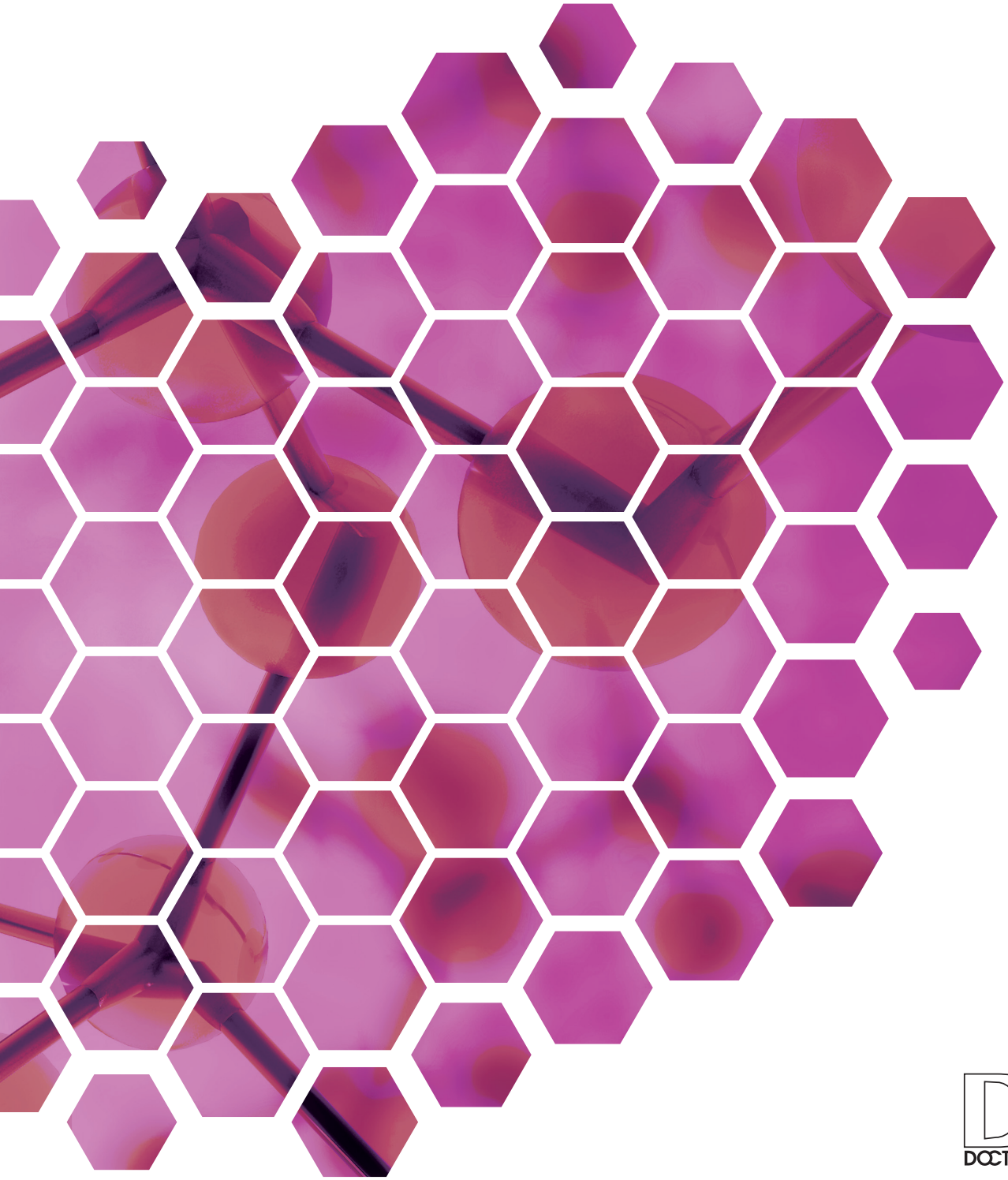
Zonulin: A biomarker and regulator of gastrointestinal tight gap junctions

RESOURCE GUIDE



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Zonulin: A biomarker and regulator of gastrointestinal tight gap junctions

Research and clinical studies of the protein zonulin and the zonulin signaling pathway demonstrate the clinical efficacy of zonulin as a biomarker of intestinal permeability. Studies also confirm that zonulin signaling is an essential mechanism in promoting healthy immune function and tolerance at the gastrointestinal mucosal barrier.

