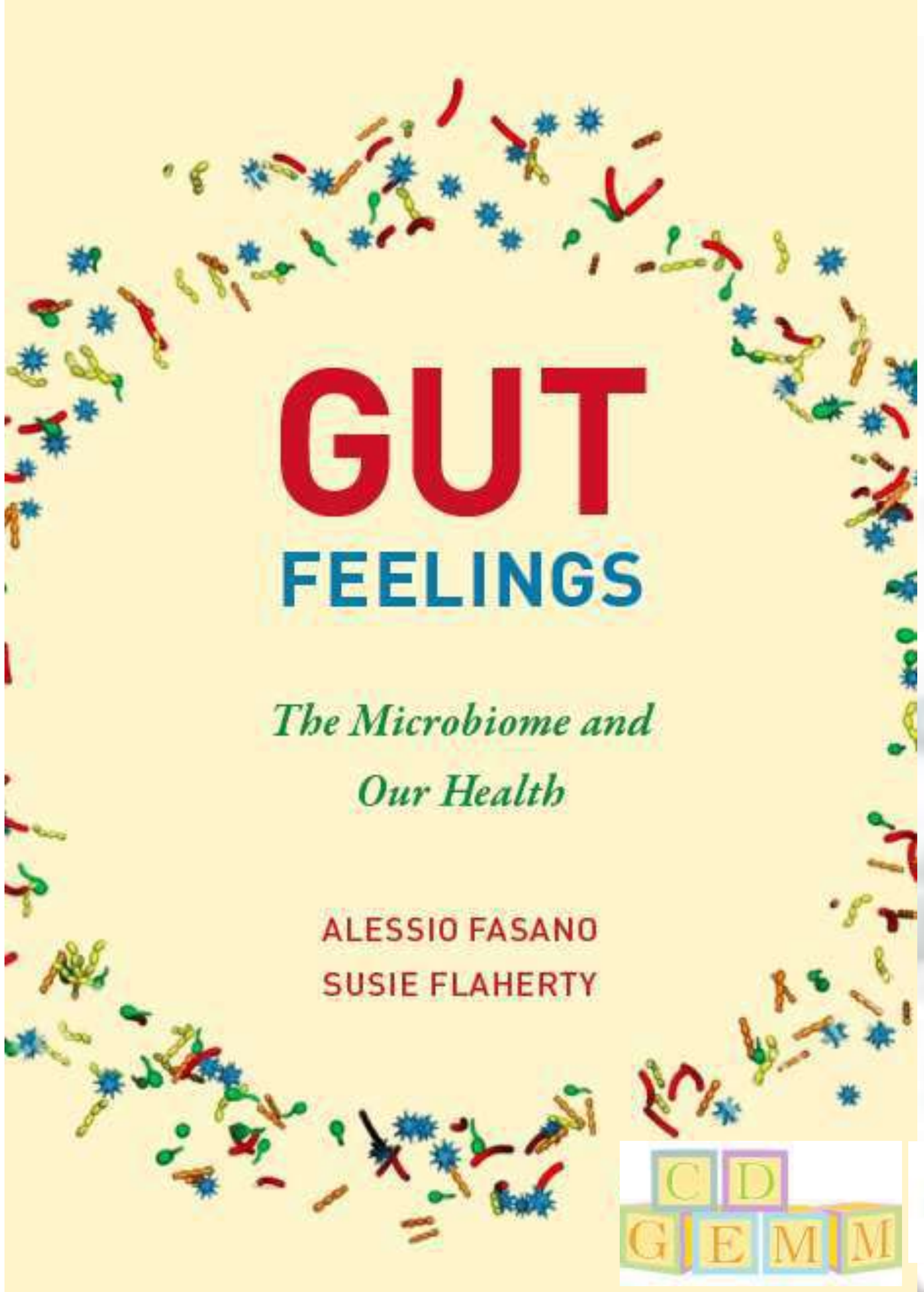


Microbiota e Zonulina, Malattie Metaboliche A Base Infiammatoria, Cenni Di Base Del Sistema Gastro-Intestinale

.

Alessio Fasano, M.D.
W. Allan Walker Chair in Pediatric Gastroenterology and Nutrition
Professor of Pediatrics Harvard Medical School
Professor of Nutrition Harvard T.H. Chen School of Public Health
Mucosal Biology and Immunology Research Center
And Center for Celiac Research
Massachusetts General Hospital for Children

LEARNING OBJECTIVES

- **Define the role of gut permeability in the pathogenesis of chronic inflammatory diseases, including metabolic disorders**

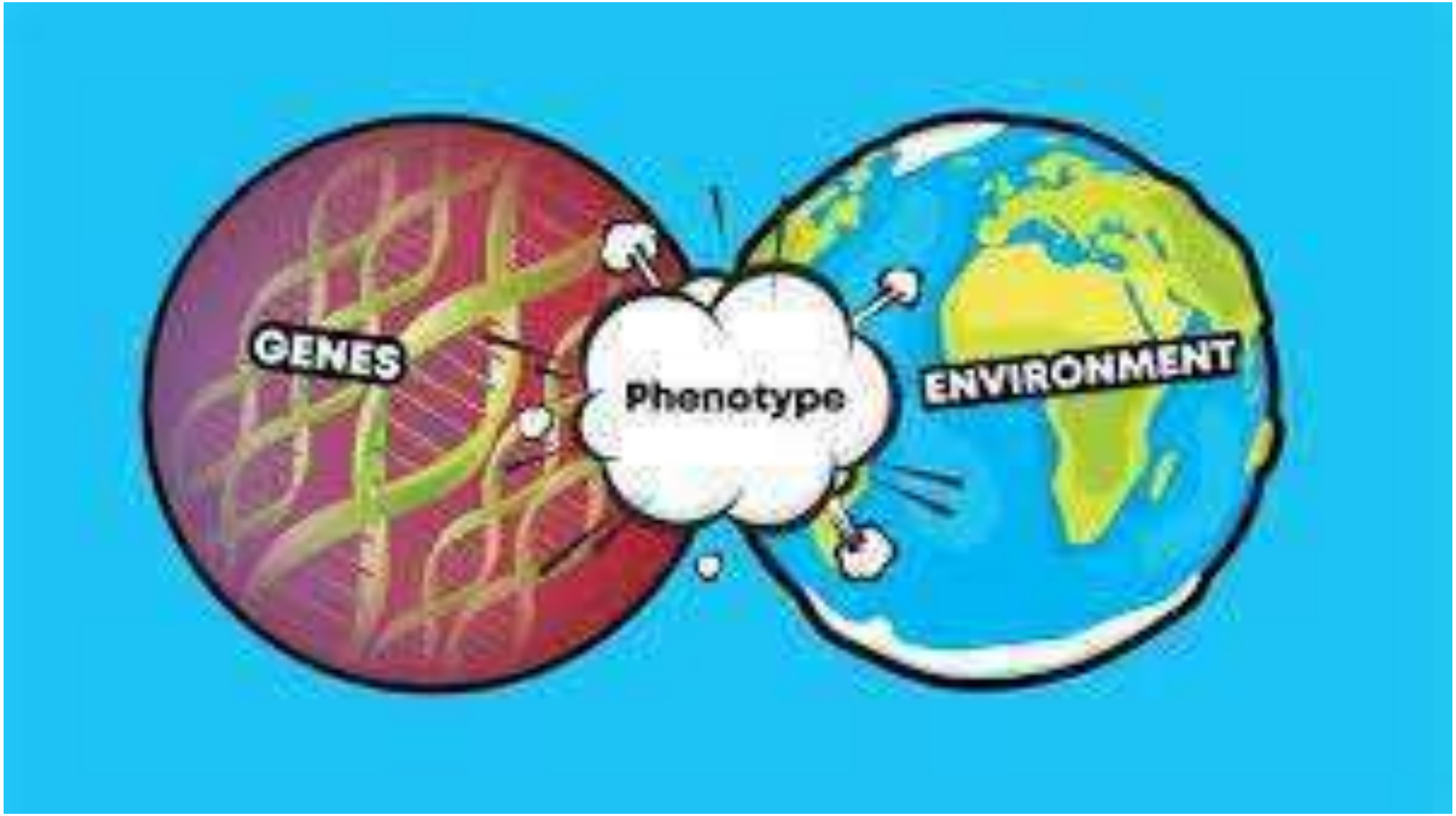
- **Outline the interconnection between antigen trafficking (gut permeability), immune system and microbiome in dictating the balance between health and disease**

- **Discuss the crucial role of gut permeability and microbiome composition and function in programming the immune system during the first 1000 days of life**

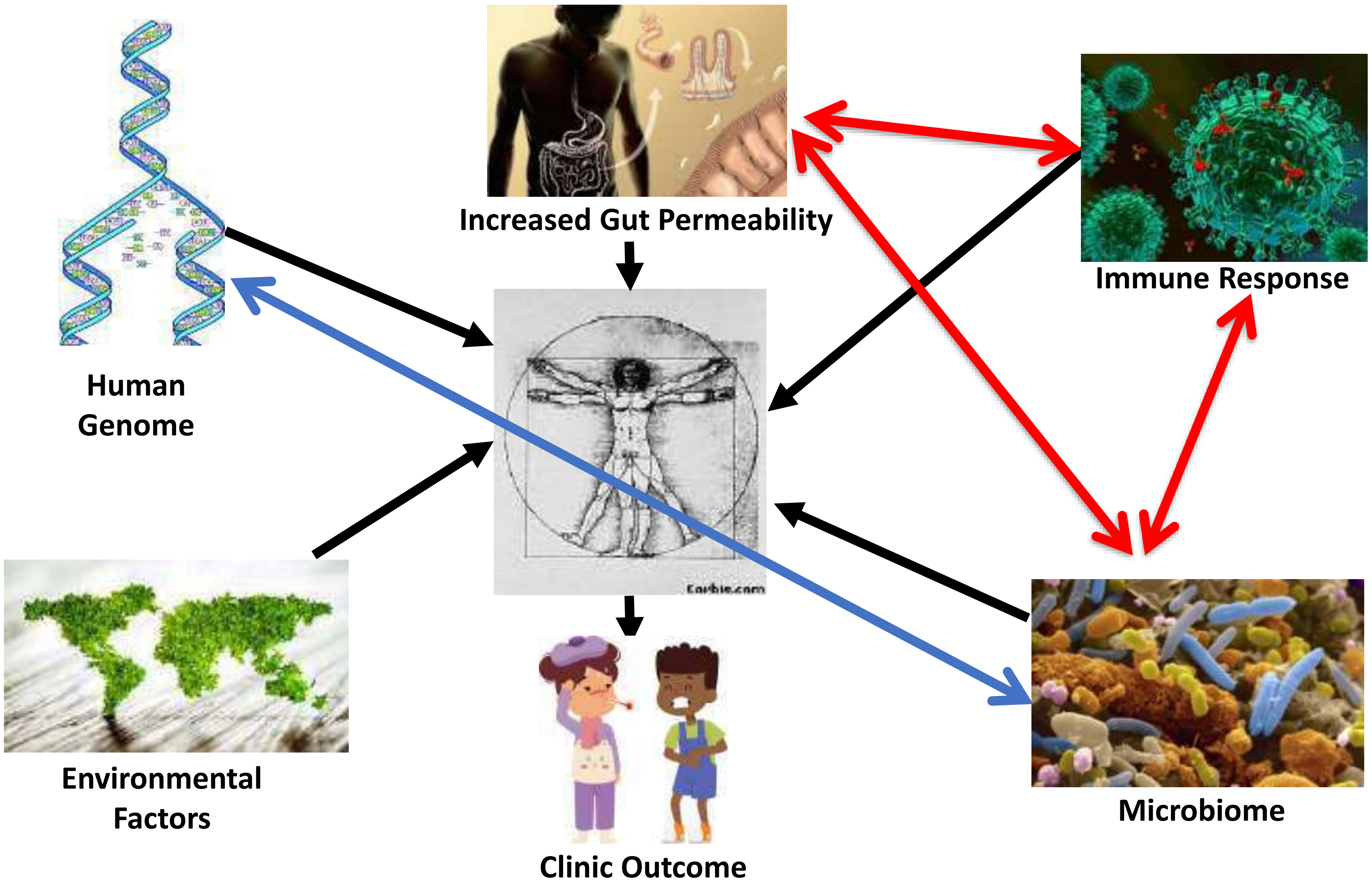
- **Provide examples of chronic inflammatory diseases in which gut permeability is at play**

- **Outline the importance of nutrition as a possible therapeutic intervention to influence microbiome composition and function, so mitigating inflammatory processes**

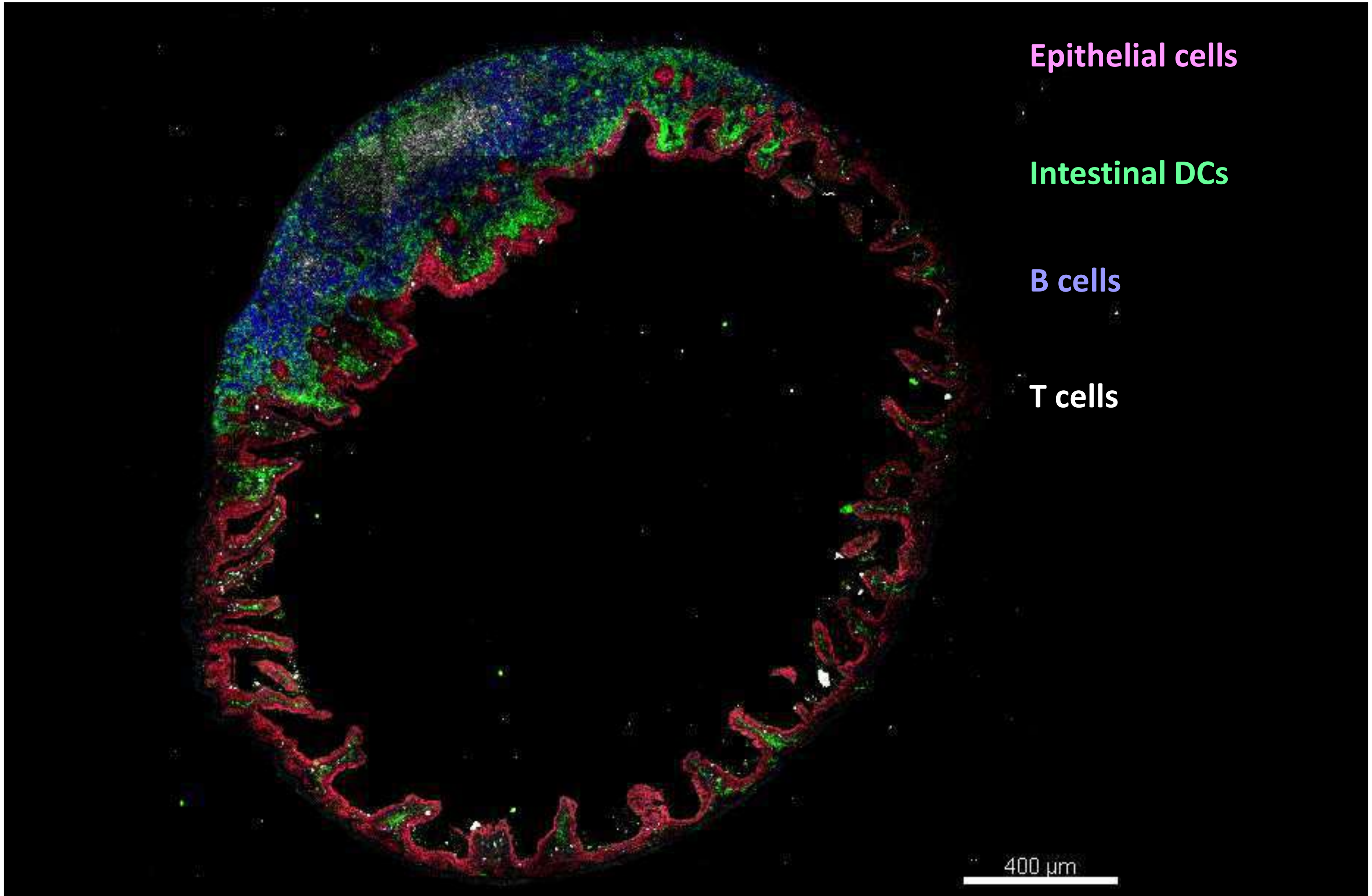
Old Theory of Pathogenesis of Human Diseases



The Yin and Yang Between Tolerance and Immune Response Leading To CID



Several Cells Play a Role in Maintaining The Immune Homeostasis



Excessive and Inappropriate Inflammatory Process Associated to a Dysfunction of Intestinal Barrier:

