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Pathophysiology of Gluten and Wheat-Related Disorders

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GI Advanced Practice Module

Disclosure Statement

Patrick Hanaway, MD disclosed he has no financial relationships with any commercial interest relevant to this activity.

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Performance Objectives

Following this activity, successful participants will be able to…

1. Explain how various wheat proteins and gluten form the basis for a variety of pathophysiologic responses.
2. Summarize other reactions to wheat and other gluten-containing grains.
3. Distinguish the pathophysiology of gluten and wheat-related disorders.

Evidence Icons: Key

Clinical Disclaimers:

Association, not causation

Lab test
(Labs not generally accepted in conventional care)

Clinical experience
(Intervention warranted by historical clinical experience of educator and/or functional medicine community of practitioners in the context of evidentiary paucity)

Clinical judgment
(Intervention warranted by clinical judgment of educator and/or functional medicine community of practitioners in the context of evidentiary paucity)

Conflict of interest

Study Types:

Animal study

In vitro study

n of 1, or single-case study

In silico *(Computerized molecular modeling)*

Reactions to Wheat Components

Components of Wheat	Effects	Associated GI Conditions
Gluten	• Damage to enterocyte tight junctions leading to intestinal permeability • Activation of CD4 T lymphocytes and pro-inflammatory cytokines (IFN- γ) • Infiltration of eosinophils • Secretion of anti-gliadin and anti-tissue-transglutaminase antibodies • Increased density of CD8 intraepithelial cells • TLR elevation • Activation of the innate immune response	Celiac disease, NCGS
Wheat protein	• Activation of pro-inflammatory cytokines • Inhibition of gut epithelial cell repair	Wheat allergy, NCWS
α-amylase and trypsin (ATI)	• Activation of TLR4 and the innate immune response • Increase in inflammation	Celiac disease, NCWS, IBS, IBD
Rapidly fermentable carbohydrates (FODMAPS)	• Fermentation of indigestible carbohydrates leading to the production of gas and short chain fatty acids	IBS, NCWS

See References: Reactions to Wheat Components

References: Reactions to Wheat Components

1. Brouns F, Rooy GV, Shewry P, Rustgi S, Jonkers D. Adverse reactions to wheat or wheat components. Comprehensive Reviews in Food Science and Food Safety. 2019; 18(5): 1437-1452.
2. Junker Y, Zeissig S, Kim SJ, et al. Wheat amylase trypsin inhibitors drive intestinal inflammation via activation of toll-like receptor 4. J Exp Med. 2012;209(13):2395-2408. doi:10.1084/jem.20102660
3. Vojdani A, Perlmutter D. Differentiation between Celiac Disease, Nonceliac Gluten Sensitivity, and Their Overlapping with Crohn's Disease: A Case Series. Case Reports Immunol. 2013;2013:248482. doi:10.1155/2013/248482
4. Parzanese I, Qehajaj D, Patrinicola F, et al. Celiac disease: From pathophysiology to treatment. World J Gastrointest Pathophysiol. 2017;8(2):27-38. doi:10.4291/wjgp.v8.i2.27
5. Aziz I, Hadjivassiliou M, Sanders DS. The spectrum of noncoeliac gluten sensitivity. Nat Rev Gastroenterol Hepatol. 2015 Sep;12(9):516-26. doi: 10.1038/nrgastro.2015.107. Epub 2015 Jun 30. PMID: 26122473.

Different pathogenic mechanisms are likely responsible for/ involved in different gluten & wheat-related conditions:

- **Wheat allergy** – gluten and potentially non gluten proteins
- **Celiac Disease and other Autoimmune conditions:** gluten proteins found in wheat, barley, and rye
- **Non-Celiac Gluten Sensitivity:** gluten proteins
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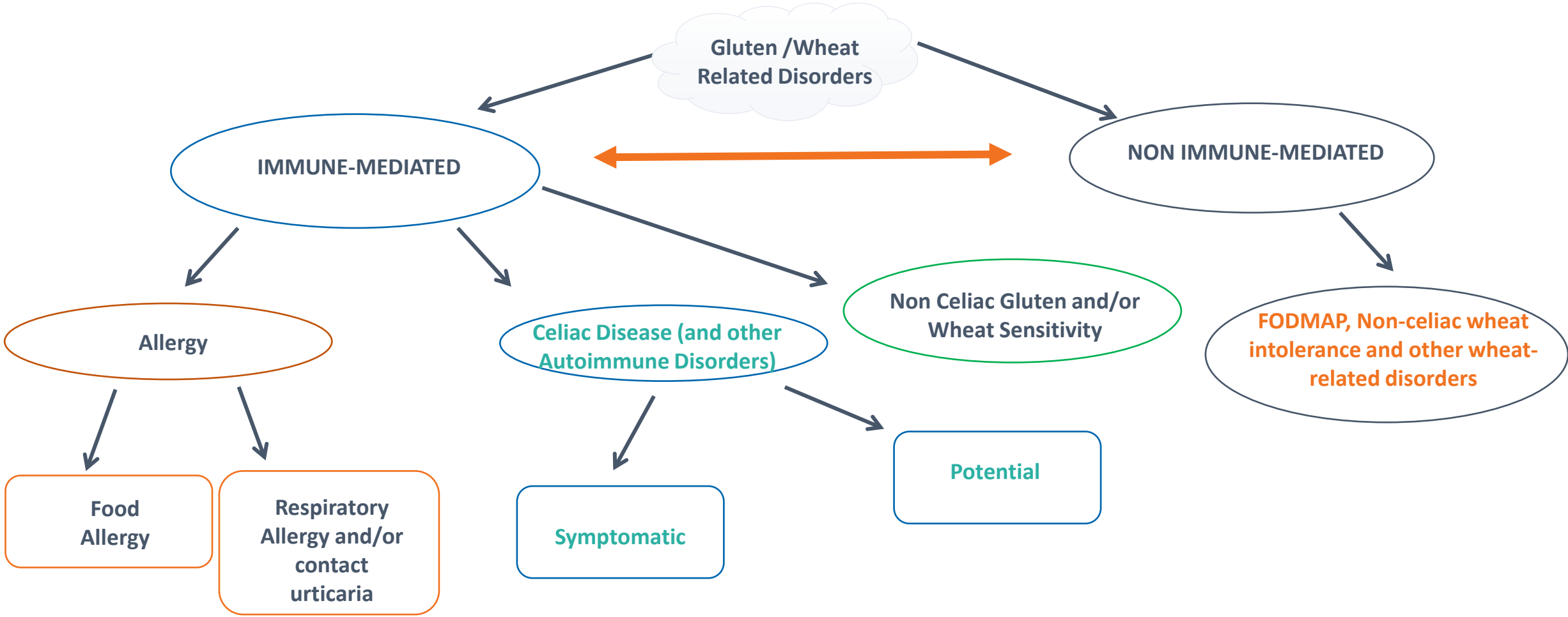
  - Albumins, globulins, Amylase-Trypsin Inhibitors (ATIs)
  - Wheat germ agglutinin (a lectin)
  - Other unidentified protein antigens/epitopes…?
- **Non-Celiac Wheat Intolerance:**
  - FODMAP reactions – intestinal sx only

Carroccio A, Rini G, Mansueto P. Non-celiac wheat sensitivity is a more appropriate label than non-celiac gluten sensitivity. Gastroenterology. 2014 Jan;146(1):320-1. doi: 10.1053/j.gastro.2013.08.061.

Abbreviations

- **EMA** – endomysial antibodies
- **tTG** – tissue transglutaminase
- **DGP** – Deamidated Gliadin Peptide
- **CD** – celiac disease
- **WA** – wheat allergy
- **NCGS** – non celiac gluten sensitivity
- **NCWS** – non celiac wheat sensitivity
- **IELs** – intestinal intraepithelial lymphocytes
- **ATIs** – amylase trypsin inhibitors
- **WGA** – wheat germ agglutinin
- **FODMaPs** – fermentable oligo-, di-, and mono-saccharides and polyols

Schematic of Gluten/Wheat Related Disorders



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Understanding Wheat: **Proteins**

- **Wheat proteins:**
  - **Gluten** = gliadins (α, β, γ, ω) + glutenins = 80% of wheat proteins
  - **Albumins** = 10% of wheat proteins
  - **Globulins** = 10% of wheat proteins

- There are **at least 50 T-cell stimulatory epitopes** (antigenic determinants) in gluten proteins.
  - These exert immunomodulatory, cytotoxic, and gut-permeating activities.
  - These epitopes have been somewhat mapped to certain domains in α-gliadin.

1. Kasarda DD. Can an increase in celiac disease be attributed to an increase in the gluten content of wheat as a consequence of wheat breeding?. J Agric Food Chem. 2013;61(6):1155–1159. doi:10.1021/jf305122s
2. Fasano A. Zonulin and its regulation of intestinal barrier function: the biological door to inflammation, autoimmunity, and cancer. Physiol Rev. 2011 Jan;91(1):151-75. doi: 10.1152/physrev.00003.2008.
3. Nikulina M, Habich C, Flohé SB, Scott FW, Kolb H. Wheat gluten causes dendritic cell maturation and chemokine secretion. J Immunol. 2004 Aug 1;173(3):1925-33.

Understanding Wheat & Gluten: **Proteins**

- The **most immunogenic peptide in gluten** is a unique 33-mer gliadin fragment.
- It is **resistant to enzymatic degradation** by gastric, pancreatic, and brush border peptidases.
- Enzyme called tissue transglutaminase (tTG) leaks from damaged intestinal cells – in CD, develop antibodies to TG2
  - Anti-tTG assays are now considered to be the most sensitive markers.

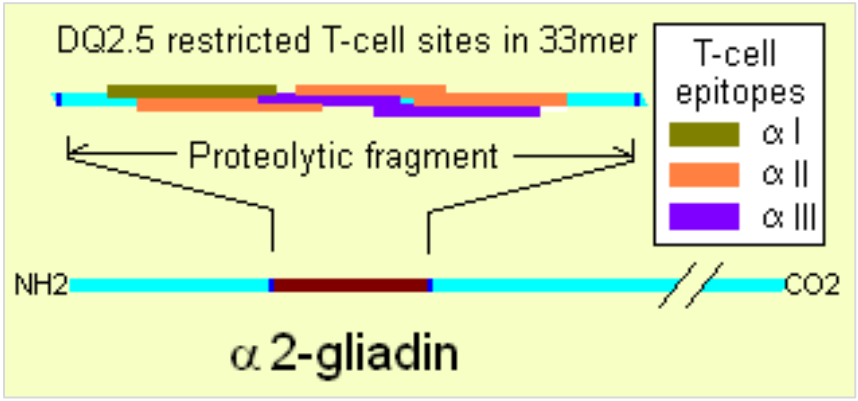


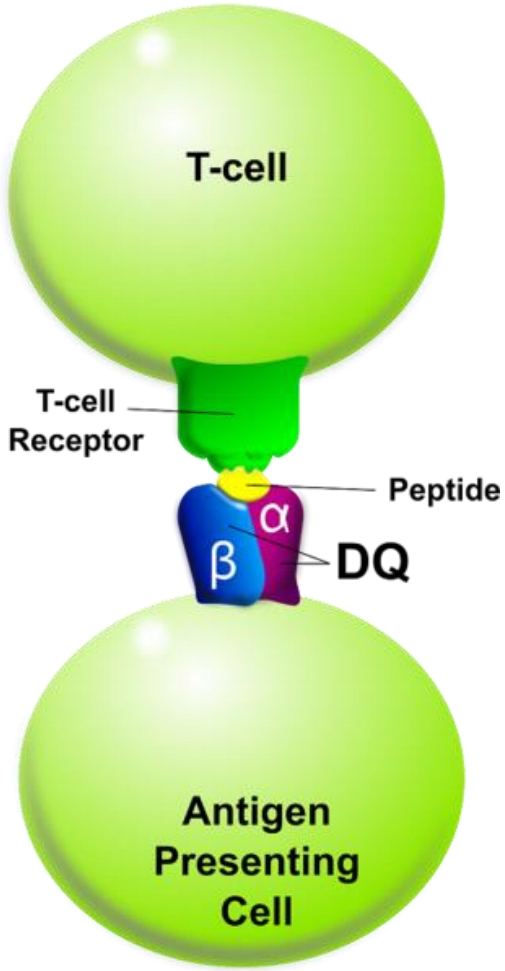
Illustration of deamidated α2-gliadin's 33mer, amino acids 56-88 in sequence, showing the overlapping of 3 varieties of T-cell epitope

Courtesy of Pdeitiker.

1. Fasano A. Zonulin and its regulation of intestinal barrier function: the biological door to inflammation, autoimmunity, and cancer. Physiol Rev. 2011;91(1):151-175. doi:10.1152/physrev.00003.2008.
2. Nikulina M, Habich C, Flohé SB, Scott FW, Kolb H. Wheat gluten causes dendritic cell maturation and chemokine secretion. J Immunol. 2004;173(3):1925-1933. doi:10.4049/jimmunol.173.3.1925

Gluten: Proteolysis Resistant

- Normal digestion: proteins broken down to individual amino acids or small chains – at most, intestine can only absorb single aa's or chains of up to 3-4 aa's
- Epitopes (antigenic determinants) usually contain between **8-11 amino acids**
- **Gluten** – most immunogenic fragment is glutamine and proline-rich 33-mer gliadin molecule



HLA DQ Receptor with bound peptide and TCR by Davide.pirolli is licensed under CC BY-SA 3.0.

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"No one can digest gluten" → *What does this really mean?*

- **Prolamin** class of proteins – very rich in amino acids **glutamine and proline**
  - Prolamins – Amino acid composition makes these **highly resistant to complete degradation via gastric, pancreatic, and intestinal proteases, leaving small peptides intact** (potential antigenic triggers).

Gluten Prolamins:
Wheat – gliadins
Rye – secalins
Barley – hordeins
Oats – avenins

- Repetitive presence of these residues makes these a preferred substrate of tTG.

Mejías JH, Lu X, Osorio C, Ullman JL, von Wettstein D, Rustgi S. Analysis of wheat prolamins, the causative agents of celiac sprue, using reversed phase high performance liquid chromatography (RP-HPLC) and matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS). Nutrients. 2014;6(4):1578-1597. Published 2014 Apr 15. doi:10.3390/nu6041578

Experimental Laboratory Tests of Gluten Sensitivity

More on testing at the GI APM!

- Current testing for Gluten Sensitivity and Celiac disease includes IgG and IgA against gliadin and tissue transglutaminase.
- These **antibodies are measured against a single component of wheat protein called alpha-gliadin.**
- **However, wheat proteins consist of alpha-gliadin, omega-gliadin, glutenin, gluteomorphin, prodynorphin and agglutinins, all of which have a capacity to challenge the immune system.**

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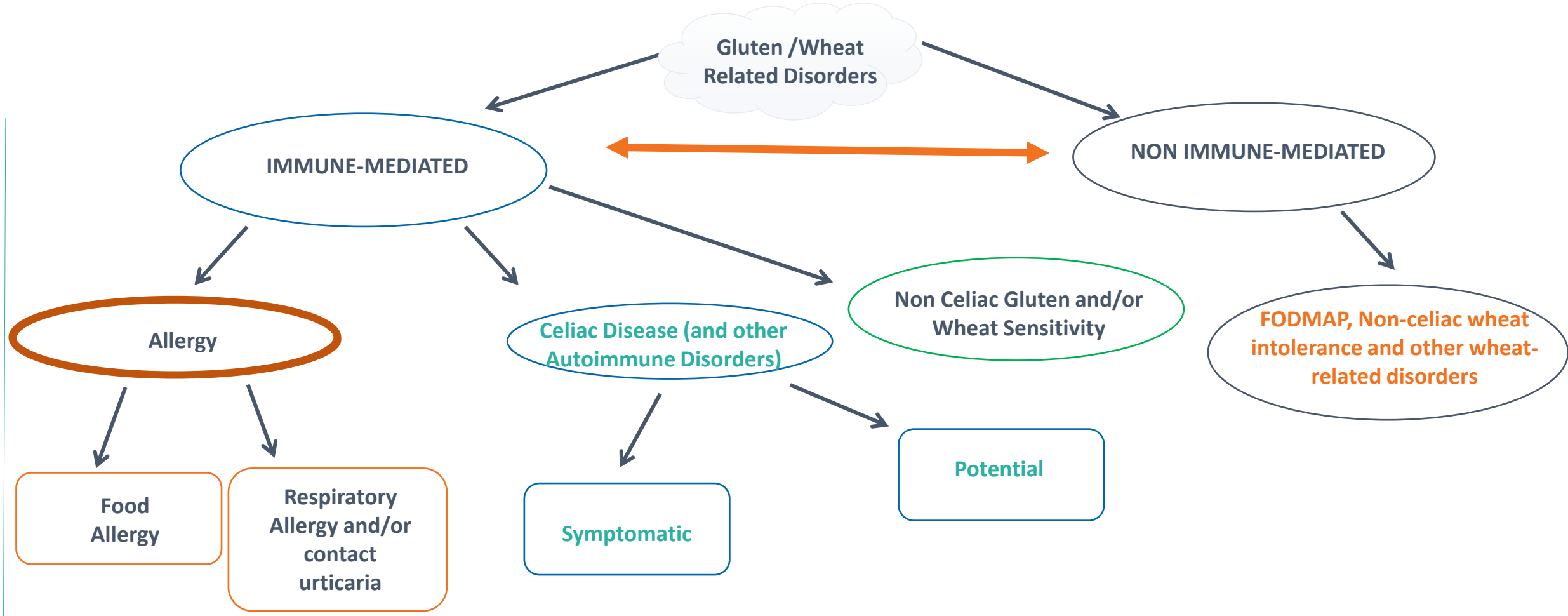

Wheat allergy

- **Wheat allergy: IgE**
  - includes classic food allergy, WDEIA (wheat-dependent, exercise-induced anaphylaxis), and baker's asthma (respiratory wheat allergy)
- **Reactions possible to *any* of the wheat proteins**
- IgE antibodies particularly reactive with one group of wheat proteins: **α-amylase inhibitors**
- Baker's asthma: Also reactive to **wheat germ agglutinin, peroxidase, and LTPs** *(non-specific lipid transfer proteins)*

Bakers are about 80 times more likely to develop occupational asthma than the average British worker.

Cianferoni A. Wheat allergy: diagnosis and management. J Asthma Allergy. 2016;9:13–25. Published 2016 Jan 29. doi:10.2147/JAA.S81550

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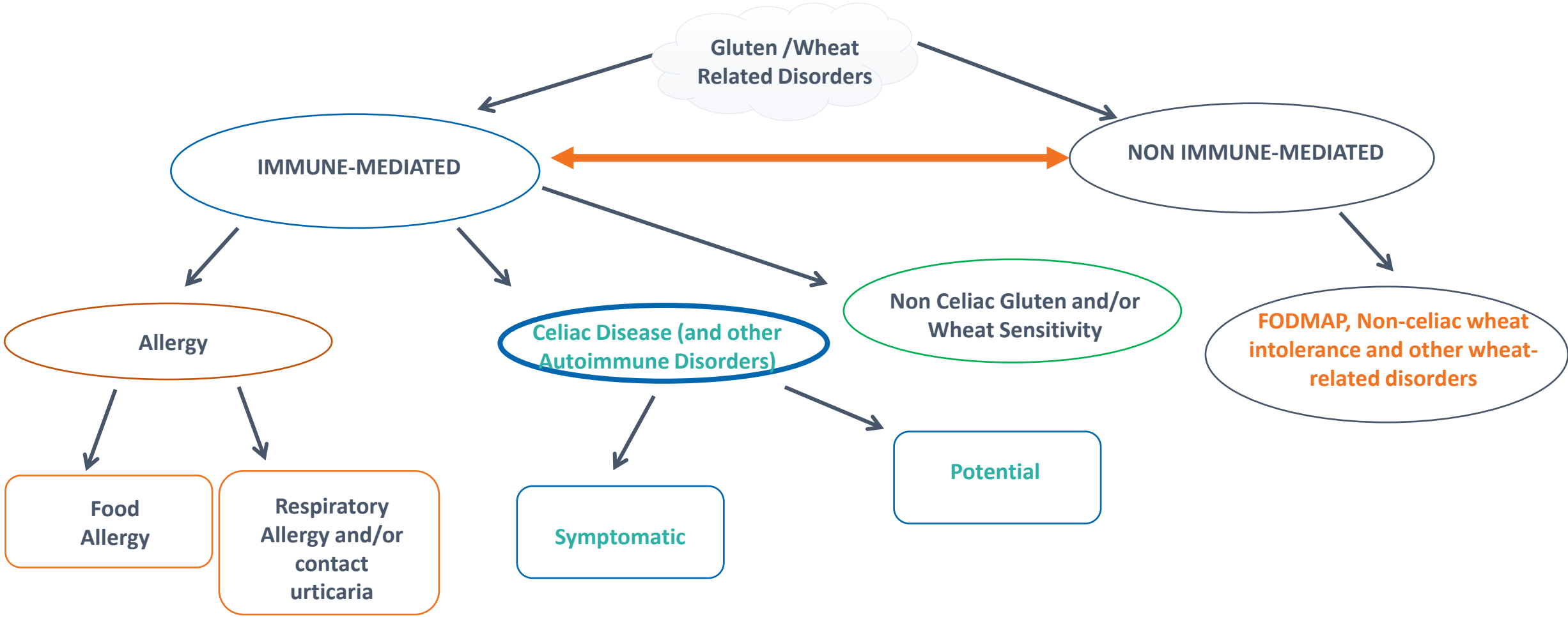
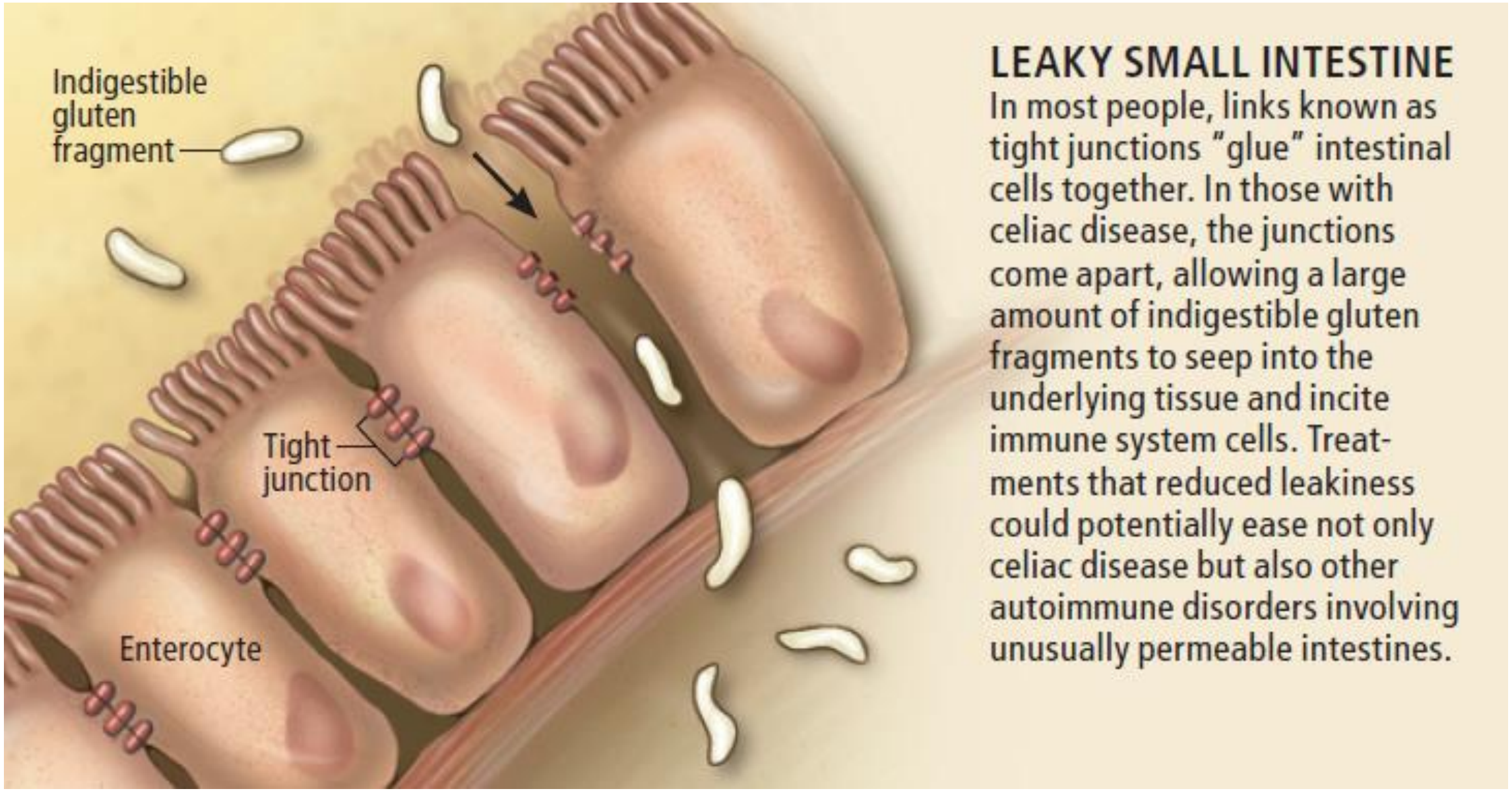


Surprises from Celiac Disease

Study of a potentially fatal food-triggered disease has uncovered a process that may contribute to many autoimmune disorders

A. Fasano



Fasano A. Surprises from celiac disease. Sci Am. 2009 Aug;301(2):54-61. doi: 10.1038/scientificamerican0809-54. PMID: 19634568.