Disregulation of the zonulin signaling pathway disrupts normal gut barrier function and alters immune responses. As a result, high levels of serum zonulin may point to the presence of increased intestinal permeability. Over time, persistent high levels of zonulin in the blood may predispose susceptible individuals to inflammatory, autoimmune, and even neoplastic disorders by increasing the paracellular permeability of the gastrointestinal mucosa. As increased intestinal permeability persists only in the presence of high zonulin levels, the biomarker may also be used to monitor therapeutic interventions designed to restore gut barrier function.

Zonulin is the only currently known reversible regulator of intestinal permeability.

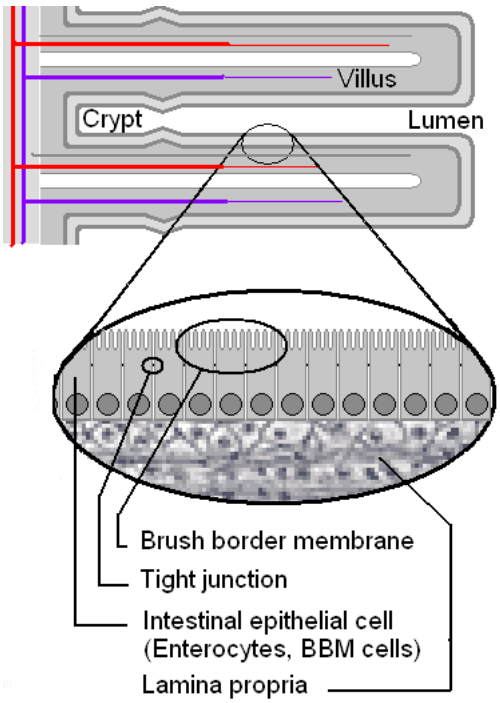
What is zonulin?

The paracellular tight junctions between the intestinal epithelial cells are a critical component of the mucosal barrier and regulate the functional state of the paracellular pathway. Zonulin is a protein that regulates the reversible permeability of tight junctions.

Zonulin is the endogenously produced analog of the *Vibrio cholerae* enterotoxin Zot. When *Zot* or zonulin bind to intestinal epithelial cells, a signal cascade is induced. The signal cascade disassembles the paracellular tight junctions between the epithelial cells of the intestinal mucosa, which increases intestinal permeability. However, the effects of *Zot* and zonulin are reversible. The tight junctions are the most apical structures to the gut lumen and are the rate-limiting factor for the paracellular migration of molecules through the intestinal barrier.

In the small intestine, epithelial cohesion is maintained by the apical junction complex, composed of tight and zonula adherens junctions. The gastrointestinal mucosa forms a barrier between the body and the contents of the gut lumen. The mucosal barrier regulates the passage of macromolecular compounds through the intestinal epithelium to the gut-associated lymphoid tissue. The competence of the paracellular pathway is regulated by the zonulin signaling pathway, the gut-associated lymphoid tissue (GALT) and neuroendocrine networks. The dendritic cells of the GALT are found in the lamina propria layer of the gut mucosa. The dendritic cells take up antigens, process them and load them onto human leukocyte antigen (HLA) proteins for presentation to T-cells. The antigens presented may promote tolerant/anti-inflammatory or proinflammatory T-cell induction.

Zonulin is a prehaptoglobulin (pre-HP2), and levels are modulated by the presence or absence of haptoglobin (HP) gene. Zonulin release in the small intestine occurs when a chemokine receptor is stimulated by gliadin or chemokines and induces proinflammatory signaling pathways in gastrointestinal epithelial cells. The released single-chain zonulin activates the cell-signaling pathway via epidermal protease-activated receptor 2 and growth factor. This results in disassembly of the tight paracellular junctions between the gut epithelial cells. When zonulin is cleaved by either complement component 1 (C1RL) in the cellular endoplasmic reticulum or by intestinal tryptase IV, it is converted into haptogloblulin, a protein with heme (iron)-binding, antioxidant and antimicrobial properties. Haptoglobin does not affect tight junctions.



