



Investigation of the Relation between Epithelial Barrier Function and Autism Symptom Severity in Children with Autism Spectrum Disorder

Kevser Nalbant¹ · Semih Erden² · Abdullah Yazar³ · İbrahim Kılınc⁴

Received: 23 February 2021 / Accepted: 5 December 2021 / Published online: 6 January 2022
© The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2021

Abstract

Autism spectrum disorder (ASD) is a common neurodevelopmental disorder characterized by limitations in mutual communication and social interaction as well as restricted, repetitive patterns of behaviors, interests, or activities. The possible role of biological abnormalities in the etiopathogenesis of this disorder arouses research interest in this area. This is a case–control study evaluating epithelial barrier function by comparing serum concentrations of occludin and zonulin in children with ASD ($n = 60$) and controls ($n = 30$). The Childhood Autism Rating Scale (CARS) was used to evaluate autism symptom levels in all children. Serum occludin and zonulin levels were analyzed using an enzyme-linked immunosorbent assay. Serum occludin was significantly lower in children with ASD than in control subjects. In children with ASD, a decrease in occludin level was significantly associated with the disorder symptom levels items mean score (CARS total scores). Our findings showed that children with ASD had alterations in epithelial barrier function compared to the control group. The investigation of the mechanism underlying the different levels of occludin between ASD and controls may be of importance in clarifying the etiopathogenesis of ASD, as well as its follow-up and treatment.

Keywords Autism spectrum disorder · Occludin · Zonulin · Epithelial barrier · Gut–brain axis

Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by limitations in mutual communication and social interaction as well as restricted, repetitive patterns of behaviors, interests, or activities (Association 2013). The prevalence of ASD has been reported to have increased, affecting approximately 2% of children (Xu et al. 2019). Despite the increasing number of studies investigating this

disorder, which negatively affects a significant part of society (Rogge and Janssen 2019), its etiopathogenesis remains uncertain and there is no satisfactory effective treatment. In addition, there is no biomarker for scanning, diagnosis, evaluation of response to treatment, or follow-up of the disorder such as blood test or radiological scan. Therefore, a growing number of studies in recent years have focused on elucidating biomarkers of ASD, and many research areas have revealed abnormal biological processes associated with ASD (Frye et al. 2019). Some previous studies have suggested that epithelial barrier dysfunction such as the gut barrier may be a pathophysiological mechanism of ASD (Erickson et al. 2005; Liu et al. 2005; de Magistris et al. 2010; Iovene et al. 2017; Fiorentino et al. 2016).

An imbalance in gut barrier integrity may trigger an immune reaction and may be associated with diseases such as intestinal inflammation diseases, rheumatoid arthritis, and multiple sclerosis (MS) (De Mey and Freund 2013; Catalioto et al. 2011). Also, increasing numbers of studies indicate a possible link between ASD and disturbed gut barrier integrity (de Magistris et al. 2010; Iovene et al. 2017; Fiorentino et al. 2016). In individuals with ASD, lactulose/mannitol

✉ Kevser Nalbant
drkevser@yahoo.com

¹ Medical Faculty, Department of Child and Adolescent Psychiatry, Hacettepe University, Ankara, Turkey

² Meram Medical Faculty, Department of Child and Adolescent Psychiatry, Necmettin Erbakan University, Konya, Turkey

³ Meram Medical Faculty, Department of Biochemistry, Necmettin Erbakan University, Konya, Turkey

⁴ Meram Medical Faculty, Department of Pediatrics, Necmettin Erbakan University, Konya, Turkey

ratio tests of gut permeability have shown increased gut permeability compared with healthy controls (de Magistris et al. 2010). However, the mechanism of increased gut permeability in individuals with ASD has not been fully elucidated. Increasing evidence from many studies suggests that occludin (Kucharzik et al. 2001; Zeissig et al. 2007) and zonulin (Fasano 2011; Tripathi et al. 2009) may be biomarkers of epithelial barrier permeability.

