“Irritable Bowel Syndrome: Advancing Our Understanding
Zonulin serum levels are increased in non-celiac gluten sensitivity and irritable bowel syndrome with diarrhea”

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Summary: Zonulin, the human analogue of the Vibrio cholerae zonula occludens toxin, is an endogenous modulator of epithelial tight junctions (TJs), and is overexpressed in disorders in which TJ dysfunction is central, including celiac disease (CD). These researchers hypothesized that loss of intestinal barrier function, secondary to the activation of the Zonulin pathway by food-derived environmental triggers or changes in gut microbiota, might be a key factor in the pathogenesis of irritable bowel syndrome (IBS) and non-celiac gluten sensitivity (NCGS). They found that Zonulin serum levels were elevated not only in CD, but also in NCGS and IBS-D patients. Interestingly, the researchers noted, Zonulin serum levels correlate with anti-TTG and anti-DGP antibody titers. Their data suggest that a Zonulin-dependent TJ dysfunction inducing “leaky gut” may play a role in the pathophysiology of NCGS and IBS. They call for further studies to demonstrate the possible role of Zonulin as a biomarker in pathologies with permeability alteration like NCGS and IBS.

Methods:

- The researchers studied 15 patients with IBS-D, 15 patients with NCGS, 15 patients with CD, and 15 healthy controls (HC).

- They used ELISA assay to evaluate Zonulin serum levels; they evaluated total levels spectrophotometrically and used them to normalize the data.

- The researchers recorded clinical data for each patient, including anti-transglutaminase (TTG) antibodies, anti-deamidated gliadin peptide (DGP) antibodies, IgE, abdominal symptoms, and bowel habits.
They evaluated the expression of the Zonulin gene in paraffin-embedded colonic biopsy samples (20 IBS and 10 HC) using quantitative real-time RT-PCR assay.

**Results:**

- Zonulin serum levels were significantly different among the four groups ($P<0.0001$).
- CD patients showed significantly higher Zonulin serum levels compared to HC ($0.033 \pm 0.004$ vs $0.007 \pm 0.001$ ng/mg total proteins, $P<0.0001$) and to IBS-D patients ($0.012 \pm 0.002$ ng/mg total proteins, $P<0.001$).
- NCGS Zonulin serum levels were significantly higher than HC ($0.030 \pm 0.006$ vs $0.007 \pm 0.001$, $P<0.05$) and IBS-D ($P<0.05$).
- IBS-D patients showed higher Zonulin serum levels than HC, without reaching statistical significance ($P=0.1$).
- Zonulin levels were positively correlated with the titer of anti-DGP antibodies ($r:0.6; P<0.05$,) and anti-TTG antibodies ($r:0.6; P<0.05$).
- mRNA expression of Zonulin was increased in the colonic mucosa of patients with IBS in comparison with HC, although the difference did not reach the level of statistical significance ($P=0.08$).

**Discussion:**

**Zonulin in autoimmune disease**

Zonulin is a type of protein (a pre-haptoglobin 2) that was discovered in 2000 by a team of researchers at Maryland School of Medicine in the USA. The protein is found within intestinal cells and it is the only human protein discovered so far that regulates the permeability of the intestine. Zonulin has been called a "tight junction regulator", as it controls the size of the gaps between the intestinal cells and orchestrates the passage of nutrients, water and cells into and out of the gut.
Scientists have found that Zonulin is produced and released by triggers including intestinal bacterial infections and gluten, and a link between Zonulin and coeliac disease has already been established. In the presence of Zonulin, the normally tight junctions between the intestinal cells remain open, creating bowel "leakiness" and initiating an inflammatory cascade that eventually damages the intestinal wall.

"Increased intestinal permeability has been implicated in a range of autoimmune conditions including coeliac disease, type 1 diabetes, rheumatoid arthritis and multiple sclerosis," explained Prof. Barbara. "Since Zonulin is a key regulator of intestinal permeability, it is possible that this protein provides a common link between all these conditions. This study has increased our understanding of Zonulin and how it might contribute to the development of these common and disabling bowel conditions. Hopefully, our work will lead to new diagnostic and therapeutic strategies for patients with these and possibly other autoimmune conditions."

References
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