The Role of Nutrition, Gut Microbiome, and Gut Permeability in Immunomodulation: The Celiac Disease and Type 1 Diabetes Paradigms

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W. Allan Walker Chair in Pediatric Gastroenterology and Nutrition

Professor of Pediatrics Harvard Medical School

Mucosal Biology and Immunology Research Center

And Center for Celiac Research

Massachusetts General Hospital for Children

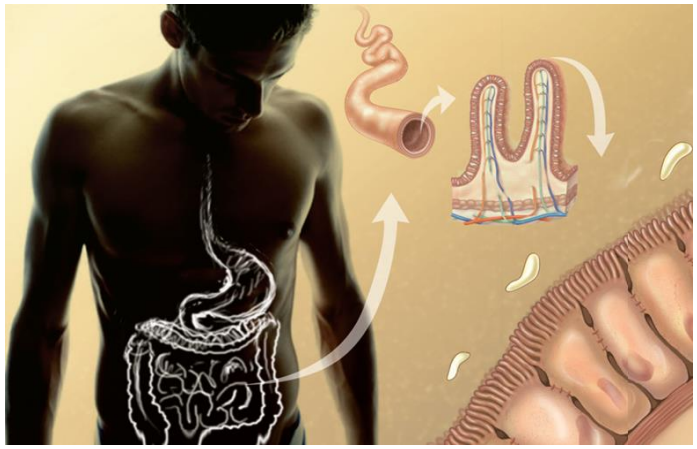
Presented in ICoMI 2017

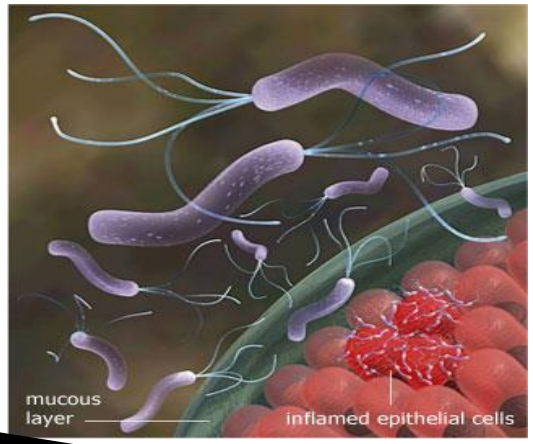
MassGeneral Hospital for Children

Harvard Medical School

Meeting Overview On New Concepts of Autoimmunity:

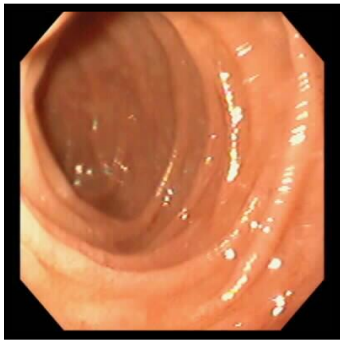
Excessive and Inappropriate Inflammatory Process Associated to a Dysfunction of Barriers

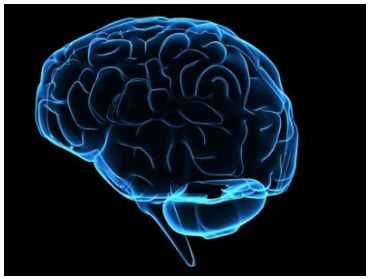


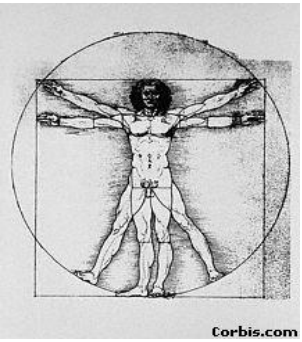


Microbiome

Environmental Factors







Clinic Outcome

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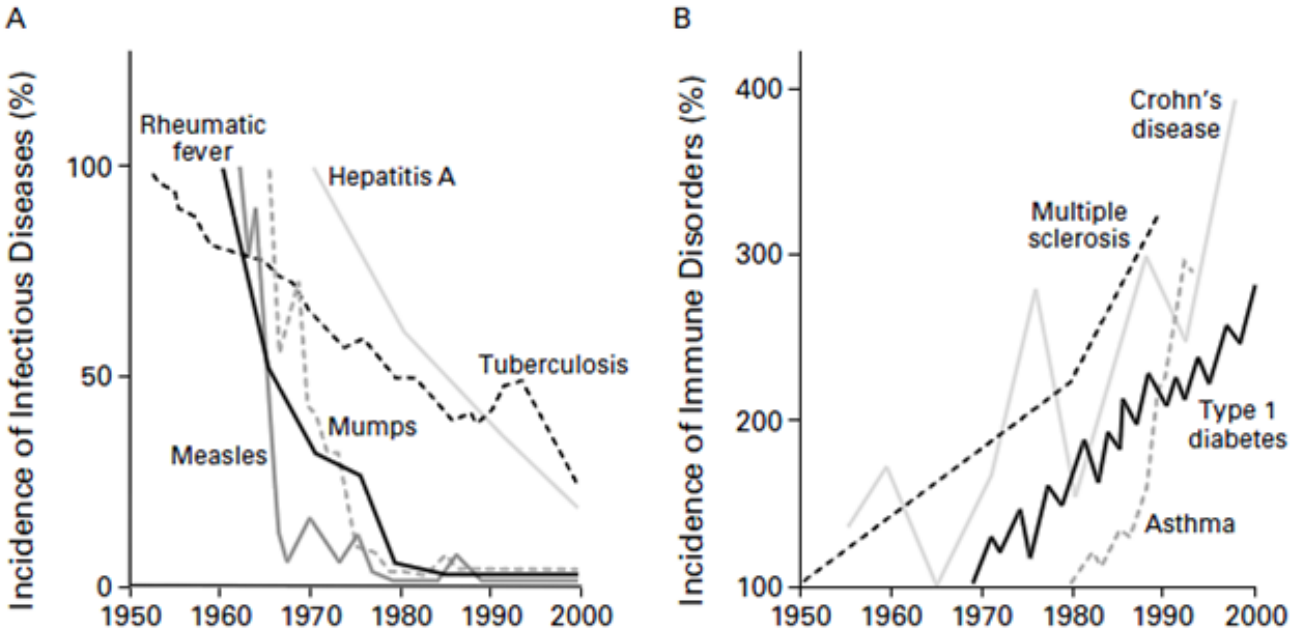
The Epidemics of Immune-Mediated Diseases In The Western Hemisphere: The Hygiene Hypothesis