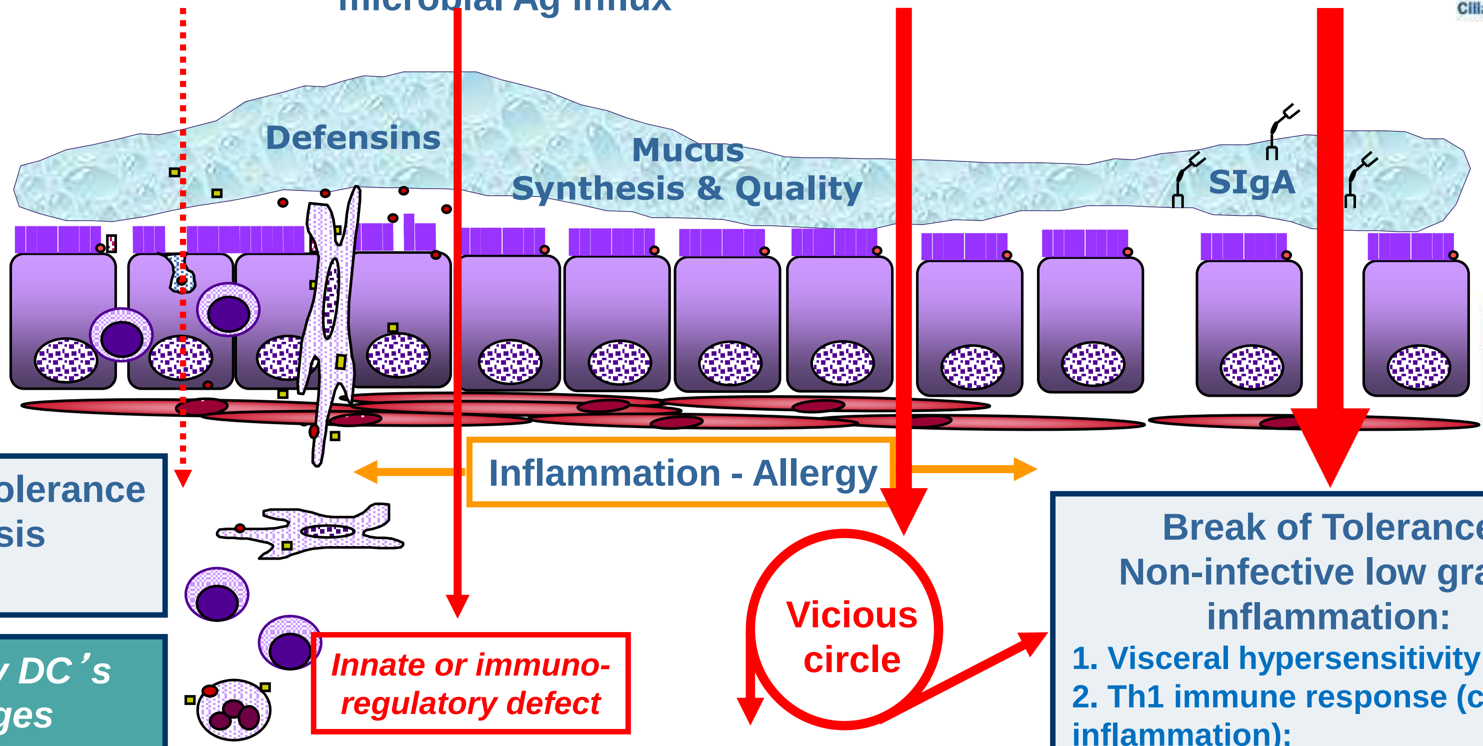
Loss of Mucosal Immune Homeostasis

❶ Normal/physiologically controlled permeability

❷ Minor Zonulin-dependent barrier defect dietary/ microbial Ag influx

❸ Increased permeability

❹ Massive dietary and microbial antigen influx



Mucosal Tolerance
Homeostasis
Anergy

Regulatory DC's
Macrophages
Tregs
IL-10/TGF-β

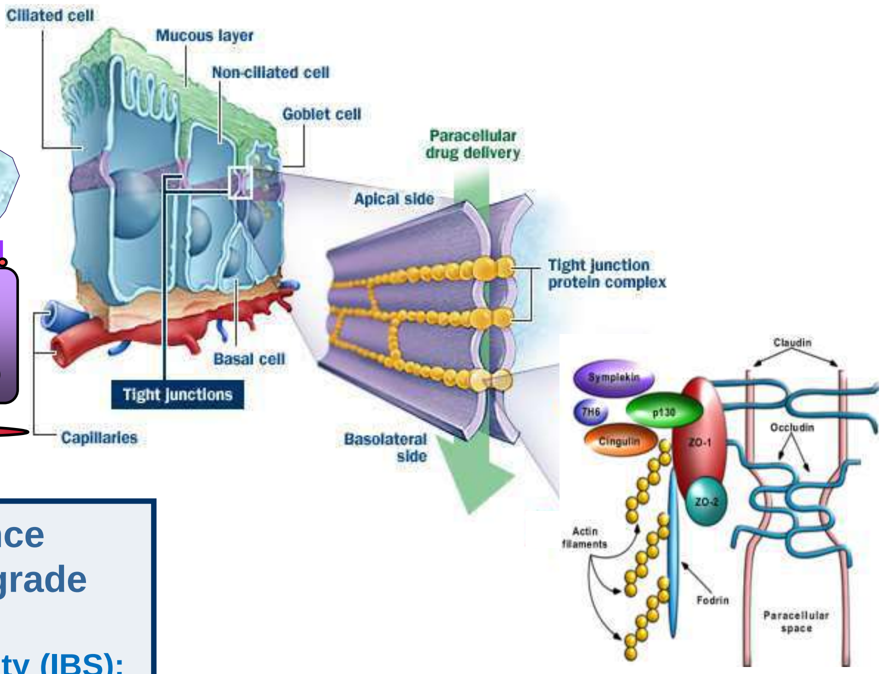
Innate or immuno-regulatory defect

Proinflammatory Allergic cytokines

Break of Tolerance
Non-infective low grade inflammation:

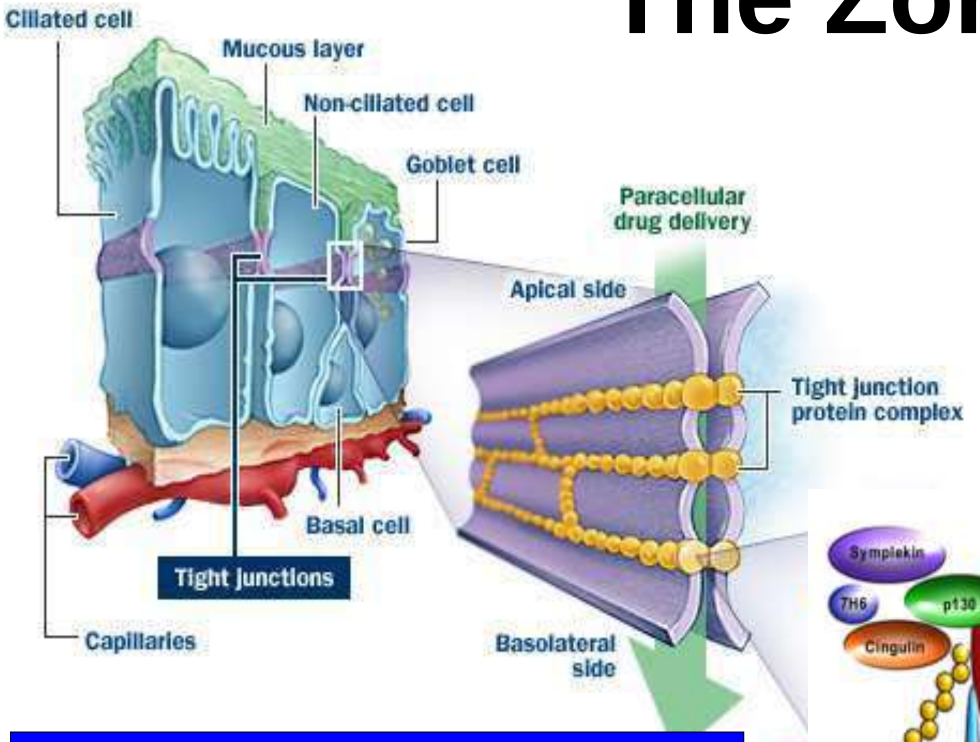
1. Visceral hypersensitivity (IBS);
2. Th1 immune response (chronic inflammation);
3. Th2 immune response (food allergies);
4. Th17 immune response (autoimmunity).

Adapted from P. Brandtzaeg, Beneficial Microbes 2010



…Tight junctions are a 'dark horse' implicated in a host of disease states, ranging from acute injury to chronic inflammation and autoimmune diseases

The Zonulin Pathway

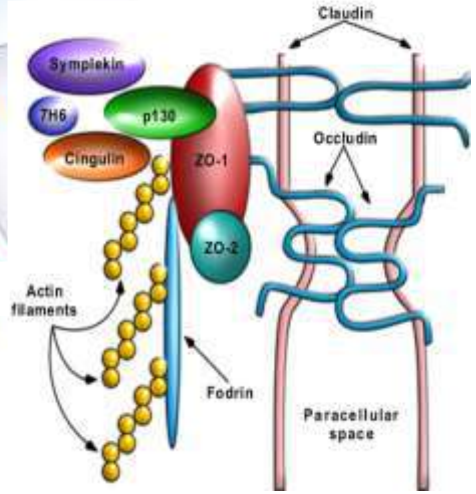


Human zonulin, a potential modulator of intestinal tight junctions

Wenle Wang[1], Sergio Uzzau[1], Simeon E. Goldblum[2] and Alessio Fasano[1,3,*]

[1]Division of Pediatric Gastroenterology and Nutrition and Gastrointestinal Pathophysiology Section, Center for Vaccine Development, [2]Division of Infectious Diseases, Department of Veterans Affairs Medical Center and [3]Department of Physiology, University of Maryland, School of Medicine, Baltimore, Maryland 21201, USA

*Author for correspondence at address 1 (e-mail: afasano@umaryland.edu)



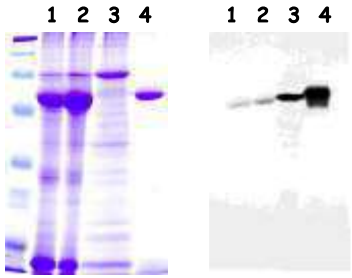
PURIFICATION PROTOCOL FROM HUMAN INTESTINE

1: Tissue lysate

2: Sephacryl-S300

3: Q-sepharose

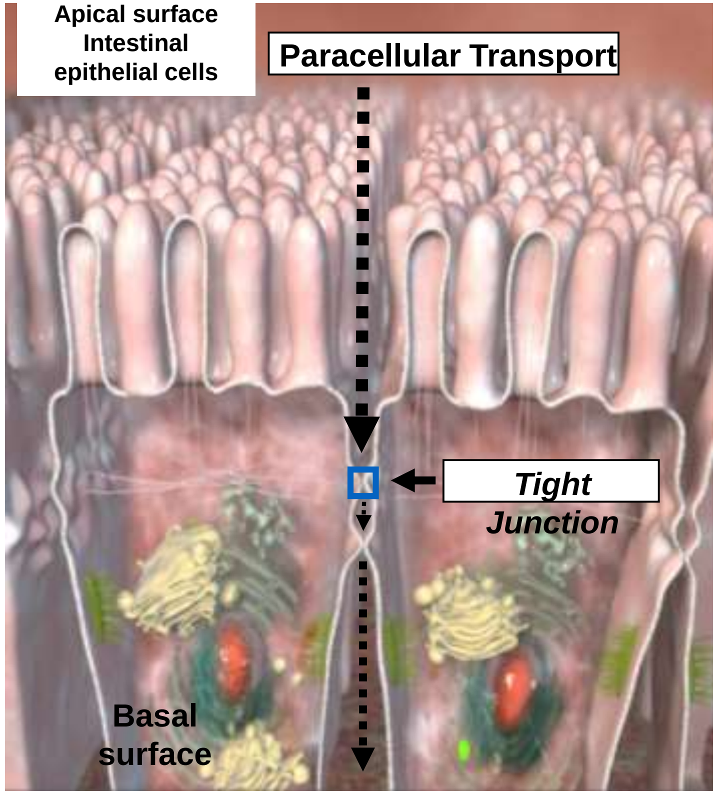
4: Immuoaffinity



Coomassie Western blot

…Tight junctions are a 'dark horse' implicated in a host of disease states, ranging from acute injury to chronic inflammation and autoimmune diseases

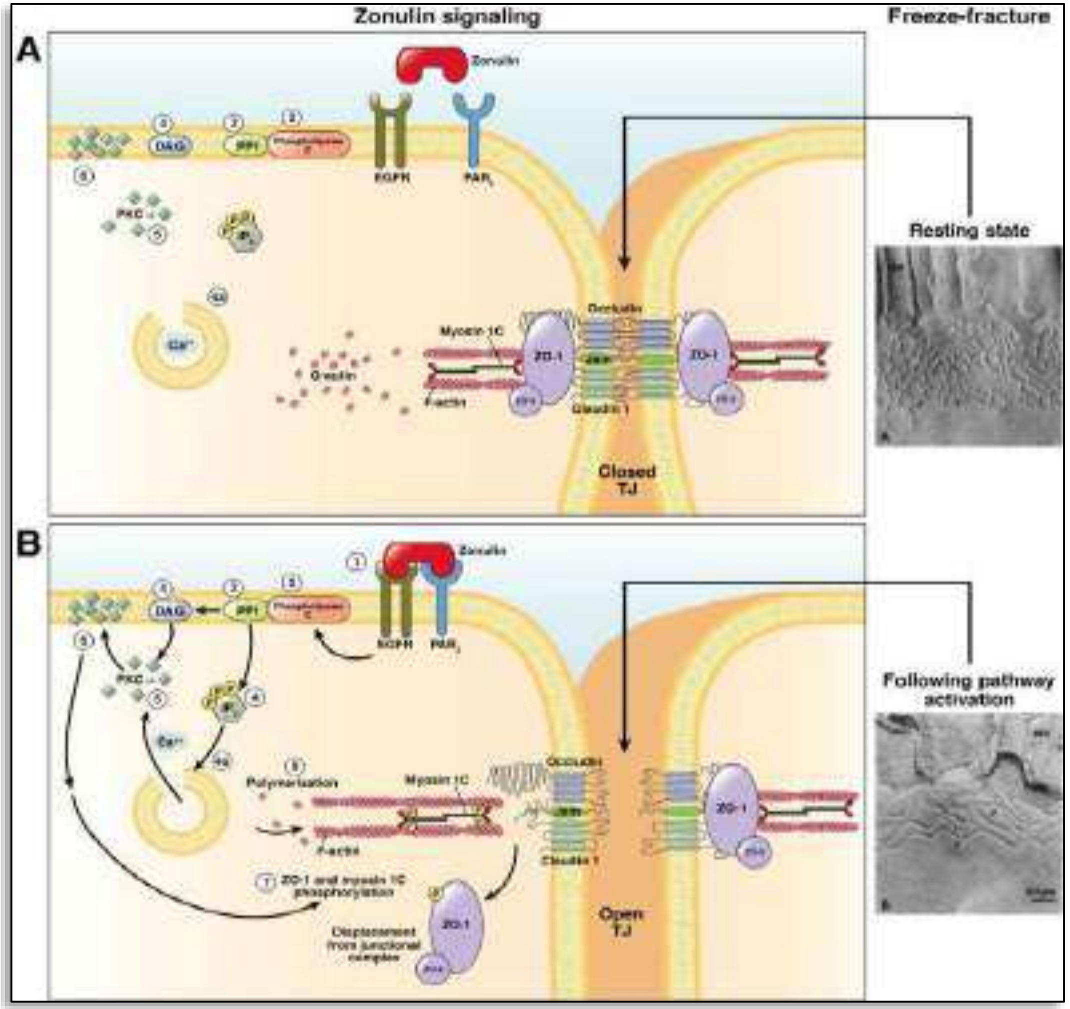
Fasano A. et al Lancet 2000;355:1518-1519.-
Wang W et al *J Cell Sci 2000;*24:4435-4440



- Tight junctions are inter-cellular "gates" that open and close in response to internal and external stimuli
  - Allows for immune surveillance
  - Modulates immune function
  - Regulates exchange of small molecules, proteins and cells across these barriers
- Zonulin is the only physiologic regulator of tight junction permeability discovered so far
- Paradigm shift in the treatment of immune mediated and chronic inflammatory diseases (e.g. Celiac Disease, T1D, MS, IBD, IBS, etc.)

Literature Report on Zonulin and Chronic Inflammatory Diseases[1,2]

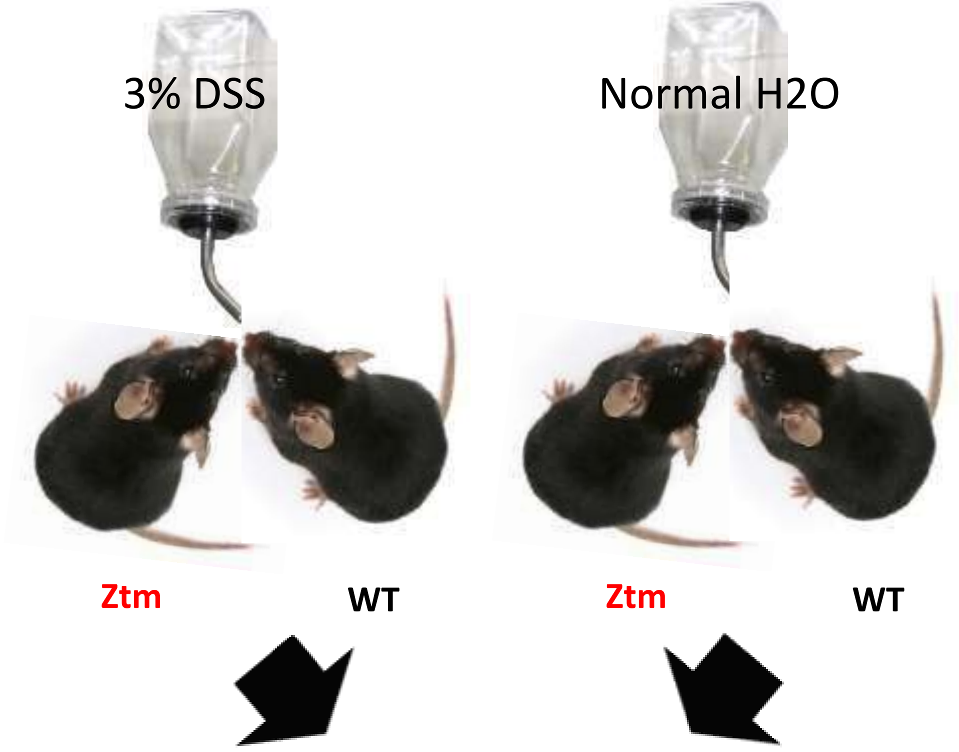
Disease	Model	Reference (PMID)	Disease	Model	Reference (PMID)
ADHD	Human	36786182	**Irritable bowel syndrome**	Human	31210949
Aging	Human	29896420	**HIV**	Human	29762690
Ankylosing spondylitis	Human	28069576	**Long COVID**	Human	1182544
Asthma	Human	34465387	**MIS-C**	Human	34032635
Autism	Human	36447452	**ME/CFS**	Human	35946099
Bipolar disorders	Human	37098666	**Multiple sclerosis**	Mouse	25184418
Celiac ddisease	Human	32162764	**Multiple sclerosis**	Human	31317818
Colitis/IBD (Crohn disease)	Human	34979917	**Necrotizing enterocolitis (NEC)**	Human	35279661
Colitis	Mouse	28423466	**Nonalcoholic fatty liver disease**	Human	32255299
Depressive disorders	Human	34320451	**Non-Celiac gluten Sensitivity**	Human	32060130
Food allergies	Human	36297068	**Obesity/insulin resistance**	Human	35666025
Gestational diabetes	Human	35994108	**Sepsis**	Human	23457771
Glioma	Human	19701495	**Type 1 diabetes**	Human	16644703
Glioma	Cells	23637756	**Type 2 diabetes**	Human	24347174



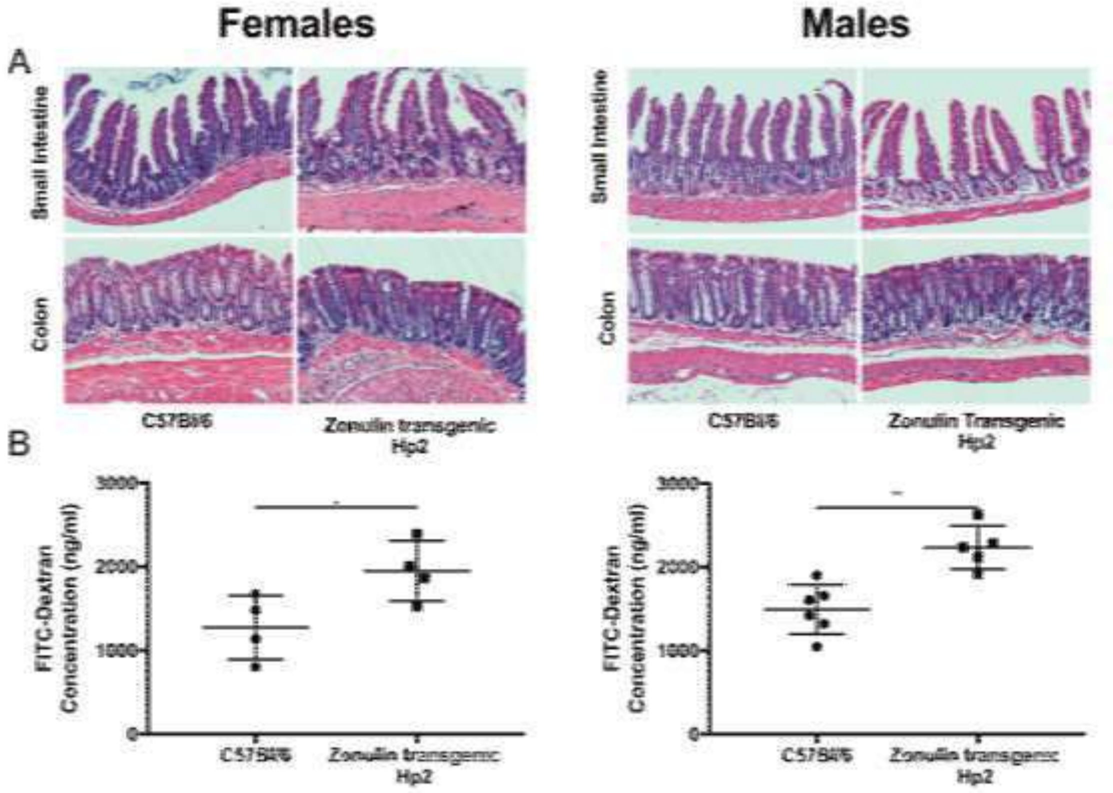
1. Fasano A. *Clin Gastroenterol Hepatol.* 2012;10(10):1096-1100. **2.** Sturgeon C, Fasano A. *Tissue Barriers.* 2016;4(4):e1251384.