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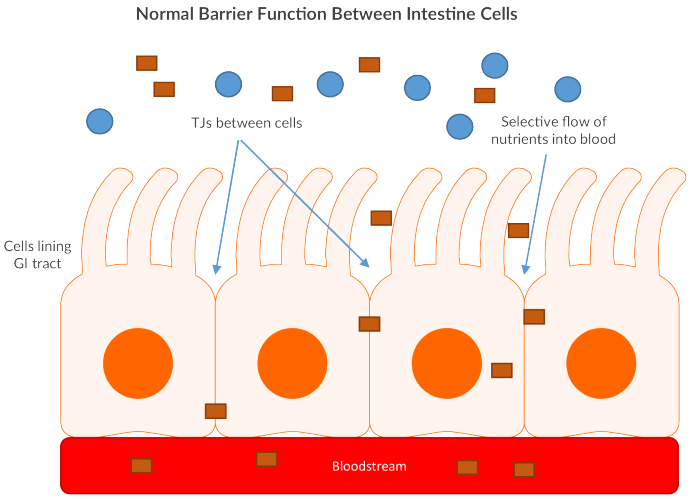
Zonulin levels may increase due to inflammation, infection, injury, diet, or disease, altering the tight junctions between the epithelial cells and allowing increased passage of macromolecular proteins, toxins, and microorganisms into systemic circulation. Loss of tight junction functions and increased intestinal permeability may alter the delicate balance of tolerance and immune response essential to health and trigger inflammatory T-cell responses. Simple sugars, sodium, emulsifiers, the food additive microbial transglutaminase, and nanoparticles may disrupt intestinal barrier function and increase zonulin levels. In susceptible individuals, inflammatory disease or autoimmunity may develop.

The connection between gluten and zonulin

Zonulin release has been triggered experimentally by the presence of gluten-derived gliadin in the gut lumen. The production of specific gliadin-derived peptides by digestive enzymes activates protease activated receptor 2 (PAR2) and epidermal growth factor (EGFR) and protease activated receptor 2 (PAR2). This signal cascade promotes disassembly of small intestine tight junctions.

The loss of tight junctions allows gliadin and other antigens to enter the lamina propria layer of the small intestine where they are presented to the enteric immune cells, inducing a shift in immune function. The shift to proinflammatory signaling results in an increased peripheral immune response to gliadin. Dendritic cells loaded with gliadin antigen can migrate from the small intestine to the mesenteric and pancreatic lymph nodes, promoting inflammatory responses in the small intestine or pancreas.



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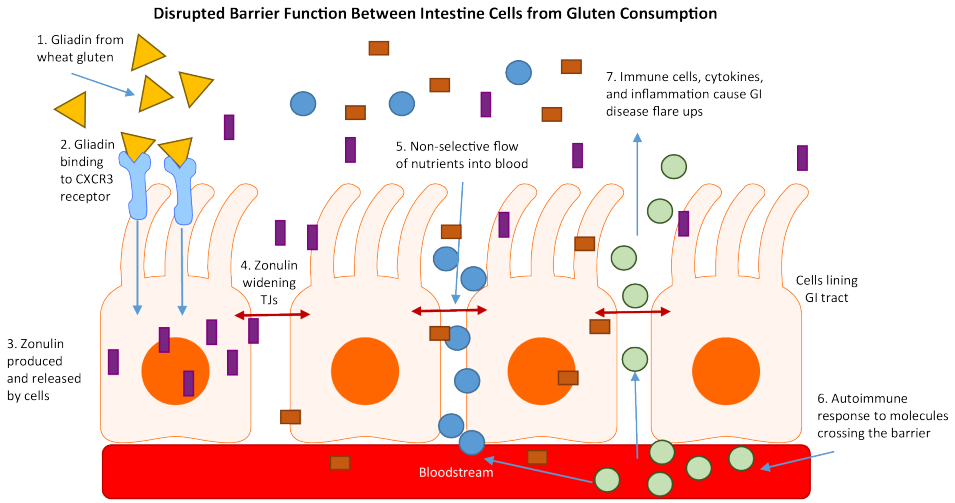

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Implementation of a gluten-free diet may prevent further activation of the zonulin pathway and the autoimmune process, however, the loss of barrier function may persist for years in cases of gastrointestinal autoimmunity such as celiac disease. The composition of the gastrointestinal microbiome, including the resident gut microbes, is frequently altered in individuals with celiac disease and other autoimmune disorders, which may promote proinflammatory signaling and enhance the translocation of gliadin into the intestinal villi.

Zonulin and bacterial adhesion

The epithelial surface of the gut mucosa is covered by a thick mucus layer. The microbiome of resident gastrointestinal bacteria and lipopolysaccharides from bacterial cell walls induce the secretion of mucus from host goblet cells. The mucus layer is protective—it prevents the direct adhesion of gastrointestinal bacteria to the epithelium, has a high concentration of secretory IgA, and provides binding sites for resident microbes. In the small intestine, the mucus layer covers both the villi and the villi tips. Disruption of the mucus layer from altered immunity, poor diet, or other causes, or alterations in resident microbes and gut ecology, may contribute to proinflammatory signaling and increased bacterial adhesion to the mucosa. The penetrability of the inner mucus layer, which normally repels bacteria, is determined at least in part by the immune system. The direct adhesion of pathogen or resident bacteria to the wall of the small intestine stimulates the release of zonulin from the gut mucosa and increases intestinal permeability.