Occludin is an important tight junction (TJ) protein known to be associated with epithelial permeability (Camara-Lemarroy et al. 2019). Occludin contributes to the stability and integrity of TJs, thus regulating and restricting the paracellular transport pathway (Feldman et al. 2005). Occludin mutant or knockout animals were found to exhibit chronic inflammation and a defective epithelial barrier despite having morphologically intact TJs, thus demonstrating that occludin plays a critical role in maintaining barrier stability rather than TJ assembly (Balda et al. 2000; Chen et al. 1997). A review of the recent literature showed that there has been no study evaluating serum occludin in ASD. When we looked at the studies on occludin, in a study that aimed to identify the biomarkers to track the active period in MS, serum levels of occludin and zonulin were found to increase during the active period of the disease, and it was emphasized that these findings supported the leaky gut hypothesis (Camara-Lemarroy et al. 2019). On the other hand, in inflammatory bowel disease studies, it has been reported that the expression of occludin decreases, and this is related to gut permeability (Kucharzik et al. 2001; Zeissig et al. 2007).

Zonulin, a protein produced by small intestine epithelium, is known to induce TJ disassembly between enterocytes and then increase gut permeability (Fasano 2011). Besides its effect on the gut, zonulin also regulates blood–brain barrier (BBB) permeability (Barber et al. 2019). Therefore, its possible use as a biomarker of epithelial barrier permeability is being investigated. Zonulin levels have been found to be high in obesity (Zak-Golab et al. 2013), type 1 and 2 diabetes (Jayashree et al. 2014; Sapone et al. 2006), and multiple sclerosis (MS) (Camara-Lemarroy et al. 2019). Also, zonulin has been studied in various psychiatric disorders such as schizophrenia (Barber et al. 2019; Usta et al. 2021), attention-deficit/hyperactivity disorder (Ozyurt et al. 2018; Aydogan Avsar et al. 2021), obsessive-compulsive disorders (Isik et al. 2020), and ASD (Esnafoglu et al. 2017; Jozefczuk et al. 2018). The results of previous clinical studies on zonulin levels in psychiatric disorders are inconsistent. Some studies have found that patients have higher levels of zonulin compared to controls (Barber et al. 2019; Ozyurt et al. 2018; Esnafoglu et al. 2017; Rose et al. 2018); other studies have found no difference between the two groups (Isik et al. 2020; Jozefczuk et al. 2018).

These contradictory results suggest that more research is needed on occludin and zonulin. Therefore, based on previous studies suggesting that the epithelial barrier permeability of occludin and zonulin may be biomarkers, we aimed to evaluate epithelial barrier function by comparing the serum concentrations of occludin and zonulin in children with ASD and controls. To the best of our knowledge, there has is study in the current literature evaluating serum occludin and zonulin levels together in ASD. We assumed that increased levels of occludin and zonulin could occur in ASD, and the severity of ASD could be related to occludin and zonulin levels. Since zonulin is a regulator of the TJ proteins, we assumed that the levels of occludin and zonulin would correlate positively with each other. We then further explored the possible causes of changes in the levels of these biomarkers.

Materials and Methods

Participants

This study was conducted at the Necmettin Erbakan University, Meram Faculty of Medicine, Department of Child and Adolescent Psychiatry and Department of Pediatrics, between March and December 2018. The diagnosis of ASD was made through a semi-structured clinical interview conducted by a child and adolescent psychiatrist based on the ASD section of the Schedule for Affective Disorders and Schizophrenia for School-Age Children–Present and Lifetime Version/Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (K-SADS-PL/DSM-5) (Unal et al. 2019). Of the children aged between 30–96 months who were diagnosed as having ASD, only those whose parents had agreed to participate in the study were included. Children newly diagnosed with ASD and children with known ASD under follow-up were divided into two groups. In order to control for confounding variables, children who took medicine, those diagnosed as having a gastrointestinal (GI) disease (e.g. Crohn's disease, ulcerative colitis) or chronic disease (e.g. diabetes mellitus, hypertension, epilepsy, cerebral palsy), and children with infections or obesity were excluded from the study. All parents in the study volunteered to participate at no cost. All children with ASD who met the inclusion criteria were invited to join the study. No parents refused to participate in the study. A total of 90 children, comprising 60 children with ASD and 30 controls, were included in this study. Among 72 children with a provisional diagnosis of ASD, six children with ASD were excluded because of a diagnosis of epilepsy, four children with ASD due to an accompanying severe medical disease, and two children with ASD who did not meet the DSM-5 diagnostic criteria for ASD.