How Zonulin-Mediated Increased Ag Trafficking Leads to Chronic Inflammation:

Insights From The Zonulin Trangenic Mouse (ztm) Model



Daily Body Weight after 7 days all mice are put on Normal drinking water

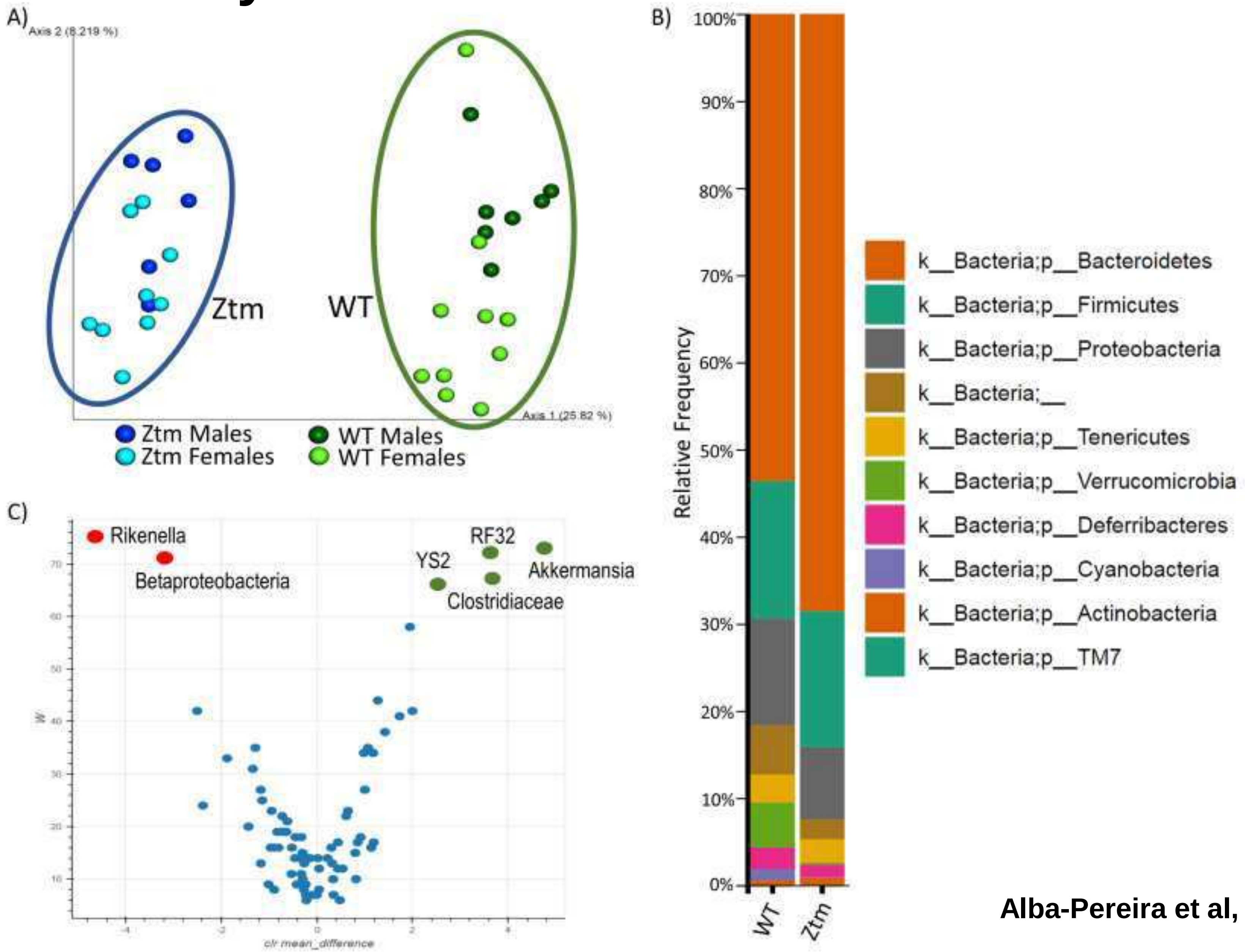


Zonulin transgenic Hp2 mice are phenotypically normal despite increased small intestinal permeability

*p<0.05

Sturgeon C et al. Ann NY Acad Sci 2017

Ztm Showed Dysbiosis Characterized by Increase pro-inflammatory Rikenella And Decreased Akkermansia



Alba-Pereira et al, Front Immunol 2019

Ztm Showed No Differences in Adaptive Immune Profiling, But Differences in Innate Immune Cell Subsets

Ztm and Ztm$_{bWT}$ showed:

-Increase in IL7R$^+$ROR$\gamma\tau^+$ innate lymphoid cells in the small intestine (no changes in the colon);

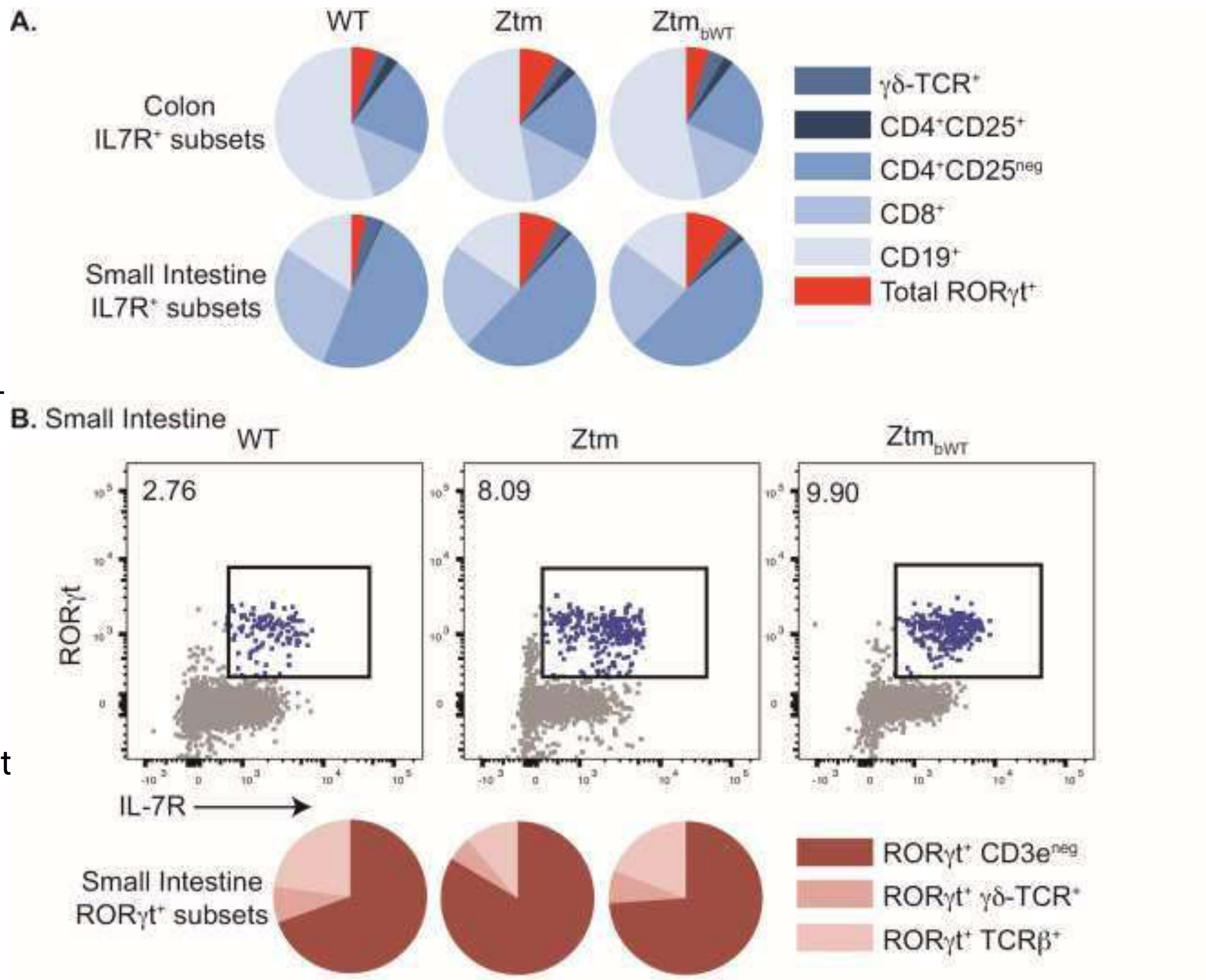
- Decreased frequency and numbers of invariant NKT (iNKT) cells (involved in mucosal immunity);

- Increased ROR$\gamma\tau$ expressing subset of iNKT cells (NKT17 cells). NKT17 cells and $\gamma\delta$-17 T cells are pro-inflammatory innate-like T cell subsets that produce IL-17 and have been implicated in the pathogenesis of various autoimmune diseases, including CD and T1D;

-Increased splenic plasmacytoid dendritic cells.

Combined, these these data suggest that altered gut permeability increases frequency of IL-17 producing T cells in mucosal tissue and in secondary lymphoid organs of Ztm mice.

The fact that the engraftment of WT microbiota did not affect the immune phenotype in Ztm$_{bWT}$ suggests that the increased antigen trafficking through an impaired gut barrier more than the function of an imbalanced microbiota primarily imprints the development of the immune system in the Ztm.



Alba-Pereira et al, Front Immunol 2019

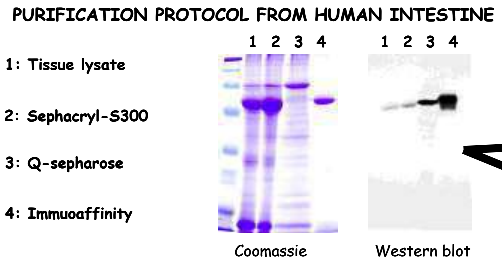
Factors Triggering Zonulin Release: Gluten

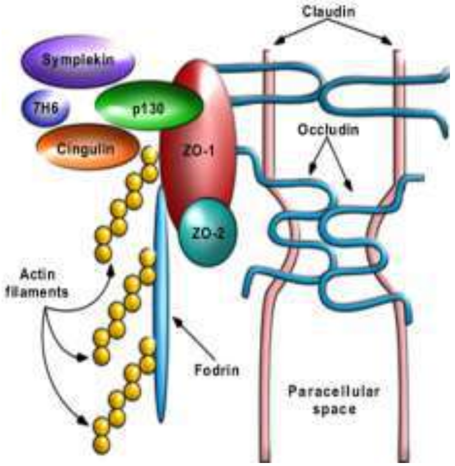
ADAPTIVE IMMINE RESPONSE
Shan L et al, *Science*. 2002; 297:2275-9.

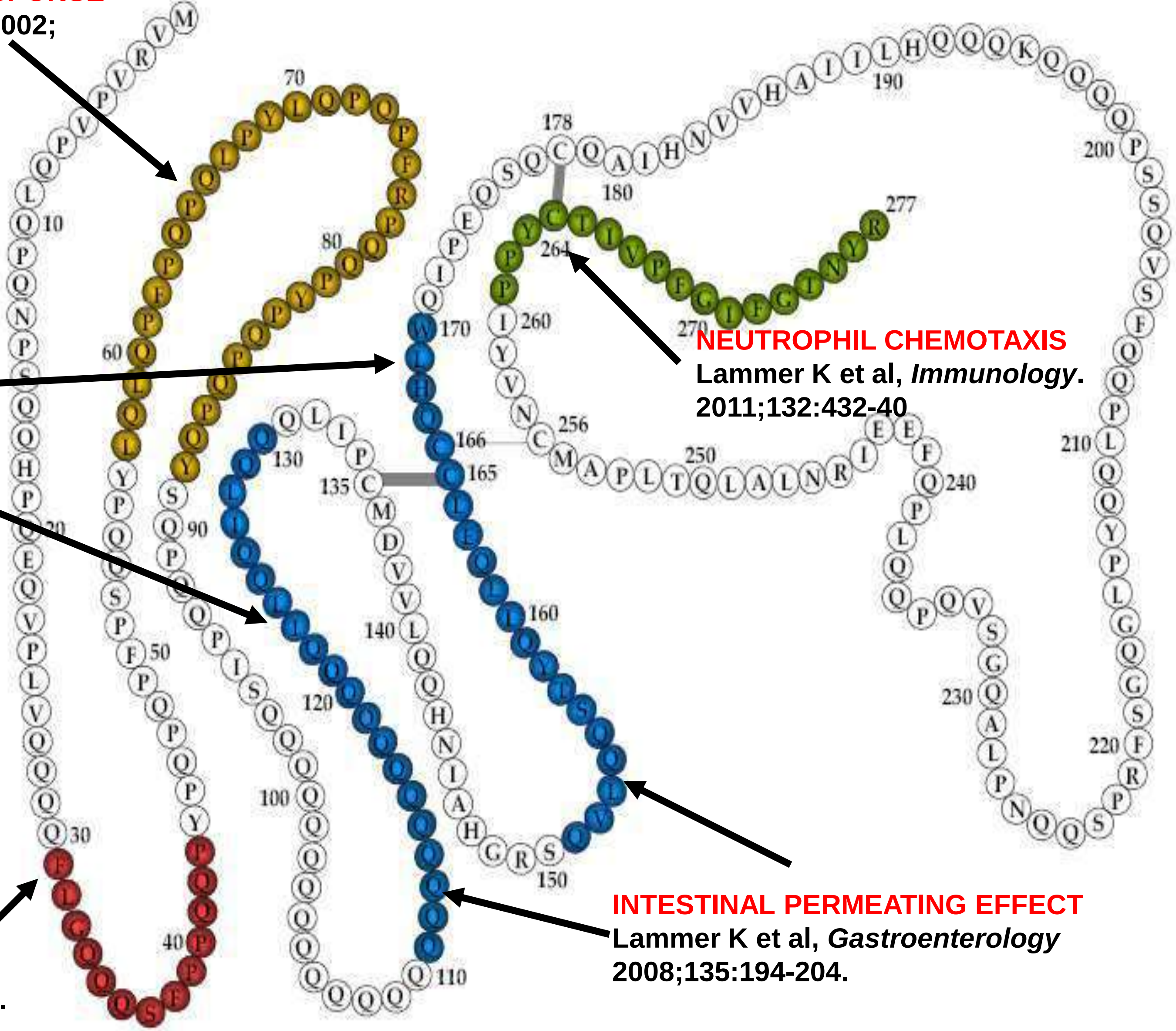

Human zonulin, a potential modulator of intestinal tight junctions

Wenie Wang[1], Sergio Uzzau[1], Simeon E. Goldblum[2] and Alessio Fasano[1,3,*]

[1]Division of Pediatric Gastroenterology and Nutrition and Gastrointestinal Pathophysiology Section, Center for Vaccine Development, [2]Division of Infectious Diseases, Department of Veterans Affairs Medical Center and [3]Department of Physiology, University of Maryland, School of Medicine, Baltimore, Maryland 21201, USA







NEUTROPHIL CHEMOTAXIS
Lammer K et al, *Immunology*. 2011;132:432-40

INTESTINAL PERMEATING EFFECT
Lammer K et al, *Gastroenterology* 2008;135:194-204.