Association of elevated zonulin with increased gut permeability and autoimmune conditions

Autoimmune disorders result from genetic predisposition, environmental exposures, and gut dysbiosis caused by altered populations of resident gut microbiota. The induction of an autoimmune response requires effector T-cells to acquire specific cytokine "fingerprints" and then migrate to target organs where they promote tissue inflammation. Increased intestinal permeability alters immune functions and increases antigen passage through paracellular channels.

Animal studies indicate that epithelial barrier dysfunction may be a causative factor in autoimmune disorders. In fact, studies in IBD animal models have shown that increased intestinal permeability occurs early in pathogenesis and precedes the development of symptomatic disease. High levels of zonulin are found in the tissues and fluids of autoimmune patients. Elevated serum levels of zonulin and increased intestinal permeability are commonly observed in patients at risk of developing disease long before the onset of symptoms (e.g. Type 1 diabetes).

Several autoimmune, inflammatory and neoplastic diseases have been associated with elevated levels of zonulin:

- **Celiac disease**—In those with the genetic predisposition (HLA-DQ2/8) and exposure to gluten, increased zonulin levels open the tight junctions and intestinal damage occurs. The breach of the intestinal barrier promotes proinflammatory T-cell signaling. Zonulin levels increase during the acute phase of the disease and decrease after gluten is removed from the diet and healing occurs. In celiac patients, serum zonulin has been correlated with lactulose/mannitol permeability testing.
- **Non-celiac gluten sensitivity (NCGS)—**A 2015 study demonstrated that individuals with NCGS have zonulin levels higher than a normal control population, but lower than values for those with celiac disease.
- **Inflammatory bowel disease (IBD)**—In children with IBD, elevated zonulin levels were associated with the presence of atopic symptoms. None of the IBD patients in the study were diagnosed with celiac disease. Interleukin-10 knockout mice, which develop chronic, patchy colitis and increased intestinal permeability, do not develop symptoms if treated with a zonulin inhibitor from weaning. Germ-free animals are also protected from symptoms, which implies trigger factors arise from the gut lumen.
- **Diarrhea-predominant irritable bowel syndrome (IBS-D)**—A 2015 study demonstrated that individuals with IBS-D have zonulin levels that are higher than a normal control population, but lower than values for those with celiac disease. Increased transit times in this population are associated with HLA-DQ2/8 genotype.
- **Type 1 diabetes (T1D)**—The autoimmune cause of pancreatic β-cell destruction has yet to be elucidated. However, 40% of Caucasian T1D patients carry a genetic predisposition (human leukocyte antigen (HLA) alleles) which, combined with environmental triggers, results in manifestation of the disease. Gliadin exposure has been linked to the expression of T1D in human and animal studies. Recent studies indicate that gastrointestinal symptoms in T1D patients are associated with increased intestinal permeability and zonulin levels which occur prior to the onset of GI complications. Human studies indicate that nondiabetic family members (with similar inheritance) commonly have increased zonulin and intestinal permeability.
- **Liver disease**—Obese adolescents and juveniles with nonalcoholic fatty liver disease had higher zonulin levels than age, gender, and BMI-matched controls.
- **Multiple sclerosis (MS)**—It has been demonstrated that that disruption of intestinal homeostasis is an early and immune-mediated event in an animal model of MS. Transfer of autoreactive T-cells from mice with experimental autoimmune encephalomyelitis (EAE) increased the infiltration of proinflammatory Th1/Th17 cells and a reduced regulatory T-cell number in the gut lamina propria, Peyer's patches, and mesenteric lymph nodes. Altered T-cell signaling increased expression of zonulin and intestinal permeability, which altered intestinal morphology. The intestinal changes were seen at 7 days (preceding the onset of neurological symptoms) and at 14 days (at the stage of paralysis) after T-cell immunization.

In many disorders associated with elevated zonulin and increased intestinal permeability, such as Crohn's diease, studies indicate that asymptomatic close relatives may often have increased zonulin and intestinal permeability, and that most develop symptomatic disease within a few years.

Normalization of zonulin levels

Probiotic use has been shown to reduce serum and fecal zonulin levels. Restoration of the gastrointestinal mucosal barrier may include dietary changes, treatment of dysbiosis, digestive supports, and anti-inflammatory supplements which may include quercetin, vitamin C, curcumin, gamma-linoleic acid, omega-3 fatty acids (EPA, DHA), and aloe vera. Other nutrients such as zinc, beta-carotene, pantothenic acid, and L-glutamine provide support for regeneration of the GI mucosa. Glutamine has specifically been shown to improve intestinal barrier function in high-stress patients. The amino acid taurine has been shown to have anti-inflammatory actions both *in vivo* and *in vitro*.