For the control group, children admitted to Necmettin Erbakan University Meram Faculty of Medicine, Department of Pediatrics outpatient clinics aged 30–96 months, whose laboratory tests (hemogram, sedimentation, C-reactive protein) and examinations were normal, who were not on medication, and who had no chronic disease (e.g. diabetes mellitus, hypertension, epilepsy, cerebral palsy), infectious disease, GI disease, or obesity were referred to Necmettin Erbakan University Meram Faculty of Medicine, Department of Child and Adolescent Psychiatry. Children without any psychiatric disease, according to the semi-structured psychiatric interview form of the Schedule for Affective Disorders and Schizophrenia for School-Age Children–Present and Lifetime Version (K-SADS-PL)/DSM-5 were included in the study (Unal et al. 2019). Written consent was obtained from the parents of all children included in the study. The sociodemographic data form and the Turkish version of the Childhood Autism Rating Scale (CARS) were completed during the clinical psychiatric evaluation by the child and adolescent psychiatrist. Among 46 children whose parents volunteered to participate in the control group, 16 were excluded because of a psychiatric disorder diagnosed through a semi-structured clinical interview (K-SADS-PL/DSM-5). The evaluation of each participant took about 2 hours.

Instruments

Sociodemographic and Clinical Information Form

The sociodemographic form included age, sex, birth history, developmental history, medical history, family history, educational status, and medications used.

Childhood Autism Rating Scale (CARS)

The CARS (Schopler et al. 1980) is a semi-structured assessment tool composed of 15 items. It is rated by physicians according to the information obtained from child observations and family interviews. A reliability and validity study of CARS for the Turkish population was conducted by İncekaş Gassaloğlu et al. (Incekas Gassalolu et al. 2016).

Schedule for Affective Disorders and Schizophrenia for School-Age Children–Present and Lifetime Version (K-SADS-PL/DSM-5)

The K-SADS-PL/DSM-5 is a semi-structured clinical interview used for determining the present and lifetime psychopathologies of children and adolescents (Kaufman et al. 2016). A reliability and validity study of K-SADS-PL/DSM-5 for the Turkish population was conducted by Ünal et al. (2019).

Serum Samples

Serum samples were obtained between 08:30 and 11:00 AM. Samples were stored at -80°C until the study. Serum occludin and zonulin levels were measured using the sandwich-enzyme-linked immunosorbent assay method (Human Zonulin ELISA Kit, catalog no. E-EL-H5560; Human Occludin ELISA Kit, Catalog no. E-EL-H1073; Elabscience Biotech, USA). Results are given in nanograms per milliliter.

Statistical Analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 15.0 software for Windows (SPSS Inc., Chicago, Illinois). The normality of the data was assessed using the Kolmogorov–Smirnov test and skewness and kurtosis statistics. For parametric data in the evaluation of the data, Student's *t*-test, analysis of variance (ANOVA), and Pearson's correlation test (for the relationship of data) were applied. For nonparametric data, the Mann–Whitney *U* test and Spearman's correlation test (for the relationship of data) were used. The chi-square (χ^2) test was used for categorical variables. *P* values less than 0.05 were considered for statistical significance in all analyses.

Results

A total of 90 children aged 30–96 months, comprising 60 children with ASD and 30 controls, were included in this study. There were no significant differences between the two groups in terms of age, sex, or socioeconomic level. Sociodemographic data and clinical characteristics are presented in Table 1. Serum occludin levels were found to be statistically significantly lower in children with ASD (0.83 ± 0.94) ($p < 0.001$). There was no statistically significant difference in serum zonulin levels (Table 1).

The serum occludin and zonulin levels of the children with newly diagnosed ASD and the children with ASD who were followed up were compared with the control group. Serum occludin levels were statistically lower in both children with new diagnoses (0.75 ± 0.81) ($p < 0.001$) and children with previously diagnosed ASD (0.80 ± 0.96) ($p < 0.001$). There was no statistically significant difference between the three groups in terms of serum zonulin levels (Table 2).

We also investigated whether there was a difference in terms of occludin and zonulin levels between males (Table 3) and females (Table 4) in children with ASD and

Table 1 Sociodemographic and clinical characteristics of participants

	ASD N/Mean (% / \pm SD)	Control N/Mean (% / \pm SD)	Statistics	<i>p</i>
Age (months)	53.40 \pm 22.65	60.60 \pm 19.69	<i>t</i> = -1.482	0.142
Sex				
Male	51 (85)	21 (70)	χ^2 = 2.813	0.094
Female	9 (15)	9 (30)		
Mother's age	32.58 (5.39)	32.70 (5.22)	<i>t</i> = 0.265	0.92
Mother's education, years	8.51 (4.02)	9.20 (4.36)	<i>t</i> = -0.94	0.56
Father's age	35.24 (5.39)	37.16 (5.62)	<i>t</i> = -1.435	0.15
Father's education, years	10.02 (4.46)	9.45 (3.92)	<i>t</i> = 2.731	0.63
Age at starting to walk (months)	15.4 \pm 5.8	14.5 \pm 4.2	<i>t</i> = 0.97	0.23
Age at starting to speak (months)	22.3 \pm 10.9	25.3 \pm 11.3	<i>t</i> = -1.20	0.78
Occludin (ng/mL)	0.83 (0.94)	2.30 (1.48)	<i>U</i> = 267.000	0.000
Zonulin (ng/mL)	8.96 (13.65)	14.91 (25.03)	<i>U</i> = 719.500	0.24