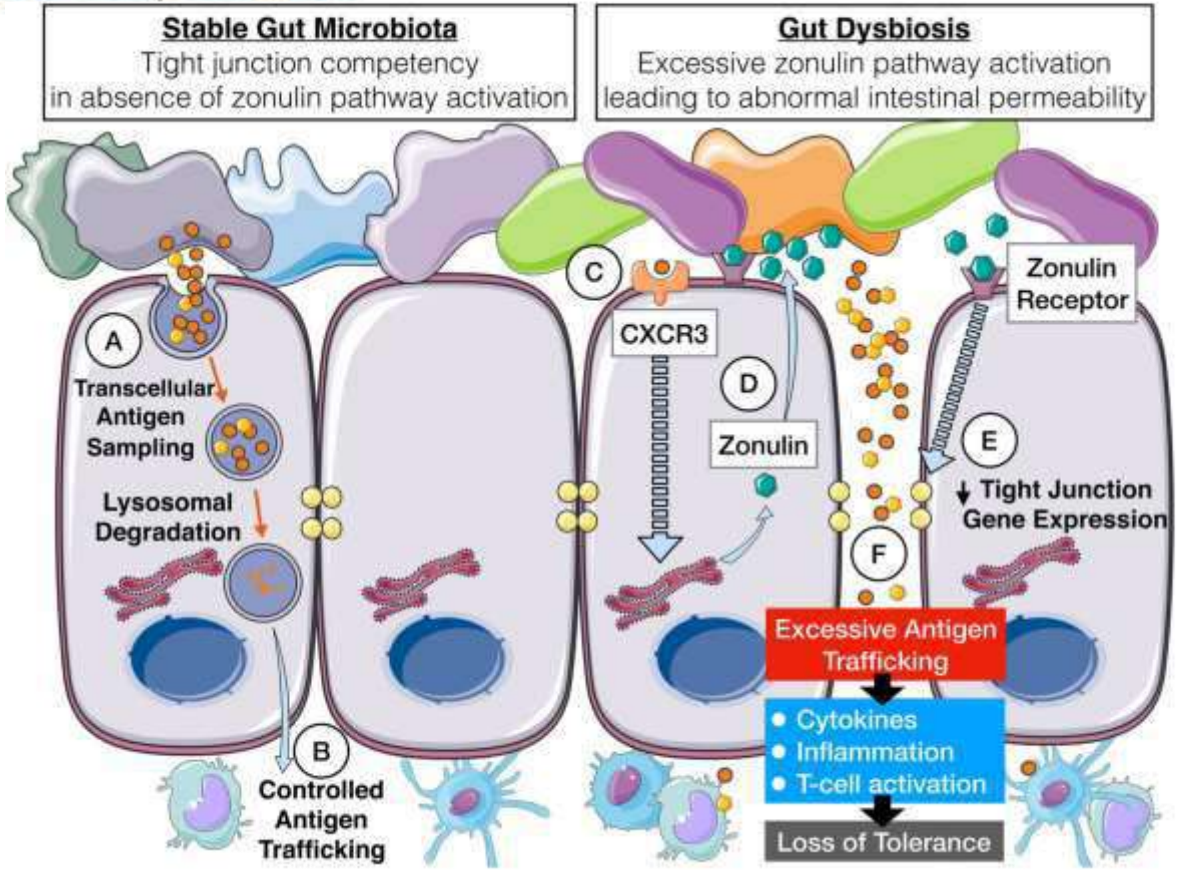
INNATE IMMUNE RESPONSE
Maiuri et al. *Scand J Gastroenterol*. 1996; 31:247-53.

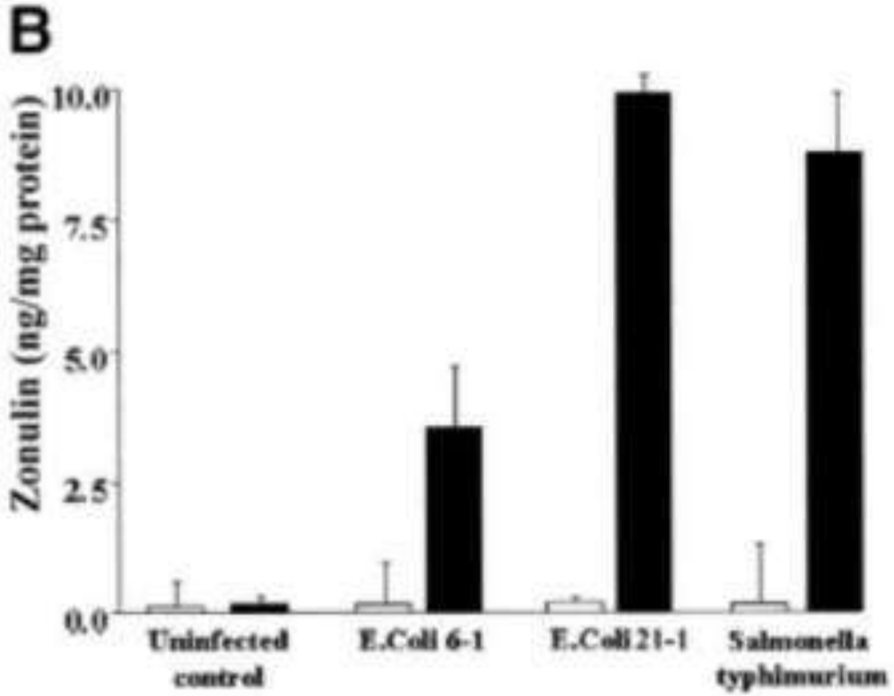
Factors Triggering Zonulin Release: Dysbiosis

> Gastroenterology. 2002 Nov;123(5):1607-15. doi: 10.1053/gast.2002.36578.

Host-dependent zonulin secretion causes the impairment of the small intestine barrier function after bacterial exposure

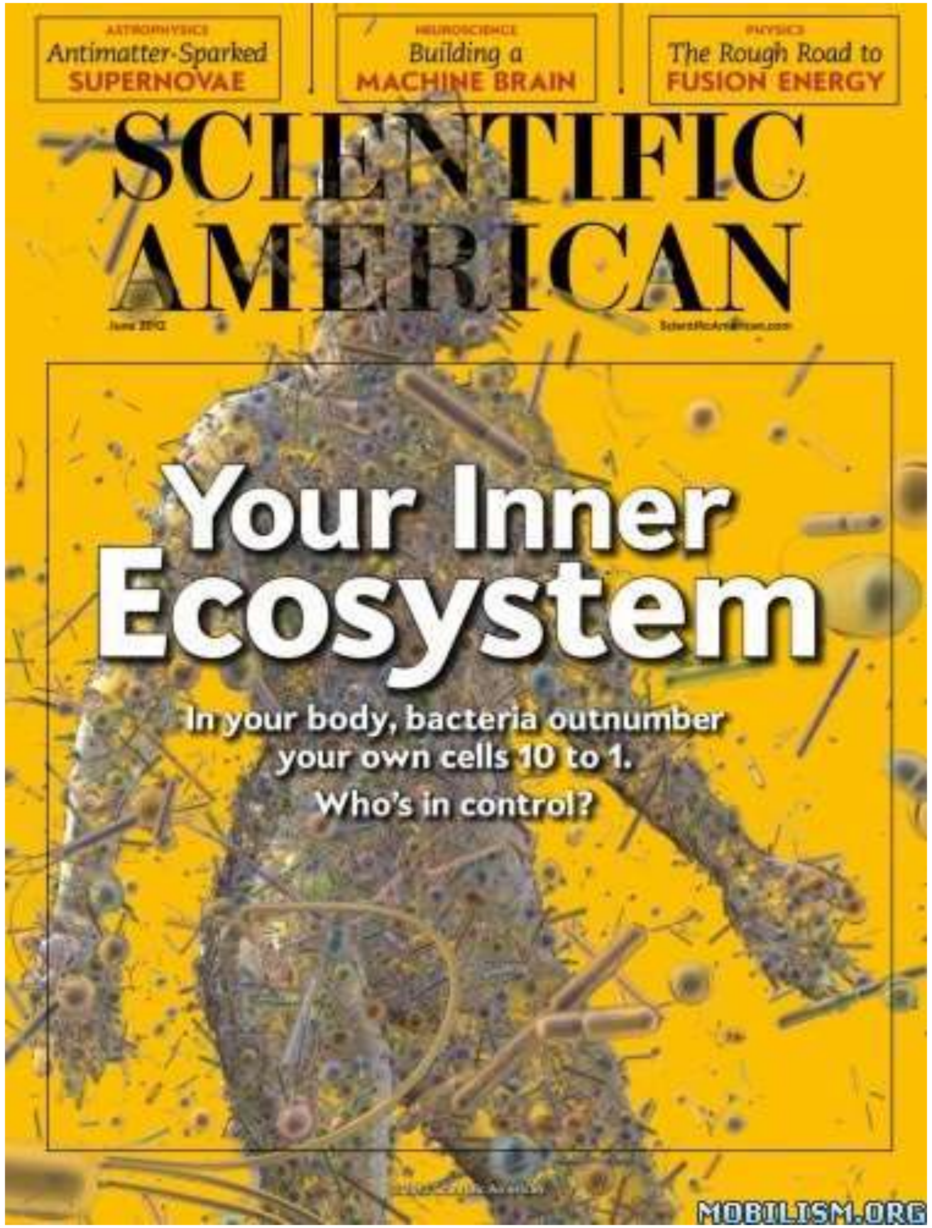
Ramzi El Asmar [1], Pinaki Panigrahi, Penelope Bamford, Irene Berti, Tarcisio Not, Giovanni V Coppa, Carlo Catassi, Alessio Fasano

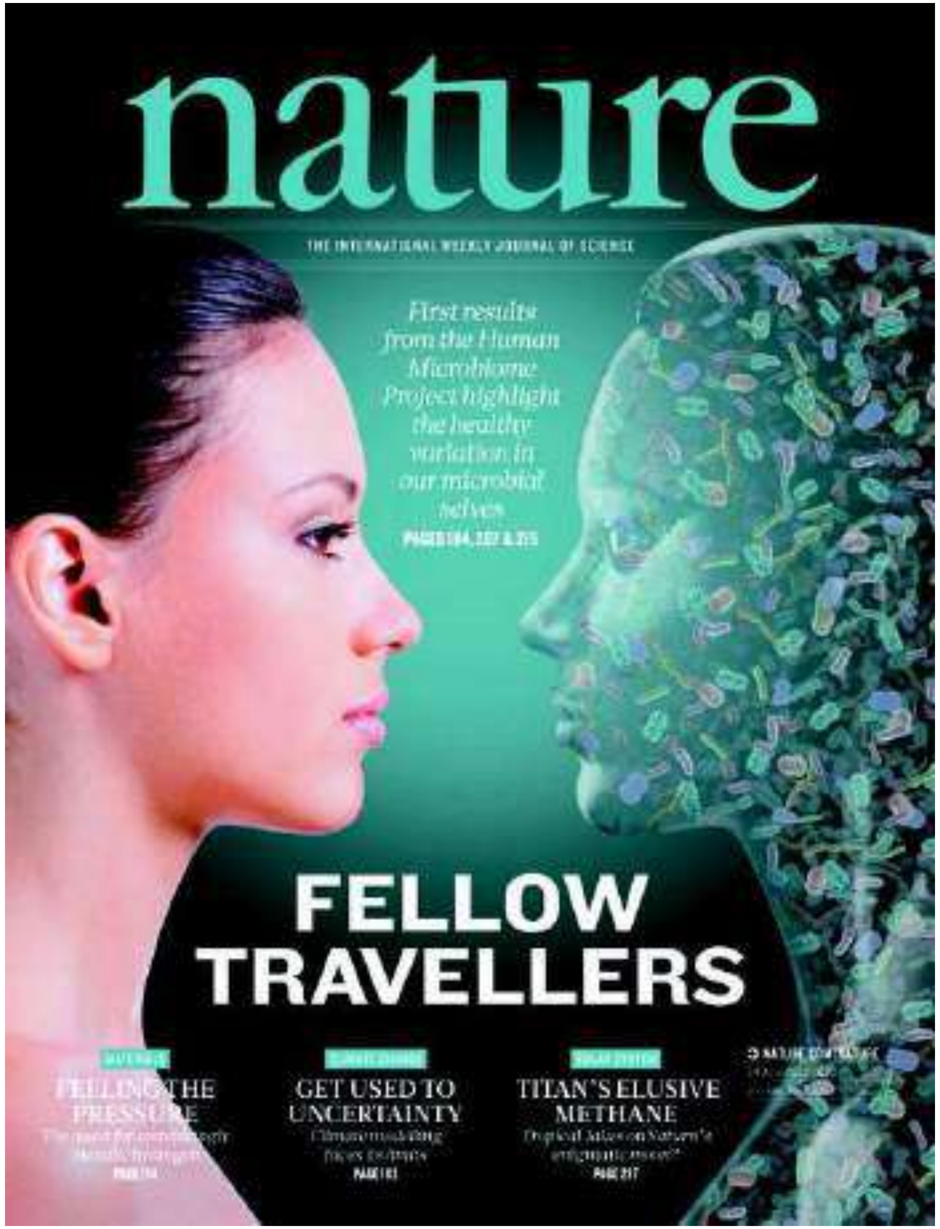




Zonulin concentration in the media collected from the lower chamber (serosal side, open bars) or upper chamber (mucosal side, closed bars) of rabbit small-intestinal tissues mounted in the micro-snapwell system and incubated for 3 hours with *E. coli* 6.1, pathogenic *E. coli* 21-1, or *S. typhimurium* added to the mucosal aspect of the intestine. Uninfected tissues are shown for comparison; n = 4.

The Changing Face Of Gut Microbes





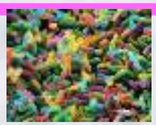
The Microbiome Is Essential To Health

100 TRILLION

The human microbiome is made up of more than 100 trillion bacteria, fungi, protozoa, and viruses that live in and on the human body

>10,000 different species of bacteria are resident in the human intestinal microbiota (400-500/person)

2-5x More



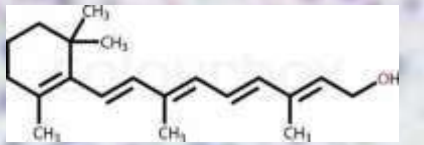
Microbial cells than human cells and the majority live in our gut

