Increased Serum Zonulin Levels as an Intestinal Permeability Marker in Autistic Subjects

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Objective To evaluate the serum levels of zonulin, which regulates tight junctions between enterocytes and is a physiological modulator controlling intestinal permeability, in patients with autism spectrum disorders (ASDs).

Study design Serum zonulin levels were determined in 32 patients with ASD and 33 healthy controls using an enzyme-linked immunosorbent assay. The severity of ASD symptoms was assessed with the Childhood Autism Rating Scale.

Results Serum zonulin levels were significantly higher in the patients with ASD (122.3 ± 98.46 ng/mL) compared with the healthy controls (41.89 ± 45.83 ng/mL). There was a positive correlation between zonulin levels and Childhood Autism Rating Scale score when all subjects were assessed ($r = 0.523$; $P < .001$).

Conclusions This study suggests that zonulin, which regulates intestinal permeability, plays a role in the development of symptoms of ASD. *(J Pediatr 2017;188:240-4).*

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Epidemiologic studies estimate that the incidence of autism spectrum disorders (ASDs) is increasing and it affects nearly 1% of the population.[1,2] To date, despite a considerable amount of research, the etiology and pathophysiology of ASD remain poorly understood.[3]

Children with ASD have a high rate of gastrointestinal (GI) symptoms like diarrhea, constipation, pickiness about food, bloating, and stomach pain.[4-6] Pathologic findings like inflammation of the GI system (esophagitis, gastritis, duodenitis, enterocolitis), lymphoid nodular hyperplasia, increased intestinal permeability, dysbiosis, and reduced digestive enzyme activity are identified in children with ASD.[7-9] Intestinal mucosal cells form an "intestinal mucosal barrier." According to the leaky gut hypothesis of ASD, the intestinal mucosa is believed to be abnormally permeable.[10] Thus, digestive products from food pass through the mucosa into blood and this way induce an antigenic response affecting the central nervous system.[10] The endorphins of gluten and casein especially have similar effects, and these are called exorphins.[11] This defect in the intestinal barrier allows central nervous system functions to be affected directly by nutritionally sourced neuroactive peptides.[12] Additionally, the immune response developed against proteins in the diet causes GI inflammation and behavior problems.[10,13] Some studies of children with ASD have identified increased intestinal permeability in support of the leaky GI hypothesis,[14,15] although others have not.[16] Clinical and animal studies have described increased intestinal permeability and mucosal damage in ASD and schizophrenia.[17] Fiorentino et al[18] compared subjects with ASD with healthy controls, and found that 75% of patients with ASD had reduced expression of barrier-forming "tight junction" (TJ) components (claudin-1, occluding, tricellulin) in the intestine and 66% had increased pore-forming claudins (claudin-2, -10 -15). The results of this study support the impaired GI barrier integrity found in ASD and shows that there are many genes controlling intestinal permeability.[18] Together with autism, intestinal permeability is increased in other diseases (inflammatory bowel disease, celiac disease, irritable bowel syndrome, type 1 diabetes, HIV, multiple sclerosis, and rheumatoid arthritis).[19]

The intestinal barrier is formed by intercellular TJ between intestinal mucosa cells. TJs are dynamic structures and there is evidence that they comply with developmental, physiological, and pathologic situations.[20] Zonulin is a physiological modulator that regulates intestinal permeability by changing TJ protein–protein interaction.[21,22] Zonulin transactivates epidermal growth factor receptor through the proteinase-activating receptor-2, with subsequent TJ disassembly and increased permeability.[20] Zonulin controls paracellular antigen trafficking by

ASD	Autism spectrum disorder
BMI	Body mass index
CARS	Childhood Autism Rating Scale
GI	Gastrointestinal
TJ	Tight junction

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The authors declare no conflicts of interest.

http://dx.doi.org10.1016/j.jpeds.2017.04.004

regulating TJs.[21,22] Some potential intestinal stimuli like intestinal bacteria and gluten increase zonulin secretion.[21] Diseases associated with zonulin include autoimmune diseases, cancers, and central nervous system diseases.[21] Zonulin has been studied as a peripheral marker of intestinal permeability in some diseases.[23] Zonulin levels have been found to be high in obesity, insulin resistance related to obesity, types 1 and 2 diabetes, and sepsis.[24-28]

To date, increased intestinal permeability in ASD has been evaluated only with the lactulose/mannitol test.[14,15] The peripheral marker of intestinal permeability of zonulin has not been researched in ASD. This study assessed the increase in intestinal permeability by measuring serum zonulin levels.