ASD autism spectrum disorder, SD standard deviation

Table 2 Occludin and zonulin levels of newly diagnosed with ASD, followed up with ASD and controls

	Newly diagnosed with ASD (<i>n</i> = 30)	Followed up with ASD (<i>n</i> = 30)	Control (<i>n</i> = 30)	Statistics	<i>p</i>
Occludin (ng/ mL), mean (\pm SD)	0.75 \pm 0.81	0.80 \pm 0.96	2.30 \pm 1.48	<i>F</i> = 26.810	0.00
Zonulin (ng/ mL), mean (\pm SD)	10.71 \pm 16.06	8.46 \pm 12.13	14.91 \pm 25.03	<i>F</i> = 0.333	0.33

ASD autism spectrum disorder, SD standard deviation

Table 3 Occludin and zonulin levels in males with ASD and controls

	ASD (<i>n</i> = 51)	Control (<i>n</i> = 21)	Statistics	<i>p</i>
Occludin (ng/mL), mean (\pm SD)	0.82 \pm 0.97	2.24 \pm 1.40	<i>U</i> = 169,500	0.000
Zonulin (ng/ mL), mean (\pm SD)	9.82 \pm 14.59	15.74 \pm 27.91	<i>U</i> = 488,000	0.55

ASD autism spectrum disorder, SD standard deviation

Table 4 Occludin and zonulin levels of females with ASD and controls

	ASD (<i>n</i> = 9)	Control (<i>n</i> = 9)	Statistics	<i>p</i>
Occludin (ng/mL), mean (\pm SD)	0.84 \pm 0.82	2.21 \pm 1.67	<i>U</i> = 18.000	0.047
Zonulin (ng/ mL), mean (\pm SD)	4.09 \pm 3.67	10.01 \pm 13.49	<i>U</i> = 30.000	0.38

ASD autism spectrum disorder, SD standard deviation

controls. Statistical analyses were found to be similar to analyses with all children with ASD and controls.

The correlation between serum occludin and zonulin levels and the CARS total scores was evaluated for the children in all groups. There was no statistically significant correlation between serum zonulin and occludin levels. A statistically significant negative and moderate correlation was found between serum occludin levels and CARS scores ($p < 0.001$). There was no statistically significant correlation between serum zonulin levels and CARS scores (Table 5).

Table 5 Correlations of CARS total score with serum occludin and zonulin levels among all participants

	Occludin	Zonulin	CARS
Occludin	1		
Zonulin	.123	1	
CARS	-.436**	-.112	1

CARS Childhood Autism Rating Scale

** $p < 0.001$

The correlation between serum occludin and zonulin levels and the CARS total scores of the children with ASD was also evaluated. There was no statistically significant correlation between serum zonulin and occludin levels ($r=0.063$, $p>0.05$). Likewise, there was no statistically significant correlation between serum occludin and zonulin levels and CARS total score ($r=0.033$, $r=-0.161$ respectively).

Discussion

In this study, serum levels of occludin, which is a paracellular TJ protein, and zonulin, which functions as the regulator of the paracellular TJ proteins, were compared between children with ASD and control children of similar age, sex, and socioeconomic level. According to our findings, the serum occludin levels in children with ASD were lower than those in the control group, but there was no significant difference between the groups in terms of serum zonulin levels. When the relationship of both markers to CARS scores was evaluated, it was found that the CARS scores increased as the occludin levels decreased, but no significant relationship was found between zonulin and CARS scores.

