiety and depression

Acute stress has been shown to increase gut permeability in rodents. In the rat, acute restraint stress induced by wrapping the animals in paper tape to partially limit their movement, induced an increase in colonic paracellular permeability.[83] In the mouse, a protocol including restraint stress together with acoustic stress induced neuroinflammation which was accompanied by an increased bacterial translocation.[84] In healthy humans, psychological stress induced by public speech also leads to increased paracellular small intestinal permeability, which was prevented by blocking mast cell activity with

disodium cromoglycate.[85] Stress also influences relapse in patients suffering from Crohn's disease, a chronic condition characterised by an exacerbated intestinal inflammation and permeability.[86]

Less evidence is available regarding the influence of altered intestinal permeability on psychological stress, anxiety or depression. Exposure to the gram-negative bacterium *Citrobacter rodentium* is commonly used as a model of transient colitis in mice. The infection is accompanied by intestinal inflammation[87] as well as enhanced colonic paracellular permeability with disruption of tight junctions at 10 days post-exposure, when the peak of infection takes place.[87, 88] One report shows that only 8 h after the challenge with *C. rodentium*, mice display an increase in anxiety-like behaviour in the hole-board open field apparatus, as evidenced by avoidance of the centre area and increased risk assessment behaviour. Plasma levels of the cytokines IFN-γ, TNF-α and IL-12 were not different from control. Also, histological examination of the colon indicated a lack of overt inflammation at the 8 h post-challenge time point, indicating that the behavioural observations were unlikely to follow from inflammation-related stress.[89] A second study failed to show behavioural changes either at the peak of infection (10 days post-challenge) or after clearance (30 days post-challenge). However, the authors did find memory alterations at both time points when infected mice were exposed to water avoidance stress.[90] Interestingly, daily treatment with probiotic bacteria (a commercially available combination of *L. rhamnosus* and *Lactobacillus helveticus* strains) prevents both the increase in intestinal permeability[87] and the memory dysfunction.[90]

Up to 65% of patients suffering from myocardial infarction experience depressive symptoms and 20% of them develop major depression.[91] This has been replicated in the rat, which displays a post-infarction behavioural syndrome that is similar to models of depression and is accompanied by increased gut permeability.[92] Treatment with the probiotic bacteria *L. helveticus* R0052 and *Bifidobacterium longum* R0175 prevented both behavioural and intestinal barrier disturbances induced by myocardial infarction in these animals.[92]

The same probiotic bacteria also attenuated the physiological response to acute stress in mice, preventing the loss of intestinal barrier integrity induced by water avoidance stress and decreasing the stress-associated elevations in plasma corticosterone.[93] It would be of interest to investigate whether restoration of stress hormone levels is consequence of intestinal barrier repair. A previous study performed in healthy human volunteers and using the same combination of *L. helveticus* and *B. longum* had shown a reduction in psychological distress after a 30 day treatment, as indicated by the Hopkins Symptom Checklist scale.[7] However, in this clinical trial intestinal barrier function was not studied.

It has been speculated that increased translocation of commensal bacteria may play a role in the pathophysiology of depression: the levels of IgA and IgM against LPS of gram-negative bacteria such as *Hafnia alvei*, *Pseudomonas aeruginosa* and *Morganella morganii* are significantly higher in patients suffering from depression than in healthy controls.[23] A larger study by the same group showed a significant difference in the IgM responses between patients with chronic depression and normal controls, but not between patients who had been depressed for less than 2 years and controls.[70] Interestingly, anti-depressants as well as mood stabilisers may have potential immunosuppresive effects in patients suffering from depression (for a review see Maes[94]). For example, one study reported that chronic treatment with fluoxetine was able to restore serum IL-6 levels,[95] although in another report, the levels of anti-LPS IgA in patients who had been treated with anti-depressants only showed a trend of reduction when compared to those who were drug free.[70] If further confirmed, the above could support the existence of an alternative mechanism for anti-depressant action.

Finally, it has been postulated that increased gut permeability might be a physiological response in individuals with bipolar disorder. Thus, intestinal inflammation might accelerate exposure of food antigens to the systemic circulation, which would in turn allow some exorphin-like compounds to gain access to the CNS. In support of this, enhanced levels of markers for intestinal damage have been reported in patients with bipolar disorder.[71]

Neurodegenerative disorders

It is believed that some neurodegenerative conditions may arise as a consequence of both genetic susceptibility and toxic environmental factors. According to this view, the GI tract would be an important source of toxins that may reach the CNS via anatomical or humoral pathways. For example, it has been hypothesised that β-amyloid, which may be present in pancreatic juice, may possibly reach the brain due to increased intestinal permeability,

thereby affecting neuronal function.[96] However, more studies are required to demonstrate causality between altered passage of luminal contents and decline of CNS function.