Genetic Propensity

Environmental Triggers

Altered Microbiome with Gut Inflammation

Increased Intestinal Permeability
and translocation of macromolecules (LPS, foods,…)

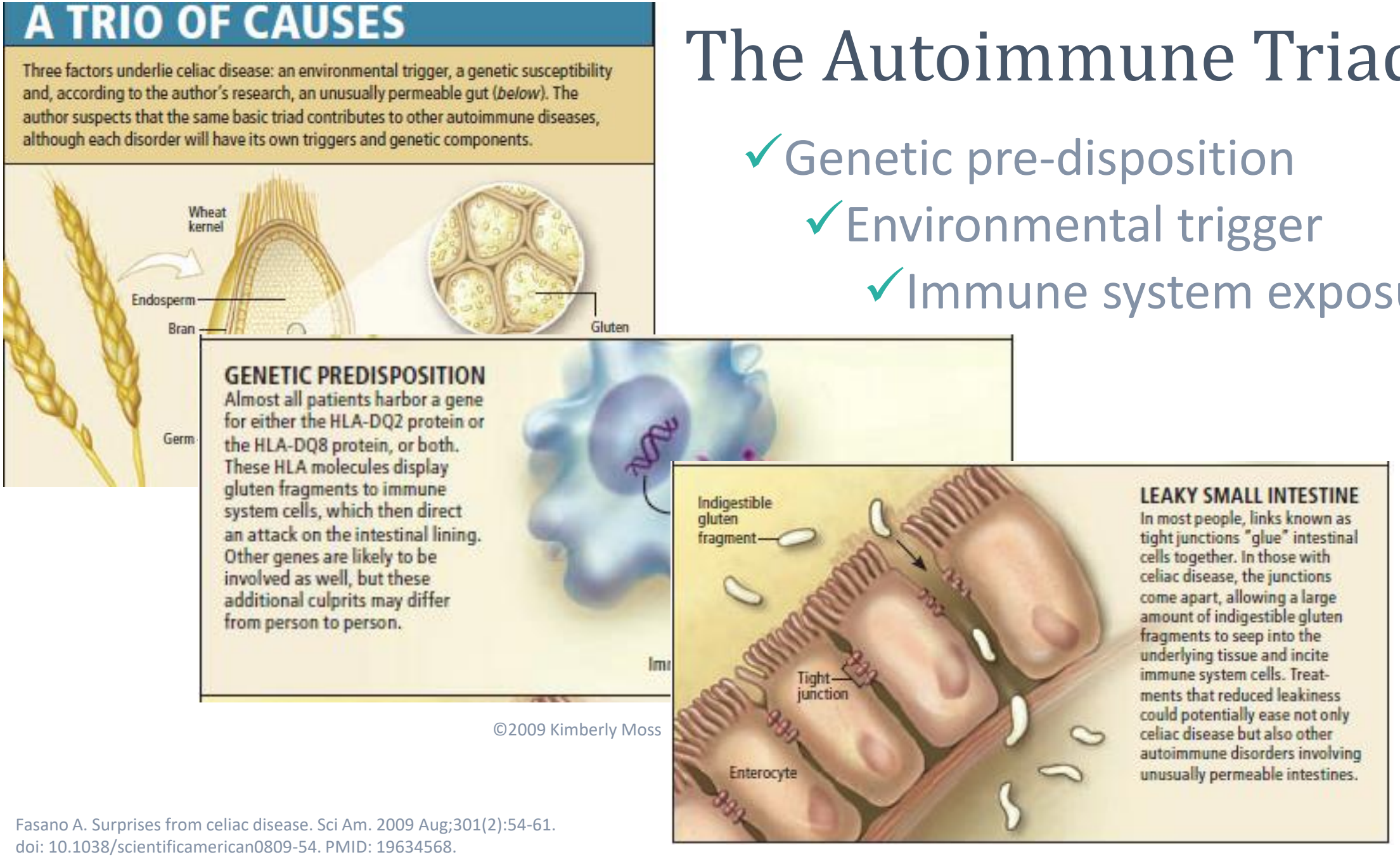
Systemic Immune Response

Initiation of the Autoimmune Spectrum

Valitutti F, Fasano A, Breaking Down Barriers: How Understanding Celiac Disease Pathogenesis Informed the Development of Novel Treatments. Dig Dis Sci. 2019 Jul;64(7):1748-1758

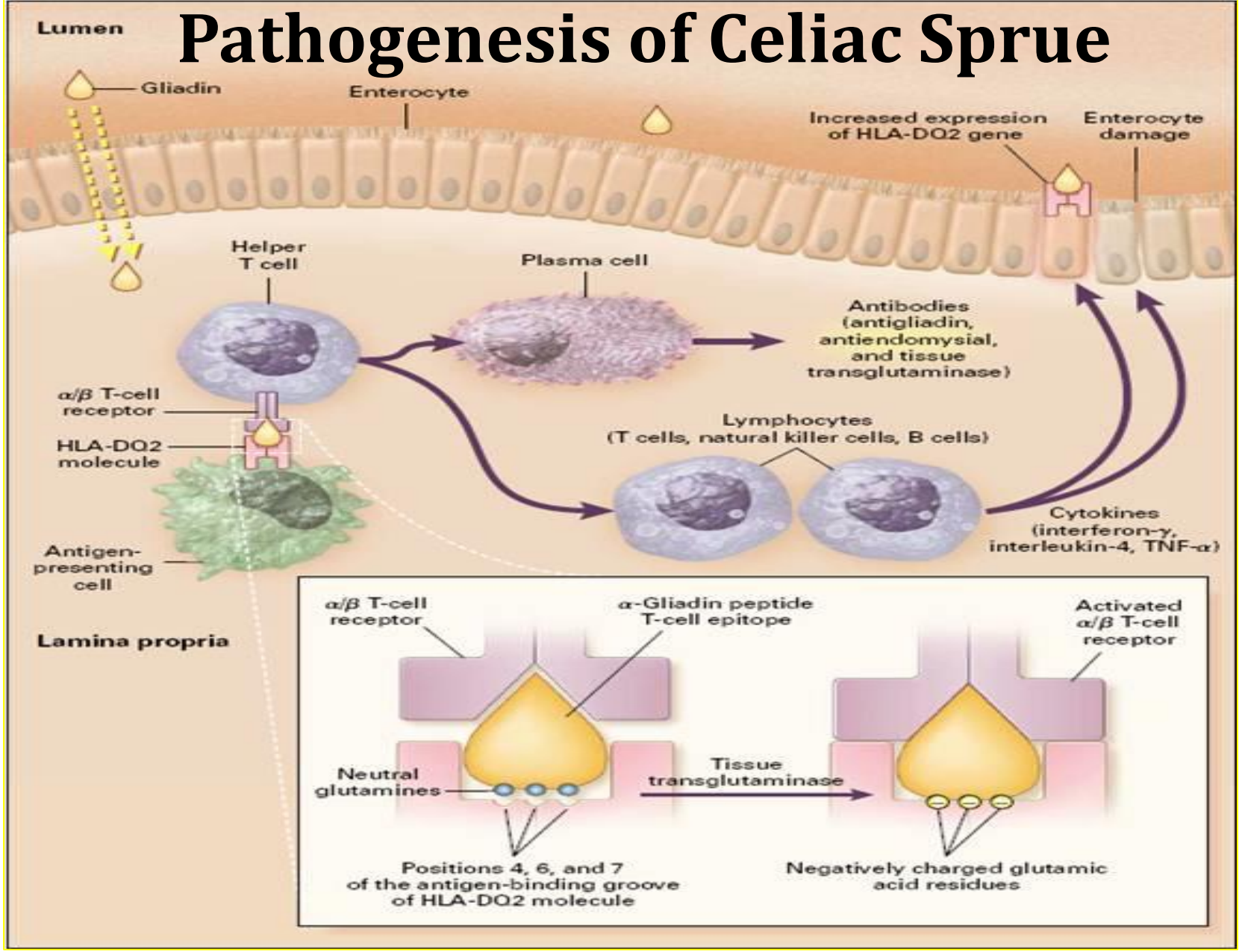
The Autoimmune Triad:

- ✓Genetic pre-disposition
- ✓Environmental trigger
- ✓Immune system exposure



Fasano A. Surprises from celiac disease. Sci Am. 2009 Aug;301(2):54-61. doi: 10.1038/scientificamerican0809-54. PMID: 19634568.

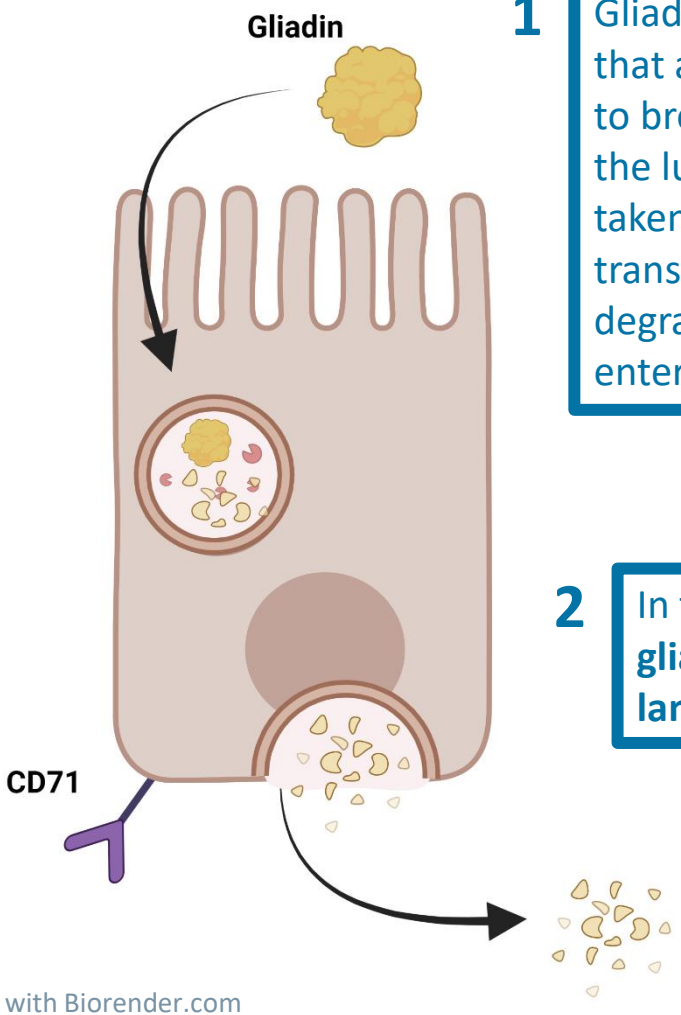
Pathogenesis of Celiac Sprue



1. Westerberg DP, Gill JM, Dave B, DiPrinzio MJ, Quisel A, Foy A. New strategies for diagnosis and management of celiac disease. J Am Osteopath Assoc. 2006 Mar;106(3):145-51. Used with permission.
2. Image: From New England Journal of Medicine, Farrell R. J., Kelly C. P., Celiac sprue, Volume 346, Page 180-8. Copyright© 2002 Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society.
3. Kagnoff MF. Celiac disease: pathogenesis of a model immunogenetic disease. *Journal of Clinical Investigation*. 2007;117(1):41-49. doi:10.1172/JCI30253.

Transport of Gliadin Peptides in Celiac Disease

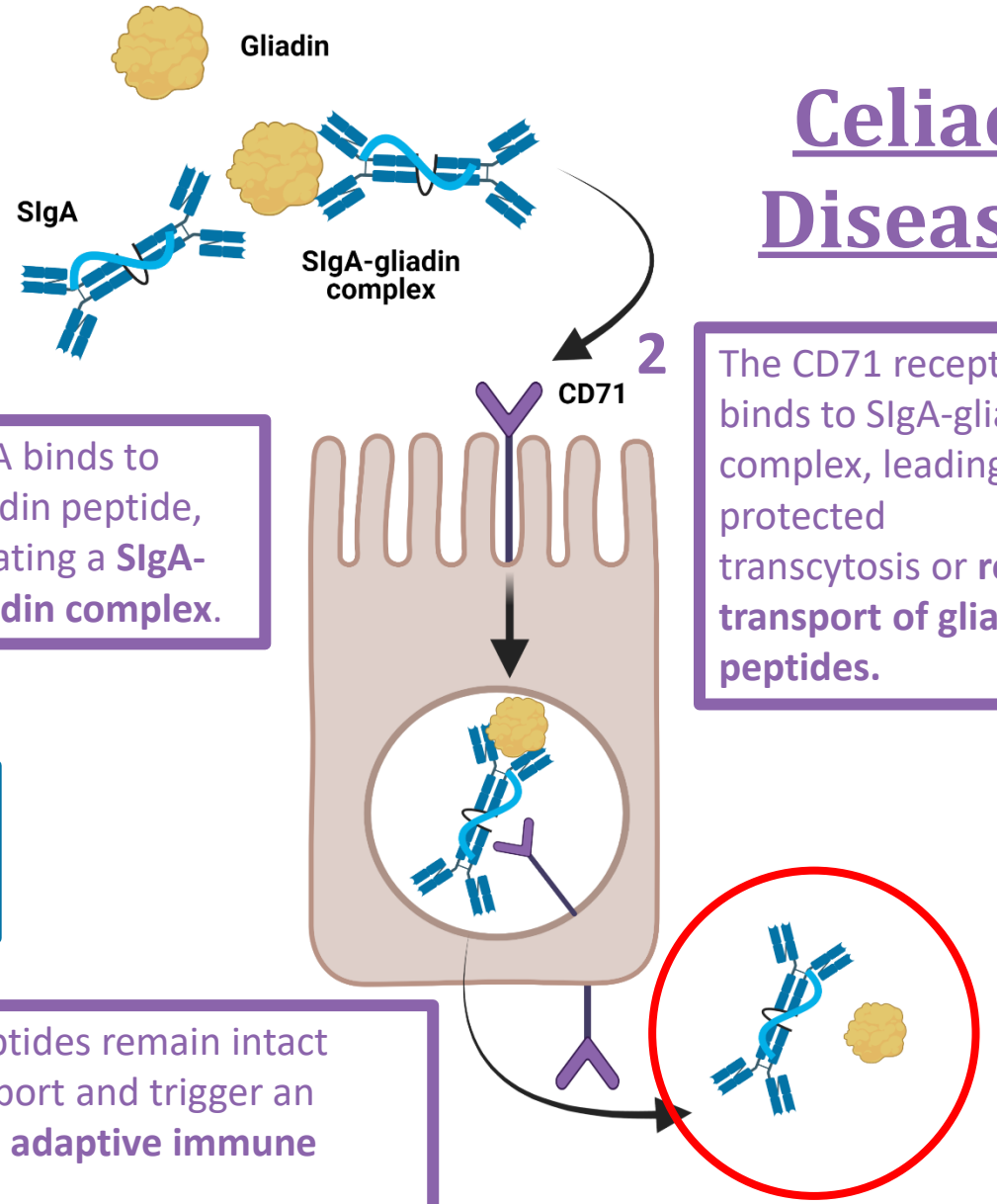
Healthy



1 Gliadin peptides, that are resistant to breakdown in the lumen, are taken up, transported, and degraded via enterocytosis

2 In this process, **very few gliadin peptides reach the lamina propria**

Celiac Disease



1 SIgA binds to gliadin peptide, creating a **SIgA-gliadin complex**.

2 The CD71 receptor binds to SIgA-gliadin complex, leading to protected transcytosis or **retro-transport of gliadin peptides.**

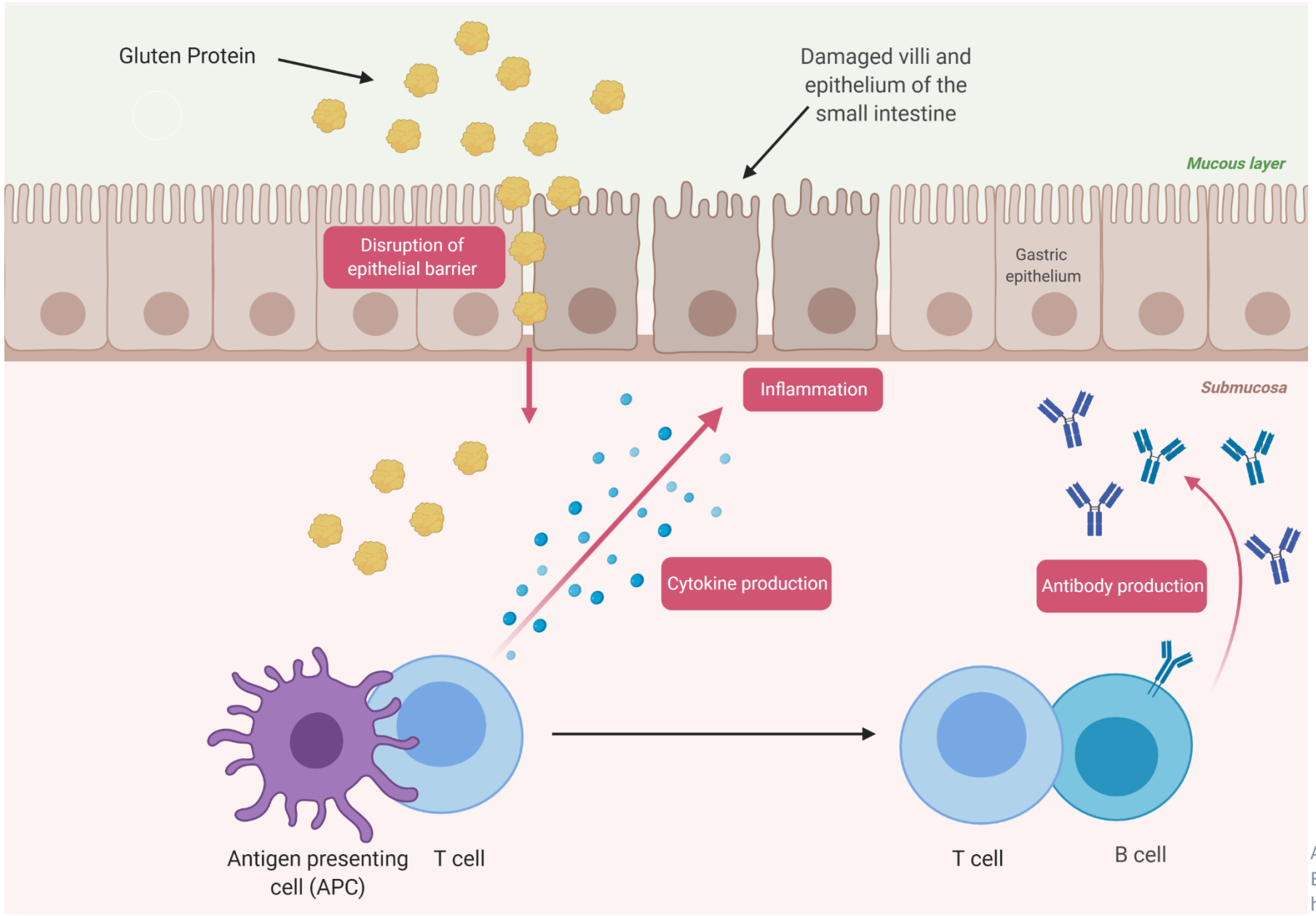
3 Gliadin peptides remain intact after transport and trigger an **innate and adaptive immune response**

Created with Biorender.com

See References: Transport of Gliadin Peptides in Celiac Disease

References: Transport of Gliadin Peptides in Celiac Disease

1. Ménard S, Cerf-Bensussan N, Heyman M. Multiple facets of intestinal permeability and epithelial handling of dietary antigens. Mucosal Immunol. 2010 May;3(3):247-59. doi: 10.1038/mi.2010.5.
2. Caio G, Volta U, Sapone A, Leffler DA, De Giorgio R, Catassi C, Fasano A. Celiac disease: a comprehensive current review. BMC Med. 2019 Jul 23;17(1):142. doi: 10.1186/s12916-019-1380-z. PMID: 31331324; PMCID: PMC6647104.
3. Matysiak-Budnik T, Moura IC, Arcos-Fajardo M, Lebreton C, Ménard S, Candalh C, Ben-Khalifa K, Dugave C, Tamouza H, van Niel G, Bouhnik Y, Lamarque D, Chaussade S, Malamut G, Cellier C, Cerf-Bensussan N, Monteiro RC, Heyman M. Secretory IgA mediates retrotranscytosis of intact gliadin peptides via the transferrin receptor in celiac disease. J Exp Med. 2008 Jan 21;205(1):143-54. doi: 10.1084/jem.20071204. Epub 2007 Dec 31. PMID: 18166587; PMCID: PMC2234361.



Adapted from "H, Pylori Pathogenesis", by BioRender.com (2020). Retrieved from https://app.biorender.com/biorender-templates

1. Bethune MT, Khosla C. Parallels between pathogens and gluten peptides in celiac sprue. PLoS Pathog. 2008 Feb;4(2):e34. doi: 10.1371/journal.ppat.0040034. PMID: 18425213; PMCID: PMC2323203.
2. Cardoso-Silva D, Delbue D, Itzlinger A, Moerkens R, Withoff S, Branchi F, Schumann M. Intestinal Barrier Function in Gluten-Related Disorders. Nutrients. 2019 Oct 1;11(10):2325. doi: 10.3390/nu11102325. PMID: 31581491; PMCID: PMC6835310.

Pathogenesis of Celiac

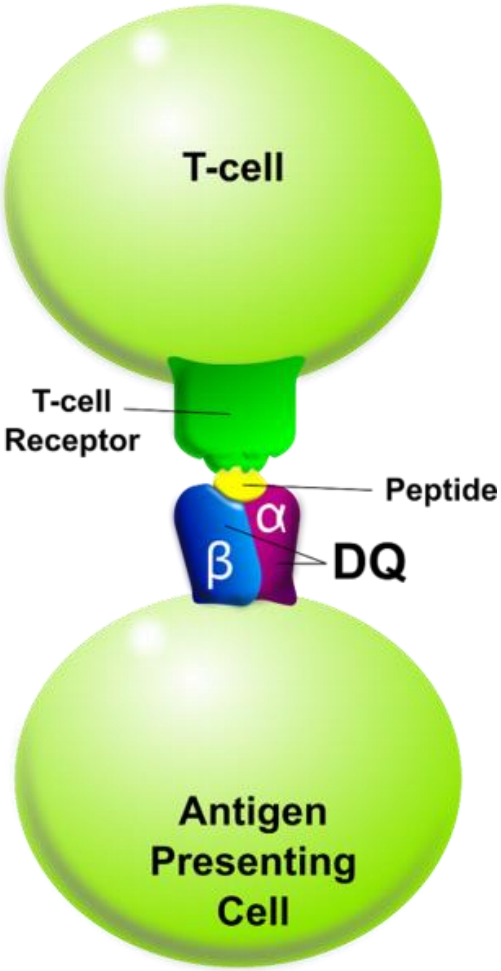
- Gliadin is absorbed into the lamina propria and presented in conjunction with HLA-DQ2 or DQ8 cell-surface antigens by antigen-presenting cells (dendritic cells), to sensitized T cells expressing the α/β T-cell receptor.
- Tissue transglutaminase deamidates gliadin peptides, generating acidic, negatively charged residues of glutamic acid from neutral glutamines (inset). Since negatively charged residues are preferred in positions 4,6, and 7 of the antigen-binding groove of HLA-DQ2, deaminated gliadin elicits a stronger T-cell response.
- These lymphocytes then activate other lymphocytes to generate cytokines, such as interferon-γ, interleukin-4, and tumor necrosis factor α (TNF-α), which damage the villi, resulting in enteritis. Induction of aberrant HLA class II cell-surface antigens on the enterocytes may permit these cells to present additional antigens to the sensitized lymphocytes.