Occludin is part of the epithelial and endothelial junction complexes, and recent data suggest that occludin regulates barrier functions of structures such as the gut and BBB (Raleigh et al. 2011). In a review of recent literature, no study investigating the relationship between occludin and ASD was found. When we looked at the studies on occludin, in a study that aimed to identify biomarkers for the active period in MS, serum levels of occludin and zonulin were found to increase during the active period of the disease, and it was emphasized that these findings supported the leaky gut hypothesis (Camara-Lemarroy et al. 2019). A study by Mankertz et al. showed that proinflammatory cytokines [tumor necrosis factor-alpha (TNF- α), interferon gamma (IFN- γ)] downregulated occludin expression, and the authors suggested that increased proinflammatory cytokines in inflammatory diseases might be an important mechanism in the increased paracellular TJ permeability by mediating the reduction of occludin expression (Mankertz et al. 2000). In two other studies, in which gut barrier function was investigated in inflammatory diseases of the intestines (Crohn's and ulcerative colitis), it was stated that occludin expression decreased during the active period of the disease, unlike other intracellular TJ proteins, and this might play a role in increased paracellular permeability (Kucharzik et al. 2001; Zeissig et al. 2007). In addition to these studies, in vivo and in vitro studies have shown that proinflammatory cytokines also disrupt TJs in the BBB (Rahman et al. 2018; Kebir et al. 2007). The results of all these studies appear to correlate with the fact that inflammatory processes disrupt TJ proteins, specifically reducing occludin expression.

Moreover, in recent years, emphasis has been increasing on the role of inflammatory processes in ASD etiopathogenesis (Shen et al. 2020). In fact, a recent meta-analysis concluded that the concentration of proinflammatory cytokines [IFN- γ , interleukin-1 (IL-1), IL-6, and TNF- α] in individuals diagnosed with ASD was higher than that of controls (Saghazadeh et al. 2019). Given these findings, although simultaneous proinflammatory cytokines were not examined in our study, it could be suggested that the low levels of occludin in children with ASD in our study may be the result of increased inflammation. However, this assumption needs to be supported by looking at the simultaneous levels of proinflammatory cytokine and occludin. In addition, the results of our study should be investigated in more detail with gut biopsy samples, in vivo studies, and in vitro studies in a larger sample.

Another biomarker, zonulin, has been suggested for use as a serologic marker for the evaluation of epithelial barrier integrity. Zonulin is produced by enterocytes under the influence of environmental stimuli (Fasano 2011), and mediates increased intestinal permeability through TJs (Fasano 2011; Tripathi et al. 2009). The results of previous clinical studies on zonulin levels in ASD are inconsistent. Some studies have reported that serum zonulin levels are higher in children with ASD compared with typically developing children (Esnafoglu et al. 2017; Rose et al. 2018), whereas other studies have reported no difference (Jozefczuk et al. 2018). In a recent study, in vitro IFN-, IL-17A, or zonulin exposure was found to increase the small intestine epithelial barrier and BBB permeability by changing the localization of zonula occludens, claudine-5, and occludin, and it was stated that these findings might help to explain the pathogenesis of neuro-inflammatory diseases associated with disorders in the gut–brain axis (Rahman et al. 2018). Given the studies reporting that intestinal permeability plays a role in ASD, it may be that zonulin contributes to the development of ASD through this mechanism. However, there was no statistically significant difference between the groups in terms of zonulin levels. This finding suggests that the rates of construction and destruction of zonulin were similar between the ASD and control groups.

Finally, since zonulin is a regulator of the TJ proteins, we assumed that the levels of occludin and zonulin would correlate positively with each other. However, no correlation was found between occludin and zonulin levels in our study. Based on our findings, we can infer that the difference in occludin levels was independent of zonulin. We believe that the involvement of occludin in both the gut barrier and the BBB may mediate our understanding of the pathogenesis of ASD associated with the gut–brain axis. In addition, clarification of the structure represented by occludin subtypes through genetic research will facilitate the interpretation of these results.

Despite the inclusion of more participants with ASD than in previous studies, our study is still limited. The main limitation of the study is its cross-sectional rather than longitudinal design. This does not permit us to determine any cause–effect relationship for our results. The absence of an examination of simultaneous proinflammatory cytokines, the lack of objective tests of intestinal permeability (lactulose/mannitol ratio tests), and the absence of an examination of intestinal biopsy and epithelial barriers were among the other limitations of our study. Further studies with larger sample sizes are needed to reach stronger conclusions. Despite all these limitations, however, our findings suggest that occludin may play a role in influencing the gut–brain axis in children with ASD.