A study by Forsyth *et al.* evaluated intestinal permeability in nine subjects recently diagnosed with Parkinson's disease (PD) and 10 age-matched healthy controls.[72] Urinary sucralose (a marker of whole gut permeability) was significantly greater in PD patients than in controls. There was also significantly more staining of *Escherichia coli* in both the epithelial and lamina propria zones of sigmoid mucosa samples from patients with PD. Plasma levels of LPS binding protein were significantly lower in PD patients, which could represent a higher risk of exposure of intestinal mucosa to luminal bacteria products. The authors also proposed that neuronal tissues from individuals susceptible to PD may be more sensitive to the effects of endotoxins.[72]

Another line of research points to the involvement of metal transporters in neurodegenerative diseases. The intestine expresses the divalent metal transporter 1 (DMT1) which mediates the transport of essential metals but also toxic metals, including cadmium and lead. A diet low in essential metals induces duodenal overexpression of DMT1[97] which might possibly increase transport of cadmium or lead. In the CNS, iron, copper, and zinc concentrations are strictly regulated to prevent oxidative damage. According to this theory, the oxidative damage found in brains from patients with Alzheimer's disease and PD could be generated by inadequate intestinal transport of either essential or toxic metals.[98] Bressler *et al.* highlight the importance of identifying transporters that mediate uptake of toxic metals as predicting risk factors for neurotoxicity.[98]

Clinical trials of dietary interventions

It has been proposed that ASD may be caused by an inappropiate central activity of opioid peptides, including gliadomorphin and casomorphin which could originate after digestion of wheat gliadin and bovine milk casein, respectively.[99, 100] In a healthy gut, brush border and cytoplasmic peptidases found in the enterocytes may break down such opioid peptides; however a deficient intestinal barrier could possibly promote their absorption.[101] This hypothesis has led families of children with autism to use the Gluten-free Casein-free (GFCF) diet. This feeding regimen has shown to reduce intestinal permeability in children with ASD: de Magistris reported that lactulose/mannitol ratio was 0.017 ± 0.012 in ASD children receiving GCFC diet vs. 0.055 ± 0.097 in ASD children under unrestricted diet ($P = 0.034$).[74] However, an association between diet, behaviour and intestinal permeability has not been demonstrated in children with ASD. Moreover, there are only limited data supporting the efficacy of the GFCF diet with regard to behavioural improvement. Although this was systematically reviewed by Mari-Bauset *et al.*,[82] we discuss two reports that stand out due to high quality evidence: one showing no effects of the elimination diet and another one with positive results.

A randomised, double blind, crossover pilot study compared the effects of the GFCF diet with a placebo diet on 13 children with ASD. The control and experimental diets were administered to the children for 6 weeks. The severity of autistic symptoms was evaluated by the Childhood Autism Rating Scale, the Ecological Communication Orientation Scale as well as in home observation of parent–child interaction. No significant changes of behaviour were observed, nor in the urinary levels of the opioid peptides derived from gluten and casein. Interestingly, the authors report that the parents from eight of the 13 participating children were not able to distinguish, whether their kids were in the placebo or the experimental diets. Parent behaviour was also evaluated, showing no detectable parental behavioural influence.[73]

Another study included 72 ASD children, of whom 38 received the GFCF diet and 34 their regular diet (control group). At 12 months, the analysis of time–treatment interaction showed that children receiving the GFCF diet improved their social interaction ($P = 0.0001$), degree of inattention ($P = 0.0007$), and hyperactivity ($P = 0.0188$).[75] A limitation was the high number of dropouts (the study ended with only 26 and 29 children per group, respectively). Because families are more likely to abandon a study when no improvement is observed, results must be carefully interpreted. Also, in this study the control group did not receive a placebo diet, which means parents were aware of the nature of their children's treatment and therefore their own behaviour may have influenced some of the observed patient responses.

In their review, Mari-Bauset *et al.* conclude that the evidence to support GFCF diets in ASD is limited and weak, and do not validate the opioid theory.[82] Finally, considering the issue of nutritional adequacy[102] due to the fact that many ASD children display unique dietary patterns,[81, 82] there is currently insufficient evidence to recommend the GFCF diet for the nutritional management of subjects with ASD.[103, 104]

CONCLUSIONS

A number of clinical observations report increased gut permeability and/or permeability-related parameters in patients with schizophrenia, ASD, depression, or PD. In the case of schizophrenia and ASD, most of the evidence supporting an association with alterations of gut barrier comes from studies that have design problems. In addition, there are conflicting findings showing no changes in intestinal permeability for schizophrenia and ASD when compared to healthy volunteers, and these studies have in general a better experimental design. Regarding depression and PD, there are very few studies and although they all show that patients display increased gut permeability or permeability-related markers, more and larger studies are necessary to confirm these observations.

The use of gluten-free casein-free diets to improve intestinal barrier function and in turn treat disorders such as autism and schizophrenia has also produced conflicting results and is therefore not yet recommended as formal treatment by the expert medical community.

With regard to pre-clinical data, it has been shown that animal models designed to replicate symptoms of autism or stress display gut barrier disturbances. In these adequately controlled, well-validated animal models, restoration of the CNS function occurs along with improvement of intestinal barrier function.

Future research efforts in the clinical field should focus on randomised, controlled trials as well as prospective studies when possible. For both clinical and animal studies, the contribution of intestinal microbiota to the development and treatment of CNS disorders appears as a promising field of research.

AUTHORSHIP

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