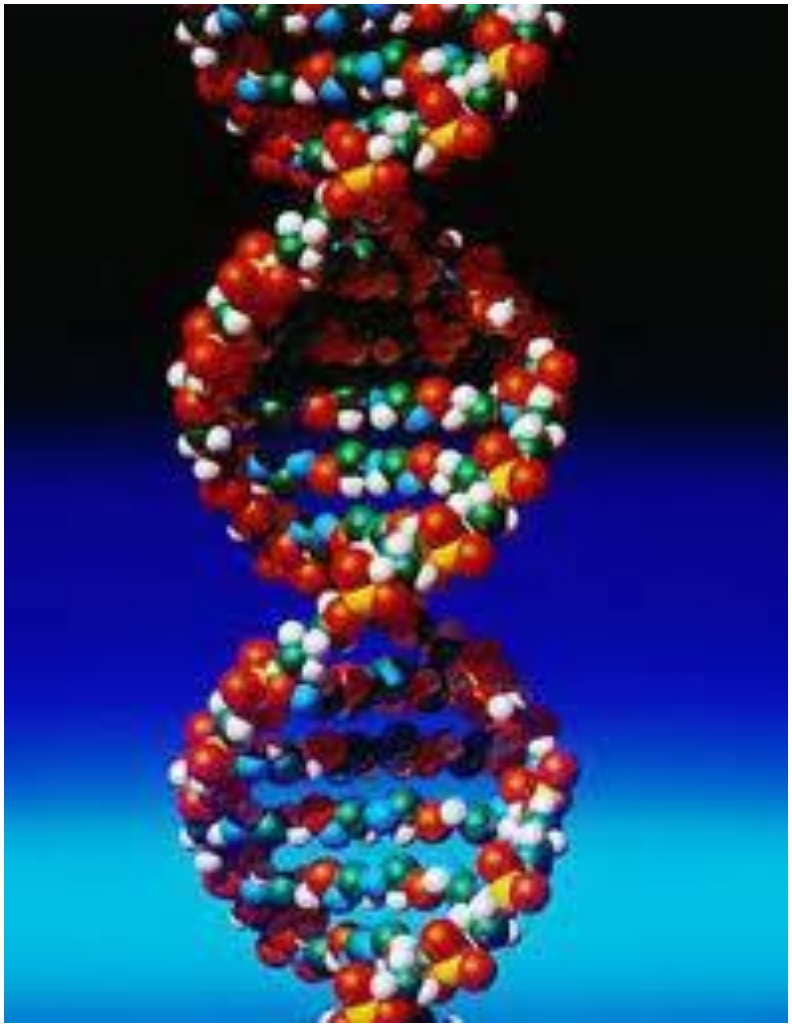


Autoimmune disorders incidence

Helminths infestation incidence

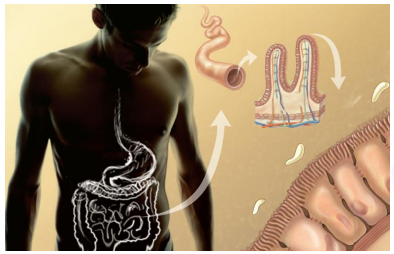
Personal communication from Dr. Joel Weinstock

We Are Not Born With The Destiny to Develop Chronic Inflammatory Diseases

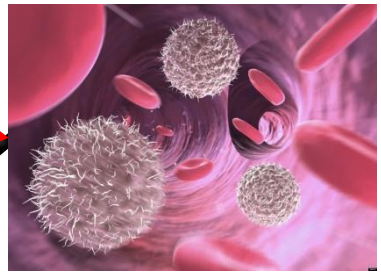


The Yin and Yang Between Tolerance and Immune Response Leading To Chronic Inflammatory Diseases: Lesson Learned From Celiac Disease