1. Bascuñán KA, Araya M, Roncoroni L, Doneda L, Elli L. Dietary Gluten as a Conditioning Factor of the Gut Microbiota in Celiac Disease. Adv Nutr. 2020;11(1):160-174. doi:10.1093/advances/nmz080
2. Yu X, Vargas J, Green PHR, Bhagat G. Innate Lymphoid Cells and Celiac Disease: Current Perspective [published online ahead of print, 2020 Dec 10]. Cell Mol Gastroenterol Hepatol. 2020;11(3):803-814. doi:10.1016/j.jcmgh.2020.12.002

The HLA effect

- Certain HLA-DQ allotypes (DQ2, DQ8) predispose to celiac disease by **presenting posttranslationally modified (deamidated) gluten peptides to CD4+ T cells.**

- The deamidation of gluten peptides is mediated by transglutaminase 2.



HLA DQ Receptor with bound peptide and TCR by Davide.pirolli is licensed under CC BY-SA 3.0.

1. Offord C. The Celiac Surge. The Scientist. June 2017. Available at: http://www.the-scientist.com/?articles.view/articleNo/49467/title/The-Celiac-Surge/. Accessed October 5, 2017.
2. Sollid LM. The roles of MHC class II genes and post-translational modification in celiac disease. Immunogenetics. 2017 Aug;69(8-9):605-616. doi: 10.1007/s00251-017-0985-7.

Genetic Predispositions for Celiac Disease

- **HLA-DQ2 and/or DQ8 haplotypes:** Considered to account for **up to 40% of the disease heritability**
  - General Population Statistics: About 1/3 of the general population carries the HLA susceptibility genes
    - However, only 2% to 5% of people with these genes develop clinically evident celiac disease.
    - >98% of those with Celiac disease have either HLA-DQ2 or –DQ8
- CD patients **also tend to have other genetic predispositions:** overproduction of IL-15 and harbor hyperactive immune cells that prime immune system to attack the gut in response to gluten.

References see: "References: Celiac Disease"

References: Celiac Disease

1. Almeida LM, Gandolfi L, Pratesi R, et al. Presence of DQ2.2 Associated with DQ2.5 Increases the Risk for Celiac Disease. *Autoimmune Diseases*. 2016;2016:5409653. doi:10.1155/2016/5409653.
2. Fasano A. Surprises from celiac disease. Sci Am. 2009 Aug;301(2):54-61.
3. Kochhar GS, Singh T, Gill A, Kirby DF. Celiac disease: an internist's perspective. Cleve Clin J Med 2016; 83:217–227.
4. Cecilio LA, Bonatto MW. The prevalence of HLA DQ2 and DQ8 in patients with celiac disease, in family and in general population. *Arq Bras Cir Dig*. 2015;28(3):183–185. doi:10.1590/S0102-67202015000300009.
5. Sollid LM. Molecular basis of celiac disease. Annu Rev Immunol 2000;18:53–81.
6. Kagnoff MF. Celiac disease: pathogenesis of a model immunogenetic disease. J Clin Invest. 2007 Jan;117(1):41-9.
7. Sciurti M, Fornaroli F, Gaiani F, Bonaguri C, Leandro G, Di Mario F, De' Angelis GL. Genetic susceptibilty and celiac disease: what role do HLA haplotypes play? Acta Biomed. 2018 Dec 17;89(9-S):17-21. doi: 10.23750/abm.v89i9-S.7953. PMID: 30561391; PMCID: PMC6502200.

Genetics in Celiac Disease

Celiac genes: **HLA DQ2 & DQ8**

>95% DQ2

>7% DQ8

- Estimated that 0.5% of celiac patients lack DQ2 and/or DQ8
- 30-40% of susceptible populations carry these variants

References see: “References: Genetics in Celiac Disease”

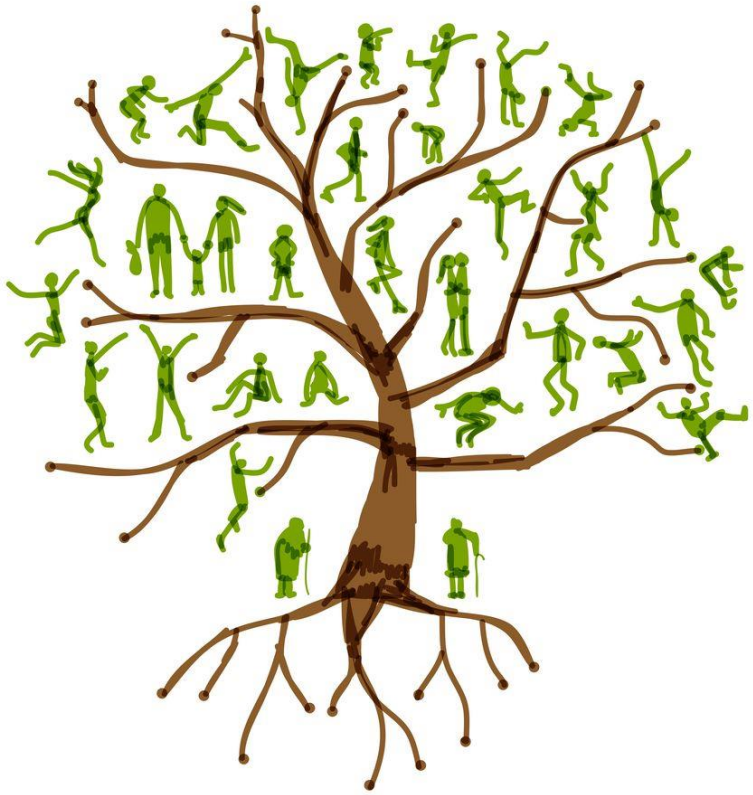
References: Genetics in Celiac Disease

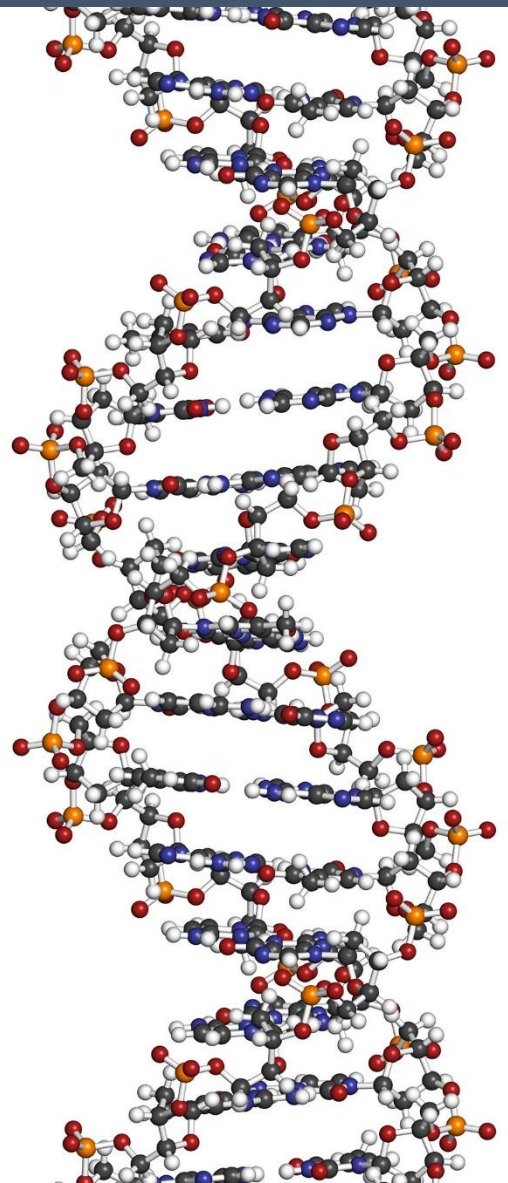
- Pietzak MM, Schofield TC, McGinniss MJ, Nakamura RM. Stratifying risk for celiac disease in a large at-risk United States population by using HLA alleles. Clin Gastroenterol Hepatol. 2009 Sep;7(9):966-71. doi: 10.1016/j.cgh.2009.05.028.
- Sollid LM, Lie BA. Celiac disease genetics: current concepts and practical applications. Clin Gastroenterol Hepatol. 2005;3(9):843–851.
- U. Lindqvist, Å. Rudsander, Å. Boström, B. Nilsson, G. Michaëlsson; IgA antibodies to gliadin and coeliac disease in psoriatic arthritis, Rheumatology, Volume 41, Issue 1, 1 January 2002, Pages 31–37, https://doi.org/10.1093/rheumatology/41.1.31
- Karell K, Louka AS, Moodie SJ, Ascher H, Clot F, Greco L, Ciclitira PJ, Sollid LM, Partanen J; European Genetics Cluster on Celiac Disease. HLA types in celiac disease patients not carrying the DQA1*05-DQB1*02 (DQ2) heterodimer: results from the European Genetics Cluster on Celiac Disease. Hum Immunol. 2003 Apr;64(4):469-77.
- Cecilio LA, Bonatto MW. The prevalence of HLA DQ2 and DQ8 in patients with celiac disease, in family and in general population. *Arq Bras Cir Dig*. 2015;28(3):183–185. doi:10.1590/S0102-67202015000300009.

There are numerous gene variants (SNPs) that ‘predispose’ a significant percentage of the population to autoimmune development. However, only a fraction go on to develop full-blown autoimmune disorders.

Other mitigating factors (triggers & mediators) must be involved…

“Genes load the gun, environment pulls the trigger”





What in the **Environment** Pulls the Trigger and leads to Gluten and/or Wheat-related Disorders?

Trigger: Modern Wheat?

- There was an understanding that modern wheat had a higher levels of 33-mer gliadin peptide and higher ATI activity.
- However, findings showed that the modern variety did not compromise barrier function or contribute to gut inflammation compared to its heirloom predecessor.
- A survey of data from the 20th and 21st centuries for the United States was carried out. **The results do not support the likelihood that wheat breeding has increased the protein content (proportional to gluten content) of wheat in the United States.**

References see: “References: Trigger of Modern Wheat?”

Modern Wheat is not a Trigger for Celiac Disease

- Modern wheat is not more toxic for celiac patients and breeding does not seem to be related with higher prevalence of celiac disease.
  - Future research should identify currently unknown environmental factors in CD.
  - Further studies are urgently required, particularly from a wider range of research groups, but also on a wider range of genotypes of ancient and modern wheat species.

References see: “References: Trigger of Modern Wheat?”

References: Trigger of Modern Wheat?

1. Ribeiro M, Nunes FM. We might have got it wrong: Modern wheat is not more toxic for celiac patients. Food Chem. 2019 Apr 25;278:820-822. doi: 10.1016/j.foodchem.2018.12.003.
2. Shewry PR. Do ancient types of wheat have health benefits compared with modern bread wheat?. J Cereal Sci. 2018;79:469–476. doi:10.1016/j.jcs.2017.11.010
3. Keirns BH, Anderson KL, Ojo BA, Washburn KF, El-Rassi GD, Lightfoot SA, Carver BF, Lucas EA, Smith BJ. A Comparative Study of Modern and Heirloom Wheat on Indicators of Gastrointestinal Health. J Agric Food Chem. 2019 Dec 26;67(51):14027-14037. doi: 10.1021/acs.jafc.9b05851. Epub 2019 Dec 16. PMID: 31771323.
4. Kasarda DD. Can an increase in celiac disease be attributed to an increase in the gluten content of wheat as a consequence of wheat breeding?. J Agric Food Chem. 2013;61(6):1155–1159. doi:10.1021/jf305122s
5. Pronin D, Börner A, Scherf KA. Old and modern wheat (Triticum aestivum L.) cultivars and their potential to elicit celiac disease. Food Chem. 2021 Mar 1;339:127952. doi: 10.1016/j.foodchem.2020.127952. Epub 2020 Sep 12. PMID: 33152854.
6. Fasano, A. et al. (2015). Nonceliac Gluten Sensitivity. *Gastroenterology*, Mar. 2015, 1-10.
7. Sapone, A. et al. (2012). Spectrum of gluten-related disorders: consensus on new nomenclature and classification. *BMC Medicine* 2012, 10:13.

Old vs. Modern Wheat Varieties: *No increase in gluten toxicity*

- **Study:** Analyzed gluten peptides generated by in vitro digestion of different old and modern Triticum varieties, using LC-MS. Peptides containing CD epitopes were quantified in all samples with LC-MS.
- **Conclusion:**
  - In fact, old varieties analyzed produced a higher quantity of peptides containing immunogenic and toxic sequences than modern ones. (Old wheat lines are not to be considered "safer" for subjects that are genetically predisposed to celiac disease.)
  - Peptide profiles were similar between modern and old Triticum varieties.

Prandi B, Tedeschi T, Folloni S, Galaverna G, Sforza S. Peptides from gluten digestion: A comparison between old and modern wheat varieties. Food Res Int. 2017 Jan;91:92-102. doi: 10.1016/j.foodres.2016.11.034.

Trigger: Modern Food Technology?

- Cereal food technology has changed dramatically.
- Modern Manufacturing: Accelerated Process
- Longer fermentations by sourdough (acidifying and proteolytic lactic acid bacteria with or without *Saccharomyces cerevisiae*) are mostly replaced by chemical and/or baker's yeast leavening agents.
- Cereal proteins are subjected to *very mild or absent degradation* during manufacturing → potentially less digestible foods compared to traditional and ancient sourdough baked goods

Gobbetti M. The sourdough microflora: Interactions of lactic acid bacteria and yeasts. Trends Food Sci. Tech. 1998;9:267–274. doi: 10.1016/S0924-2244(98)00053-3.

Trigger: Modern Food Technology? Microwave treatment of gluten

- The microwave treatment increased the amount of potentially toxic epitopes released after peptic and tryptic digestion, showing inefficiency as a treatment to detoxify the gluten for celiac disease patients.
- Gluten secondary structure was affected by the microwave treatment and related to the polymer's disaggregation phenomenon observed.

Mahroug H, et al. How microwave treatment of gluten affects its toxicity for celiac patients? A study on the effect of microwaves on the structure, conformation, functionality and immunogenicity of gluten. Food Chem. 2019 Nov 1;297:124986. doi: 10.1016/j.foodchem.2019.124986.

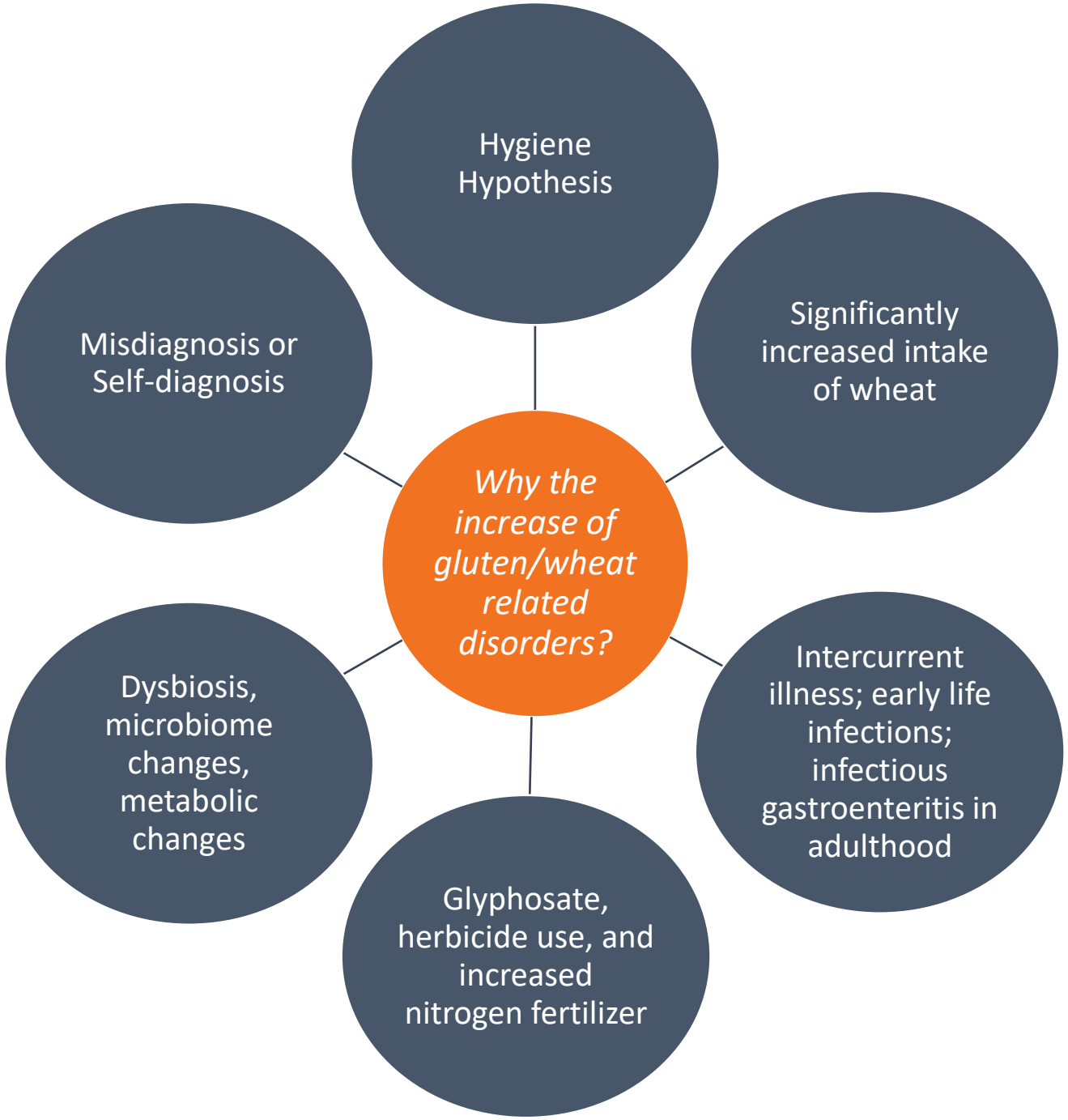
Trigger: Timing of Exposure?

…NOT TIMING

- Several *retrospective studies* have suggested that breastfeeding, modality of delivery, and time of gluten introduction in the diet of infants at risk for CD may affect the incidence of the disease.
  - **However, the data supporting the role of these factors in the risk of developing CD is limited.**
- **Neither the early introduction of gluten nor breast-feeding increased the risk of celiac disease among at-risk infants.**
  - The early introduction of gluten (@ 4 months) was associated with a decreased incidence of celiac disease.
  - Although, the later introduction of gluten was associated with a delayed onset of disease.

References – See "References: More Gluten as Trigger"

So what is it?

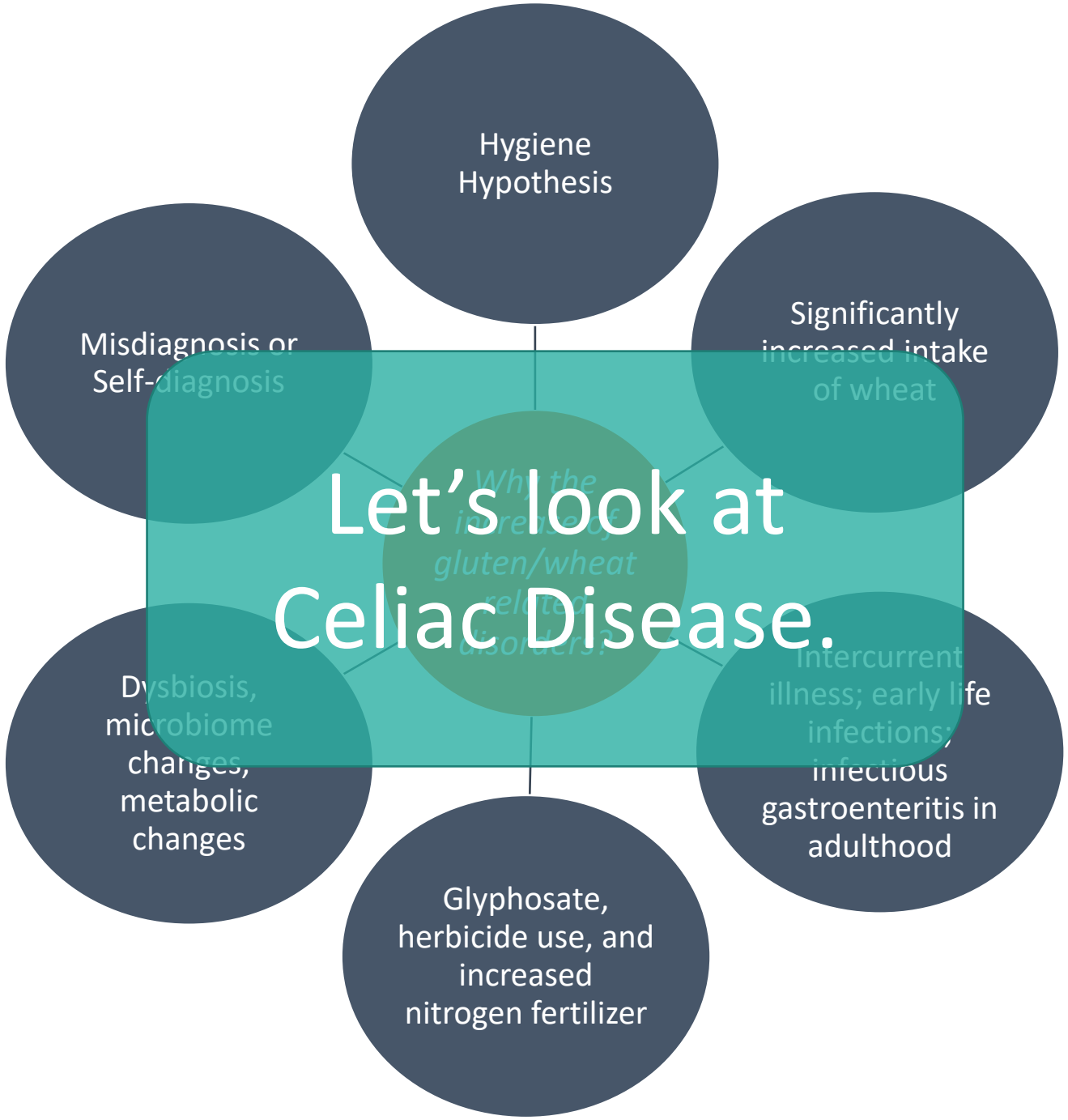


See References: "References: Why the Increase Graphic"