Increased fiber consumption may improve mucus production in the large and small intestine, as fiber consumption increases bacterial synthesis of short-chain fatty acids which improve intestinal barrier integrity.

Conclusion

The disregulation of the zonulin signaling pathway disrupts normal gut barrier function and alters immune responses. High levels of serum zonulin may alert clinicians to the presence of increased intestinal permeability in their patients. As zonulin is the only known regulator of reversible intestinal permeability, the biomarker may be used to monitor therapeutic interventions designed to restore gut barrier function.

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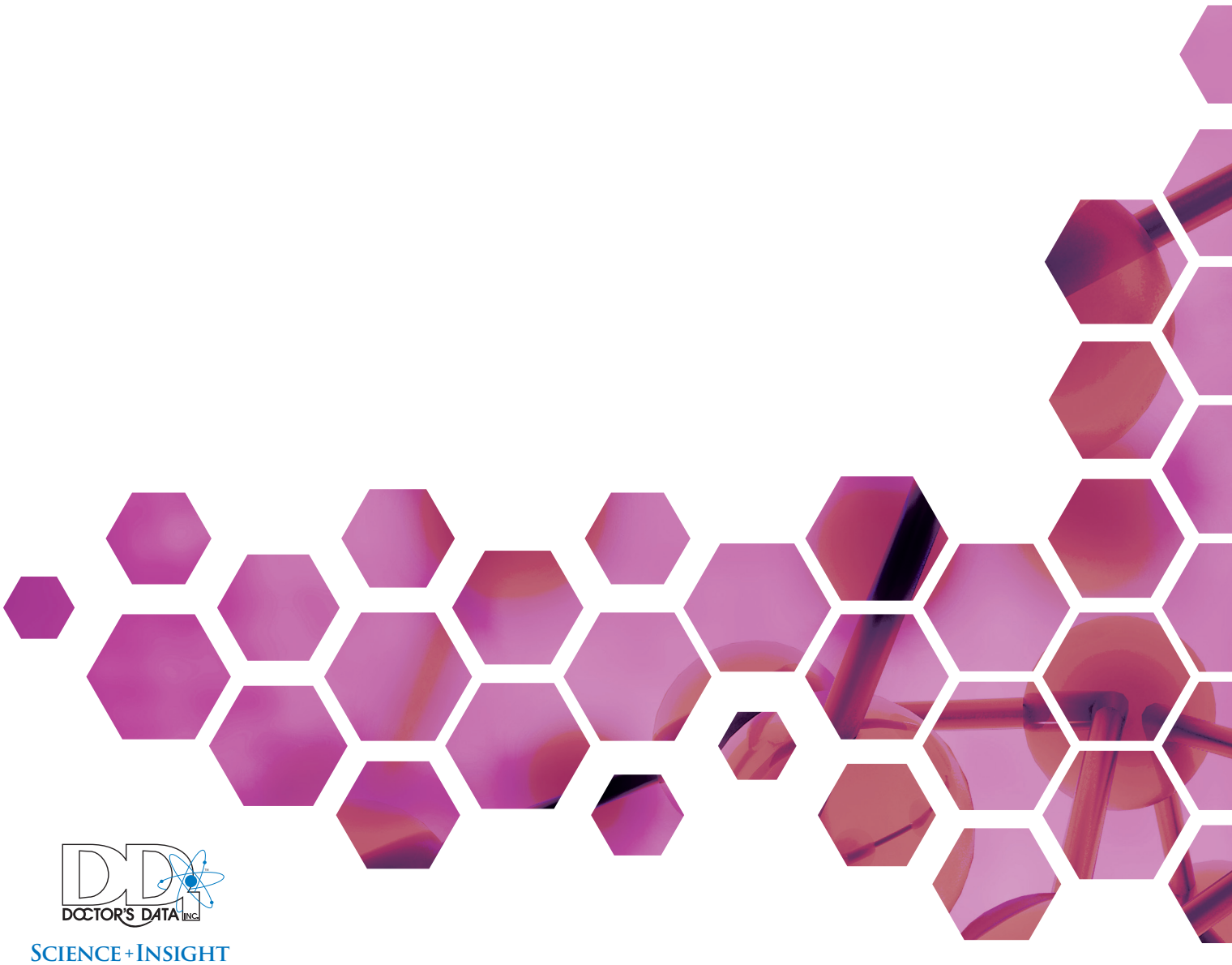

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