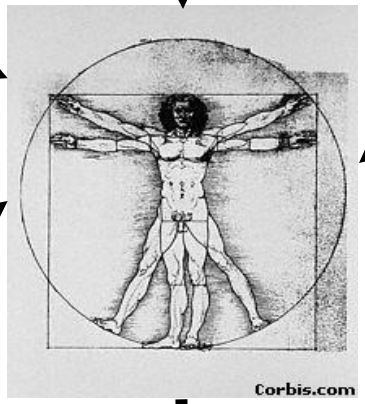
Human Genome



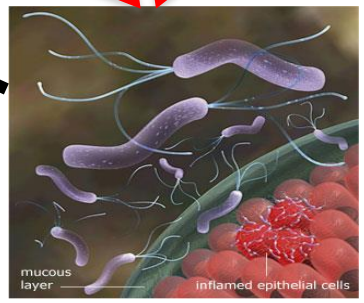
Increased Gut Permeability



Immune Response



Environmental Factors



Microbiome

Clinic Outcome

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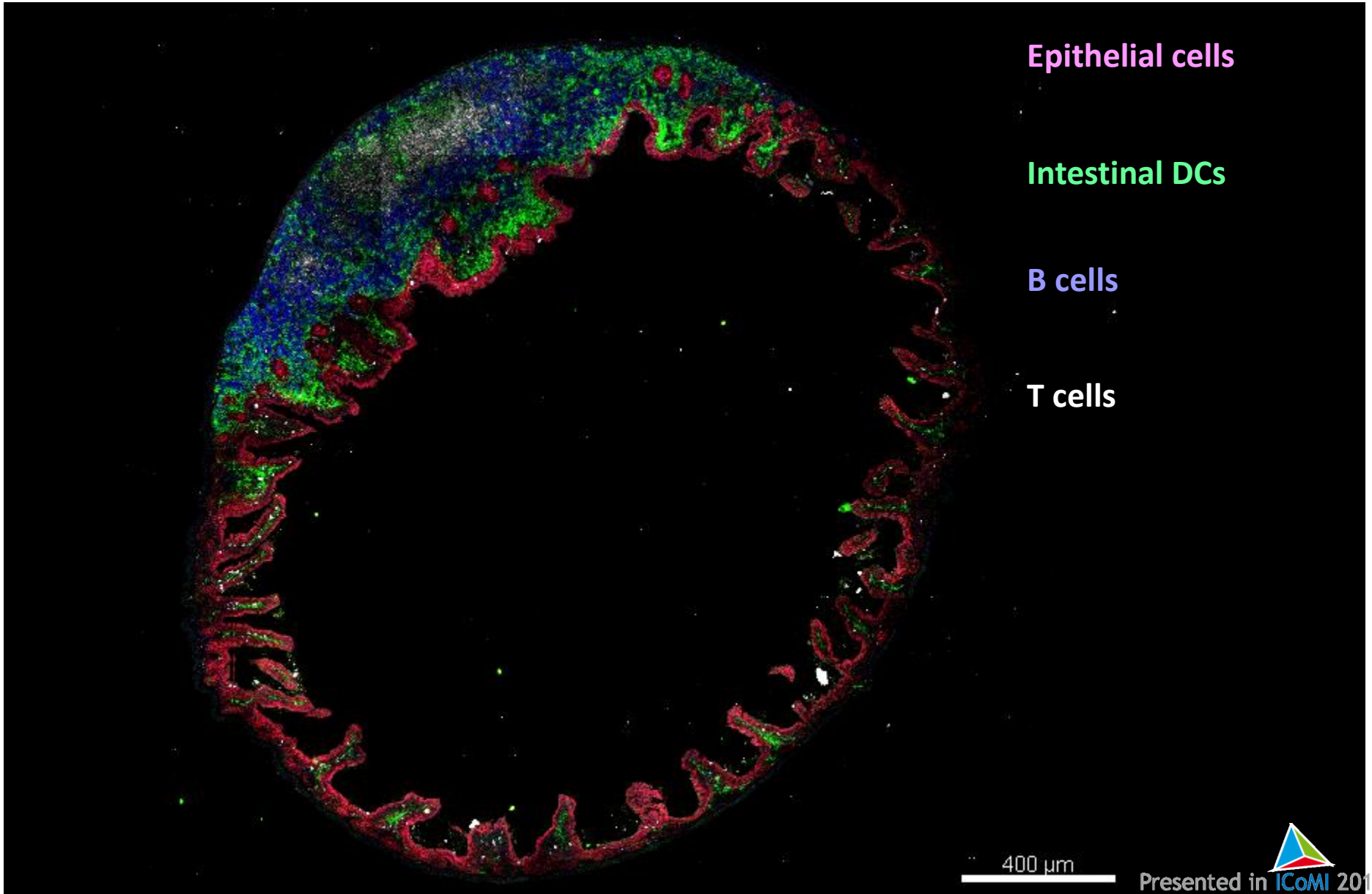
Several Cells Play a Role in Maintaining The Immune Homeostasis