References: Why the Increase

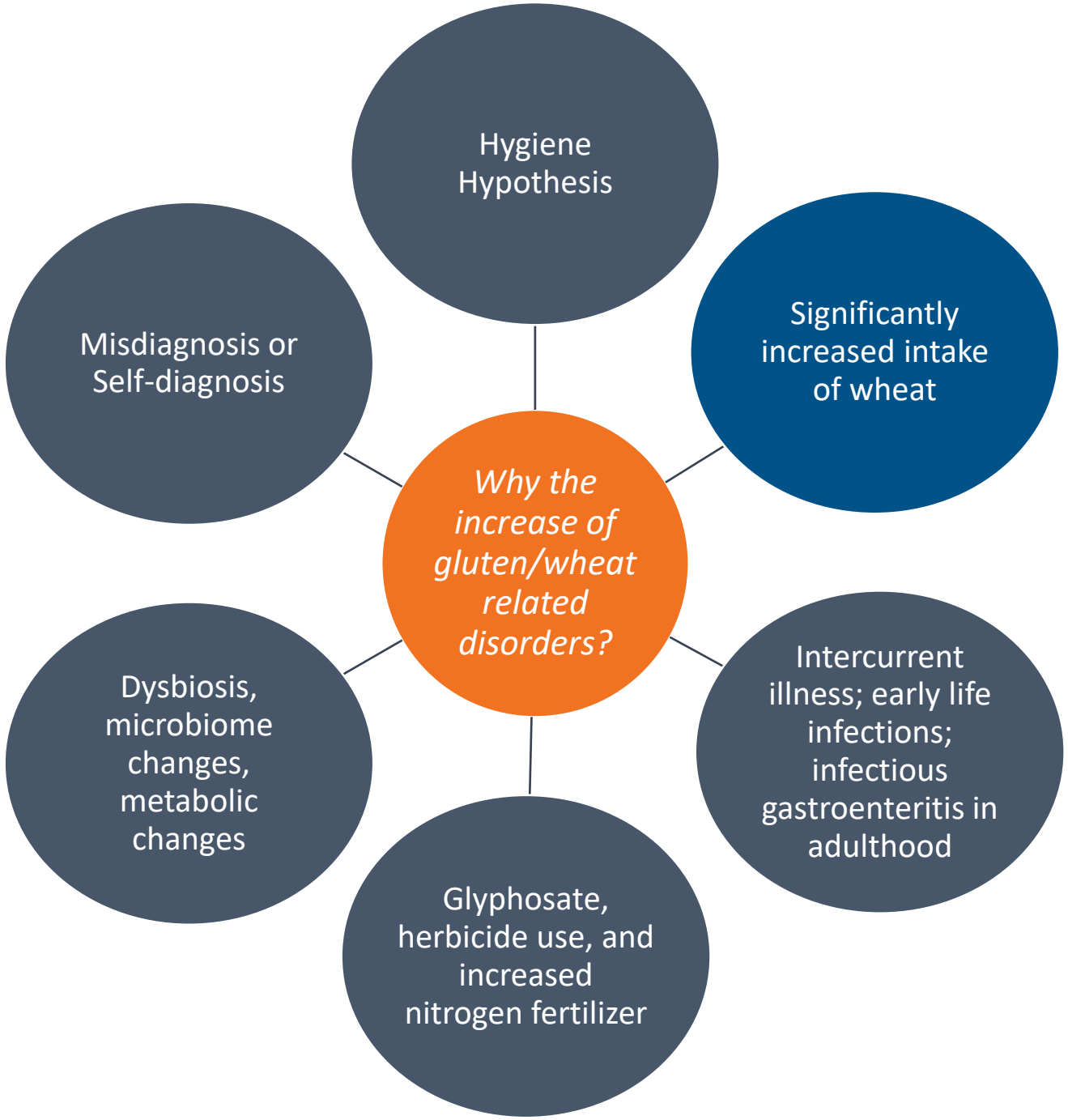
1. Hygiene Hypothesis: Riddle MS, Murray JA, Porter CK. The Incidence and Risk of Celiac Disease in a Healthy US Adult Population. The American journal of gastroenterology. 2012;107(8):1248-1255. doi:10.1038/ajg.2012.130.
2. Dr. FAQ: Stefano Guandalini on the Rise of Celiac Disease. Science Life. 2017. Available at: https://sciencelife.uchospitals.edu/2014/02/05/dr-faq-stefano-guandalini-on-the-rise-of-celiac-disease/. Accessed October 4, 2017.
3. Samsel A, Seneff S. Glyphosate, pathways to modern diseases II: Celiac sprue and gluten intolerance. Interdisciplinary Toxicology. 2013;6(4):159-184. doi:10.2478/intox-2013-0026.
4. Rubio-Tapia A, Kyle RA, Kaplan EL, et al. Increased Prevalence and Mortality in Undiagnosed Celiac Disease. Gastroenterology. 2009;137(1):88-93. doi:10.1053/j.gastro.2009.03.059.
5. Green PH, Cellier C. Celiac disease. N Engl J Med 2007; 357:1731–1743.
6. Andrén Aronsson C, Lee HS, Hård Af Segerstad EM, Uusitalo U, Yang J, Koletzko S, Liu E, Kurppa K, Bingley PJ, Toppari J, Ziegler AG, She JX, Hagopian WA, Rewers M, Akolkar B, Krischer JP, Virtanen SM, Norris JM, Agardh D; TEDDY Study Group. Association of Gluten Intake During the First 5 Years of Life With Incidence of Celiac Disease Autoimmunity and Celiac Disease Among Children at Increased Risk. JAMA. 2019 Aug 13;322(6):514-523. doi: 10.1001/jama.2019.10329.
7. Sapone, A. et al. (2012). Spectrum of gluten-related disorders: consensus on new nomenclature and classification. *BMC Medicine* 2012, 10:13.
8. Increased consumption: Fasano, A. et al. (2015). Nonceliac Gluten Sensitivity. Gastroenterology, Mar. 2015, 1-10.
9. Increased consumption: Khamsi, R. (2014). The Trouble with Gluten. Scientific American, Feb. 2014.
10. Increased consumption: Koning F. Celiac disease: quantity matters. Seminars in Immunopathology. 2012;34(4):541-549. doi:10.1007/s00281-012-0321-0.
11. Glyphosate/Herbicide: Samsel A, Seneff S. Glyphosate, pathways to modern diseases II: Celiac sprue and gluten intolerance. Interdisciplinary Toxicology. 2013;6(4):159-184. doi:10.2478/intox-2013-0026.
12. Microbiome Changes: Fasano, A. (2015). Celiac Disease and Gluten-Related Disorders: A Clinical Conversation. *Alternative and Complementary Therapies*, 2015 Feb, Vol. 21:1, 18-21.
13. Infections: Stene LC et al. Rotavirus infection frequency and risk of celiac disease autoimmunity in early childhood: a longitudinal study. Am J Gastroenterol. 2006 Oct;101(10):2333-40.

So what is it?



See References: "References: Why the Increase Graphic"

So what is it?



See References: "References: Why the Increase Graphic"

Increased Consumption

- There have been changes in the per capita consumption of wheat flour and the use of vital gluten as a food additive.
- Wheat and wheat isolates that contain deamidated gluten proteins and/or microbial tTG are pervasive in Westernized diets.

1. Kasarda DD. Can an increase in celiac disease be attributed to an increase in the gluten content of wheat as a consequence of wheat breeding?. J Agric Food Chem. 2013;61(6):1155–1159. doi:10.1021/jf305122s
2. Koning F. Celiac disease: quantity matters. Seminars in Immunopathology. 2012;34(4):541-549. doi:10.1007/s00281-012-0321-0.
3. Andrén Aronsson C, Lee HS, Hård Af Segerstad EM, Uusitalo U, Yang J, Koletzko S, Liu E, Kurppa K, Bingley PJ, Toppari J, Ziegler AG, She JX, Hagopian WA, Rewers M, Akolkar B, Krischer JP, Virtanen SM, Norris JM, Agardh D; TEDDY Study Group. Association of Gluten Intake During the First 5 Years of Life With Incidence of Celiac Disease Autoimmunity and Celiac Disease Among Children at Increased Risk. JAMA. 2019 Aug 13;322(6):514-523. doi: 10.1001/jama.2019.10329.
4. Khamsi, R. (2014). The Trouble with Gluten. *Scientific American*, Feb. 2014.

Trigger: Dose Matters

DOSE MATTERS…

- The higher the level of gluten presentation, the higher the chance to develop CD.
- Reduction of gluten intake may thus be an effective approach to prevent or delay the development of CD.

Koning F. Celiac disease: quantity matters. Seminars in Immunopathology. 2012;34(4):541-549. doi:10.1007/s00281-012-0321-0.

References: More Gluten as Trigger

1. Andrén Aronsson C, Lee HS, Hård Af Segerstad EM, Uusitalo U, Yang J, Koletzko S, Liu E, Kurppa K, Bingley PJ, Toppari J, Ziegler AG, She JX, Hagopian WA, Rewers M, Akolkar B, Krischer JP, Virtanen SM, Norris JM, Agardh D; TEDDY Study Group. Association of Gluten Intake During the First 5 Years of Life With Incidence of Celiac Disease Autoimmunity and Celiac Disease Among Children at Increased Risk. JAMA. 2019 Aug 13;322(6):514-523. doi: 10.1001/jama.2019.10329.
2. Risk of developing CD is limited: Caio G, Volta U, Sapone A, et al. Celiac disease: a comprehensive current review. BMC Med. 2019;17(1):142. Published 2019 Jul 23. doi:10.1186/s12916-019-1380-z
3. Later intro associated with delayed onset: Lionetti E, et al. Introduction of gluten, HLA status, and the risk of celiac disease in children. N Engl J Med. 2014 Oct 2;371(14):1295-303. doi: 10.1056/NEJMoa1400697.
4. Earlier intro associated with decreased incidence: Logan K, et al. Early Gluten Introduction and Celiac Disease in the EAT Study: A Prespecified Analysis of the EAT Randomized Clinical Trial. JAMA Pediatr. 2020;174(11):1041–1047. doi:10.1001/jamapediatrics.2020.2893
5. Lionetti E, et al. Mode of Delivery and Risk of Celiac Disease: Risk of Celiac Disease and Age at Gluten Introduction Cohort Study. J Pediatr. 2017 May;184:81-86.e2. doi: 10.1016/j.jpeds.2017.01.023.

Quantity Matters

Especially under conditions where there is an *increased inflammatory T cell response*…

EXAMPLE: Loss of tolerance to gluten may occur during an infection in the GI tract through the following:

- **Increased HLA Expression:** Pathogen-specific T cells release IFNγ. IFNγ Increases HLA expression on antigen-presenting cells, increasing the level of gluten presentation.
- **Increased active tissue transglutaminase enzyme (TG2):** TG2 is simultaneously released due to local tissue damage, increasing the amount of gluten peptides that can be presented and thus works in concert with the increased HLA expression.
- **Presence of gluten-reactive T cells in the mesenteric lymph nodes:** under the conditions mentioned previously, the pro-inflammatory environment would lead to their activation and, ultimately to **loss of tolerance to gluten.**

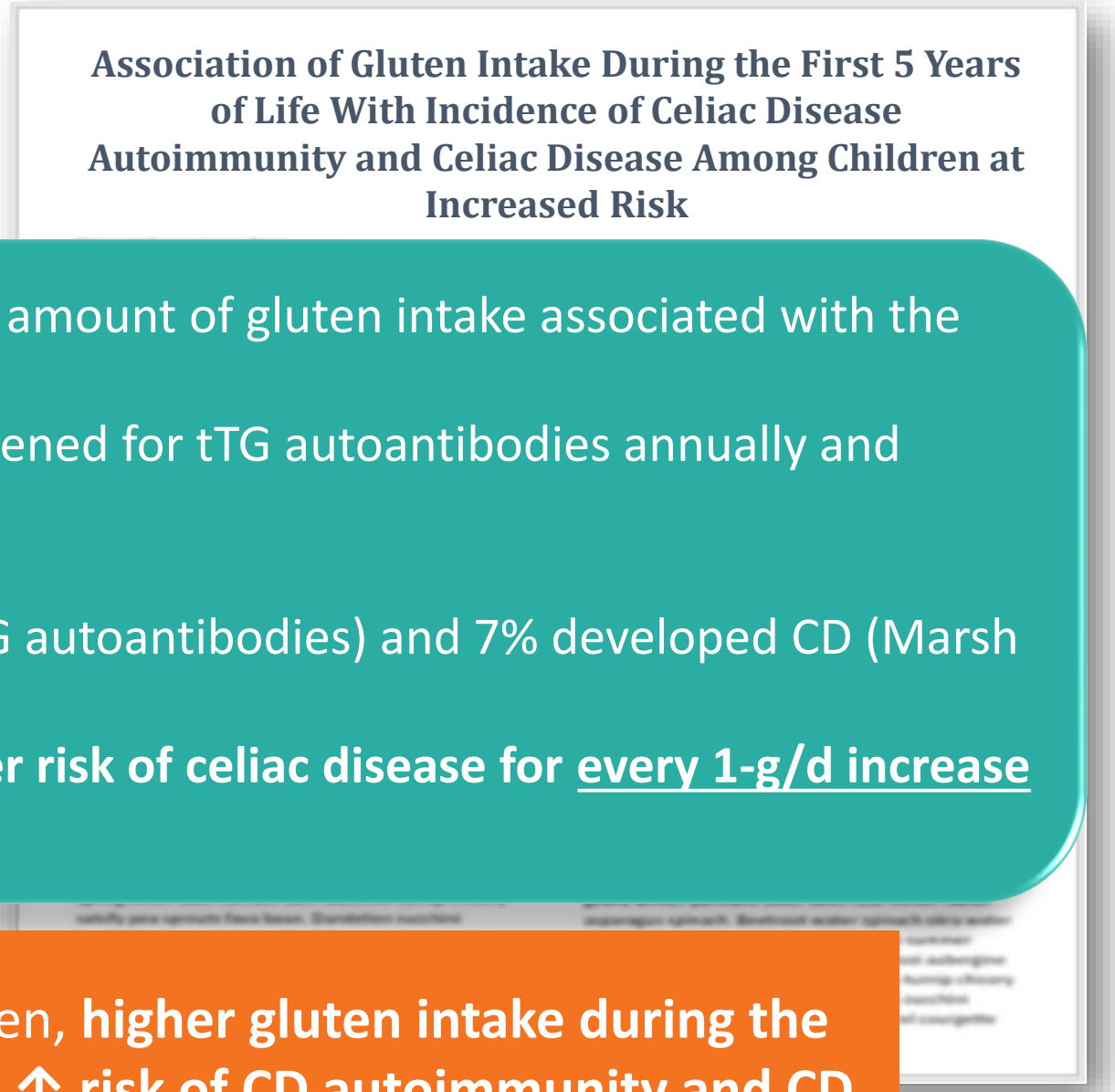
Koning F. Celiac disease: quantity matters. Seminars in Immunopathology. 2012;34(4):541-549. doi:10.1007/s00281-012-0321-0.

Therefore, 3 factors may be involved in the development of celiac disease:

1. High levels of gluten
2. Infection in the GI tract
3. Presence of naïve gluten-reactive T cells in mucosal tissue

Koning F. Celiac disease: quantity matters. Seminars in Immunopathology. 2012;34(4):541-549. doi:10.1007/s00281-012-0321-0.

Quantity Matters


Association of Gluten Intake During the First 5 Years of Life With Incidence of Celiac Disease Autoimmunity and Celiac Disease Among Children at Increased Risk

Prospective observational study investigating the amount of gluten intake associated with the development T1DM and CD in at risk infants.

- N = 6605 infants with HLA genotype were screened for tTG autoantibodies annually and gluten intake was tracked over 5 years.

Results:

- 18% developed CD autoimmunity (positive tTG autoantibodies) and 7% developed CD (Marsh score of >2 on biopsy).
- **Daily gluten intake was associated with higher risk of celiac disease for <u>every 1-g/d increase in gluten consumption.</u>**

Among genetically predisposed children, **higher gluten intake during the first 5 years of life was associated with ↑ risk of CD autoimmunity and CD.**

Andrén Aronsson C, Lee HS, Hård Af Segerstad EM, Uusitalo U, Yang J, Koletzko S, Liu E, Kurppa K, Bingley PJ, Toppari J, Ziegler AG, She JX, Hagopian WA, Rewers M, Akolkar B, Krischer JP, Virtanen SM, Norris JM, Agardh D; TEDDY Study Group. Association of Gluten Intake During the First 5 Years of Life With Incidence of Celiac Disease Autoimmunity and Celiac Disease Among Children at Increased Risk. JAMA. 2019 Aug 13;322(6):514-523. doi: 10.1001/jama.2019.10329.

Quantity Matters…

- In 2019, the University of Chicago Celiac Disease Center recommended new guidelines for introducing gluten to infants at risk for CD.
- At risk infants:
  - First degree family member
  - Newborn with HLADQ 2.5 or HLADQ8 genes
- They concluded that **consuming large amounts of gluten** in the first 2 years of life favors the onset of CD in at risk infants.

Guandalini S. New Infant? New Guidelines: Feeding Infants at Risk for Celiac Disease. Impact. 2019.

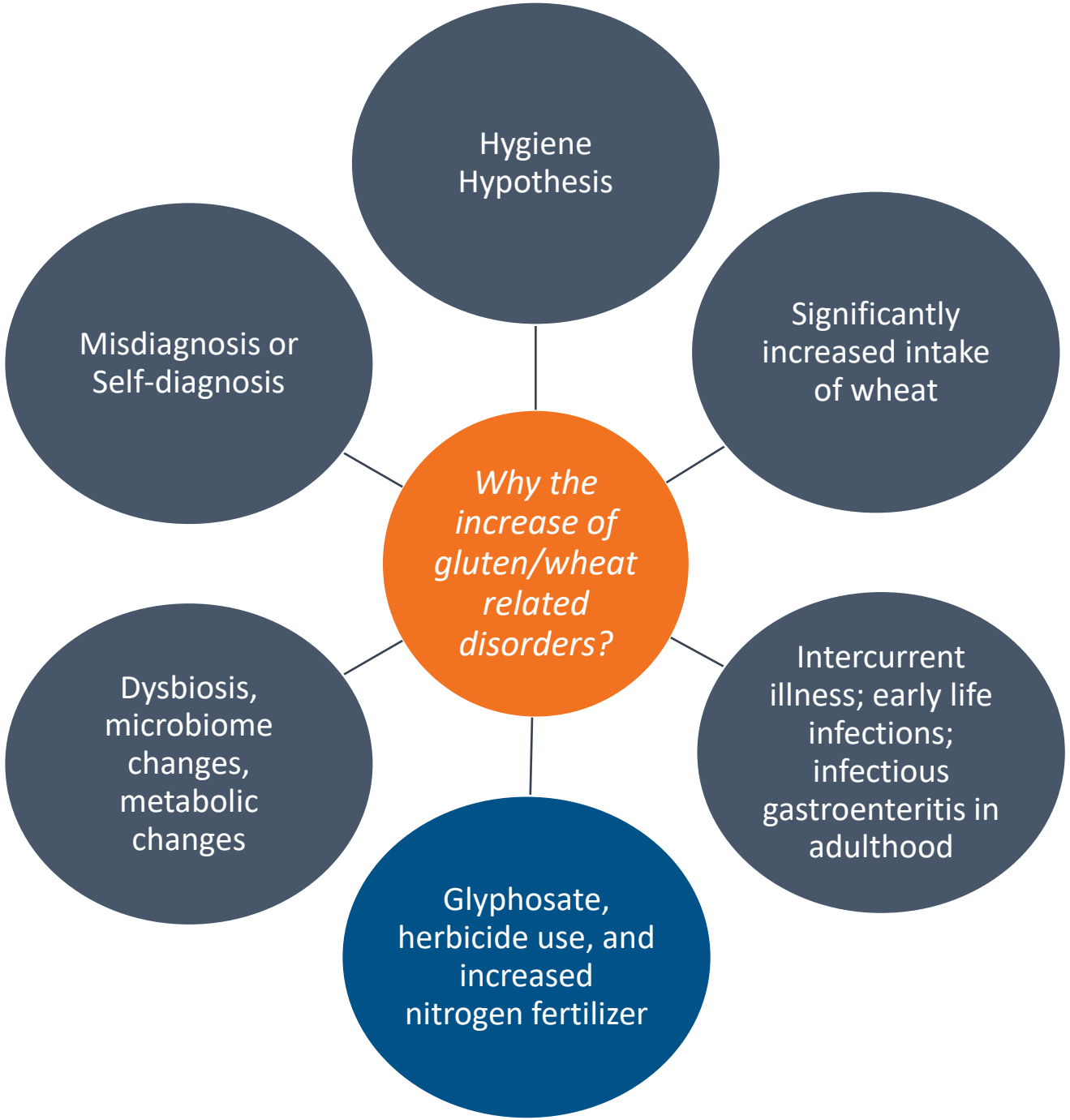
Quantity Matters…

Gluten introduction in infants at risk for celiac disease:

- **2010 recommendation:** gluten should be introduced into the diet of infants 4-6 months old, but no particular quantity is recommended.

- **2019 recommendation: less than 5 grams of gluten per day** at the start of food introduction (at 4-to-6 months) through the age of 2 years old is recommended .

1. Radlovic NP, Mladenovic MM, Lekovic ZM, Stojsic ZM, Radlovic VN. Influence of early feeding practices on celiac disease in infants. *Croat Med J*. 2010;51(5):417-422. doi:10.3325/cmj.2010.51.417
2. Guandalini S. New Infant? New Guidelines: Feeding Infants at Risk for Celiac Disease. Impact. 2019.



See References: "References: Why the Increase Graphic"

Increased Application of Nitrogen Fertilizer to Wheat

- Increased application of nitrogen fertilizer to wheat which has been shown to increase proportion of gliadin proteins and dough extensibility.
- A global meta-analysis indicated that:
  - wheat plants growing in soils receiving higher doses of N fertilizer leads to higher yield but also grains and flours that contain higher total gluten, total gliadin, α/β-gliadin, γ-gliadin and ω-gliadin contents and higher gliadin transcription in their grain.
  - Per capita annual average intake of gliadins from wheat and derived foods and found that it increased from 1961 to 2010 from approximately 2.4 to 3.8 kg y-1 per capita (or 58 ± 7.5%).
    - And this increase was positively correlated with the increase in the rates of coeliac disease in all the available studies with temporal series of coeliac disease.

1. Meta-analysis: Penuelas J, Gargallo-Garriga A, Janssens IA, Ciais P, Obersteiner M, Klem K, Urban O, Zhu YG, Sardans J. Could Global Intensification of Nitrogen Fertilisation Increase Immunogenic Proteins and Favour the Spread of Coeliac Pathology? Foods. 2020 Nov 4;9(11):1602. doi: 10.3390/foods9111602. PMID: 33158083; PMCID: PMC7694225.
2. Godfrey D, Hawkesford MJ, Powers SJ, Millar S, Shewry PR. Effects of crop nutrition on wheat grain composition and end use quality. J Agric Food Chem. 2010 Mar 10;58(5):3012-21. doi: 10.1021/jf9040645.

Increased Use of Chemicals/Pesticides

Glyphosate/herbicide use prior to harvest: microbiome changes may change gliadin immunogenicity and alter microbiome.

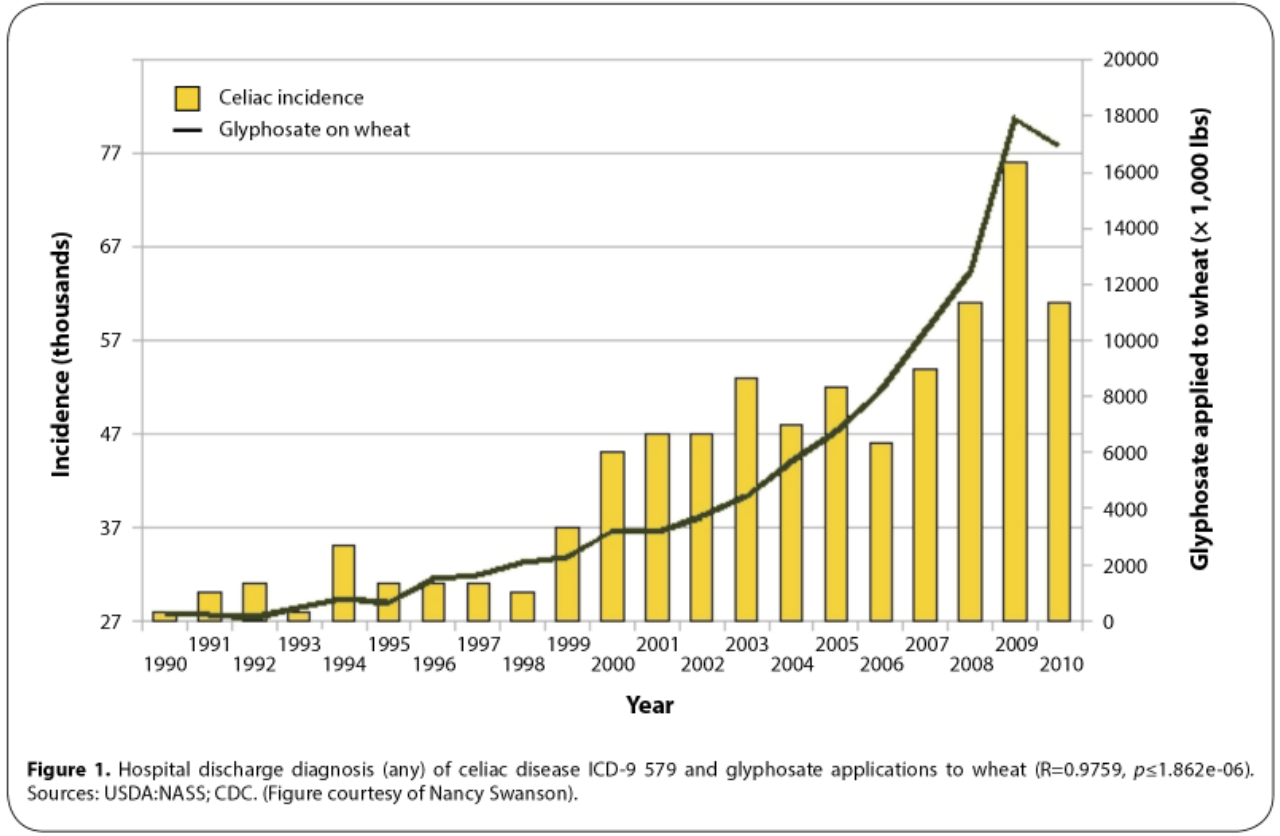


Figure 1. Hospital discharge diagnosis (any) of celiac disease ICD-9 579 and glyphosate applications to wheat (R=0.9759, $p \leq 1.862e\text{-}06$). Sources: USDA:NASS; CDC. (Figure courtesy of Nancy Swanson).