150x More

Genes than the human genome

Energy From Food

Regulates Metabolism



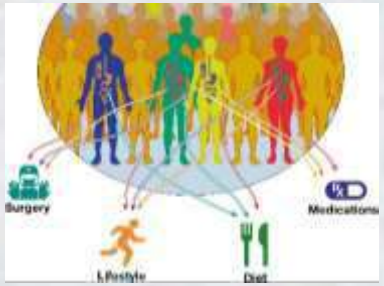
Producing Essential Vitamins

Regulate Immune System

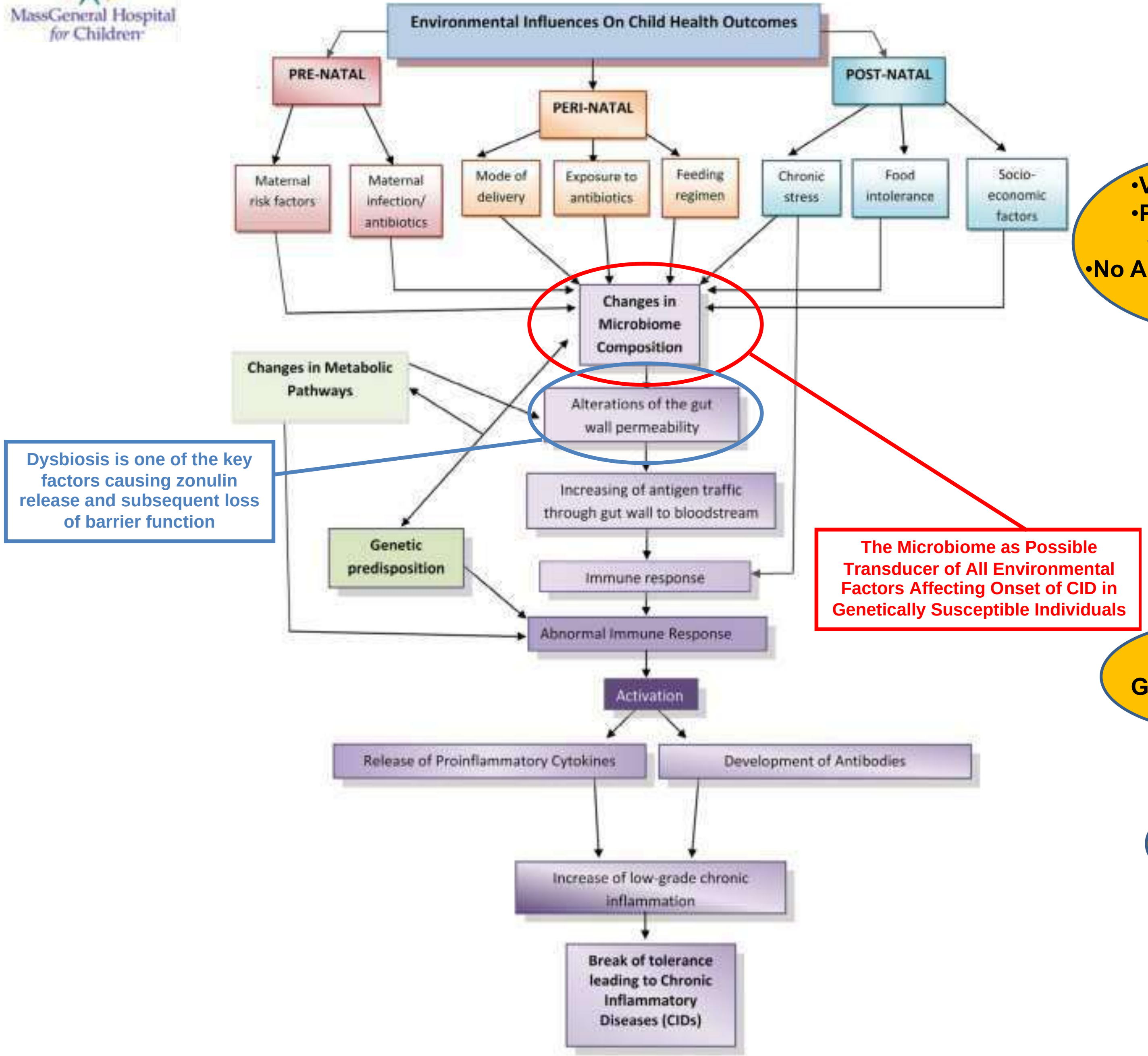
Protection from pathogenic bacteria

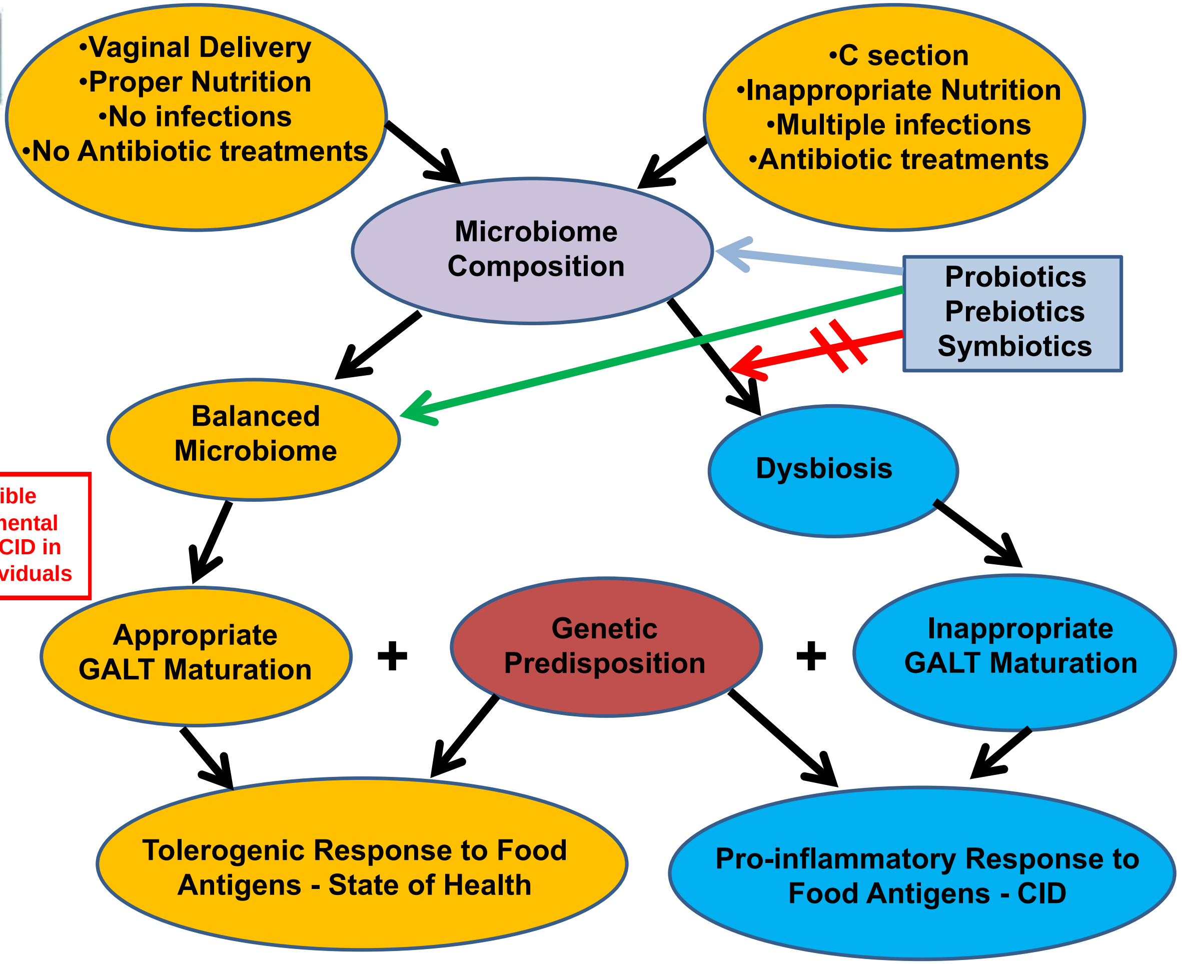
Symbiotic

Personalized



Why The First 1000 Days Of Life Are Instrumental For Clinical Destiny



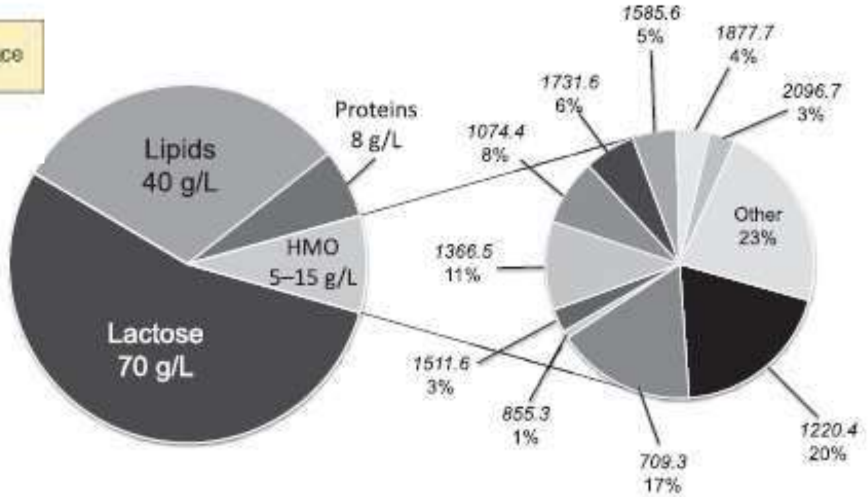


Martin VJ, et al J Pediat 2017

Role of Breastmilk

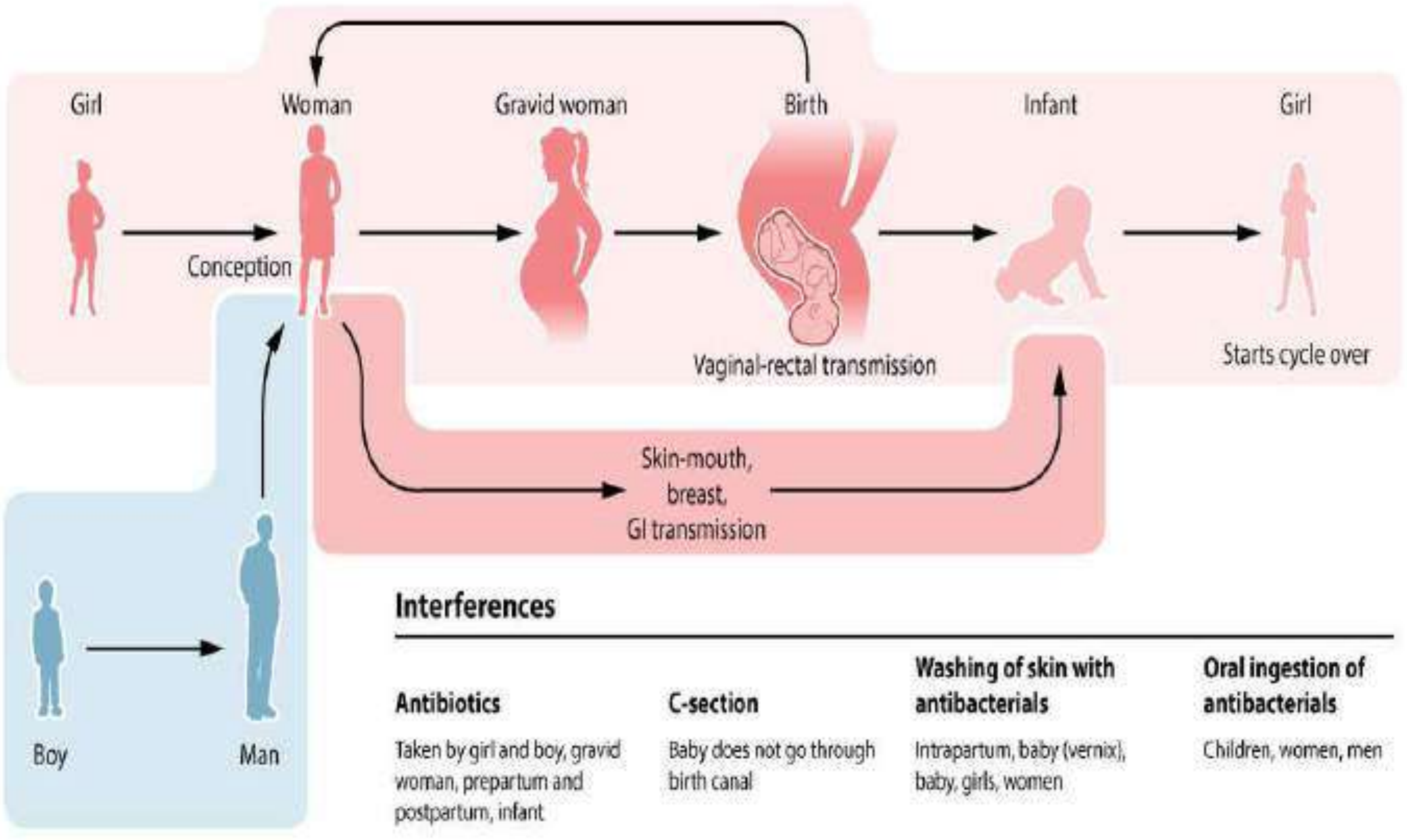
http://www.nature.com

Impact of human milk glycobiome on the infant intestinal microbiota



Zivkovica AM, et al. *PNAS* 2011;108: 4653-58

Cycle of Microbiota Transmission

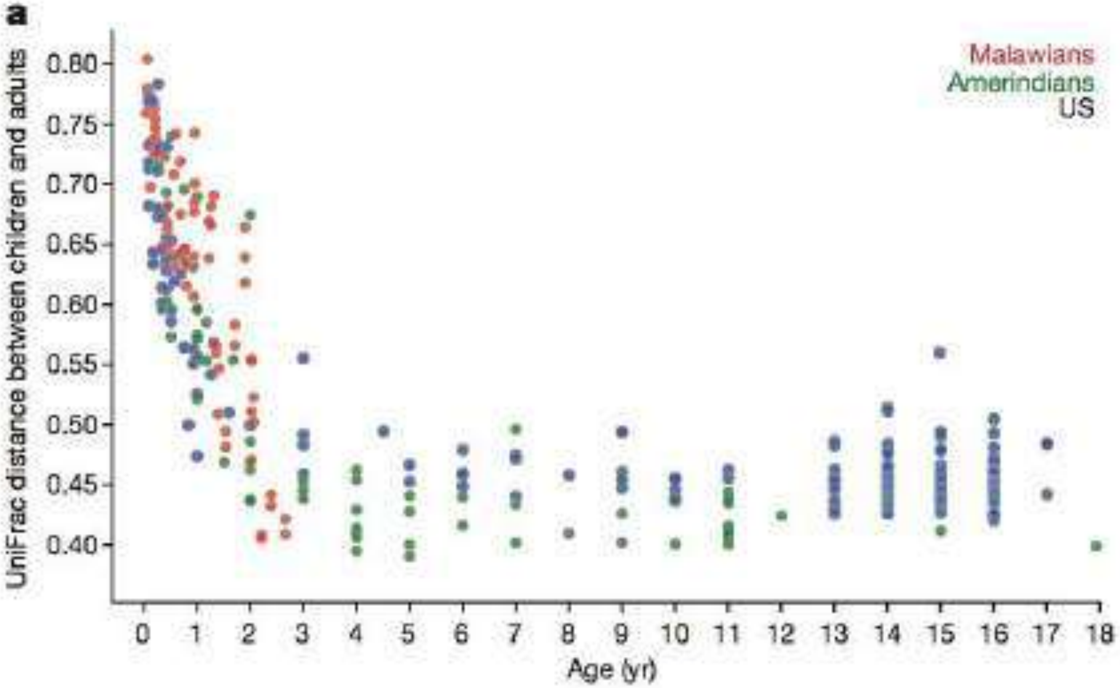


Dominguez-Bello et al Science Trans Med 2015;7:307-39
Fischbach et al Cell 2016;164:1288-1300

Baby's first bacteria

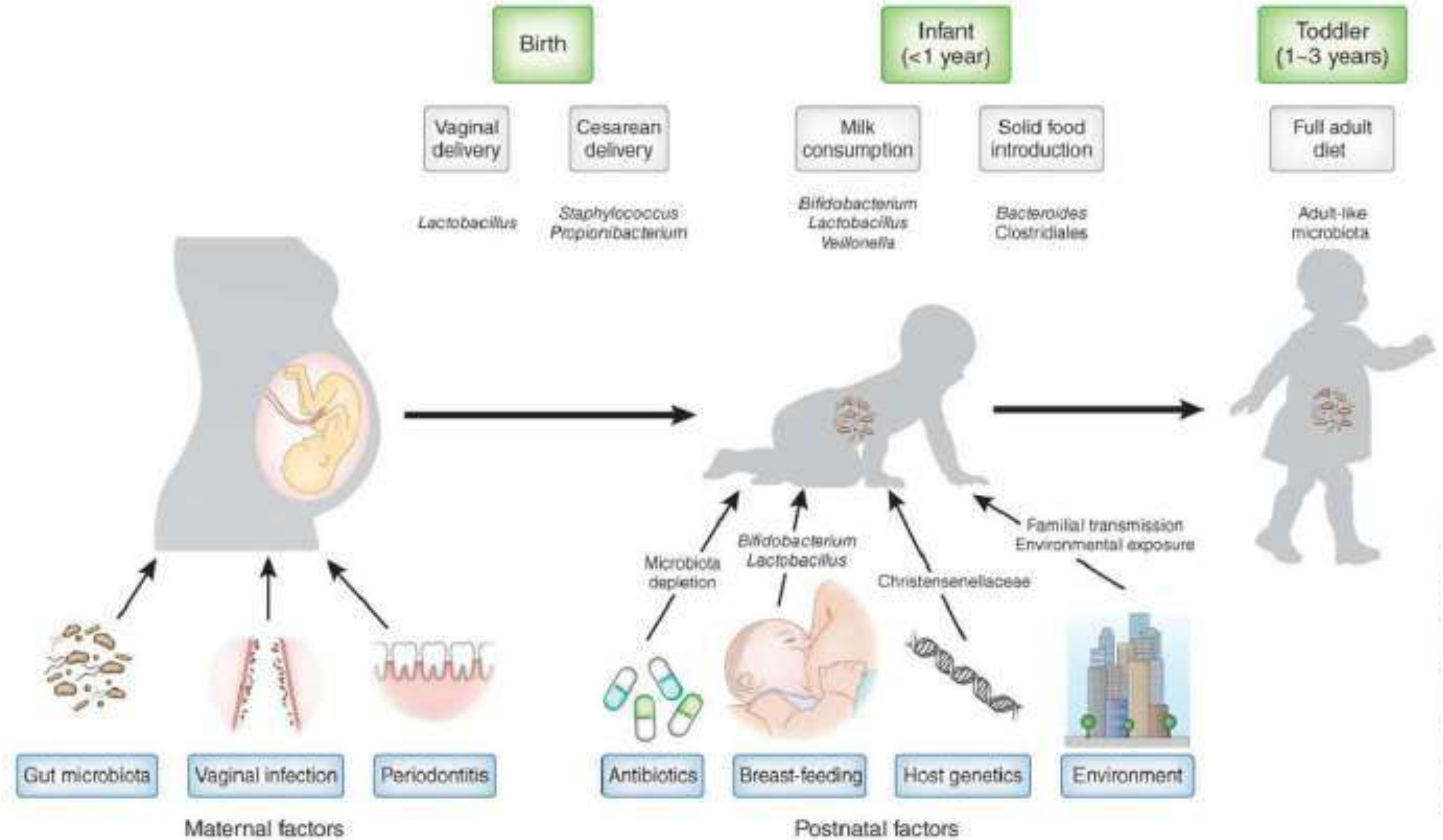
THE WOMB WAS THOUGHT TO BE STERILE. SOME SCIENTISTS ARGUE IT'S WHERE THE MICROBIOME BEGINS.

- Exactly when an infant is first exposed to microbes is still under debate
- Largest microbial transfer occurs at birth
- Microbial colonization of the newborn intestine contributes to the development of the host's immune function
- The first 1-3 years of an infant's microbiome development is characterized by chaotic and dramatic shifts until stabilization at approximately age 3



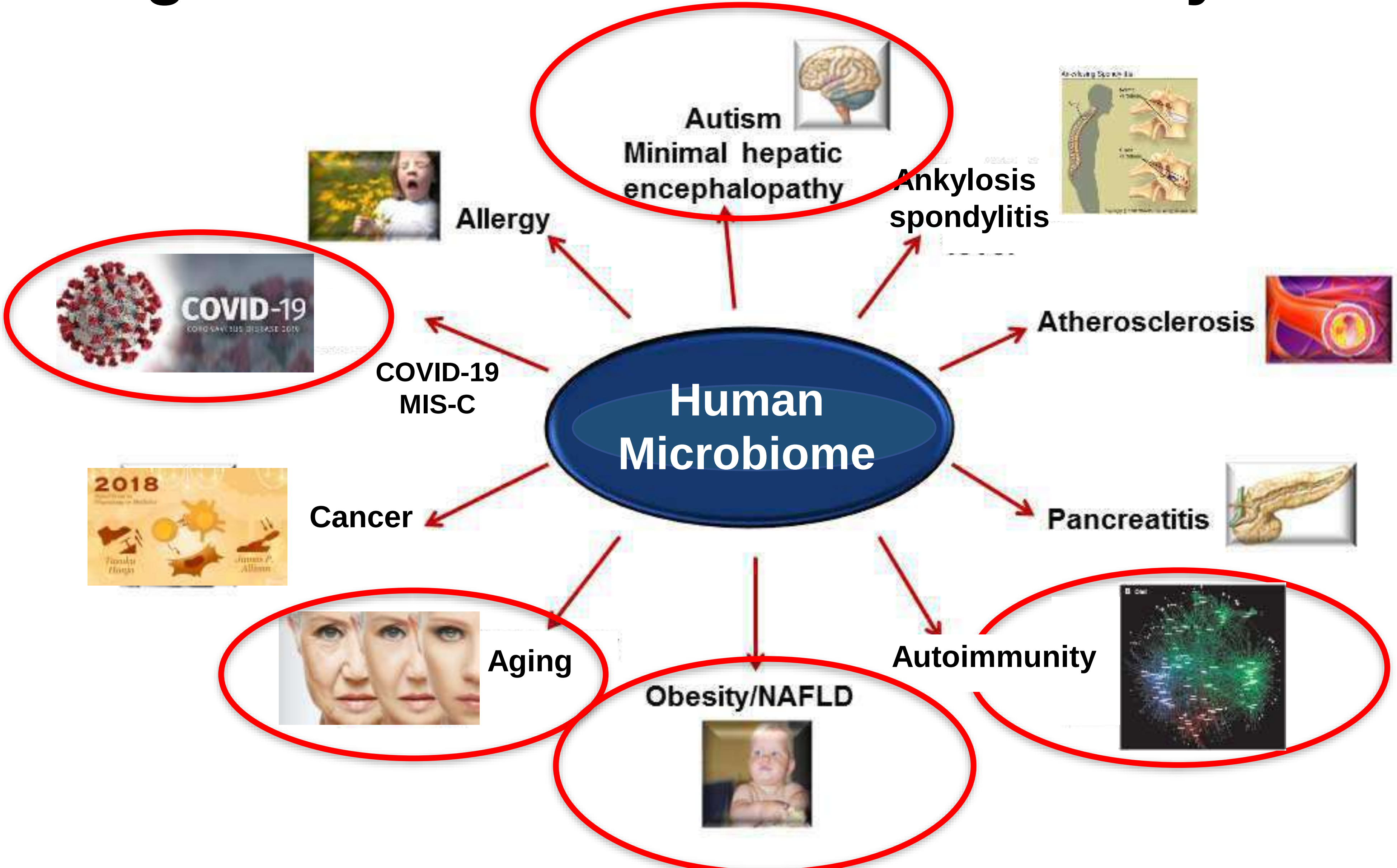
Nature, News Feature 1/18/2018
Yatsunenko, *Nature* ,2012