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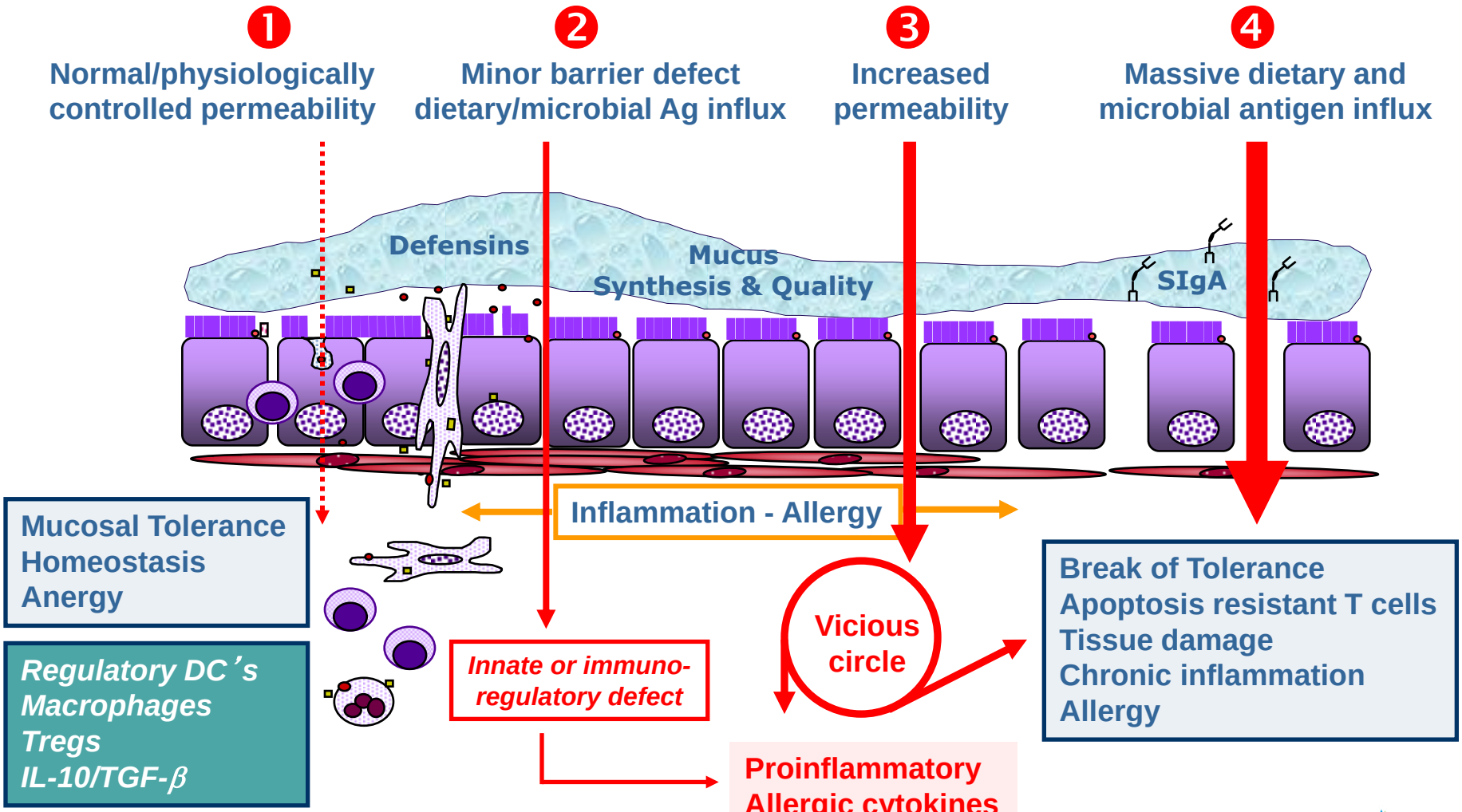
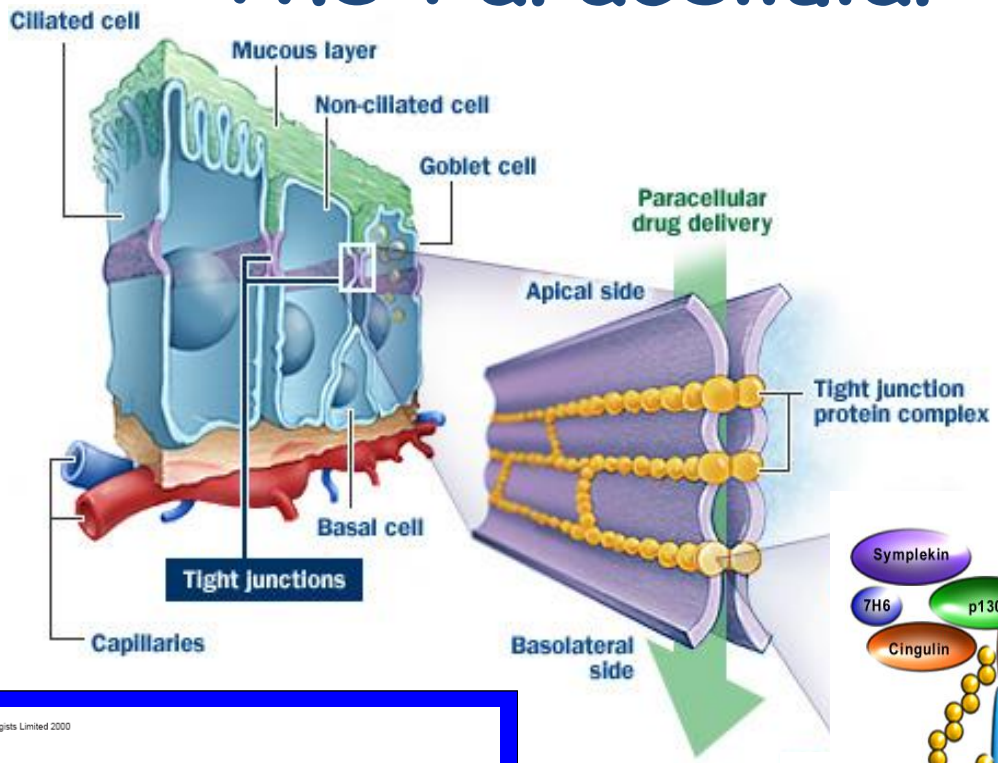
Excessive and Inappropriate Inflammatory Process Associated to a Dysfunction of Barriers:

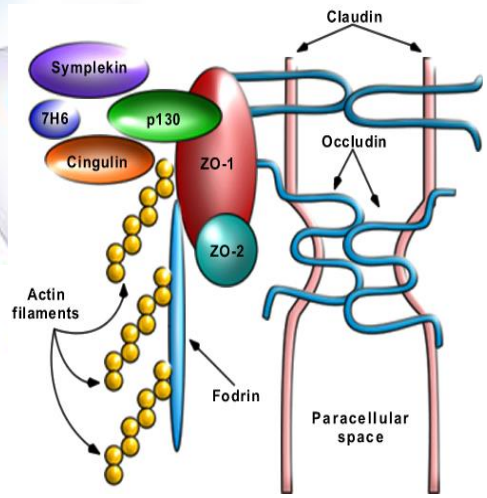
Loss of Mucosal Immune Homeostasis



Adapted from P. Brandtzaeg, Beneficial Microbes 2010

The Paracellular Pathway





Journal of Cell Science 113, 4435-4440 (2000)
Printed in Great Britain © The Company of Biologists Limited 2000
JCS1508