The serum levels of occludin and zonulin were detected in this study. Our findings have shown that there are alterations of the occludin levels in children with ASD. Further studies are needed to investigate the role of occludin in influencing the gut–brain axis in children with ASD. Previous studies suggest that biological abnormalities may be associated with ASD, and these abnormalities may be important as potential biomarkers in diagnosis, follow-up, and/or treatment. The investigation of the mechanism underlying the different levels of occludin between ASD and controls may be of importance in clarifying the etiopathogenesis of ASD, as well as its follow-up and treatment.

Acknowledgements We wish to thank all subjects who participated in this study.

Author Contributions All authors have made a significant contribution to the findings, writing, and methods in the paper.

Funding Funding for this study was provided by a grant from the Scientific Research Project Coordination Unit of the Necmettin Erbakan University, Meram Faculty of Medicine (Project no: 181218011).

Availability of Data The data used to support the findings of this study are available from the corresponding author upon request.

Declarations

Ethics Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This research was approved by the Ethics Committee of the Non-Interventional Clinical Research of the Necmettin Erbakan University, Meram Faculty of Medicine.

Informed Consent Informed consent was obtained from all individual participants included in the study.

Conflict of Interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

- Association AP (2013) Diagnostic and statistical manual of mental disorders (DSM-5®). American Psychiatric Pub
- Aydogan Avsar P, Isik U, Aktepe E, Kilic F, Doguc DK, Buyukbayram HI (2021) Serum zonulin and claudin-5 levels in children with attention-deficit/hyperactivity disorder. *Int J Psychiatry Clin Pract* 25(1):49–55. <https://doi.org/10.1080/13651501.2020.1801754>
- Balda MS, Flores-Maldonado C, Cerejido M, Matter K (2000) Multiple domains of occludin are involved in the regulation of paracellular permeability. *J Cell Biochem* 78(1):85–96
- Barber GS, Sturgeon C, Fasano A, Cascella NG, Eaton WW, McMahon RP et al (2019) Elevated zonulin, a measure of tight-junction permeability, may be implicated in schizophrenia. *Schizophr Res* 211:111–112. <https://doi.org/10.1016/j.schres.2019.07.006>
- Camara-Lemarroy CR, Silva C, Greenfield J, Liu WQ, Metz LM, Yong VW (2019) Biomarkers of intestinal barrier function in multiple sclerosis are associated with disease activity. *Mult Scler* 1352458519863133. <https://doi.org/10.1177/1352458519863133>
- Catalioto RM, Maggi CA, Giuliani S (2011) Intestinal epithelial barrier dysfunction in disease and possible therapeutical interventions. *Curr Med Chem* 18(3):398–426. <https://doi.org/10.2174/092986711794839179>
- Chen Y, Merzdorf C, Paul DL, Goodenough DA (1997) COOH terminus of occludin is required for tight junction barrier function in early *Xenopus* embryos. *J Cell Biol* 138(4):891–899. <https://doi.org/10.1083/jcb.138.4.891>
- de Magistris L, Familiari V, Pascotto A, Sapone A, Frolli A, Iardino P et al (2010) Alterations of the intestinal barrier in patients with autism spectrum disorders and in their first-degree relatives. *J Pediatr Gastroenterol Nutr* 51(4):418–424. <https://doi.org/10.1097/MPG.0b013e3181dccc4a5>
- De Mey JR, Freund JN (2013) Understanding epithelial homeostasis in the intestine: An old battlefield of ideas, recent breakthroughs and remaining controversies. *Tissue Barriers* 1(2):e24965. <https://doi.org/10.4161/tisb.24965>
- Erickson CA, Stigler KA, Corkins MR, Posey DJ, Fitzgerald JF, McDougle CJ (2005) Gastrointestinal factors in autistic disorder: a critical review. *J Autism Dev Disord* 35(6):713–727. <https://doi.org/10.1007/s10803-005-0019-4>
- Esnafoglu E, Cirrik S, Ayyildiz SN, Erdil A, Erturk EY, Dagli A et al (2017) Increased Serum Zonulin Levels as an Intestinal Permeability Marker in Autistic Subjects. *J Pediatr* 188:240–244. <https://doi.org/10.1016/j.jpeds.2017.04.004>
- Fasano A (2011) Zonulin and its regulation of intestinal barrier function: the biological door to inflammation, autoimmunity, and cancer. *Physiol Rev* 91(1):151–175. <https://doi.org/10.1152/physrev.00003.2008>
- Feldman GJ, Mullin JM, Ryan MP (2005) Occludin: structure, function and regulation. *Adv Drug Deliv Rev* 57(6):883–917. <https://doi.org/10.1016/j.addr.2005.01.009>
- Fiorentino M, Sapone A, Senger S, Camhi SS, Kadzielski SM, Buie TM et al (2016) Blood-brain barrier and intestinal epithelial barrier alterations in autism spectrum disorders. *Mol Autism* 7:49. <https://doi.org/10.1186/s13229-016-0110-z>
- Frye RE, Vassall S, Kaur G, Lewis C, Karim M, Rossignol D (2019) Emerging biomarkers in autism spectrum disorder: a systematic review. *Ann Transl Med* 7(23):792. <https://doi.org/10.21037/atm.2019.11.53>
- Incekas Gassaloglu S, Baykara B, Avcil S, Demiral Y (2016) Validity and Reliability Analysis of Turkish Version of Childhood Autism Rating Scale. *Turk Psikiyatri Derg* 27(4):266–274
- Iovene MR, Bombace F, Maresca R, Sapone A, Iardino P, Picardi A et al (2017) Intestinal Dysbiosis and Yeast Isolation in Stool