The Wisdom of Microscopic Species



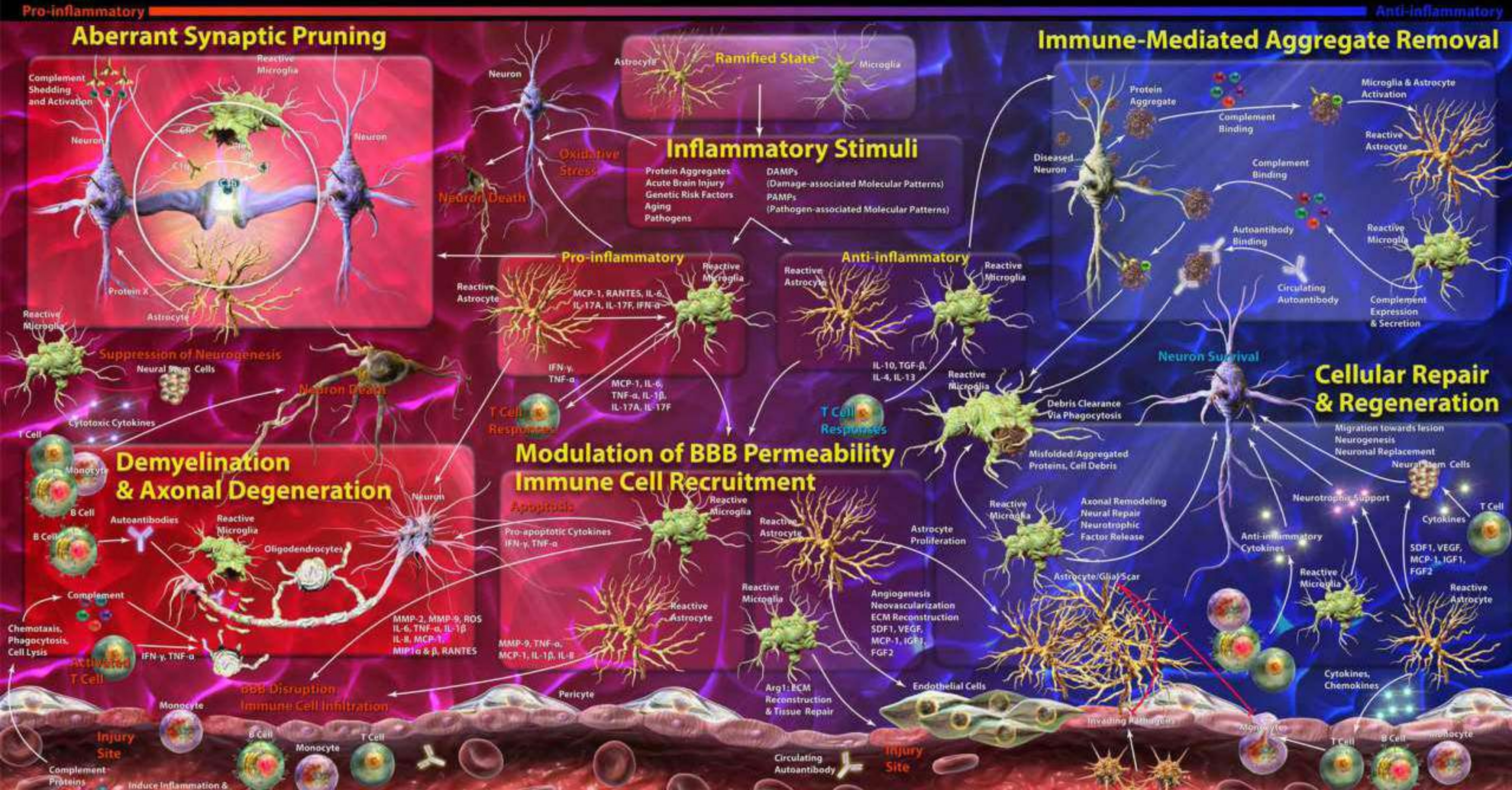
Tamburini, *Nature* 2016.

Role of Zonulin-Immune System-Microbiome Triangulation In Chronic Inflammatory Diseases

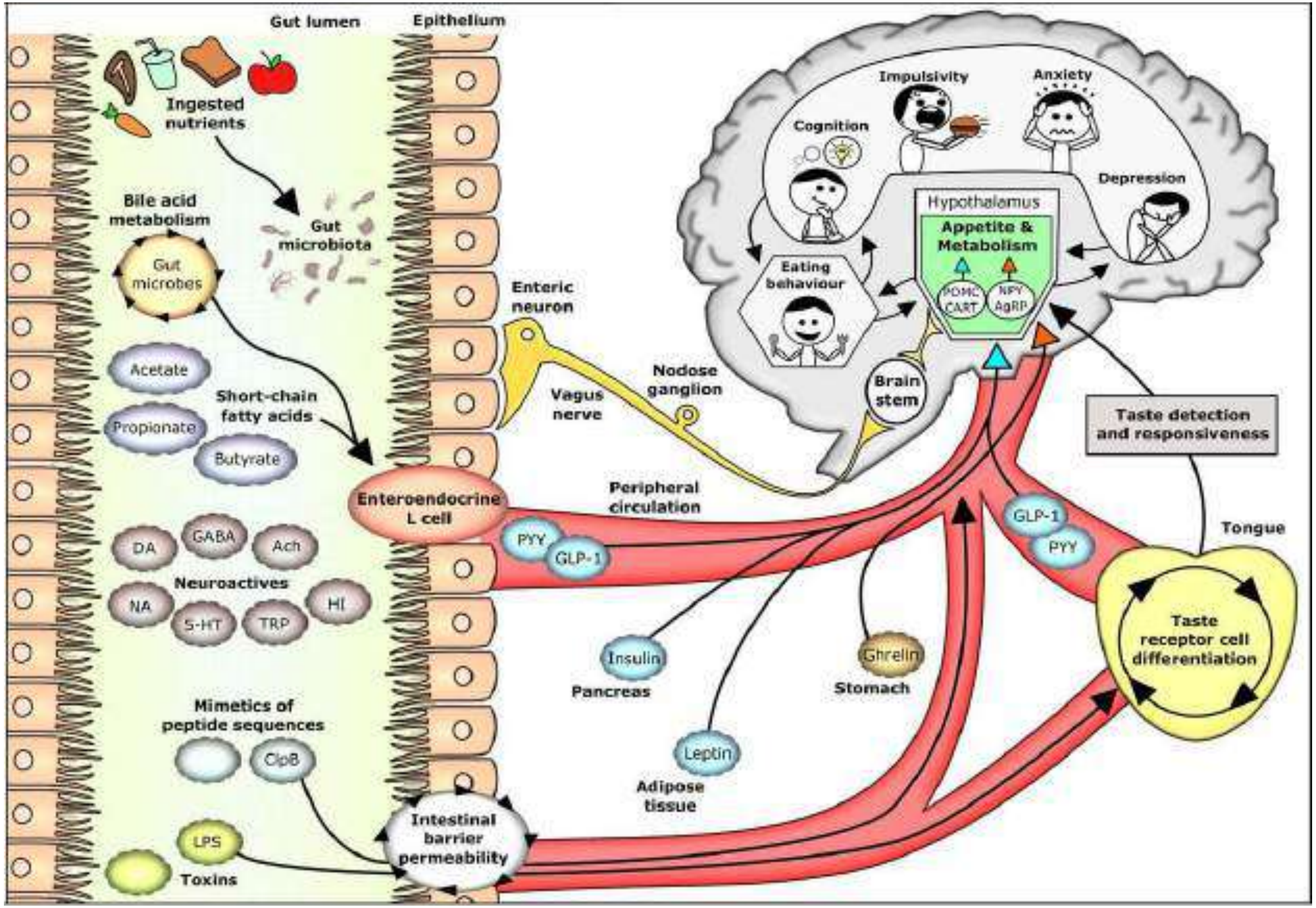


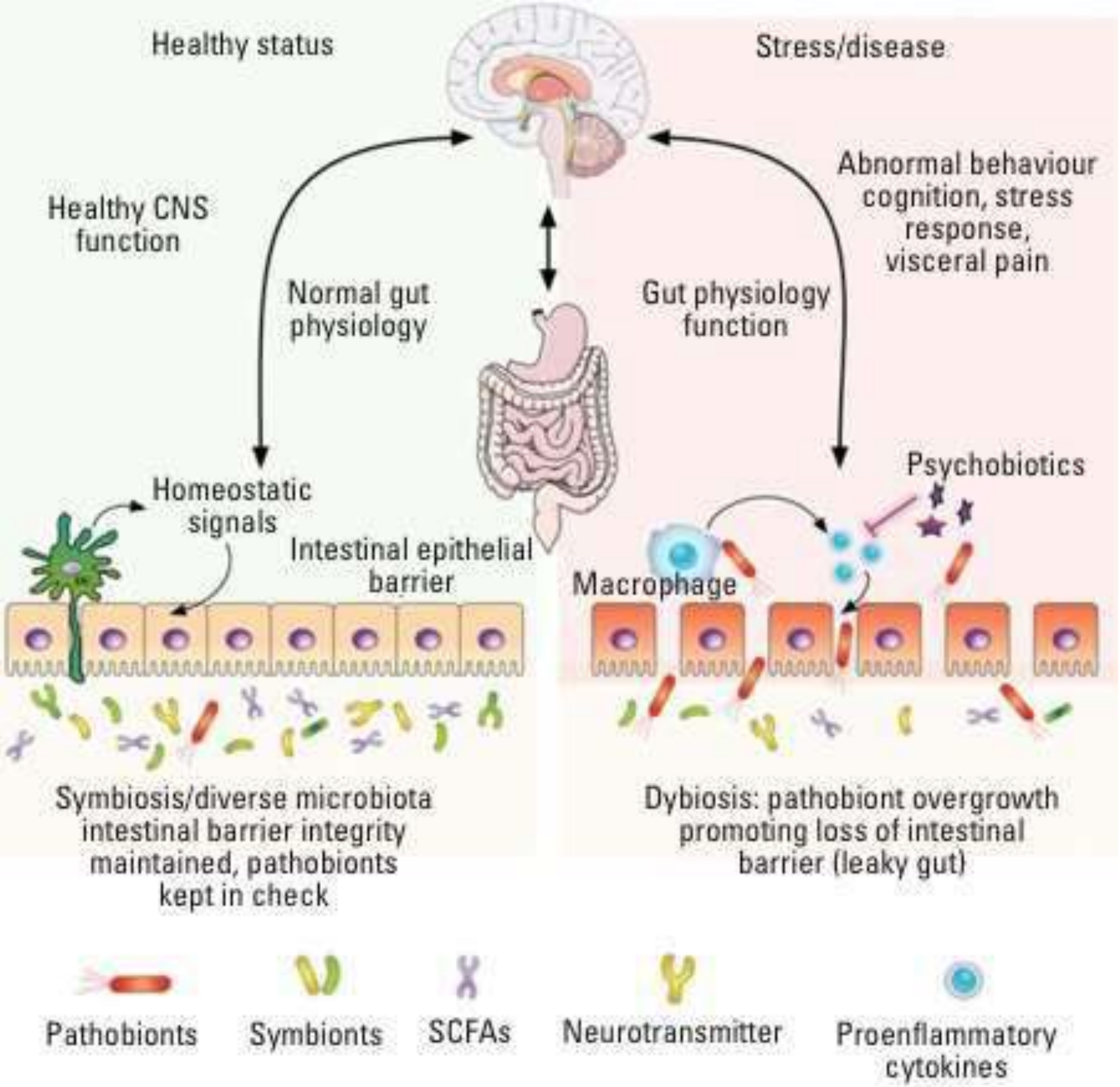

Autism

Neuroinflammation



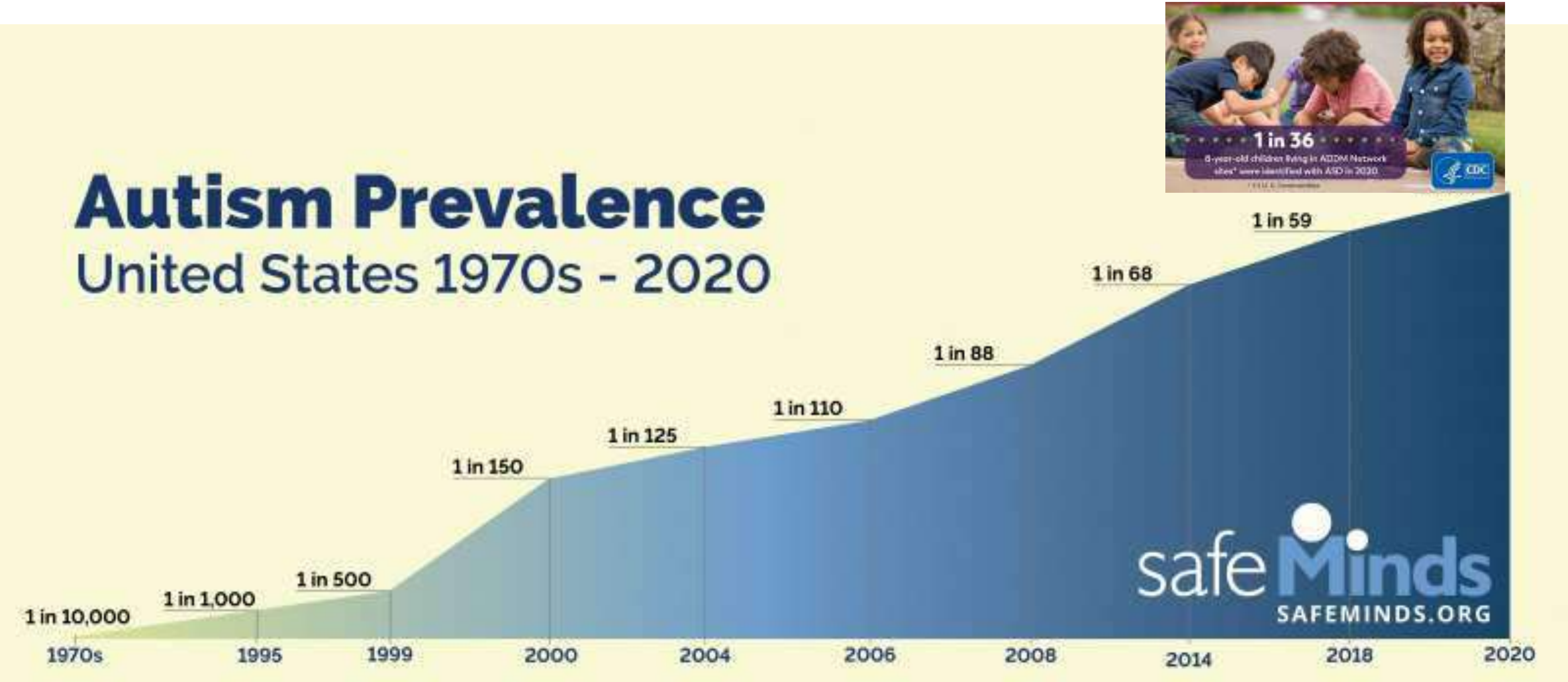
How The Gut Controls Behavior





the brain foundation

Autism Spectrum Disorder (ASD) "Epidemics"



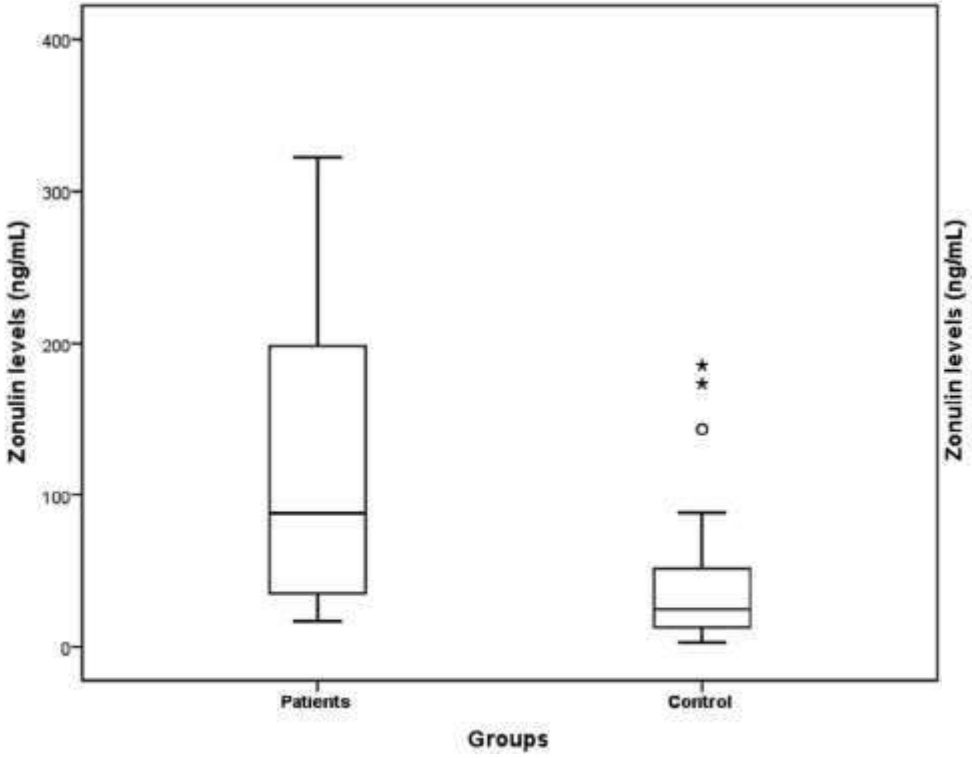
www.jpeds.com • THE JOURNAL OF PEDIATRICS

2017

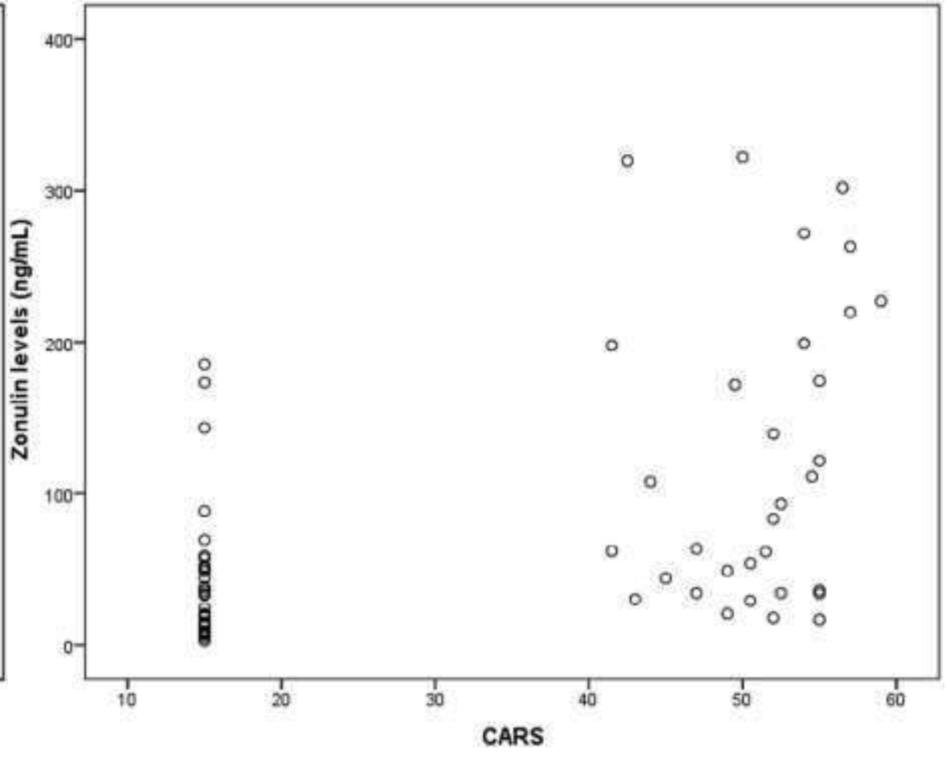
Increased Serum Zonulin Levels as an Intestinal Permeability Marker in Autistic Subjects