Methods

Patients with ASD (10 girls, 22 boys; age 7.46 ± 3.63) were included in the study from Ordu University Faculty of Medicine Education and Research Hospital Pediatric and Adolescent Psychiatry outpatient clinics. ASD diagnosis was according to *Diagnostic and Statistical Manual of Mental Disorders, 5th edition* criteria. Healthy controls (9 girls, 24 boys; age 6.96 ± 3.28) were included from those applying to the pediatric and adolescent psychiatry and pediatric outpatient clinics for minor reasons at the same hospital (**Table**). All subjects completed a sociodemographic form and the Turkish version of the Childhood Autism Rating Scale (CARS).[29] The sociodemographic form included age, sex, birth history, medical history, family history, duration of breastfeeding, educational situation, medications used, body mass index (BMI), and GI complaints (nausea, vomiting, reflux, bloating, stomach pain, diarrhea, constipation). GI complaints were accepted as present if they occurred at least once a week. Additionally, all subjects had laboratory tests performed (hemogram, routine biochemistry, thyroid function tests, sedimentation, C-reactive protein, vitamin B_{12}, folate, and vitamin D). Subjects were examined by an expert pediatrician. To limit the variability and confounding factors, individuals using medication, with GI disease, with chronic disease, with infections, or with obesity were excluded from the study. Serum samples were obtained between 08:30 and 11:00 in the morning. Samples were stored at −80°C until study. Serum zonulin levels were measured using the sandwich-enzyme-linked immunosorbent assay method with the Human Zonulin ELISA kit (Elabscience, Wuhan, Hubei Province, China). Serum samples were diluted to a 1/10 ratio. Written consent was obtained from the family of each subject. The study received permission from Ordu University Faculty of Medicine ethics committee.

Table. Characteristics of patients and control groups

	Patient group (n = 32)	Control group (n = 33)	*P* value
Sex (female/male)	10/22	9/24	.789*
Age ± SD	7.46 ± 3.63	6.96 ± 3.28	.563†
CARS ± SD	50.92 ± 4.85	15	<.001†
BMI ± SD	19.10 ± 2.26	18.23 ± 1.32	.061†
Zonulin (ng/mL), mean ± SD	122.3 ± 98.46	41.89 ± 45.83	<.001‡
Median (IQR)	88.32 (163.96)	24.66 (40.74)	

*χ^2 test.
†Student *t* test.
‡Mann-Whitney U test.

Statistical Analyses

All statistical analyses were performed with SPSS 17.0 for Windows software (SPSS Inc, Chicago, Illinois). Data are presented as the number of cases and percent for categorical variables (sex), and as mean ± SD for ages, CARS, BMI, and zonulin values. The difference between the patient and control groups in terms of ages, CARS, and BMI values was determined using the Student *t* test. The χ^2 test was used for the categorical variable. The zonulin levels were compared between the 2 groups using the Mann-Whitney U test. Direct relationships between continuous variables were examined with Pearson and Spearman correlation tests. $P < .05$ was considered significant.

Results

There was no difference between the groups in terms of age, sex, and BMI ($P = .563$, .789, and .061, respectively). There was a significant difference between the CARS scores in the 2 group ($P < .001$). All subjects in the healthy control group had no autistic symptoms and received scores of 15 on CARS. Serum zonulin levels in the subjects with ASD (122.30 ± 98.46 ng/mL) were significantly higher ($P < .001$) than those of normal control subjects (41.89 ± 45.83 ng/mL) (**Table** and **Figure 1**). There

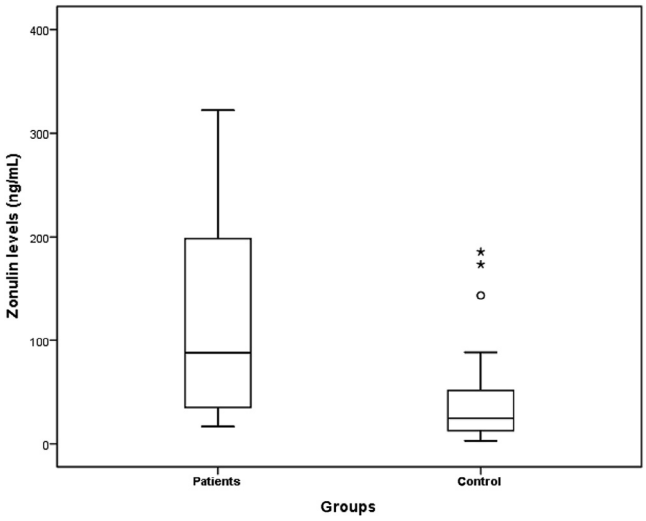

Figure 1. Distribution of zonulin levels in the groups. The upper and lower edges of the boxes show the 25th and 75th percentile values. Horizontal lines in the box are median value, asterisk (*) are extreme values, and open circle (o) are outliers. The vertical lines extending from the boxes extend to larger and smaller values that are not outliers.