Samsel A, Seneff S. Glyphosate, pathways to modern diseases II: Celiac sprue and gluten intolerance. Interdisciplinary Toxicology. 2013;6(4):159-184. doi:10.2478/intox-2013-0026.

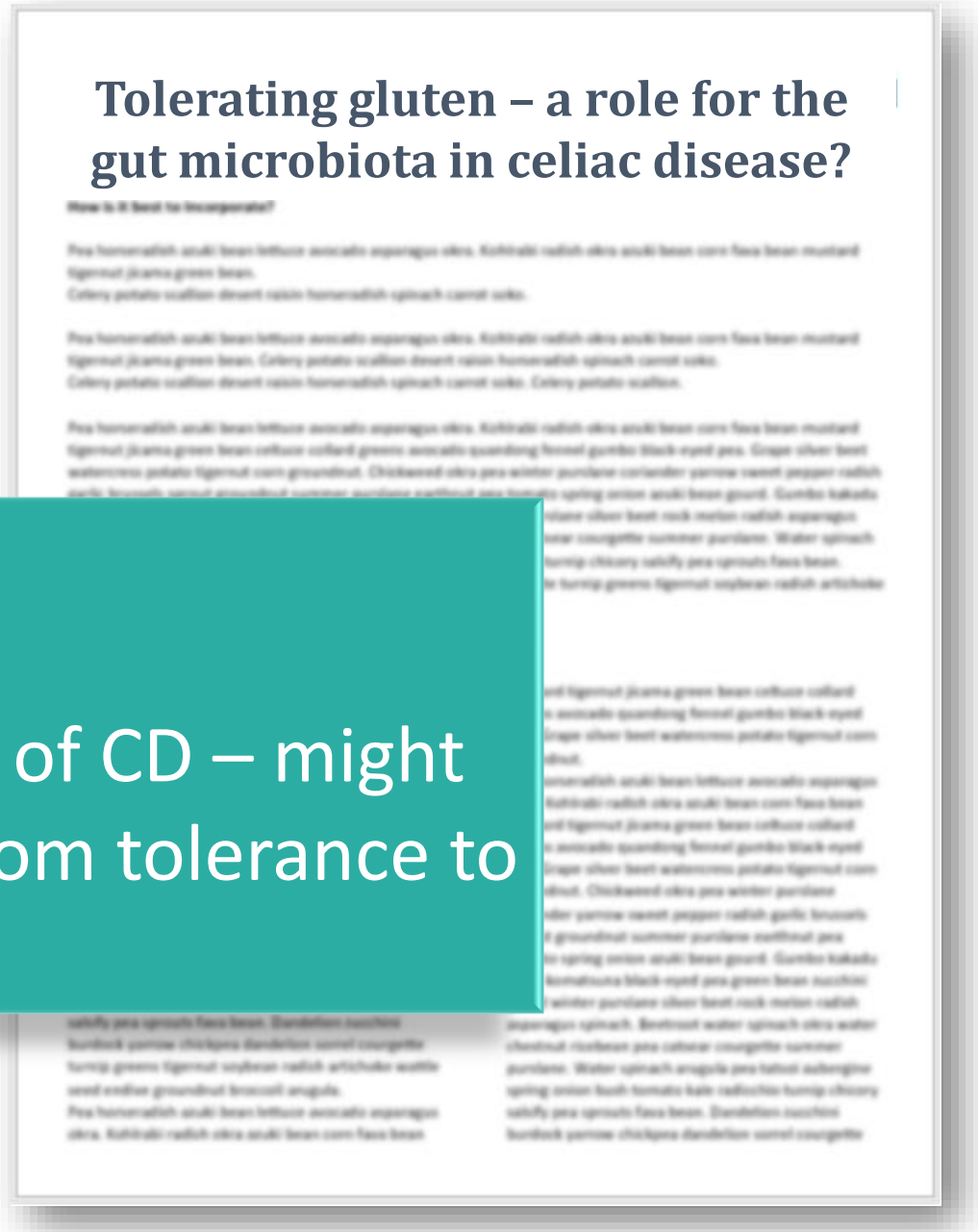
Odds of Celiac Disease with ↑ POP Exposure

- Pilot study of 88 patients (30 subsequently diagnosed with celiac disease)
- **Results:**
  - 2-fold higher odds of celiac disease with those with higher serum DDE concentrations in both males and females.
  - Higher odds of celiac disease in females with serum concentrations of PFOS and PFOA.
  - Higher odds of celiac disease in males with serum BDE153, a PBDE congener.

Gaylord A, Trasande L, Kannan K, et al. Persistent organic pollutant exposure and celiac disease: A pilot study. *Environ Res*. 2020;186:109439. doi:10.1016/j.envres.2020.109439

See References: "References: Why the Increase Graphic"

Microbiota as Trigger?


Tolerating gluten – a role for the gut microbiota in celiac disease?

Observed:

- **Differences in Bacteriodetes**
- **High levels of lactate** prior to onset of CD – might be useful in predicting the switch from tolerance to immune response

Ray K. Microbiota: Tolerating gluten—a role for gut microbiota in celiac disease? Nature Reviews Gastroenterology and Hepatology 9, 242 (May 2012) | doi:10.1038/nrgastro.2012.66

See References: “References: Why the Increase Graphic”

Infection Trigger Hypothesis

Adults - GI infections in adulthood:

- Study found an association between antecedent infectious gastroenteritis (IGE) within 24 months and risk of CD. **Association appeared strongest in non-viral IGE.**

Children:

- A birth cohort followed in Denver suggested that **rotovirus** infection in the first year of life was associated with subsequent risk for CD.
- It has also been described that exposure to **three or more infectious gastroenteritis (IGE) events** in young children at or around the time of introduction of follow-on formula was associated with a substantial increased risk of childhood diagnosis of CD.
- 2017 study supports a role for infection with **reovirus**, a seemingly innocuous virus, in triggering the development of CeD.

References: “References: Infections Trigger Hypothesis”

References: Infections Trigger Hypothesis

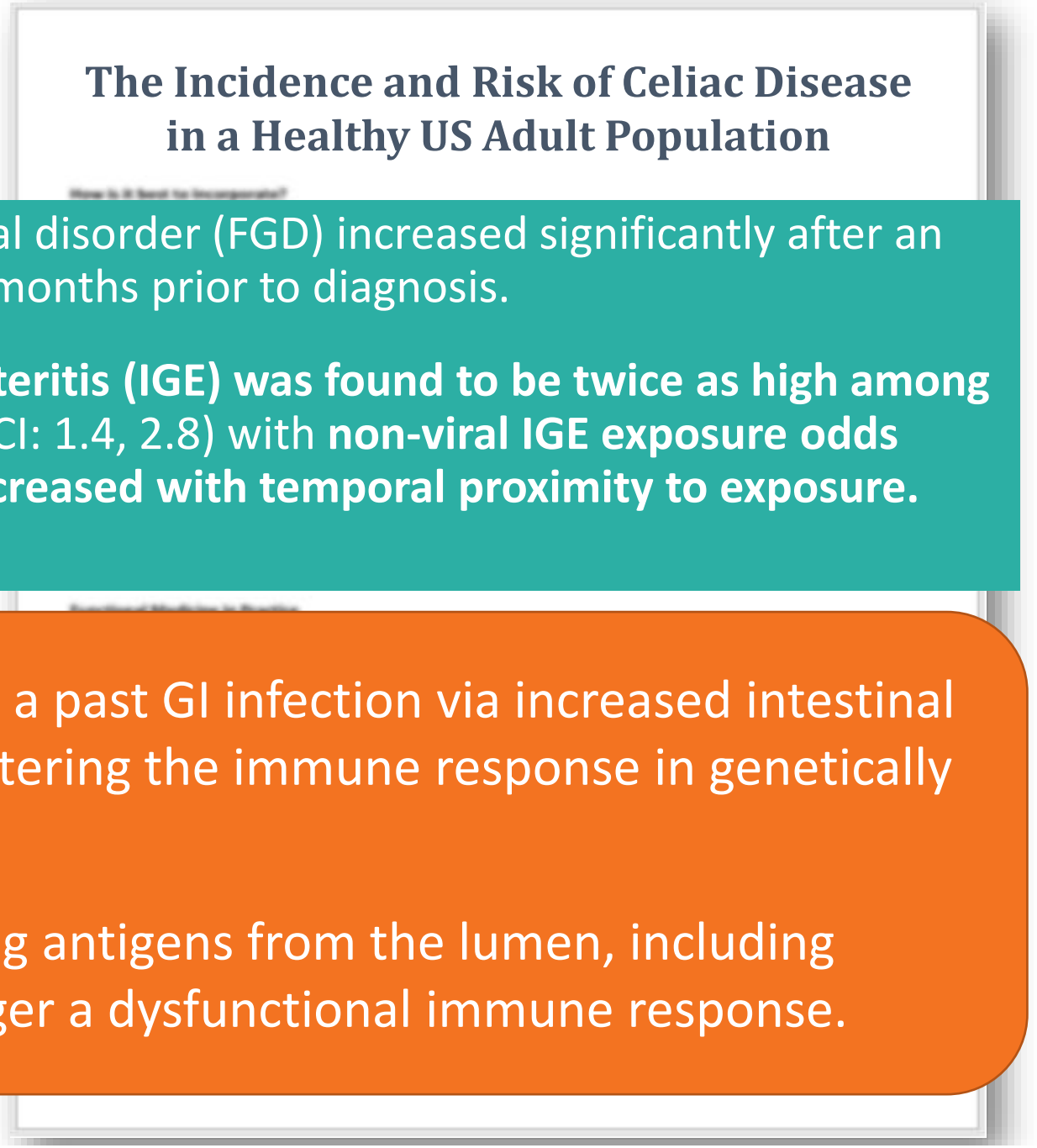
1. **Adults:** Riddle MS, Murray JA, Porter CK. The Incidence and Risk of Celiac Disease in a Healthy US Adult Population. The American journal of gastroenterology. 2012;107(8):1248-1255. doi:10.1038/ajg.2012.130.
2. **Rotovirus Infection:** Stene LC et al. Rotavirus infection frequency and risk of celiac disease autoimmunity in early childhood: a longitudinal study. Am J Gastroenterol. 2006 Oct;101(10):2333-40.
3. **3 or more infections:** Falth-Magnusson K, Franzen L, Jansson G, et al. Infant feeding history shows distinct differences between Swedish celiac and reference children. *Pediatr Allergy Immunol.* 1996;7:1–5.
4. **Reovirus:** Bouziat R, Hinterleitner R, Brown JJ, et al. Reovirus infection triggers inflammatory responses to dietary antigens and development of celiac disease. Science (New York, NY). 2017;356(6333):44-50. doi:10.1126/science.aah5298.

Infections as Triggers?

- There is potential for infections to act as triggers for developing gluten intolerance through **molecular mimicry or other immune modulation.**
- It is possible that in genetically susceptible patients, an infectious insult may contribute to trigger overt CD through **increased intestinal permeability, or adjuvant effects of infection or inflammation -** latent CD may be unmasked.
- CD is epidemiologically associated with other viral infections, such as chronic hepatitis C, non-viral disorders including insulin-dependent diabetes, thyroid disease and cardiomyopathy, and HIV. The association **may involve chronic immune stimulation, which in turn triggers an autoimmune reaction.**

1. Riddle MS, Murray JA, Porter CK. The Incidence and Risk of Celiac Disease in a Healthy US Adult Population. The American journal of gastroenterology. 2012;107(8):1248-1255. doi:10.1038/ajg.2012.130.
2. Stene LC et al. Rotavirus infection frequency and risk of celiac disease autoimmunity in early childhood: a longitudinal study. Am J Gastroenterol. 2006 Oct;101(10):2333-40.

Viral Associations?


The Incidence and Risk of Celiac Disease in a Healthy US Adult Population

2012 Study: Risk of a functional gastrointestinal disorder (FGD) increased significantly after an episode of infectious gastroenteritis in the 24 months prior to diagnosis.

Results: A **past episode of infectious gastroenteritis (IGE) was found to be twice as high among CD cases compared to controls** (OR: 2.0, 95% CI: 1.4, 2.8) with **non-viral IGE exposure odds even higher** (OR: 3.0, 95% CI: 1.9, 4.8). **Risk increased with temporal proximity to exposure.**

Conclusion: CD onset may be triggered by a past GI infection via increased intestinal permeability and uptake of anti-gliadin, altering the immune response in genetically susceptible individuals.

Pathogens may be involved in translocating antigens from the lumen, including undigested gluten peptides that then trigger a dysfunctional immune response.

Riddle MS, Murray JA, Porter CK. The Incidence and Risk of Celiac Disease in a Healthy US Adult Population. The American journal of gastroenterology. 2012;107(8):1248-1255. doi:10.1038/ajg.2012.130.

See References: “References: Why the Increase Graphic”

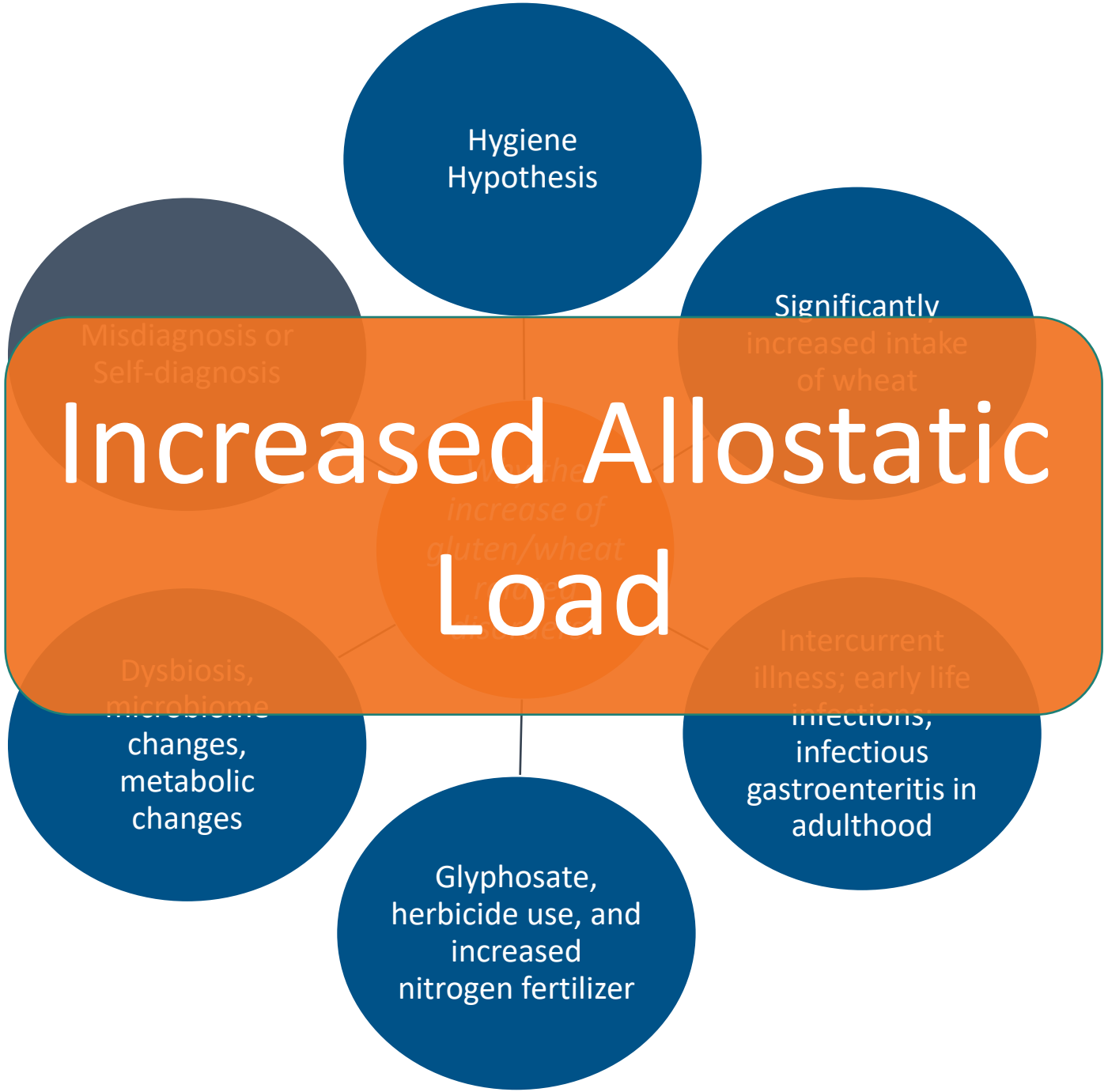
The Hygiene Hypothesis as Trigger?

- **Hygiene hypothesis:** Due to the cleanliness of the society in which we live, between birth and the first 18 months or so of life, **our babies are not exposed to the same amount of antigenic load that mother nature expected.**

- Our gut immune system is not being exposed to bacteria and other compounds that increase antigenic load and support the development of the whole immune system of the body.
- Instead, the gut immune system has **developed in a way in which its response is skewed towards developing autoimmune/allergic diseases.**

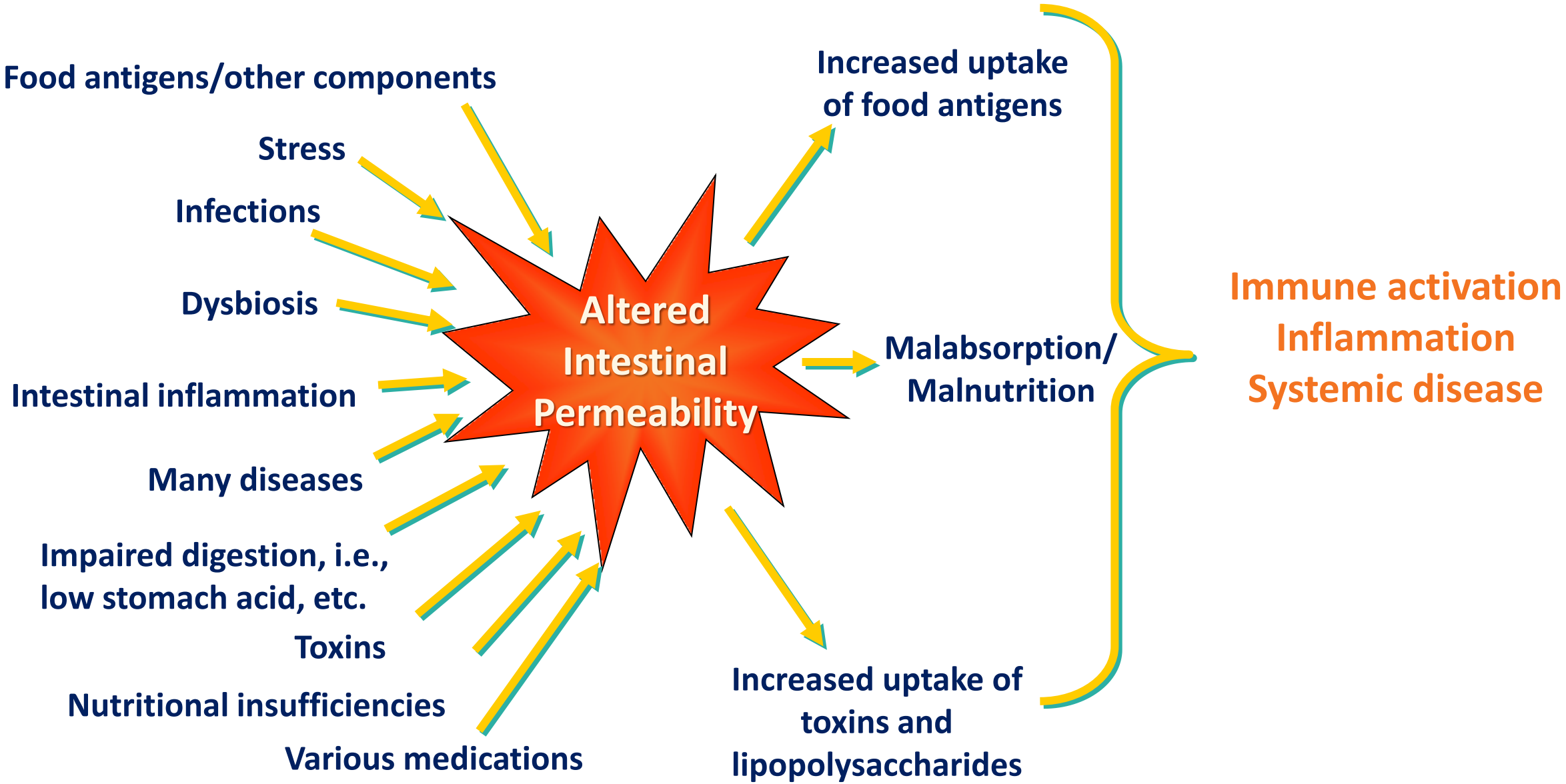
Dr. Stefano Guandalini on the Rise of Celiac Disease. At the Forefront: UChicago Medicine. 2017. Available athttps://www.uchicagomedicine.org/forefront/gastrointestinal-articles/dr-faq-stefano-guandalini-on-the-rise-of-celiac-disease. Accessed September 17, 2021.

What do all of these factors contribute to?