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)

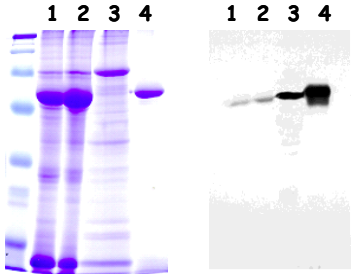
PURIFICATION PROTOCOL FROM HUMAN INTESTINE

1: Tissue lysate

2: Sephacryl-S300

3: Q-sepharose

4: Immunoaffinity



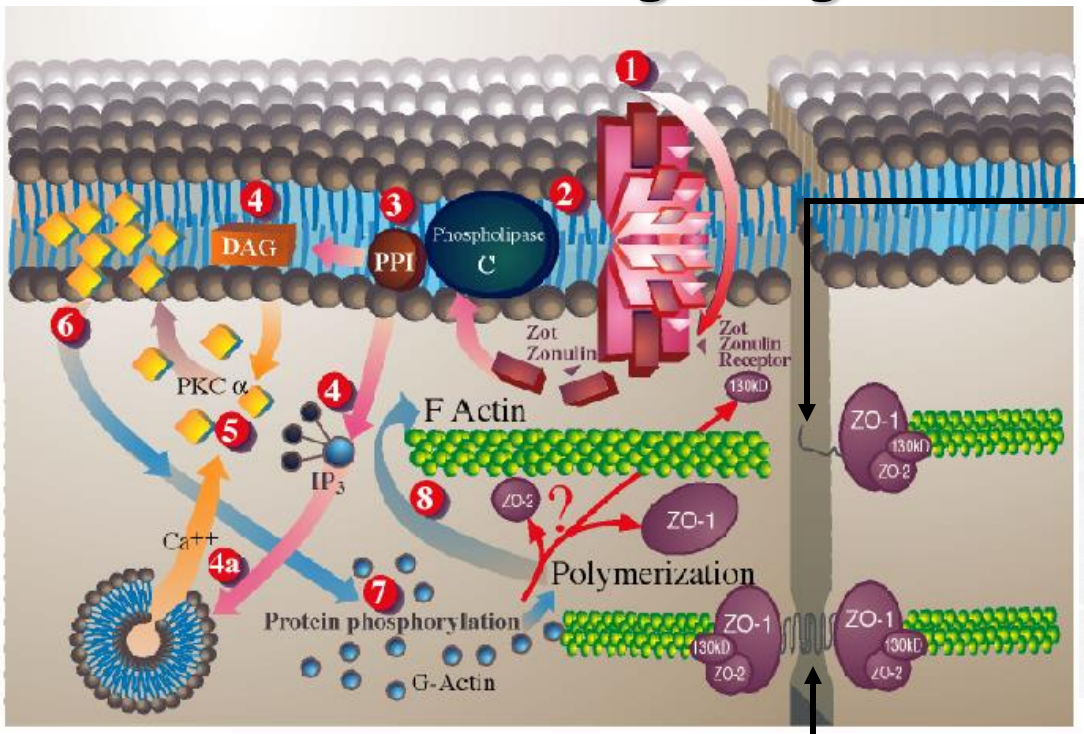
Coomassie Western blot

…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

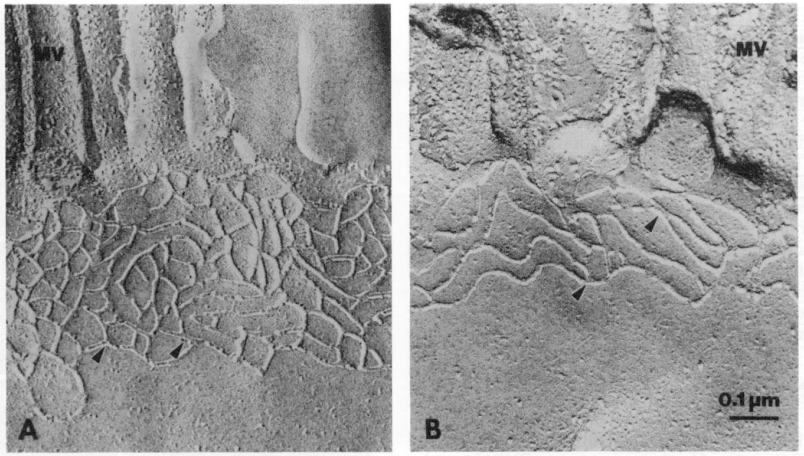
Zonulin Characterization and Signaling

Zonulin signaling



Freeze-Fracture

Following Pathway Activation



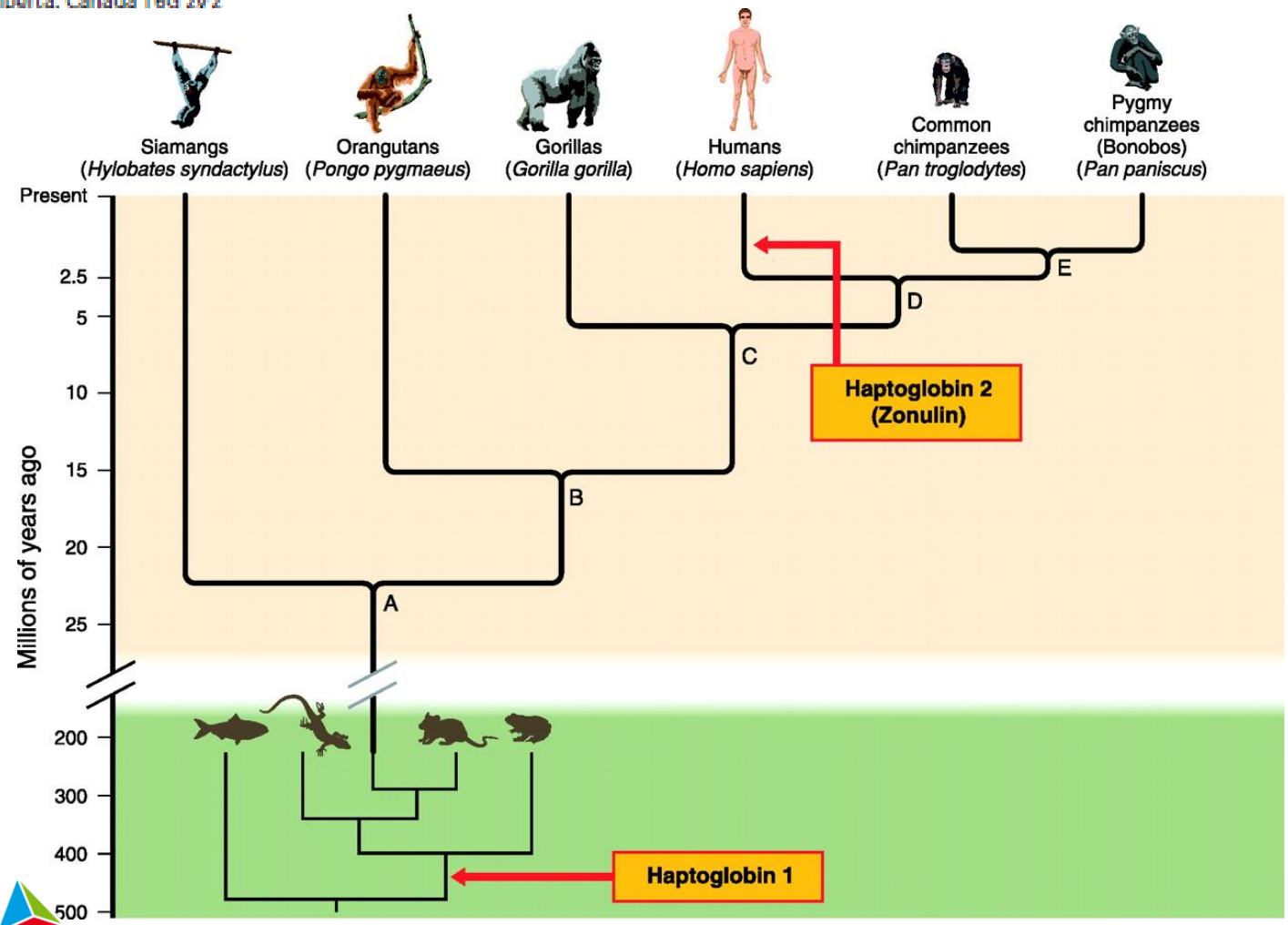
Resting State

Fasano et al, J Clin Invest 1995;96:710-20; el Asmar et al Gastroenterology 2002;123:1607-15

Identification of human zonulin, a physiological modulator of tight junctions, as prehaptoglobin-2

Amit Tripathi[a], Karen M. Lammers[a], Simeon Goldblum[a], Terez Shea-Donohue[a], Sarah Netzel-Arnett[b], Marguerite S. Buzza[b], Toni