- of Subjects with Autism Spectrum Disorders. *Mycopathologia* 182(3–4):349–363. <https://doi.org/10.1007/s11046-016-0068-6>
- Isik U, Aydogan Avsar P, Aktepe E, Doguc DK, Kilic F, Buyukbayram HI (2020) Serum zonulin and claudin-5 levels in children with obsessive-compulsive disorder. *Nord J Psychiatry* 1–6. <https://doi.org/10.1080/08039488.2020.1715474>
- Jayashree B, Bibin YS, Prabhu D, Shanthirani CS, Gokulakrishnan K, Lakshmi BS et al (2014) Increased circulatory levels of lipopolysaccharide (LPS) and zonulin signify novel biomarkers of proinflammation in patients with type 2 diabetes. *Mol Cell Biochem* 388(1–2):203–210. <https://doi.org/10.1007/s11010-013-1911-4>
- Jozefczuk J, Konopka E, Bierla JB, Trojanowska I, Sowinska A, Czarnecki R et al (2018) The Occurrence of Antibodies Against Gluten in Children with Autism Spectrum Disorders Does Not Correlate with Serological Markers of Impaired Intestinal Permeability. *J Med Food* 21(2):181–187. <https://doi.org/10.1089/jmf.2017.0069>
- Kaufman J, Birmaher B, Axelson D, Pereplitchikova F, Brent D, Ryan N. (2016) The KSADS-PL DSM-5. Kennedy Krieger Institute, Baltimore, MD. <https://www.kennedykrieger.org/sites/default/files/library/documents/faculty/ksads-dsm-5-screener.pdf>. Accessed 6 January 2022
- Kebir H, Kreymborg K, Ifergan I, Dodelet-Devillers A, Cayrol R, Bernard M et al (2007) Human TH17 lymphocytes promote blood-brain barrier disruption and central nervous system inflammation. *Nat Med* 13(10):1173–1175. <https://doi.org/10.1038/nm1651>
- Kucharzik T, Walsh SV, Chen J, Parkos CA, Nusrat A (2001) Neutrophil transmigration in inflammatory bowel disease is associated with differential expression of epithelial intercellular junction proteins. *Am J Pathol* 159(6):2001–2009. [https://doi.org/10.1016/S0002-9440\(10\)63051-9](https://doi.org/10.1016/S0002-9440(10)63051-9)
- Liu Z, Li N, Neu J (2005) Tight junctions, leaky intestines, and pediatric diseases. *Acta Paediatr* 94(4):386–393. <https://doi.org/10.1111/j.1651-2227.2005.tb01904.x>
- Mankertz J, Tavalali S, Schmitz H, Mankertz A, Riecken EO, Fromm M et al (2000) Expression from the human occludin promoter is affected by tumor necrosis factor alpha and interferon gamma. *J Cell Sci* 113(Pt 11):2085–2090
- Ozyurt G, Ozturk Y, Appak YC, Arslan FD, Baran M, Karakoyun I et al (2018) Increased zonulin is associated with hyperactivity and social dysfunctions in children with attention deficit hyperactivity disorder. *Compr Psychiatry* 87:138–142. <https://doi.org/10.1016/j.comppsy.2018.10.006>
- Raleigh DR, Boe DM, Yu D, Weber CR, Marchiando AM, Bradford EM et al (2011) Occludin S408 phosphorylation regulates tight junction protein interactions and barrier function. *J Cell Biol* 193(3):565–582. <https://doi.org/10.1083/jcb.201010065>
- Rahman MT, Ghosh C, Hossain M, Linfield D, Rezaee F, Janigro D et al (2018) IFN-gamma, IL-17A, or zonulin rapidly increase the permeability of the blood-brain and small intestinal epithelial barriers: Relevance for neuro-inflammatory diseases. *Biochem Biophys Res Commun* 507(1–4):274–279. <https://doi.org/10.1016/j.bbrc.2018.11.021>
- Rogge N, Janssen J (2019) The Economic Costs of Autism Spectrum Disorder: A Literature Review. *J Autism Dev Disord* 49(7):2873–2900. <https://doi.org/10.1007/s10803-019-04014-z>