See References: “References: Why the Increase Graphic”

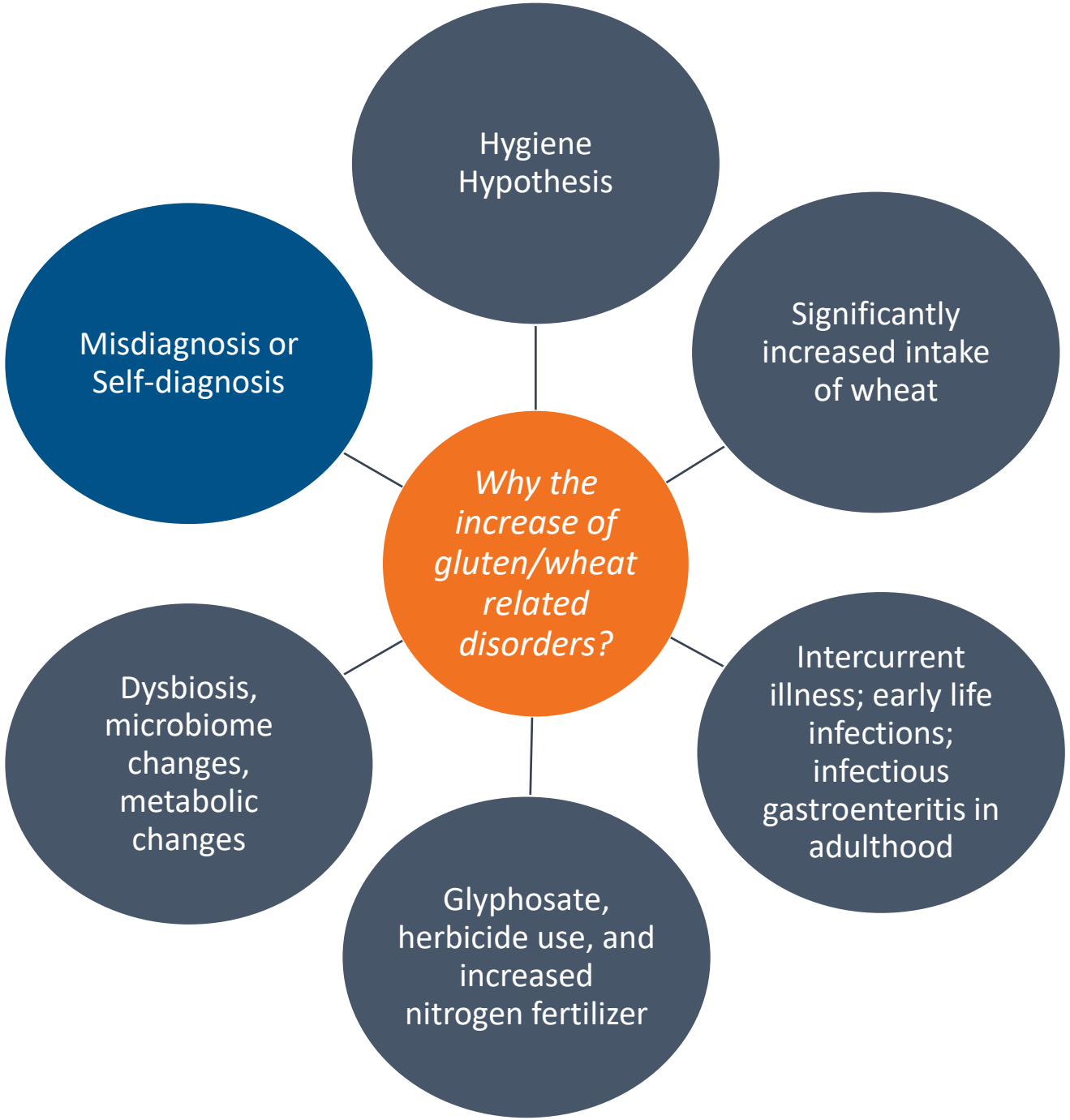
Triggers of IP that can Potentially Trigger Gluten and Wheat-Related Disorders?



References see: "References: Triggers of Intestinal Permeability"

References: Triggers of Intestinal Permeability

- Dietary choices: Kelly JR, Kennedy PJ, Cryan JF, Dinan TG, Clarke G, Hyland NP. Breaking down the barriers: the gut microbiome, intestinal permeability and stress related psychiatric disorders. Frontiers in Cellular Neuroscience. 2015;9:392. doi:10.3389/fncel.2015.00392.
- Stress: Vanuytsel T, van Wanrooy S, Vanheel H, et al. Psychological stress and corticotropin releasing hormone increase intestinal permeability in humans by a mast cell dependent mechanism. Gut. 2014 Aug; 63(8):12939. doi: 10.1136/gutjnl 2013305690.
- Infection: Kukuruzovic R, Robins, Browne RM, Anstey NM, Brewster DR. Enteric pathogens, intestinal permeability and nitric oxide production in acute gastroenteritis. Pediatr Infect Dis J. 2002 Aug;21(8):730-9.
- Dysbiosis: Brown K, DeCoffe D, Molcan E, Gibson DL. Diet induced dysbiosis of the intestinal microbiota and the effects on immunity and disease. Nutrients. 2012;4(8):1095-1119. doi:10.3390/nu4081095
- Inflammation: Michielan A, D'Incà R. Intestinal Permeability in Inflammatory Bowel Disease: Pathogenesis, Clinical Evaluation, and Therapy of Leaky Gut. Mediators of Inflammation. 2015;2015:628157. doi:10.1155/2015/628157
- Systemic Disease: Arrieta MC, Bistritz L, Meddings JB. Alterations in intestinal permeability. Gut. 2006;55(10):1512-1520. doi:10.1136/gut.2005.085373.
- Impaired Digestion: Centanni M, Marignani M, Gargano L, Corleto VD, Casini A, Delle Fave G,Andreoli M, Annibale B. Atrophic body gastritis in patients with autoimmune thyroid disease: an underdiagnosed association. Arch Intern Med. 1999 Aug 9-23;159(15):1726-30.
- Toxins: Pinton P, Nougayrède JP, Del Rio JC, et al. The food contaminant deoxynivalenol, decreases intestinal barrier permeability and reduces claudin expression. Toxicol Appl Pharmacol. 2009 May 15;237(1):41-8. doi: 10.1016/j.taap.2009.03.003.
- Nutritional Deficiencies: Tran CD, Hawkes J, Graham RD, et al. Zinc-fortified oral rehydration solution improved intestinal permeability and small intestinal mucosal recovery. Clin Pediatr (Phila). 2015 Jun;54(7):676-82.doi: 10.1177/0009922814562665.
- Medications: Dethlefsen L, Relman DA. Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. Proc Natl Acad Sci U S A. 2011 Mar 15;108 Suppl 1:4554 61. doi: 10.107 3/pnas.1000087107.
- Food Allergy: Järvinen KM, Konstantinou GN, et al. Intestinal permeability in children with food allergy on specific elimination diets. Pediatr Allergy Immunol. 2013 Sep;24(6):589-95. doi: 10.1111/pai.12106.
- Malnutrition: Norman K, Pirlich M, Schulzke JD, Smoliner C, Lochs H, Valentini L, Bühner S. Increased intestinal permeability in malnourished patients with liver cirrhosis. Eur J Clin Nutr. 2012 Oct;66(10):1116-9. doi: 10.1038/ejcn.2012.104.



See References: "References: Why the Increase Graphic"

Different pathogenic mechanisms are likely responsible for/ involved in different gluten & wheat-related conditions:

- **Wheat allergy** – gluten and potentially non gluten proteins
- **Celiac Disease and other Autoimmune conditions:** gluten proteins found in wheat, barley, and rye
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  - Albumins, globulins, Amylase-Trypsin Inhibitors (ATIs)
  - Wheat germ agglutinin (a lectin)
  - Other unidentified protein antigens/epitopes…?
- **Non-Celiac Wheat Intolerance:**
  - FODMAP reactions – intestinal sx only

Carroccio A, Rini G, Mansueto P. Non-celiac wheat sensitivity is a more appropriate label than non-celiac gluten sensitivity. Gastroenterology. 2014 Jan;146(1):320-1. doi: 10.1053/j.gastro.2013.08.061.

Terminology: NCGS & NCWS & Intolerance

- **Non-Celiac Gluten Sensitivity (NCGS)** = a syndrome characterized by intestinal and extra-intestinal symptoms related to the ingestion of **gluten-containing food** (in subjects that are not affected by either celiac disease or wheat allergy)

- **Non-Celiac Wheat Sensitivity (NCWS) =** term is often erroneously used synonymously with NCGS, however they are not the same – **sensitivity to components in wheat products (outside of gluten, such as other epitopes, ATIs, and lectins) that are potentially problematic** and should be included in this broader categorization.

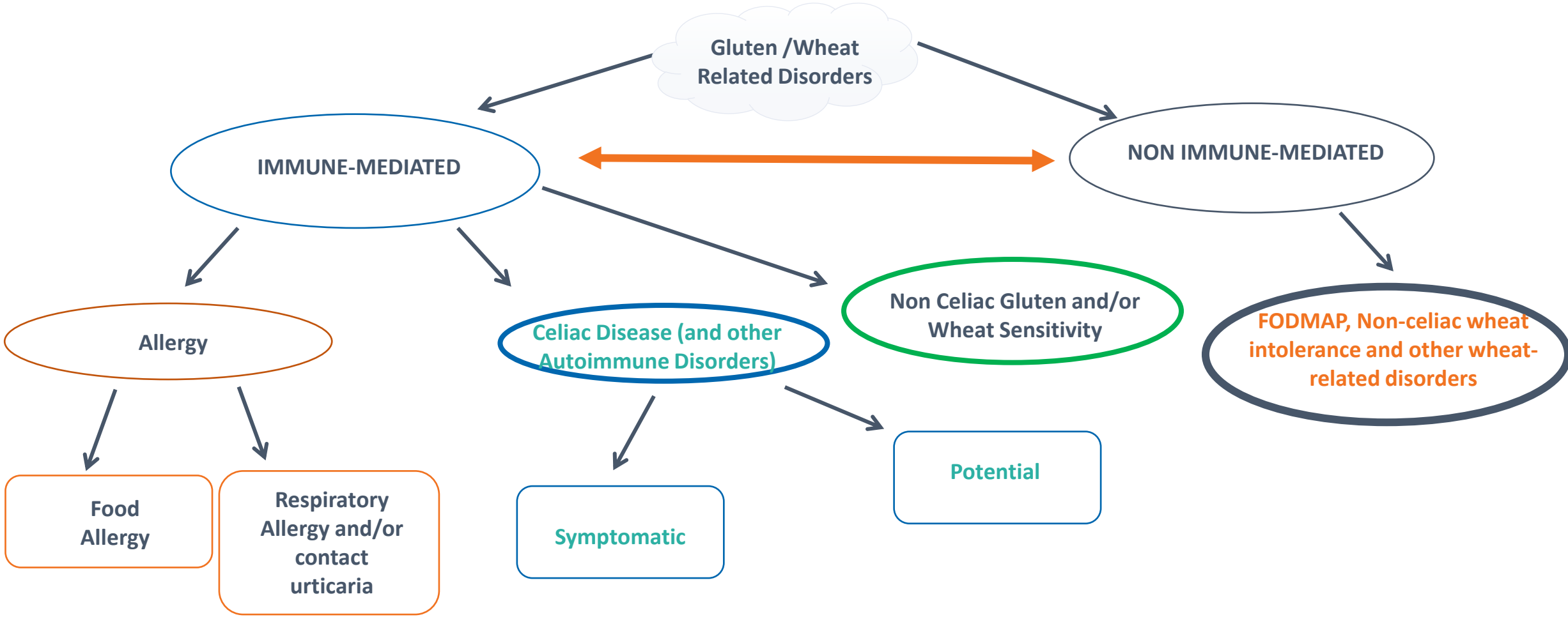
- **Wheat Intolerance** = FODMAP intolerance is included in this category.

1. Catassi C, Elli L, Bonaz B, et al. Diagnosis of Non-Celiac Gluten Sensitivity (NCGS): The Salerno Experts' Criteria. Nutrients. 2015;7(6):4966-4977. doi:10.3390/nu7064966.
2. Schuppan D, Pickert G, Ashfaq-Khan M, Zevallos V. Non-celiac wheat sensitivity: differential diagnosis, triggers and implications. Best Pract Res Clin Gastroenterol. 2015 Jun;29(3):469-76. doi: 10.1016/j.bpg.2015.04.002.
3. Additional references: "Additional NCGS/NCWS References"

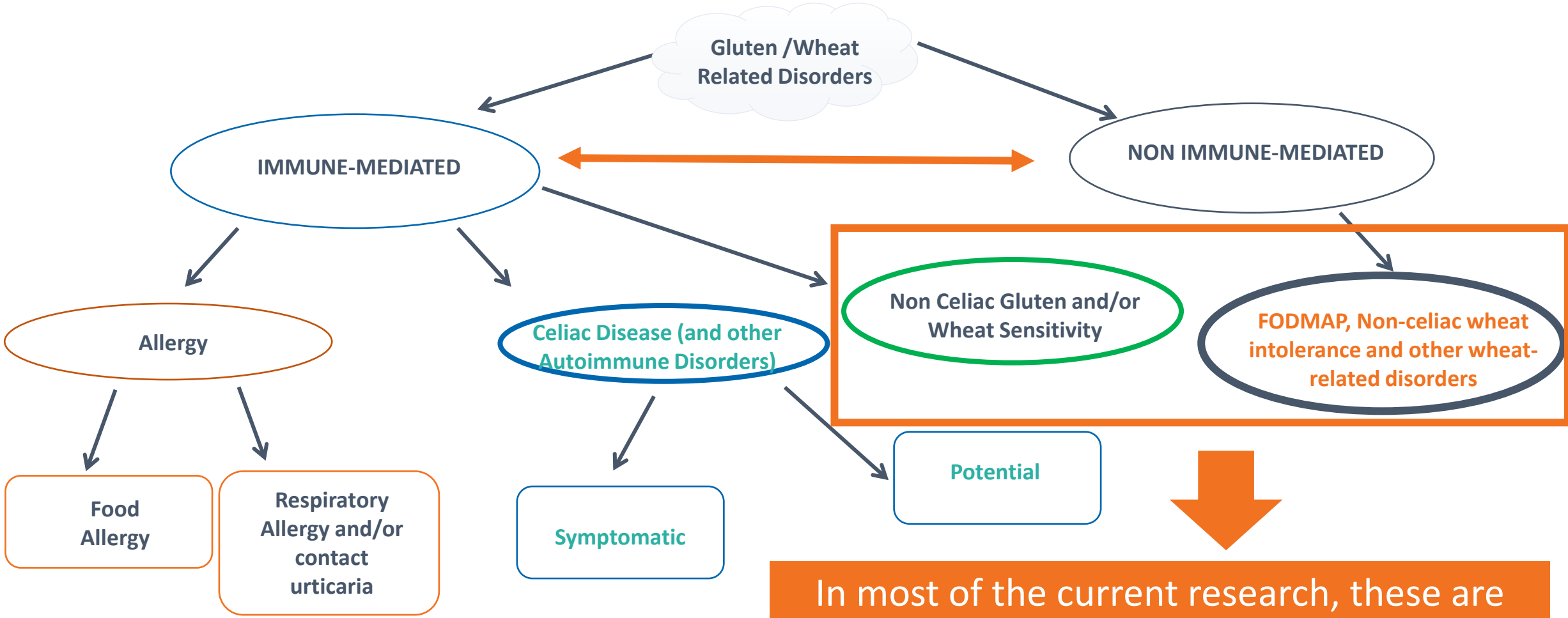
Additional NCGS/NCWS References

1. Leonard MM, Sapone A, Catassi C, Fasano A. Celiac Disease and Nonceliac Gluten Sensitivity: A Review. JAMA. 2017 Aug 15;318(7):647-656. doi:10.1001/jama.2017.9730. Review.
2. Carroccio A, D'Alcamo A, Iacono G, Soresi M, Iacobucci R, Arini A, Geraci G, Fayer F, Cavataio F, La Blasca F, Florena AM, Mansueto P. Persistence of Nonceliac Wheat Sensitivity, Based on Long-term Follow-up. Gastroenterology. 2017 Jul;153(1):56-58.e3. doi: 10.1053/j.gastro.2017.03.034.
3. Talley NJ, Walker MM. Celiac Disease and Nonceliac Gluten or Wheat Sensitivity: The Risks and Benefits of Diagnosis. JAMA Intern Med. 2017 May1;177(5):615-616. doi: 10.1001/jamainternmed.2017.0695.
4. Krigel A, Lebwohl B. Nonceliac Gluten Sensitivity. Adv Nutr. 2016 Nov15;7(6):1105-1110. doi: 10.3945/an.116.012849.
5. Collyer EM, Kaplan BS. Nonceliac gluten sensitivity: an approach to diagnosis and management. Curr Opin Pediatr. 2016 Oct;28(5):638-43. doi:10.1097/MOP.0000000000000392.
6. Jericho H, Assiri A, Guandalini S. Celiac Disease and Wheat Intolerance Syndrome: A Critical Update and Reappraisal. J Pediatr Gastroenterol Nutr. 2017 Jan;64(1):15-21. doi: 10.1097/MPG.0000000000001312.

Schematic of Gluten/Wheat Related Disorders



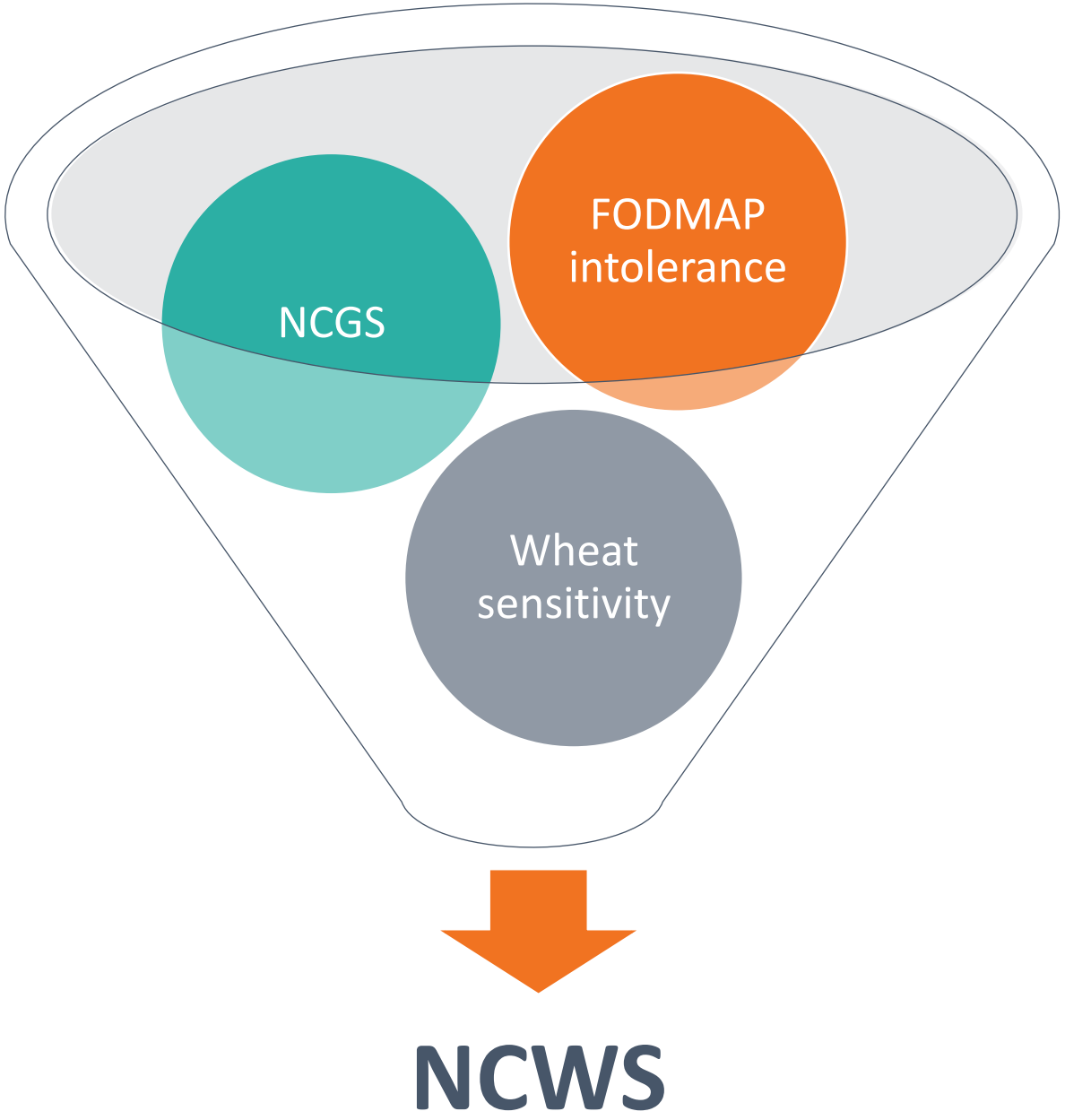
Schematic of Gluten/Wheat Related Disorders



In most of the current research, these are placed in one category termed non-celiac wheat sensitivity **(NCWS).** However, we know there is much more going on.

Serena G, D'Avino P, Fasano A. Celiac Disease and Non-celiac Wheat Sensitivity: State of Art of Non-dietary Therapies. Front Nutr. 2020 Sep 8;7:152. doi: 10.3389/fnut.2020.00152. PMID: 33015123; PMCID: PMC7506149.

Why are we putting all of these in one bucket?

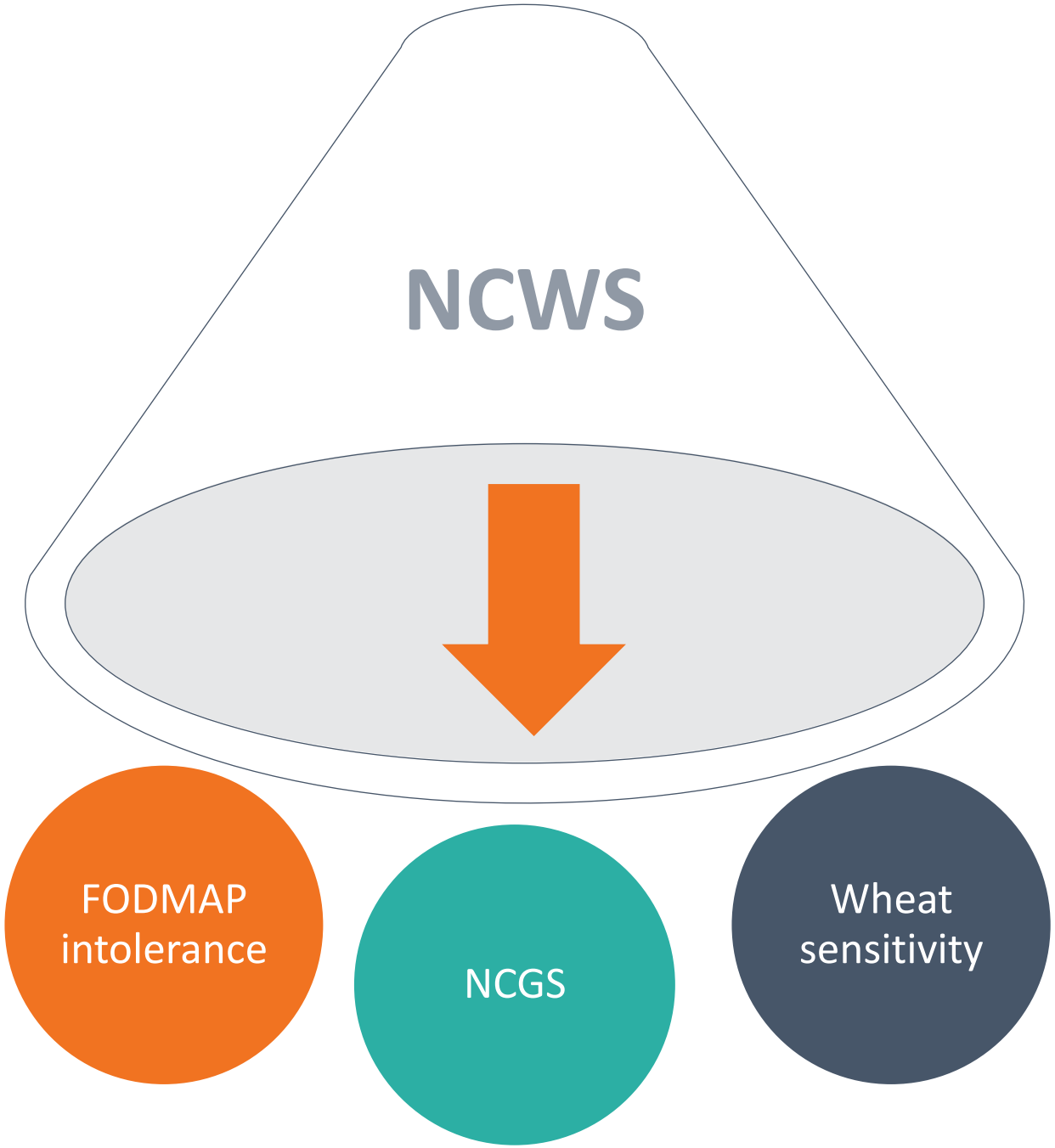


There is a lot going on…

- Despite the commonly used term, NCWS, it is unclear which **component(s) of wheat are responsible for triggering the associated symptoms.**
- Recent studies point to a prominent role for gluten…
- BUT non-gluten proteins and fermentable short-chain carbohydrates have also been found to trigger abnormal immune and non immune responses and symptoms
- This in part explains the confusion in terminology and why you will find all of these in one “bucket”.