M. Antalis[a,b], Stefanie N. Vogel[a,c], Aiping Zhao[a], Shiqi Yang[a], Marie-Claire Arrietta[c], Jon B. Meddings[d], and Alessio Fasano[a,1]

[a]Mucosal Biology Research Center, [b]Center for Vascular and Inflammatory Diseases and Department of Physiology, and [c]Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, MD 20201; and [d]Department of Medicine, University of Alberta, Edmonton, Alberta, Canada T6G 2V2



Presented in ICoMI 2017

DSS Mouse Models to Establish The Link Between Distribution of Haptoglobin Alleles And Susceptibility to Inflammation

C57Bl/6 wild type mouse

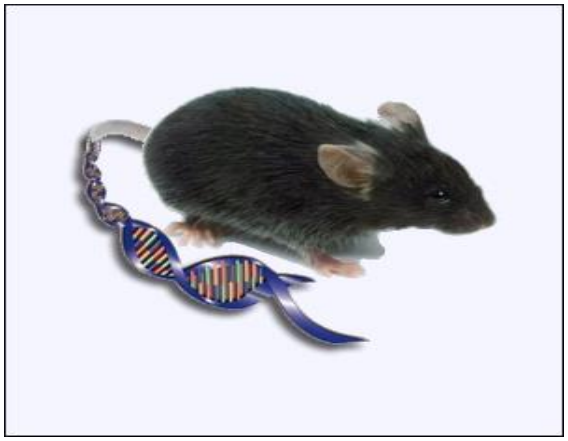
HP 1-1 (no zonulin gene)

C57Bl/6 mouse transfected with human HP2 gene

HP 1-2 (1 zonulin gene)

Transgenic C57Bl/6 mouse engineered by duplicating α chain (α1 ⇒ α2)

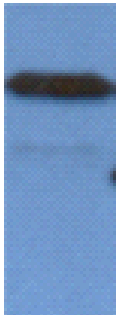
HP 2-2 (2 zonulin genes)