Erman Esnafoglu, MD[1], Selma Cırrık, PhD[2], Sema Nur Ayyıldız, MD[3], Abdullah Erdil, MD[4], Emine Yurdakul Ertürk, MD[4], Abdullah Daglı, MD[4], and Tevfik Noyan, MD[3]

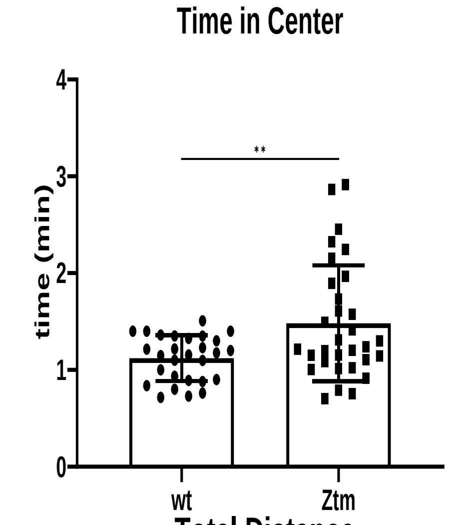
Distribution of serum zonulin levels in ASD patients and controls *p<0.001

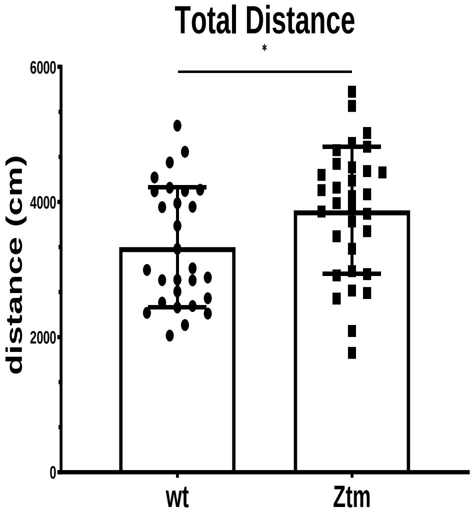


Scattergram of zonulin levels according to Childhood Autism Rating Scale (SCAR)



The Ztm mouse displays behavioral alterations and changes in BBB and pro-inflammatory genes expression

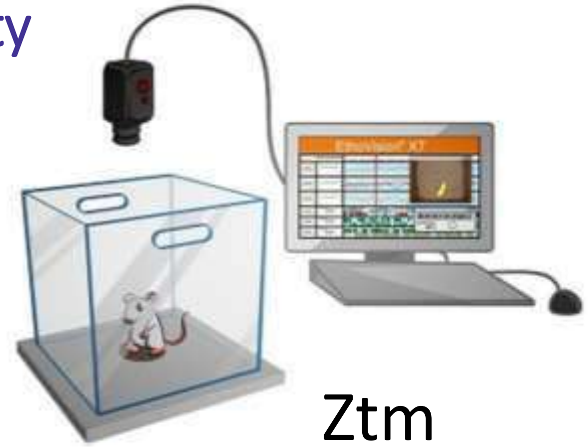




Open Field

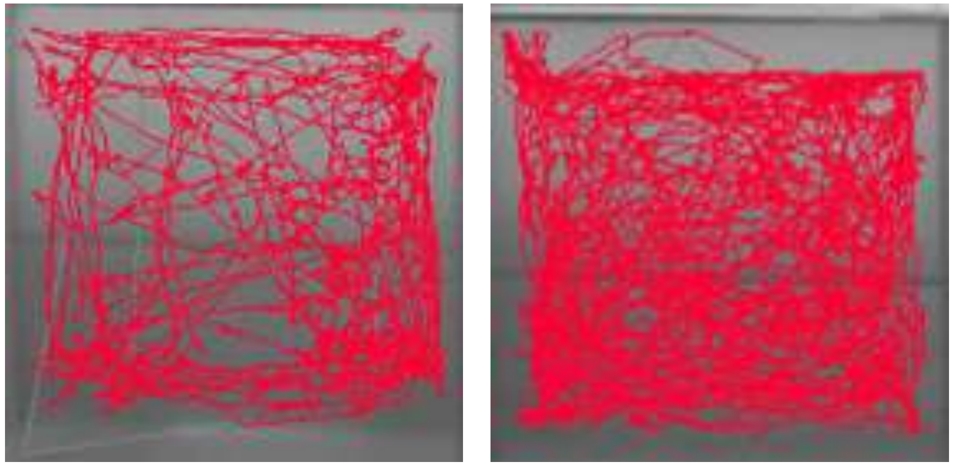
measure of anxiety and/or hyperactivity

BRAIN	MALES		FEMALES	
	FC	p	FC	p
CD11b	**2.56**	**0.006**	1.1	>0.9999
CLDN-1	0.63	0.274	**0.31**	**0.038**
CLDN-3	0.7	0.662	**0.17**	**0.002**
CLDN-5	1.29	0.081	**0.6**	**0.038**
GABA	**1.16**	**0.024**	0.41	0.571
IL1β	**1.65**	**0.004**	1.12	0.556
OCCLN	**1.8**	**0.043**	0.97	0.852
veCad	**1.55**	**0.022**	0.55	0.145



WT

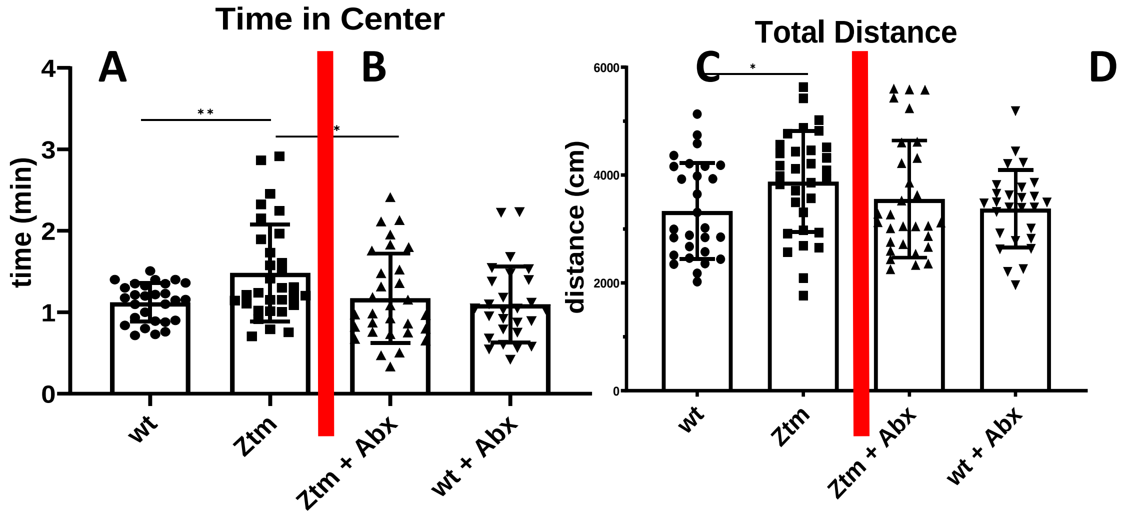
Ztm

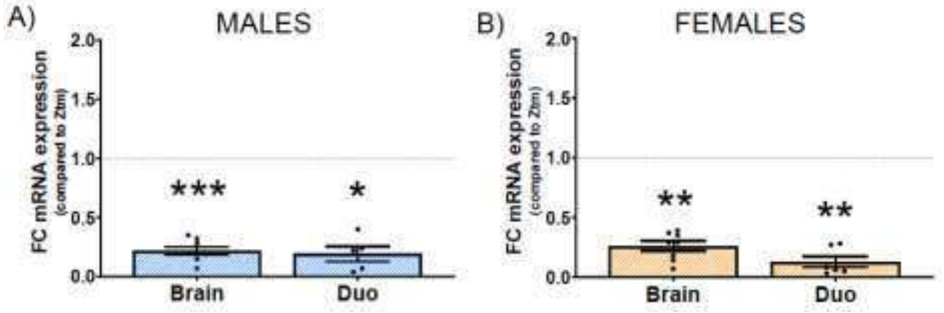


Alba-Pereira et al, Tissue Barriers 2022

the brain foundation

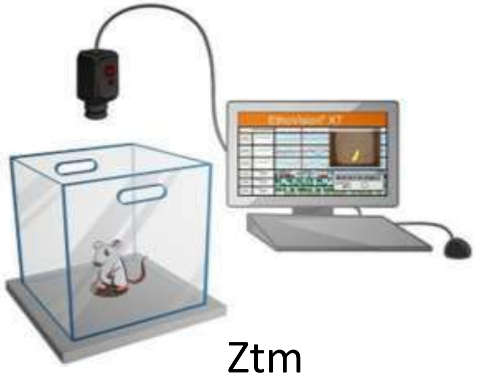
The Ztm mouse behavioral alterations and changes in BBB and pro-inflammatory genes expression is reverted by Abx treatment





Zonulin gene expression

Open Field

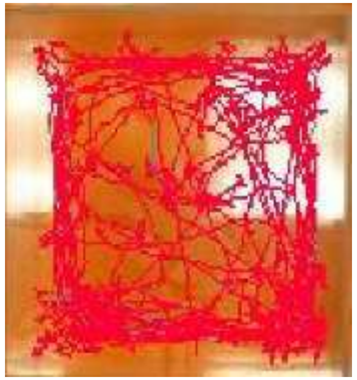


Vs untreated	BRAIN	MALES		FEMALES	
		FC	p	FC	p
↓	CD11b	0.25	0.000	0.27	0.000
↑	CLND-1	2.14	0.038	2.60	0.002
↑	CLND-3	9.30	0.000	6.14	0.001
↑	CLDN-5	4.68	0.000	2.46	0.001
↓	GABA	0.23	0.000	0.19	0.000
↓	IL1β	1.45	0.130	1.15	0.195
↓	OCCLN	1.53	0.105	1.57	0.022
↓	veCadh	0.28	0.000	0.24	0.000

Wt

Ztm

Ztm + Abx



Alba-Pereira et al, Tissue Barriers 2022

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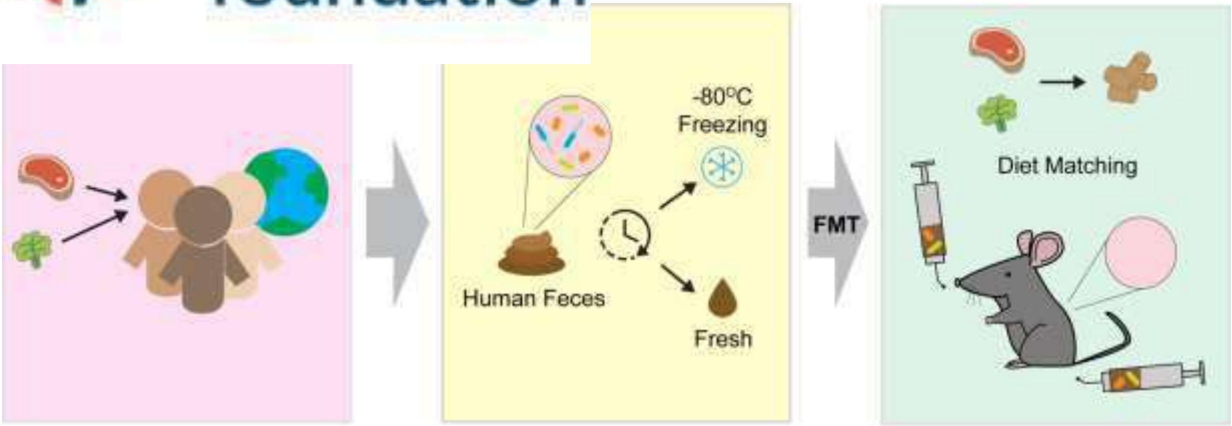
The Brain Foundation Project: Ztm ASD Humanized Model



Who?
- Health Condition
- Geography
- Ethnicity
- Diet

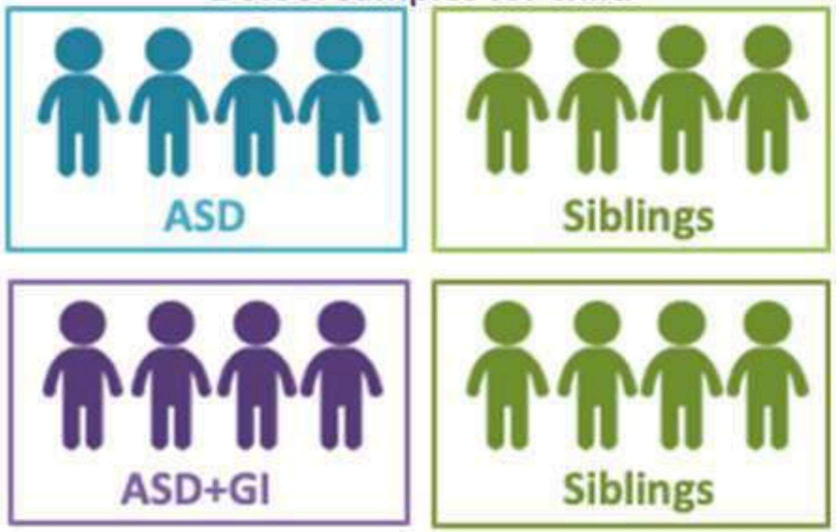
Processing
- Fresh vs Frozen
- Handling Duration
- Oral vs Rectal Adminsitration

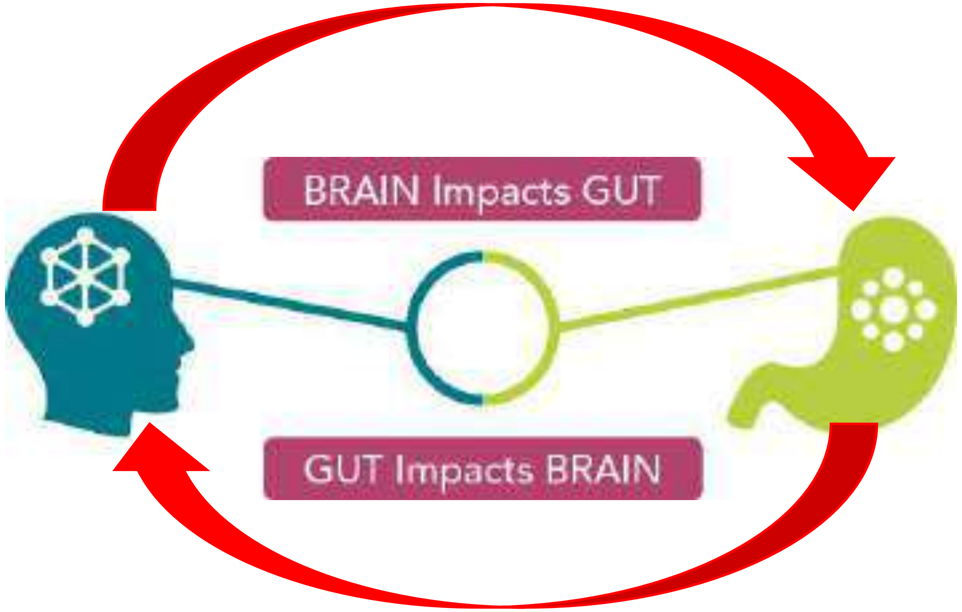
Germ-Free Mouse
- Genetic Background
- Vendor Source
- Diet Matching
- Succession

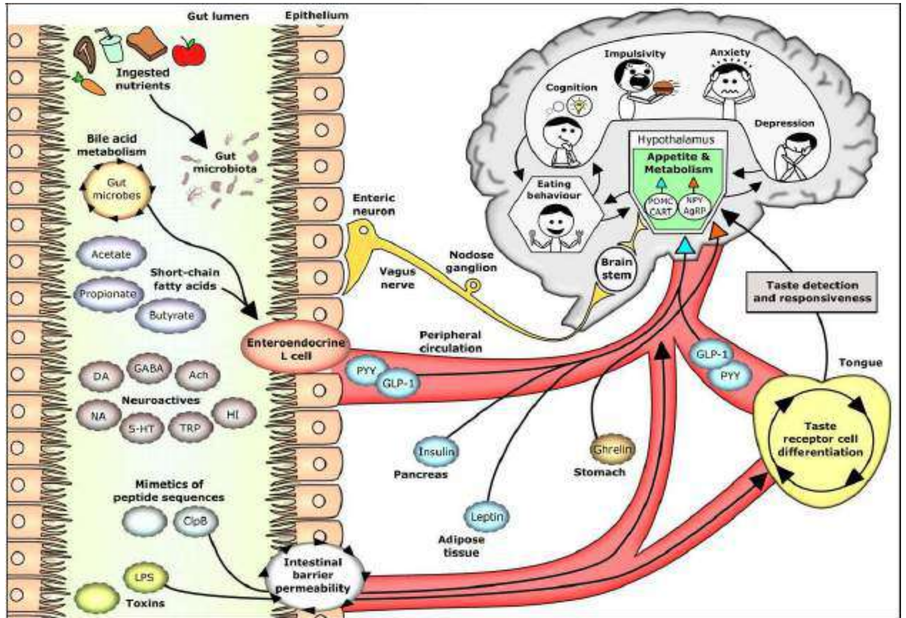
Investigation of the mechanistic pathways of microbiota-immune-system-induced behavioral changes

Test pre, pro-, or synbiotics combinations

2 stool samples for child

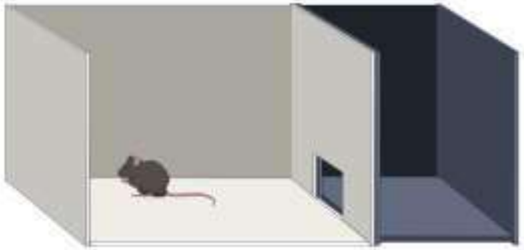






Results In The Ztm ASD Humanized Model

Light/Dark Box

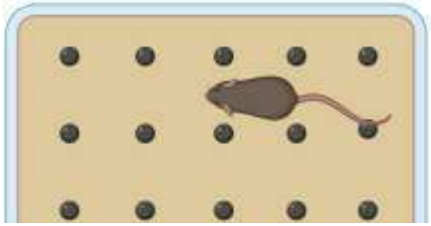


Maternal separation stress and the HP2-2 genotype increase “impulsivity and/or hyperactivity” and anxiety-like behavior