- Rose DR, Yang H, Serena G, Sturgeon C, Ma B, Careaga M et al (2018) Differential immune responses and microbiota profiles in children with autism spectrum disorders and co-morbid gastrointestinal symptoms. *Brain Behav Immun* 70:354–368. <https://doi.org/10.1016/j.bbi.2018.03.025>
- Saghazadeh A, Ataeinia B, Keynejad K, Abdolalizadeh A, Hirbod-Mobarakeh A, Rezaei N (2019) A meta-analysis of pro-inflammatory cytokines in autism spectrum disorders: Effects of age, gender, and latitude. *J Psychiatr Res* 115:90–102. <https://doi.org/10.1016/j.jpsychires.2019.05.019>
- Sapone A, de Magistris L, Pietzak M, Clemente MG, Tripathi A, Cucca F et al (2006) Zonulin upregulation is associated with increased gut permeability in subjects with type 1 diabetes and their relatives. *Diabetes* 55(5):1443–1449. <https://doi.org/10.2337/db05-1593>
- Shen Y, Li Y, Shi L, Liu M, Wu R, Xia K et al (2020) Autism spectrum disorder and severe social impairment associated with elevated plasma interleukin-8. *Pediatr Res* <https://doi.org/10.1038/s41390-020-0910-x>
- Schopler E, Reichler RJ, DeVellis RF, Daly K (1980) Toward objective classification of childhood autism: Childhood Autism Rating Scale (CARS). *J Autism Dev Disord*
- Tripathi A, Lammers KM, Goldblum S, Shea-Donohue T, Netzel-Arnnett S, Buzza MS et al (2009) Identification of human zonulin, a physiological modulator of tight junctions, as prehaemoglobin-2. *Proc Natl Acad Sci USA* 106(39):16799–16804. <https://doi.org/10.1073/pnas.0906773106>
- Unal F, Oktem F, Cetin Cuhadaroglu F, Cengel Kultur SE, Akdemir D, Foto Ozdemir D et al (2019) Reliability and Validity of the Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version, DSM-5 November 2016-Turkish Adaptation (K-SADS-PL-DSM-5-T). *Turk Psikiyatri Derg* 30(1):42–50
- Usta A, Kilic F, Demirdas A, Isik U, Doguc DK, Bozkurt M (2021) Serum zonulin and claudin-5 levels in patients with schizophrenia. *Eur Arch Psychiatry Clin Neurosci* 271(4):767–773. <https://doi.org/10.1007/s00406-020-01152-9>
- Ünal F, Öktem F, Çetin Çuhadaroğlu F, Çengel Kültür SE, Akdemir D, Foto Özdemir D et al (2019) Reliability and Validity of the Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version, DSM-5 November 2016-Turkish Adaptation (K-SADS-PL-DSM-5-T). *Turk J Psychiatry* 30(1)
- Xu G, Strathearn L, Liu B, O'Brien M, Kopelman TG, Zhu J et al (2019) Prevalence and Treatment Patterns of Autism Spectrum Disorder in the United States, 2016. *JAMA Pediatr* 173(2):153–159. <https://doi.org/10.1001/jamapediatrics.2018.4208>
- Zak-Golab A, Kocelak P, Aptekorz M, Zientara M, Juszczyk L, Martirosian G et al (2013) Gut microbiota, microinflammation, metabolic profile, and zonulin concentration in obese and normal weight subjects. *Int J Endocrinol* 2013:674106. <https://doi.org/10.1155/2013/674106>
- Zeissig S, Burgel N, Gunzel D, Richter J, Mankertz J, Wahnschaffe U et al (2007) Changes in expression and distribution of claudin 2, 5 and 8 lead to discontinuous tight junctions and barrier dysfunction in active Crohn's disease. *Gut* 56(1):61–72. <https://doi.org/10.1136/gut.2006.094375>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.