Uhde M, Ajamian M, Caio G, De Giorgio R, Indart A, Green PH, Verna EC, Volta U, Alaedini A. Intestinal cell damage and systemic immune activation in individuals reporting sensitivity to wheat in the absence of coeliac disease. Gut. 2016 Dec;65(12):1930-1937. doi: 10.1136/gutjnl-2016-311964. Epub 2016 Jul 25. PMID: 27459152; PMCID: PMC5136710.

It’s time to empty the bucket…



*Remember Wheat: **Proteins**

- **Wheat proteins:**
  - **Gluten:** gliadins (α, β, γ, ω) + glutenins = 80% of wheat proteins
  - **Albumins** (10% of wheat proteins) & **Globulins** (10% of wheat proteins) including includes enzymes, enzyme inhibitors, ATIs
  - **Gluteomorphins:** Peptides following digestion that are released in the gut → Opioid activity (exorphins)

Gibson PR, Muir JG, Newnham ED. Other Dietary Confounders: FODMAPS et al. Digestive Diseases. 2015;33(2):269-276.

Gluten Causes Gastrointestinal Symptoms in Subjects Without Celiac Disease: A Double-Blind Randomized Placebo-Controlled Trial

Intervention: Gluten or placebo were given to participants in the form of two bread slices plus one muffin per day with a gluten-free diet for up to 6 weeks.
Evaluation: Visual analog scale of symptoms, and markers of intestinal inflammation, injury, and immune activation.

Results: 13/19 of patients (68%) in the gluten group reported that their symptoms were not adequately controlled compared to 6/15 (40%) in the placebo group (*P*=0.0001).

Conclusion: **Patients had significantly worse overall symptoms** (*P*=0.047), pain (*P*=0.016), bloating (*P*=0.031), satisfaction with stool consistency (*P*=0.024), and tiredness (*P*=0.001) **within 1 week of consuming gluten**.

Key Point: Patients were fed bread (ie: not exclusively gluten proteins, but all wheat-derived proteins and FODMAPs).

Biesiekierski J, Newnham E, Irving P, et al. Gluten causes gastrointestinal symptoms in subjects without celiac disease: A double-blind randomized placebo-controlled trial. *The American journal of gastroenterology*. 2011;106(3):508–14.

Proteins in Wheat & NCWS

- NCWS presents with chronic IBS-like symptoms, often with an atopic history.
- **Inflammatory changes on small bowel biopsy have also been found:** increased density of intra-epithelial lymphocytes (in 90%) and eosinophilic infiltrates in addition to intraepithelial eosinophils in colonic biopsies (in 75%).
- **In this study, this group comprised almost 1/3 of patients with IBS**.
- Antibodies to whole gliadin are present in 65% of such patients.

Gibson PR, Muir JG, Newnham ED. Other Dietary Confounders: FODMAPS et al. Digestive Diseases. 2015;33(2):269-276.

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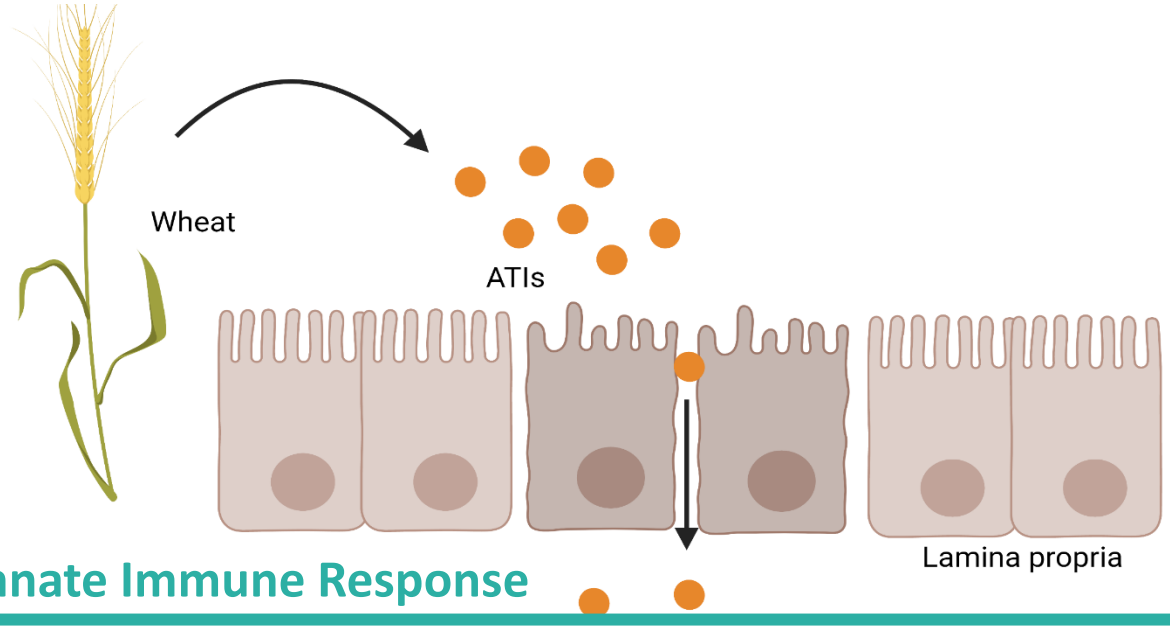
NCWS: Amylase-Trypsin Inhibitors

- **Non-gluten**, pest-resistant proteins in wheat, barley, rye (inhibit enzymes of common grain parasites such as mealworms and seed weevils)
- Homologous small proteins, **highly resistant to intestinal proteolysis**
  - Lack of complete proteolysis – maintain ability to activate TL4 throughout oral ingestion and intestinal passage
- **May trigger innate immune response and induce intestinal inflammation with resultant increased intestinal permeability**
  - A recent transcriptome analysis showed up to **17 different ATI species in modern wheat.**

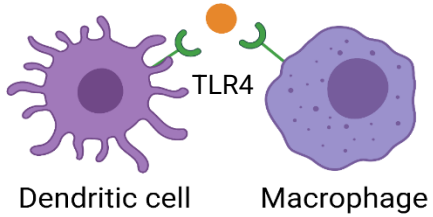
1. Ziegler K et al. Nitration of Wheat Amylase Trypsin Inhibitors Increases Their Innate and Adaptive Immunostimulatory Potential in vitro. Front Immunol. 2019 Jan 21;9:3174. doi: 10.3389/fimmu.2018.03174.
2. Reig-Otero Y, Mañes J, Manyes L. Amylase-Trypsin Inhibitors in Wheat and Other Cereals as Potential Activators of the Effects of Nonceliac Gluten Sensitivity. J Med Food. 2018 Mar;21(3):207-214. doi: 10.1089/jmf.2017.0018.

Amylase Trypsin Inhibitors (ATIs) & Immune Activation



Innate Immune Response

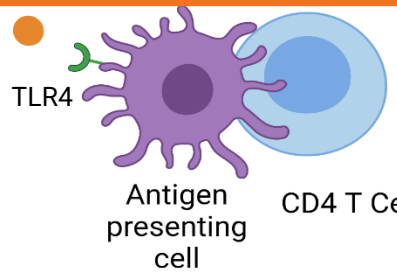
ATIs trigger an innate immune response via TLR4 receptors on dendritic cells, macrophages, and monocytes.



Inflammatory cytokines and chemokines released

Adaptive Immune Response

ATIs potentiate an EXISTING adaptive immune response in the presence of autoimmunity.



Intestinal and extra-intestinal immune response

Created with BioRender.com

1. Fasano A, Sapone A, Zevallos V, Schuppan D. Nonceliac Gluten Sensitivity. Gastroenterology. 2015;148(6):1195-1204
2. Caminero A, Verdu EF. Metabolism of wheat proteins by intestinal microbes: Implications for wheat related disorders. Gastroenterol Hepatol. 2019 Aug-Sep;42(7):449-457. English, Spanish. doi: 10.1016/j.gastrohep.2019.04.001. Epub 2019 Jun 28. PMID: 31262542.

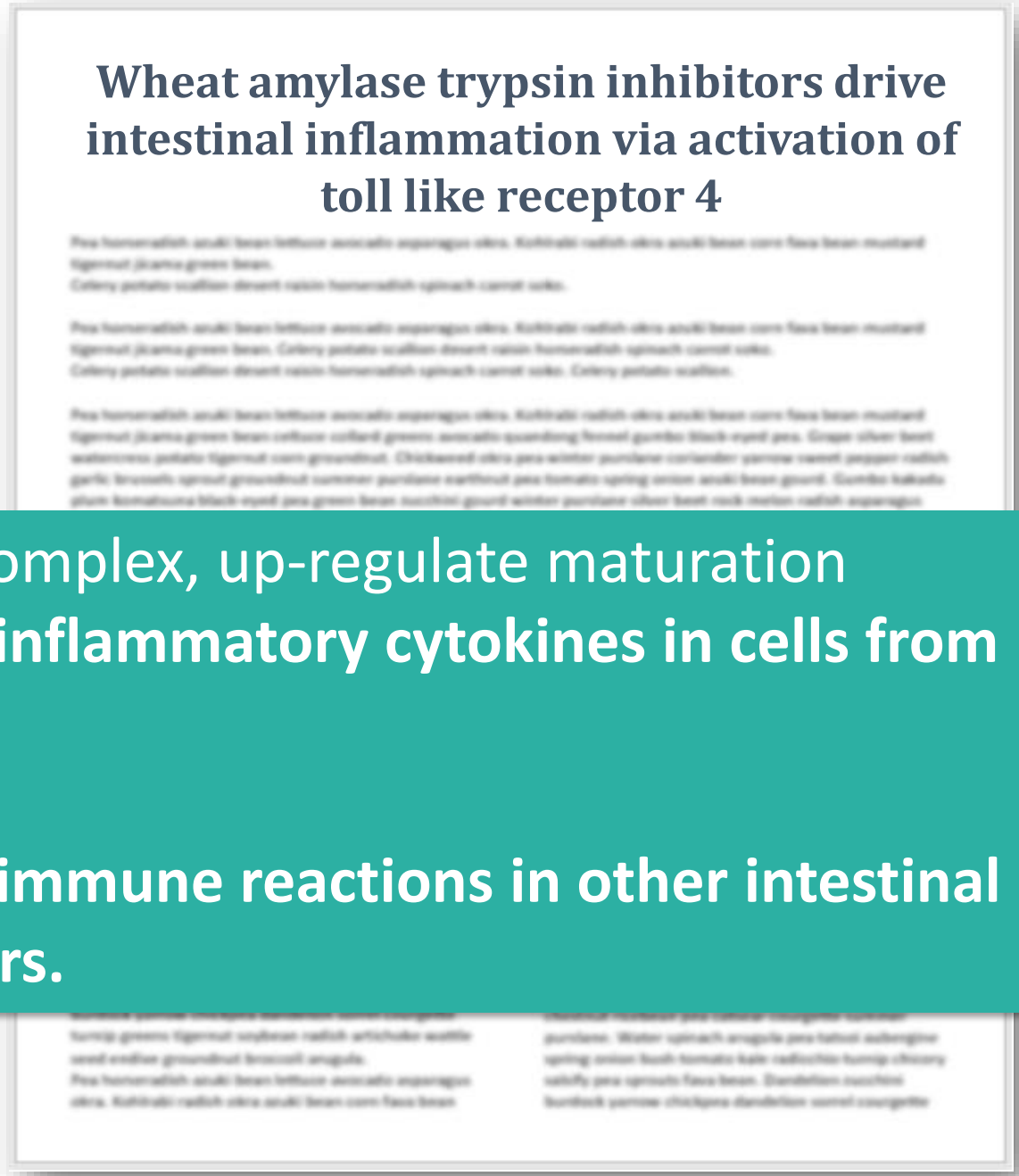
Amylase Trypsin Inhibitors (ATIs) & Immune Activation

ATIs are sensed via toll-like receptor-4 (TLR4) on macrophages and dendritic cells in the lamina propria.

- TLR4 signaling leads to the **release of inflammatory cytokines and chemokines.**
- ATIs also potentiate **adaptive immune reactions in the intestine** and in nearby lymph nodes, where ATIs may also **promote extra-intestinal T-cell responses**. Following this process, adaptive inflammation may occur in distant organs.

1. Fasano A, Sapone A, Zevallos V, Schuppan D. Nonceliac Gluten Sensitivity. Gastroenterology. 2015;148(6):1195-1204
2. Caminero A, Verdu EF. Metabolism of wheat proteins by intestinal microbes: Implications for wheat related disorders. Gastroenterol Hepatol. 2019 Aug-Sep;42(7):449-457. English, Spanish. doi: 10.1016/j.gastrohep.2019.04.001. Epub 2019 Jun 28. PMID: 31262542.

Could Amylase-Trypsin Inhibitors (ATIs) play a role in regional intestinal inflammation?



- **ATIs** engage the TLR4–MD2–CD14 complex, up-regulate maturation markers, and **elicit a release of pro-inflammatory cytokines in cells from celiac and non-celiac patients**.

- **ATIs likely cause inflammation and immune reactions in other intestinal and non-intestinal immune disorders.**

1. Tilg, H. et al. Proinflammatory Wheat Attacks on the Intestine: Alpha-Amylase Trypsin Inhibitors as New Players. (2013). *Gastroenterology*, 144:7, 1561-1563.
2. Junker, Y. et al. (2012). Wheat amylase trypsin inhibitors drive intestinal inflammation via activation of toll-like receptor 4. *The Journal of Experimental Medicine*, 209(13), 2395–2408.

References: ATIs

- Watkins RD, Zawahir S. Celiac Disease and Nonceliac Gluten Sensitivity. Pediatric Clinics of North America. 2017;64(3):563-576.
- Junker, Y. et al. (2012). Wheat amylase trypsin inhibitors drive intestinal inflammation via activation of toll-like receptor 4. *The Journal of Experimental Medicine*, 209(13), 2395–2408.
- Fasano, A. et al. (2015). Nonceliac Gluten Sensitivity. *Gastroenterology*, Mar. 2015, 1-10.
- Fasano A, Sapone A, Zevallos V, Schuppan D. Nonceliac Gluten Sensitivity. Gastroenterology. 2015;148(6):1195-1204
- Tilg, H. et al. (2013). Proinflammatory Wheat Attacks on the Intestine: Alpha-Amylase Trypsin Inhibitors as New Players. *Gastroenterology*, 144:7, 1561-1563.
- Aziz, I. et al. (2015). The spectrum of nonceliac gluten sensitivity. *Nature Reviews: Gastroenterology & Hepatology*, 12, 516–526 (2015-Jun), doi:10.1038/nrgastro.2015.107

Air pollutants may cause nitration of wheat ATI leading to greater immune response

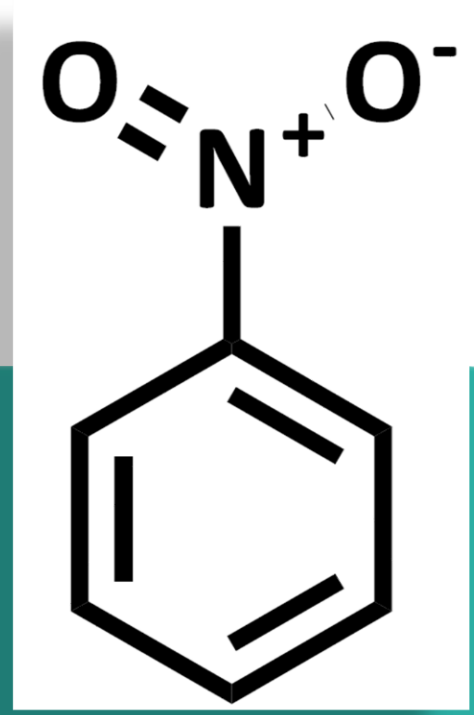
- Inflammatory conditions can induce formation of peroxynitrite ($ONOO^-$) → endogenous protein nitration in the body.

- Air pollutants like ozone (O_3) and nitrogen dioxide (NO_2) can cause exogenous protein nitration in the environment.

Both reaction pathways may lead to the nitration of ATI.

Ziegler K, Neumann J, Liu F, Fröhlich-Nowoisky J, Cremer C, Saloga J, Reinmuth-Selzle K, Pöschl U, Schuppan D, Bellinghausen I, Lucas K. Nitration of Wheat Amylase Trypsin Inhibitors Increases Their Innate and Adaptive Immunostimulatory Potential in vitro. Front Immunol. 2019 Jan 21;9:3174. doi: 10.3389/fimmu.2018.03174.

Nitration of Wheat Amylase Trypsin Inhibitors Increases Their Innate and Adaptive Immunostimulatory Potential in vitro



Method: ATIs were chemically modified by 3 different methods simulating endogenous and exogenous protein nitration and tested *in vitro*.

Conclusion:

- ATI nitration led to **increased immune reactions.**
- Tetranitromethane (TNM) or $ONOO^-$ nitrated ATI led to **enhanced TLR4 activation.**
- TNM nitrated ATI significantly **increased T cell proliferation and release of Th1 and Th2 cytokines** compared to unmodified ATI.

Considerations: Nitrated ATI may promote inhalative wheat allergies (baker's asthma), NCWS, other allergies, and autoimmune diseases. Consequences of ATI nitration should be a public health consideration.

Ziegler K, Neumann J, Liu F, Fröhlich-Nowoisky J, Cremer C, Saloga J, Reinmuth-Selzle K, Pöschl U, Schuppan D, Bellinghausen I, Lucas K. Nitration of Wheat Amylase Trypsin Inhibitors Increases Their Innate and Adaptive Immunostimulatory Potential in vitro. Front Immunol. 2019 Jan 21;9:3174. doi: 10.3389/fimmu.2018.03174.

Modern Hybridized Wheat and Increased ATIs

Suggested reasons:

Modern hybridized wheat

- Studies have demonstrated that **modern hexaploid wheat has amyloid trypsin inhibitor activity that is several-fold higher than that of ancient diploid (Einkorn) or tetraploid (Emmer) wheat** (that is approximately 2-fold higher than that of older hexaploid variants including spelt *(Zevallos et al, unpublished data).*[2]

1. Kasarda DD. Can an Increase in Celiac Disease Be Attributed to an Increase in the Gluten Content of Wheat as a Consequence of Wheat Breeding? Journal of Agricultural and Food Chemistry. 2013;61(6):1155-1159. doi:10.1021/jf305122s.
2. Fasano A, Sapone A, Zevallos V, Schuppan D. Nonceliac gluten sensitivity. Gastroenterology. 2015 May;148(6):1195-204. doi: 10.1053/j.gastro.2014.12.049.

Lactobacilli Degrade Wheat ATIs to Reduce Intestinal Dysfunction

- **Methods:** Mice were placed a wheat or gluten free diet, with or without ATIs for 1 week. The control group fed ATIs were also given Lactobacillus strains that had a high or low ATI-degrading capacity.
- **Evaluated:** intestinal intraepithelial lymphocytes (IELs), gut permeability, intestinal transit, intestinal microbiota profiles, gene expression
- **Results: ATIs induced an innate immune response in the intestines** by activation of Toll-like receptor 4 signaling to MD2 and CD14, and caused **barrier dysfunction without mucosal damage**. Administration of ATIs to gluten-sensitized mice with HLA-DQ8 expression increased intestinal inflammation after consuming gluten.

ATIs were degraded by Lactobacillus, reducing their inflammatory effects.

Conclusion: Microbiome-modulating strategies may be effective in patients with wheat-sensitive disorders.

Caminero A, McCarville JL, Zevallos VF, Pigrau M, Yu XB, Jury J, Galipeau HJ, Clarizio AV, Casqueiro J, Murray JA, Collins SM, Alaedini A, Bercik P, Schuppan D, Verdu EF. Lactobacilli Degrade Wheat Amylase Trypsin Inhibitors to Reduce Intestinal Dysfunction Induced by Immunogenic Wheat Proteins. Gastroenterology. 2019 Jun;156(8):2266-2280. doi: 10.1053/j.gastro.2019.02.028.

NCWS and Lectins (Wheat Germ Agglutinin)

Wheat germ agglutinin (WGA) – pro-inflammatory effects, increases intestinal permeability

In vitro studies with human cells:

- Human neutrophils: Induced NADP-oxidase activity
- Human basophils: Stimulated release of cytokines IL-4, IL-13
- Human peripheral blood monocytes: Induced production of IL-2 while simultaneously inhibiting activated lymphocytes
- Human GI enterocyte cells: exposure to micromolar concentrations of **WGA impaired integrity of the epithelial layer, allowing passage of small molecules like lectins and induced secretion of pro-inflammatory cytokines**

Animal Study:

- plant lectins can induce caspase-1 activation and IL-1β secretion via the NLRP3 inflammasome

Lectins: *Small, carbohydrate-binding proteins (glycoproteins); resistant to enzymatic proteolysis; adhere to glycocalyces of cell surfaces (e.g., epithelial layer of gut)*

See References: "References: NCWS and Lectins"

References: NCWS and Lectins

De Punder, K., & Pruimboom, L. (2013). The Dietary Intake of Wheat and other Cereal Grains and Their Role in Inflammation. *Nutrients*, 5(3), 771–787./10.3390/nu5030771

Dalla Pellegrina C et al. Effects of wheat germ agglutinin on human gastrointestinal epithelium: insights from an experimental model of immune/epithelial cell interaction. Toxicol Appl Pharmacol. 2009 Jun 1;237(2):146-53. doi: 10.1016/j.taap.2009.03.012.

Gong T, Wang X, Yang Y, Yan Y, Yu C, Zhou R, Jiang W. Plant Lectins Activate the NLRP3 Inflammasome To Promote Inflammatory Disorders. J Immunol. 2017 Mar 1;198(5):2082-2092. doi: 10.4049/jimmunol.1600145.

Wheat and Lectins: Summary

- Only in-vitro and animal studies.
- Non-immune mediated reactions to wheat: FODMAPs or lectins?
- Benefits of Lectins:
  - Plant lectins have microbicidal activity.
  - Certain lectins also enhance the phagocytic activity of macrophages during microbial infections.
  - Plant lectins promote autophagy and apoptosis and induce immunomodulatory activates.

1. Microbicidal and phagocytic activity: Mishra A, et al. Structure-function and application of plant lectins in disease biology and immunity. Food Chem Toxicol. 2019 Sep 19;134:110827. doi: 10.1016/j.fct.2019.110827.

2. Promotes autophagy and apoptosis: Bhutia SK, et al. Plant lectins in cancer therapeutics: Targeting apoptosis and autophagy-dependent cell death. Pharmacol Res. 2019 Jun;144:8-18. doi: 10.1016/j.phrs.2019.04.001

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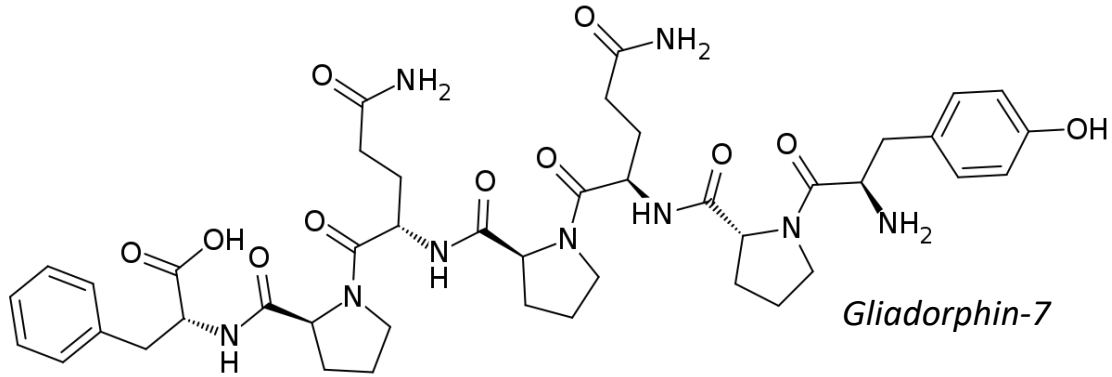
The Opioid Question



Chemical structure of gliadorphin-7 by Edgar181

- Many peptides released from proteins during fermentation or digestion by proteolytic enzymes can exert various biologic activities, including opiate-like activity.
- Most of the dietary proteins demonstrating biological activity that have been investigated originate from milk, such as casomorphins.
- Gliadin in gluten: partial digestion can yield **gluten exorphins** which possess opiate-like activity (**gliadorphin** aka **gluteomorphin**); 5 distinct exorphins of gluten have been identified.