WB

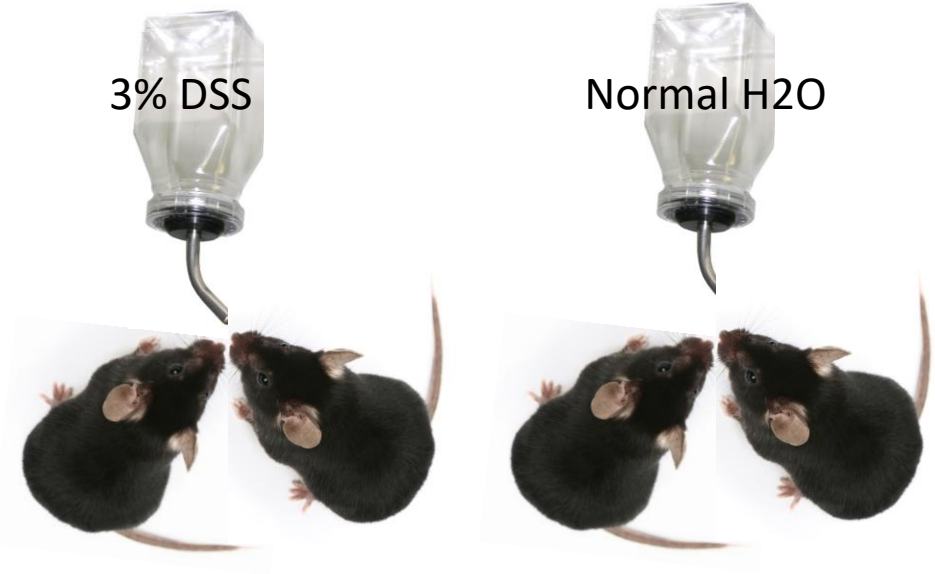
α2 →

α1 →

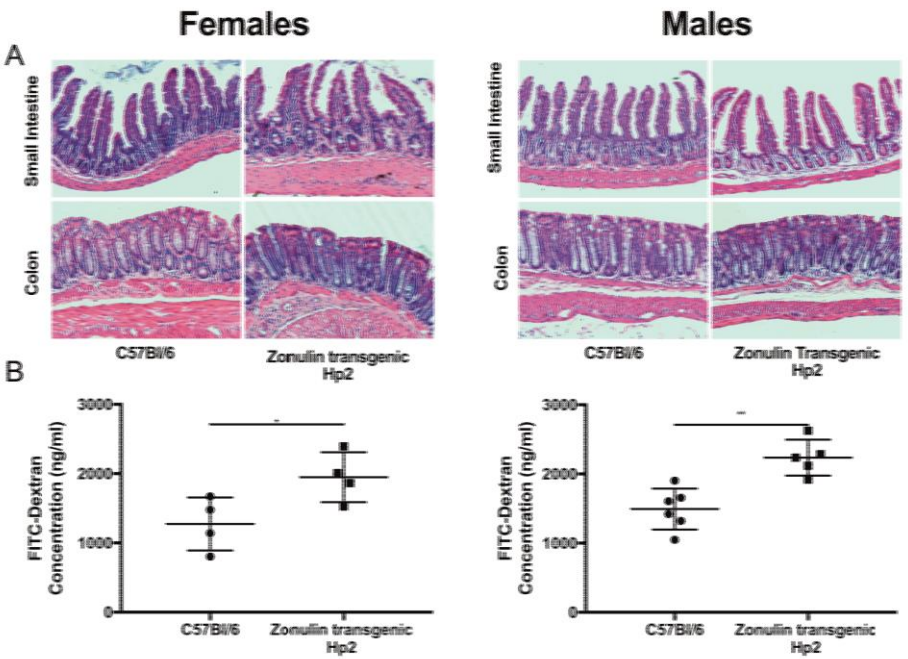


How Zonulin-Mediated Increased Ag Trafficking Leads to Chronic Inflammation:

Insights From The Zonulin Trangenic Mouse Model



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

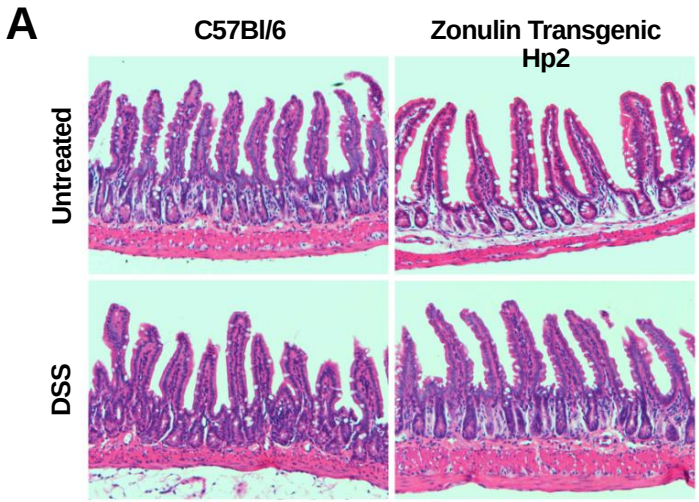


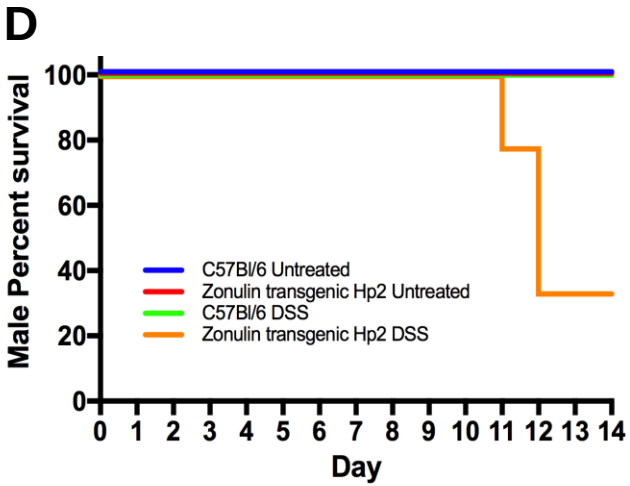
Zonulin transgenic Hp2 mice are phenotypically normal despite increased small intestinal permeability