Elevated zero maze

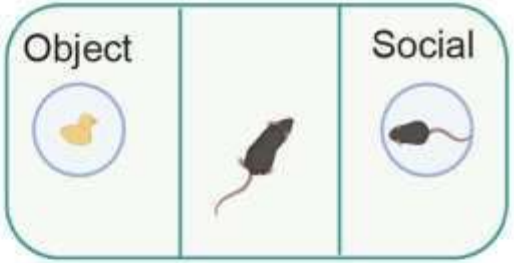
Maternal separation stress (MS), the HP2-2 genotype, and ASD FMT increase anxiety-like behavior in females

Marble burying



Maternal separation stress (MS) and the HP2-2 genotype decrease marble burying

Sociability

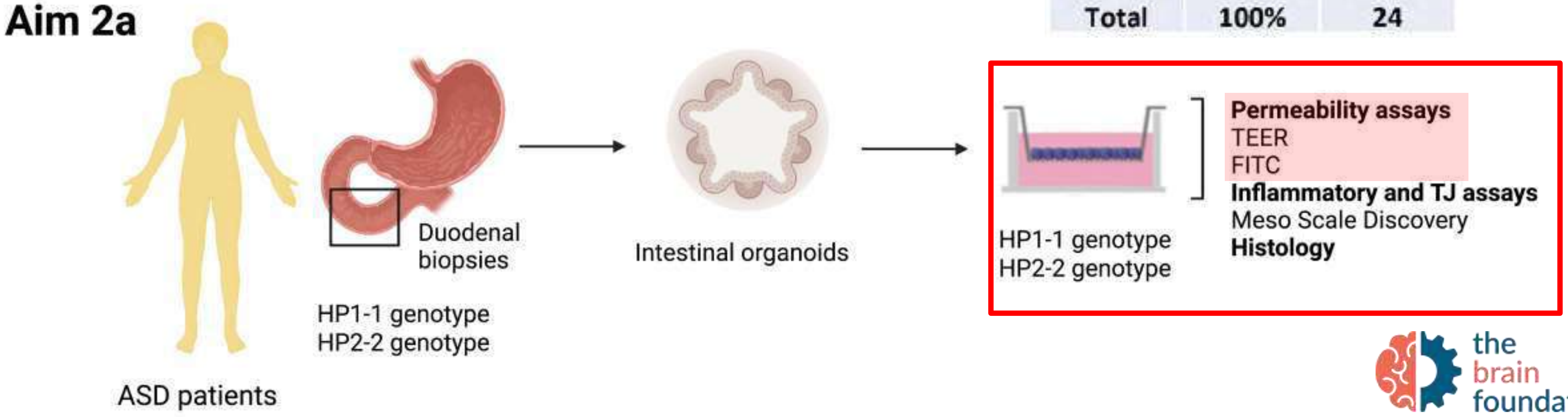


Results of Sociability Assay reveal deficit in processing stimuli

To determine if fecal conditioned media derived from children with ASD with GI symptoms exacerbates permeability, transepithelial antigen trafficking and inflammation in human intestinal tissue from ASD patients with a HP2-2 genotype as compared to an HP1-1 genotype.

Within our ASD patients with GI symptoms undergoing clinically indicated endoscopies, **we observed an over-representation of HP2-2 genotype** as compared to the general population.

	Male ASD GI	# subjects	HC
HP1-1	17%	4	~15%
HP1-2	38%	9	~50%
HP2-2	46%	11	~35%
Total	100%	24	



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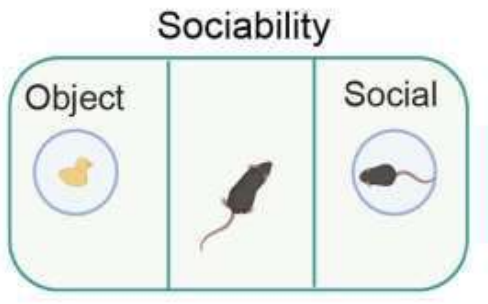
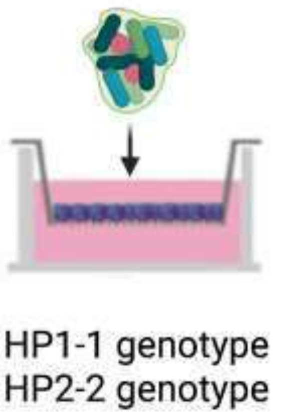
Summary

[Zonulin transgenic mouse model:]() Maternal separation stress (MS), the HP2-2 genotype, and ASD FMT showed ASD-like behavior (increase anxiety-like behavior, hyperactivity/impulsivity, and changes in sociability)



[Male ASD human intestinal monolayers:]() Compared to HP1-1 monolayers, HP2-2 monolayers are characterized by decreased barrier integrity and increased permeability at baseline. Treatment with ASD fecal conditioned media further increases the intestinal permeability of HP2-2 monolayers but not HP1-1 monolayers.



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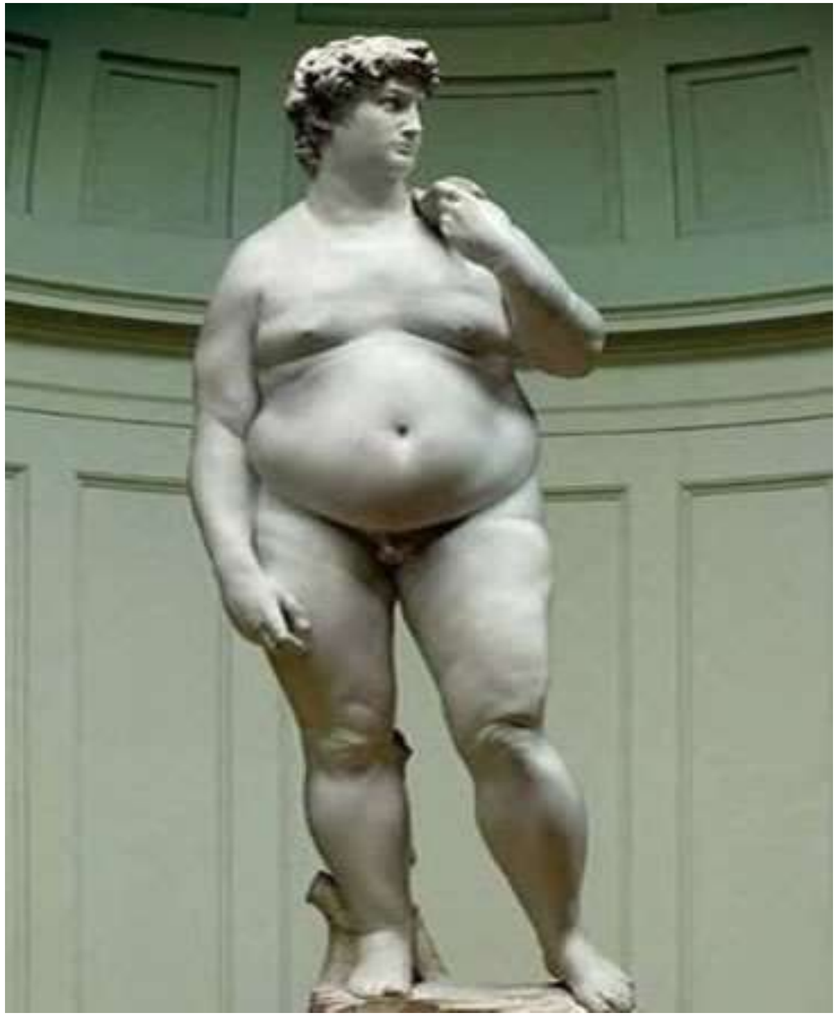
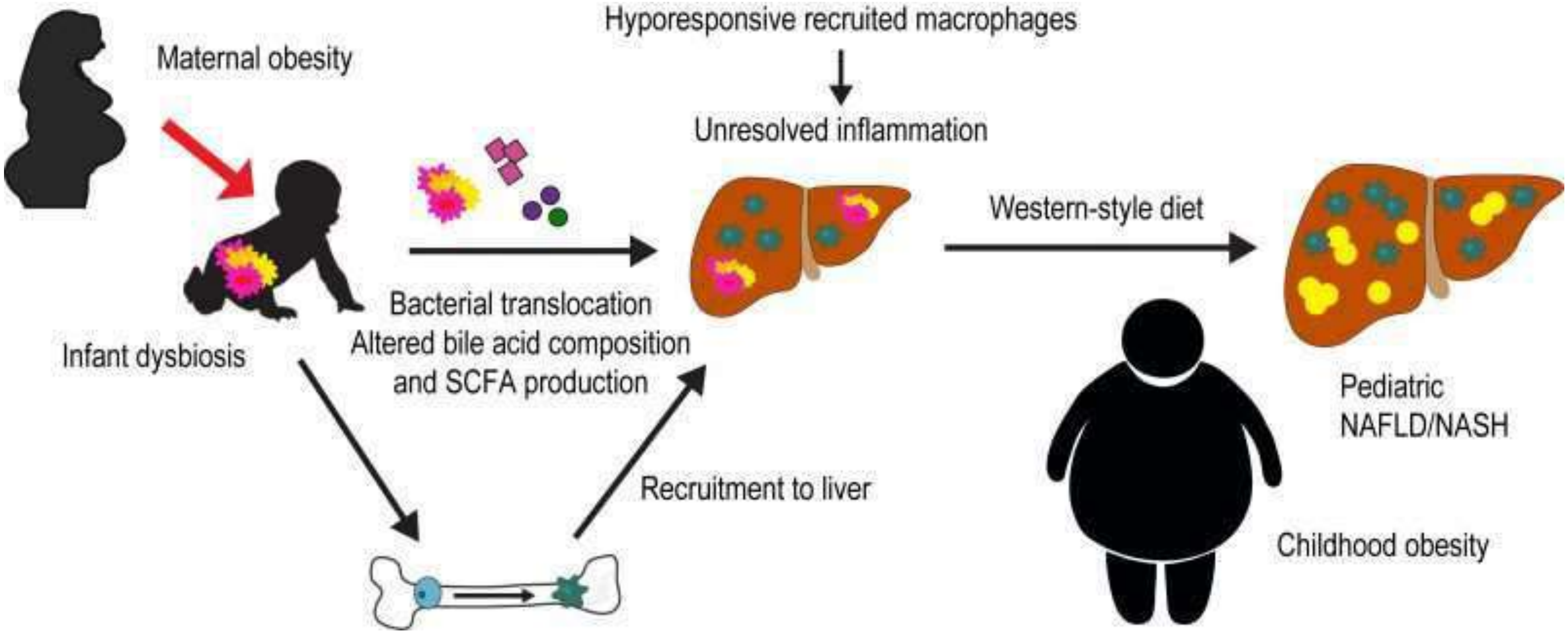
Obesity



Why A Leaky Gut Together With Dysbiosis Can Make Us Fat



RATIONALE

An increase in intestinal permeability is considered to be associated with gut inflammatory tone and development of obesity, fatty liver (typical of obese subjects) and type 2 diabetes

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PLoS one

Circulating Zonulin, a Marker of Intestinal Permeability, Is Increased in Association with Obesity-Associated Insulin Resistance

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Department of Diabetes, Endocrinology and Nutrition, Institut d'Investigació Biomèdica de Girona (IdIBGi), CIBEROBN (CB06/03/010) and Instituto de Salud Carlos III (ISCIII), Girona, Spain

Abstract

Zonulin is the only physiological mediator known to regulate intestinal permeability reversibly by modulating intercellular tight junctions. To investigate the relationship between intestinal permeability and obesity-associated metabolic disturbances in humans, we aimed to study circulating zonulin according to obesity and insulin resistance. Circulating zonulin (ELISA) was measured in 123 caucasian men in association with inflammatory and metabolic parameters (including minimal model-measured insulin sensitivity). Circulating zonulin increased with body mass index (BMI), waist to hip ratio (WHR), fasting insulin, fasting triglycerides, uric acid and IL-6, and negatively correlated with HDL-cholesterol and insulin sensitivity. In multiple regression analysis, insulin sensitivity ($p = 0.002$) contributed independently to circulating zonulin variance, after controlling for the effects of BMI, fasting triglycerides and age. When circulating IL-6 was added to this model, only BMI ($p = 0.01$) contributed independently to circulating zonulin variance. In conclusion, the relationship between insulin sensitivity and circulating zonulin might be mediated through the obesity-related circulating IL-6 increase.

Citation: Moreno-Navarrete JM, Sabater M, Ortega F, Ricart W, Fernández-Real JM (2012) Circulating Zonulin, a Marker of Intestinal Permeability, Is Increased in Association with Obesity-Associated Insulin Resistance. PLoS ONE 7(5): e37160. doi:10.1371/journal.pone.0037160

Editor: Massimo Federici, University of Tor Vergata, Italy

J Clin Res Pediatr Endocrinol. 2016 Dec 23. doi: 10.4274/jcrpe.3682. [Epub ahead of print]

The Relationship of Serum Zonulin Level with Clinical and Laboratory Parameters in Childhood Obesity.

Küme T, Acar S, Tuhan H, Çatlı G, Anık A, Gürsoy Çalan Ö, Böber E, Abacı A.

Abstract

OBJECTIVE: The aim of this study was to investigate the relationship between zonulin and clinical laboratory parameters in childhood obesity.

METHODS: The study included obese children with a body mass index >95th percentile and healthy children who were similar age and gender distribution. Clinical (body mass index, waist circumferences, mid arm circumference, triceps skin fold, percentage of body fat, systolic blood pressure, diastolic blood pressure) and biochemical (glucose, insulin, lipids, thyroid function tests, cortisol, zonulin and leptin levels) parameters were measured.

RESULTS: A total of 43 obese subjects (23 males, mean age: 11.1±3.1 yrs) and 37 healthy subjects (18 males, mean age: 11.5±3.5 yrs) were included in this study. Obese children had significantly higher insulin, HOMA-IR, TG, TC, LDL-C, HDL-C, zonulin and leptin levels than those of the healthy children ($p < 0.05$), while glucose levels were not different ($p > 0.05$). Comparison of the obese children regarding the insulin resistance showed no statistically significant differences for zonulin levels ($p > 0.05$).

CONCLUSION: To the best of our knowledge, the present study is the first study to compare serum zonulin levels between obese and non-obese children. The results of the study showed that zonulin was significantly higher in obese children when compared to healthy children, which is indicating a potential role of zonulin in the obesity etiopathogenesis and related disturbances.

PMID: 28008865 DOI: 10.4274/jcrpe.3682

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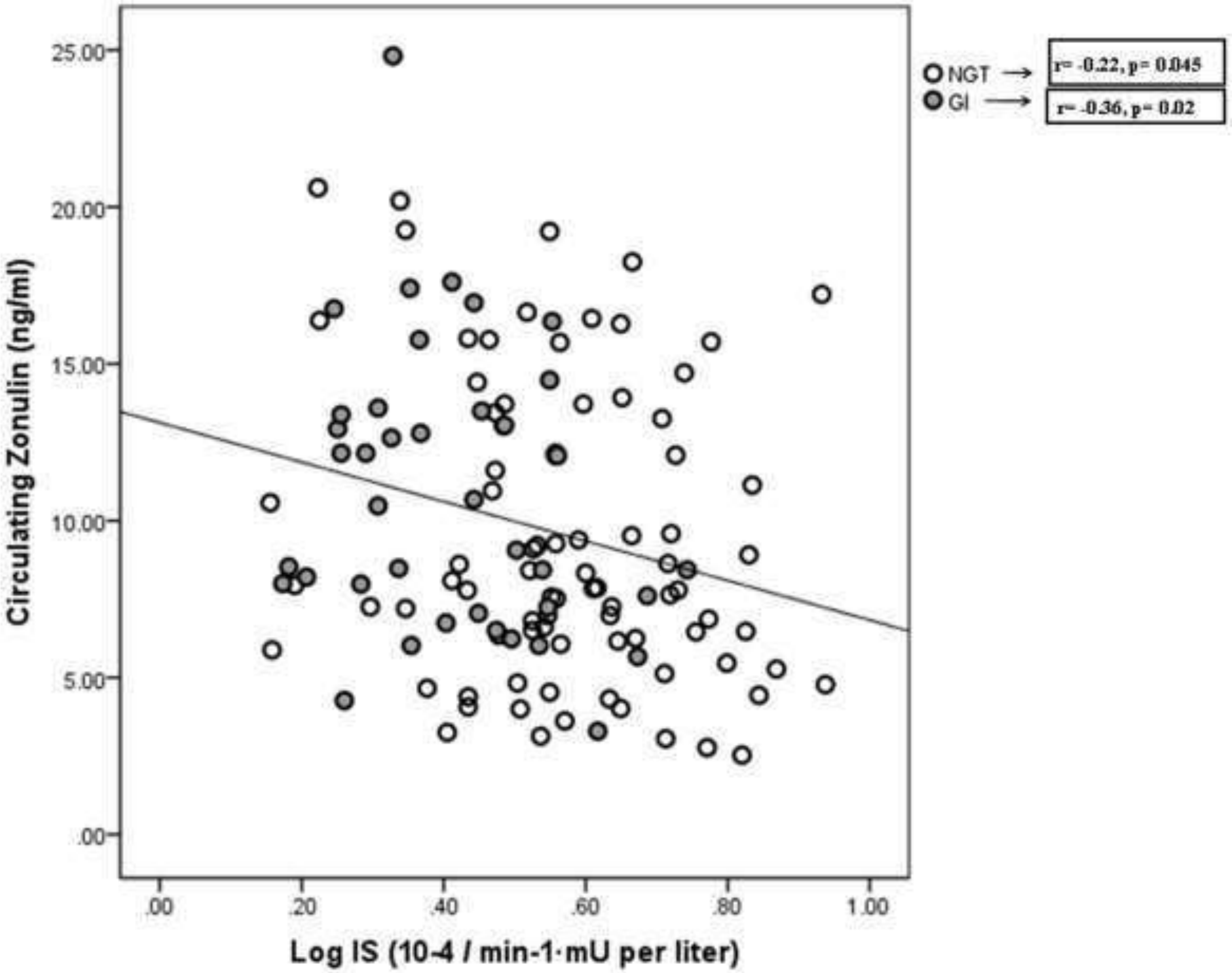
Circulating Zonulin, a Marker of Intestinal Permeability, Is Increased in Association with Obesity-Associated Insulin Resistance



The correlation between insulin sensitivity and circulating zonulin in participants with normal glucose tolerance (NGT, n = 82) and with glucose intolerance (GI, n = 41).