1. Walther B, Sieber R. Bioactive proteins and peptides in foods. Int J Vitam Nutr Res. 2011 Mar;81(2-3):181-92. doi: 10.1024/0300-9831/a000054.
2. De Noni I et al. Review of the potential health impact of β-casomorphins and related peptides. Scientific Report of European Food Safety Authority. Report of the DATEX Working Group on β-casomorphins. EFSA Scientific Report (2009) 231, 1-107.
3. Kohlstadt I. Advancing Medicine With Food And Nutrients, Second Edition. Hoboken: Taylor and Francis; 2013.

The Opioid Question, Peptide Absorption

1. A prerequisite for opioid activity after oral ingestion is that the peptides must pass the intestinal epithelial barrier.
2. In addition, subsequent biotransformation in the liver and stability in plasma may be factors determining the ultimate biological activity.
3. Finally, passage through the blood-brain-barrier is, in principle, needed for an activity in the central nervous system.

1. Walther B, Sieber R. Bioactive proteins and peptides in foods. Int J Vitam Nutr Res. 2011 Mar;81(2-3):181-92. doi: 10.1024/0300-9831/a000054.
2. De Noni I et al. Review of the potential health impact of β-casomorphins and related peptides. Scientific Report of European Food Safety Authority. Report of the DATEX Working Group on β-casomorphins. EFSA Scientific Report (2009) 231, 1-107.

The Opioid Question, Peptide Absorption

In order for exorphins to function as opioid peptides in the CNS in vivo, they must:

a) be produced in the GI tract,
b) survive degradation by intestinal proteases,
c) be absorbed- without degradation – into the bloodstream,
d) cross the blood-brain barrier and thereby reach central opiate receptors, and
e) interact as opiates with these receptors

Kohlstadt I. Advancing Medicine With Food And Nutrients, Second Edition. Hoboken: Taylor and Francis; 2013.

The Opioid Question, Peptide Absorption

It is often stated in popular literature that gluteomorphins readily cross the BBB into the brain and **exert opioid effects via binding to opioid receptors.**

- Food-derived exomorphins are oligo-peptides; most gluteomorphins are 4-7 amino acids in length.
- **There is virtually no small intestine absorption of peptides longer than four amino acids** (however, there is abundant absorption of di- and tripeptides).
- Once inside the enterocyte, the vast bulk of absorbed di- and tripeptides are digested into amino acids by cytoplasmic peptidases and exported from the cell into blood. **Only a very small number of these small peptides enter blood intact.**

1. De Noni I et al. Review of the potential health impact of β-casomorphins and related peptides. Scientific Report of European Food Safety Authority. Report of the DATEX Working Group on β-casomorphins. EFSA Scientific Report (2009) 231, 1-107. DOI: 10.2903/j.efsa.2009.231r
2. Absorption of Amino Acids and Peptides. Vivocolostateedu. 2017. Available at: http://www.vivo.colostate.edu/hbooks/pathphys/digestion/smallgut/absorb_aacids.html. Accessed October 9, 2017.

The Opioid Issue: Autism link?

- **Hypothesis: some symptoms of Autism Spectrum Disorder (ASD) may be caused by opioid peptides** created from the incomplete breakdown of foods containing gluten and casein.
- **A higher percentage of abnormal intestinal permeability tests** (found using the lactulose/ mannitol ratio) has been observed among patients with autism (36.7%) and their relatives (21.2%) compared to healthy individuals (4.8%).
- **Children with ASD have significantly higher levels of IgG antibodies (but not IgA) to gliadin** compared to healthy individuals.
    - IgG antibodies directed against food antigens are associated increased intestinal permeability.

1. Catassi C, Bai JC, Bonaz B, et al. Non-Celiac Gluten Sensitivity: The New Frontier of Gluten Related Disorders. Nutrients. 2013;5(10):3839-3853. doi:10.3390/nu5103839.
2. de Magistris L et al. Alterations of the intestinal barrier in patients with autism spectrum disorders and in their first-degree relatives. J Pediatr Gastroenterol Nutr. 2010. Oct;51(4):418-24. doi: 10.1097/MPG.0b013e3181dcc4a5.

Different pathogenic mechanisms are likely responsible for/ involved in different gluten & wheat-related conditions:

- **Wheat allergy** – gluten and potentially non gluten proteins
- **Celiac Disease and other Autoimmune conditions:** gluten proteins found in wheat, barley, and rye
- **Non-Celiac Gluten Sensitivity:** gluten proteins
- **Non-Celiac Wheat Sensitivity:** non-gluten proteins and reactants in wheat
  - Albumins, globulins, Amylase-Trypsin Inhibitors (ATIs)
  - Wheat germ agglutinin (a lectin)
  - Other unidentified protein antigens/epitopes…?
- **Non-Celiac Wheat Intolerance:**
  - FODMAP reactions – intestinal sx only

Carroccio A, Rini G, Mansueto P. Non-celiac wheat sensitivity is a more appropriate label than non-celiac gluten sensitivity. Gastroenterology. 2014 Jan;146(1):320-1. doi: 10.1053/j.gastro.2013.08.061.

Could NCGS be a FODMAP Issue?

When a gluten free diet resolves GI symptoms (bloating, abdominal pain, diarrhea), patients will often self-report and suspect a 'gluten sensitivity'.

- **NCGS may be related to FODMAPs in some patients reporting IBS symptoms.**
- People with NCGS can have IBS-like sx, but also extra-intestinal sx that cannot be explained with FODMAPs.

1. Capili, B., Chang, M., & Anastasi, J. K. (2014). A clinical update: Nonceliac gluten sensitivity--is it really the gluten? The Journal for Nurse Practitioners, 10(9), 666-673. doi:http://dx.doi.org/10.1016/j.nurpra.2014.07.036
2. Fasano, A. et al. (2015). Nonceliac Gluten Sensitivity. *Gastroenterology*, Mar. 2015, 1-10.
3. Fasano, A. (2015). Celiac Disease and Gluten-Related Disorders: A Clinical Conversation. *Alternative and Complementary Therapies*, 2015 Feb, Vol. 21:1, 18-21.
4. Priyanka P, Gayam S, Kupec JT. The Role of a Low Fermentable Oligosaccharides, Disaccharides, Monosaccharides, and Polyol Diet in Nonceliac Gluten Sensitivity. Gastroenterol Res Pract. 2018 Aug 6;2018:1561476. doi: 10.1155/2018/1561476.

…So are the carbohydrates the cause?

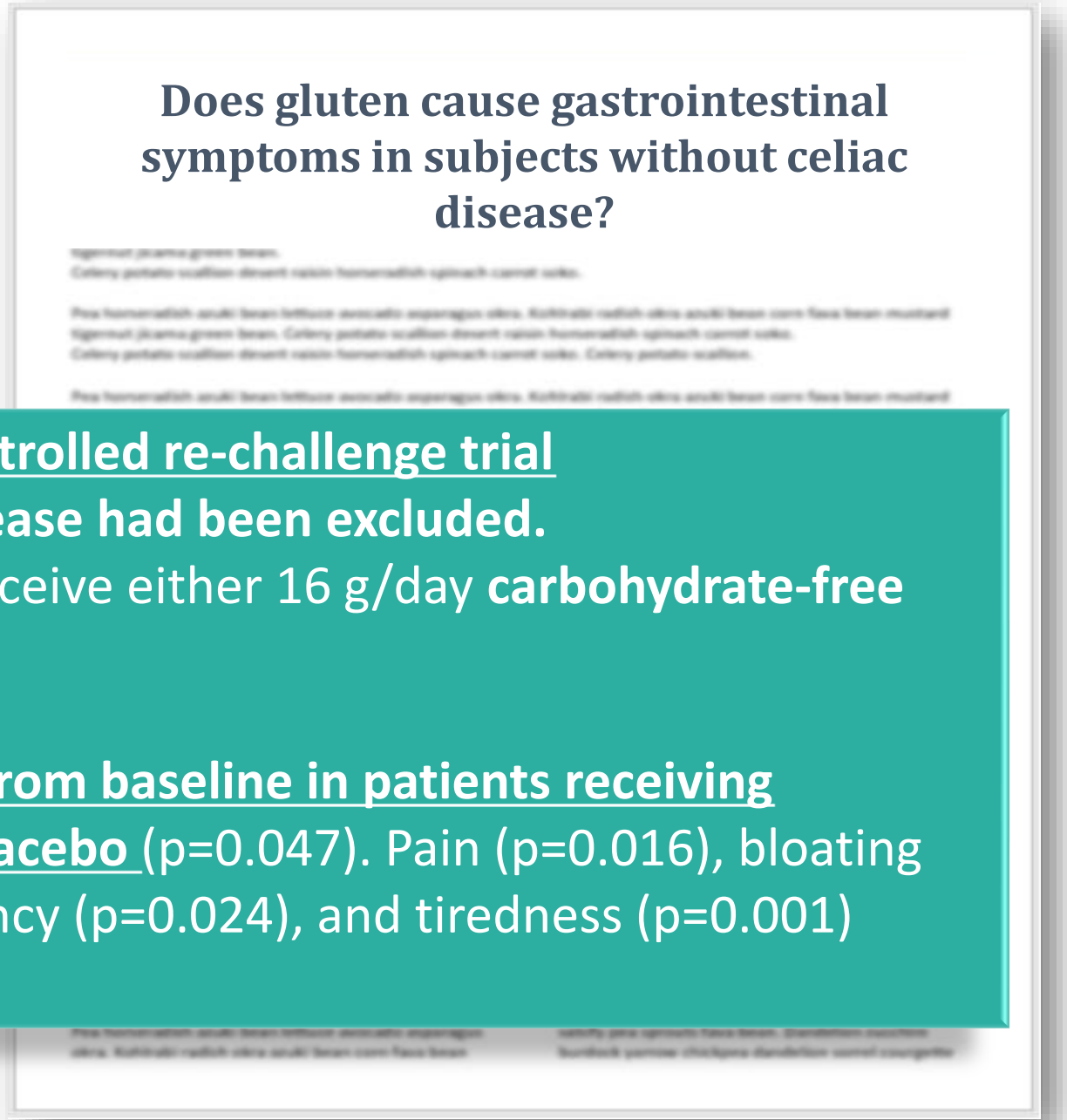
- FODMAPs can cause GI symptoms (bloating, etc.), but **they are known to inhibit, rather than trigger, intestinal inflammation.**
- This results in beneficial changes in the intestinal microbiome, including the generation of short-chain fatty acids (SCFAs).
- It is important to note that wheat and rye, when consumed in normal quantities, are only minor sources of FODMAPs.

"FODMAPs cause mild wheat intolerance at most, limited to intestinal symptoms."

(Fasano et al., 2015)

Fasano A, Sapone A, Zevallos V, Schuppan D. Nonceliac Gluten Sensitivity. *Gastroenterology*. 2015;148(6):1195-1204.

Study using **carbohydrate-free** gluten, not wheat



A double-blinded, randomized, placebo-controlled re-challenge trial

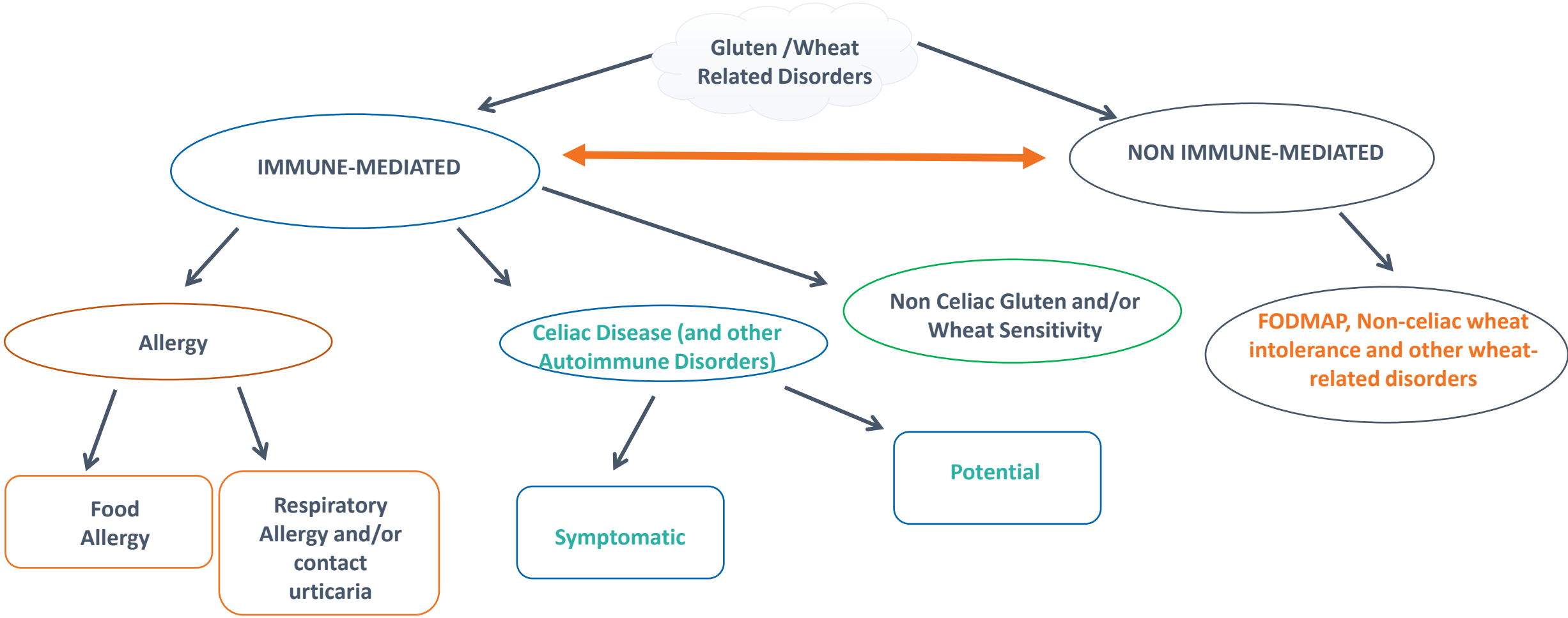
- **Participants: patients in whom celiac disease had been excluded.**
- Participants were randomly assigned to receive either 16 g/day **carbohydrate-free gluten** or placebo for six weeks.

- **Symptom severity changed significantly from baseline in patients receiving carbohydrate-free gluten compared to placebo** (p=0.047). Pain (p=0.016), bloating (p=0.031), satisfaction with stool consistency (p=0.024), and tiredness (p=0.001) worsened significantly after 1 week.

Newnham E. Does gluten cause gastrointestinal symptoms in subjects without coeliac disease? Journal of gastroenterology and hepatology. 2011;26:132–4.

SUMMARY POINTS

Schematic of Gluten/Wheat Related Disorders



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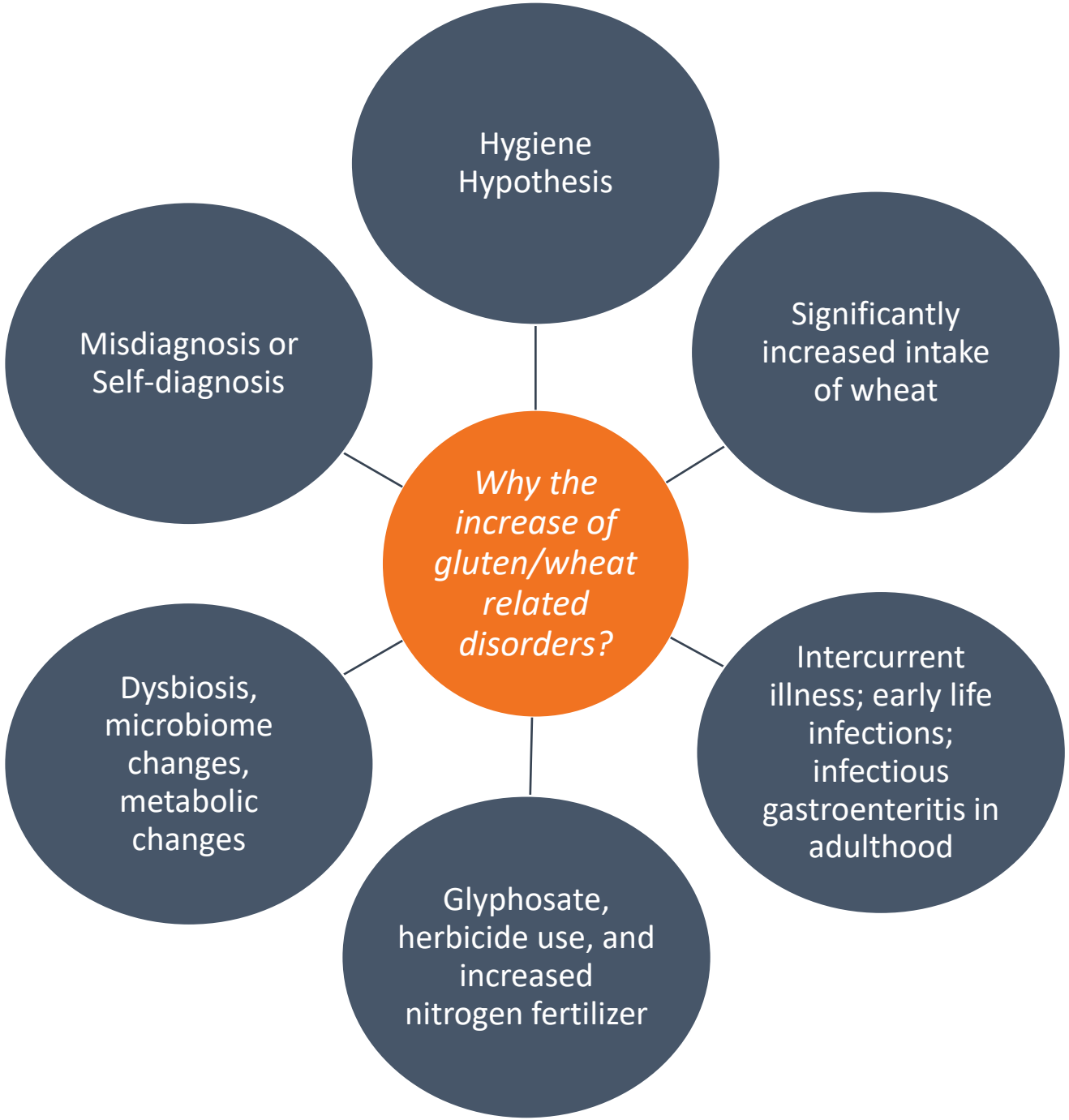
Reactions to Wheat Components

Components of Wheat	Effects	Associated GI Conditions
Gluten	• Damage to enterocyte tight junctions leading to intestinal permeability • Activation of CD4 T lymphocytes and pro-inflammatory cytokines (IFN- γ) • Infiltration of eosinophils • Secretion of anti-gliadin and anti-tissue-transglutaminase antibodies • Increased density of CD8 intraepithelial cells • TLR elevation • Activation of the innate immune response	Celiac disease, NCGS
Wheat protein	• Activation of pro-inflammatory cytokines • Inhibition of gut epithelial cell repair	Wheat allergy, NCWS
α-amylase and trypsin (ATI)	• Activation of TLR4 and the innate immune response • Increase in inflammation	Celiac disease, NCWS, IBS, IBD
Rapidly fermentable carbohydrates (FODMAPS)	• Fermentation of indigestible carbohydrates leading to the production of gas and short chain fatty acids	IBS, NCWS

See References: Reactions to Wheat Components

References: Reactions to Wheat Components

1. Brouns F, Rooy GV, Shewry P, Rustgi S, Jonkers D. Adverse reactions to wheat or wheat components. Comprehensive Reviews in Food Science and Food Safety. 2019; 18(5): 1437-1452.
2. Junker Y, Zeissig S, Kim SJ, et al. Wheat amylase trypsin inhibitors drive intestinal inflammation via activation of toll-like receptor 4. J Exp Med. 2012;209(13):2395-2408. doi:10.1084/jem.20102660
3. Vojdani A, Perlmutter D. Differentiation between Celiac Disease, Nonceliac Gluten Sensitivity, and Their Overlapping with Crohn's Disease: A Case Series. Case Reports Immunol. 2013;2013:248482. doi:10.1155/2013/248482
4. Parzanese I, Qehajaj D, Patrinicola F, et al. Celiac disease: From pathophysiology to treatment. World J Gastrointest Pathophysiol. 2017;8(2):27-38. doi:10.4291/wjgp.v8.i2.27
5. Aziz I, Hadjivassiliou M, Sanders DS. The spectrum of noncoeliac gluten sensitivity. Nat Rev Gastroenterol Hepatol. 2015 Sep;12(9):516-26. doi: 10.1038/nrgastro.2015.107. Epub 2015 Jun 30. PMID: 26122473.



See References: "References: Why the Increase Graphic"

References: Why the Increase Graphic

1. Hygiene Hypothesis: Riddle MS, Murray JA, Porter CK. The Incidence and Risk of Celiac Disease in a Healthy US Adult Population. The American journal of gastroenterology. 2012;107(8):1248-1255. doi:10.1038/ajg.2012.130.
2. Dr. FAQ: Stefano Guandalini on the Rise of Celiac Disease. Science Life. 2017. Available at: https://sciencelife.uchospitals.edu/2014/02/05/dr-faq-stefano-guandalini-on-the-rise-of-celiac-disease/. Accessed October 4, 2017.
3. Rubio-Tapia A, Kyle RA, Kaplan EL, et al. Increased Prevalence and Mortality in Undiagnosed Celiac Disease. Gastroenterology. 2009;137(1):88-93. doi:10.1053/j.gastro.2009.03.059.
4. Green PH, Cellier C. Celiac disease. N Engl J Med 2007; 357:1731–1743.
5. Andrén Aronsson C, Lee HS, Hård Af Segerstad EM, Uusitalo U, Yang J, Koletzko S, Liu E, Kurppa K, Bingley PJ, Toppari J, Ziegler AG, She JX, Hagopian WA, Rewers M, Akolkar B, Krischer JP, Virtanen SM, Norris JM, Agardh D; TEDDY Study Group. Association of Gluten Intake During the First 5 Years of Life With Incidence of Celiac Disease Autoimmunity and Celiac Disease Among Children at Increased Risk. JAMA. 2019 Aug 13;322(6):514-523. doi: 10.1001/jama.2019.10329.
6. Sapone, A. et al. (2012). Spectrum of gluten-related disorders: consensus on new nomenclature and classification. *BMC Medicine* 2012, 10:13.
7. Increased consumption: Fasano, A. et al. (2015). Nonceliac Gluten Sensitivity. Gastroenterology, Mar. 2015, 1-10.
8. Increased consumption: Khamsi, R. (2014). The Trouble with Gluten. Scientific American, Feb. 2014.
9. Increased consumption: Koning F. Celiac disease: quantity matters. Seminars in Immunopathology. 2012;34(4):541-549. doi:10.1007/s00281-012-0321-0.
10. Glyphosate/Herbicide: Samsel A, Seneff S. Glyphosate, pathways to modern diseases II: Celiac sprue and gluten intolerance. Interdisciplinary Toxicology. 2013;6(4):159-184. doi:10.2478/intox-2013-0026.
11. Microbiome Changes: Fasano, A. (2015). Celiac Disease and Gluten-Related Disorders: A Clinical Conversation. *Alternative and Complementary Therapies*, 2015 Feb, Vol. 21:1, 18-21.
12. Infections: Stene LC et al. Rotavirus infection frequency and risk of celiac disease autoimmunity in early childhood: a longitudinal study. Am J Gastroenterol. 2006 Oct;101(10):2333-40.

Stay Tuned!

- **At the GI APM, we will discuss the clinically important follow up to this topic:**
  - Prevalence
  - Presentation
  - Diagnostic criteria
  - Clinically significant lab markers