*$p<0.05$

Sturgeon C et al. Ann NY Acad Sci 2017 Apr 19 (Epub ahead print)

Presented in ICoMI 2017



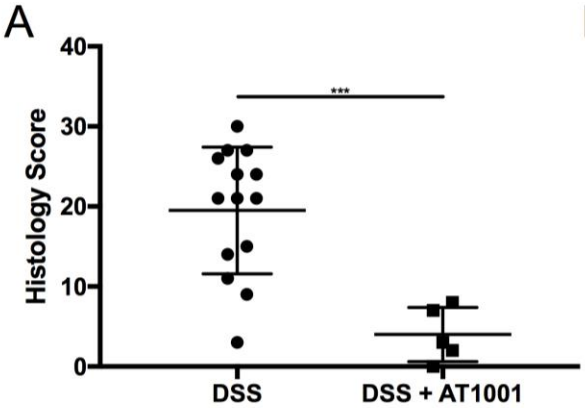


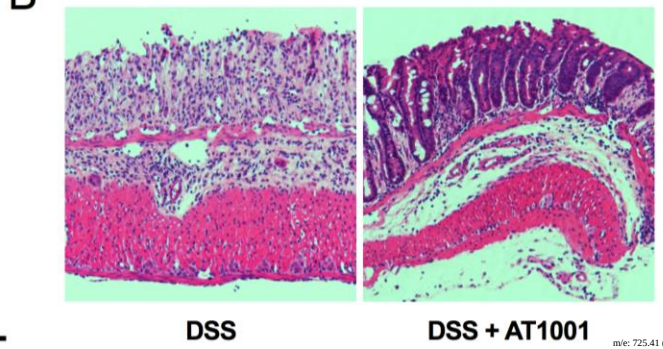
Ztm have increased morbidity (colonic mucosal damage) and mortality (70%) following DSS induced colitis

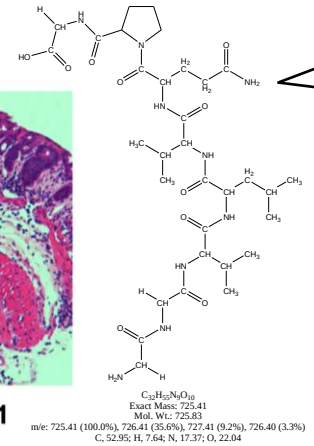
Sturgeon C et al. Ann NY Acad Sci 2017 Apr 19 (Epub ahead print)

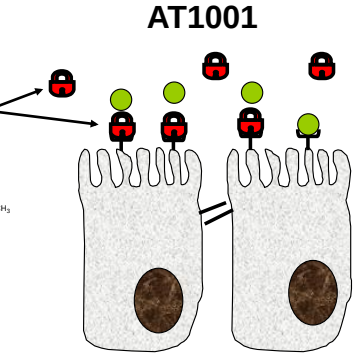
Presented in ICoMI 2017

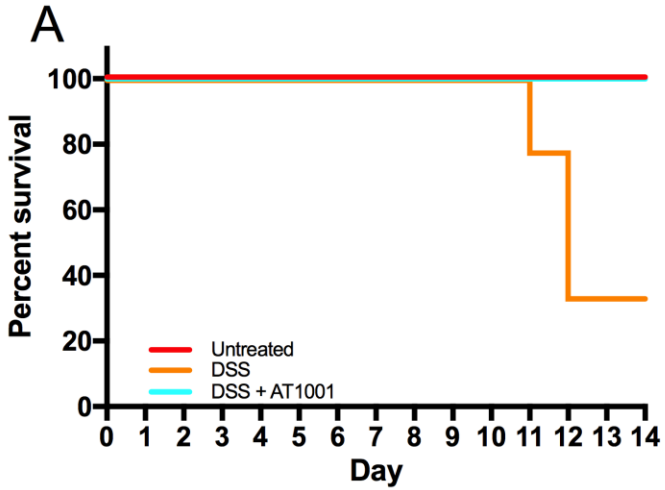
The Zonulin Inhibitor AT1001 (Larazotide Acetate) Treatment Ameliorates Increased DSS Induced Morbidity and Mortality in Ztm

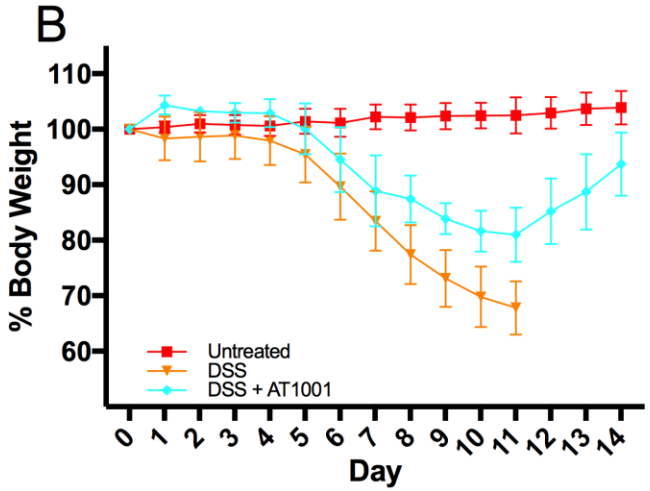












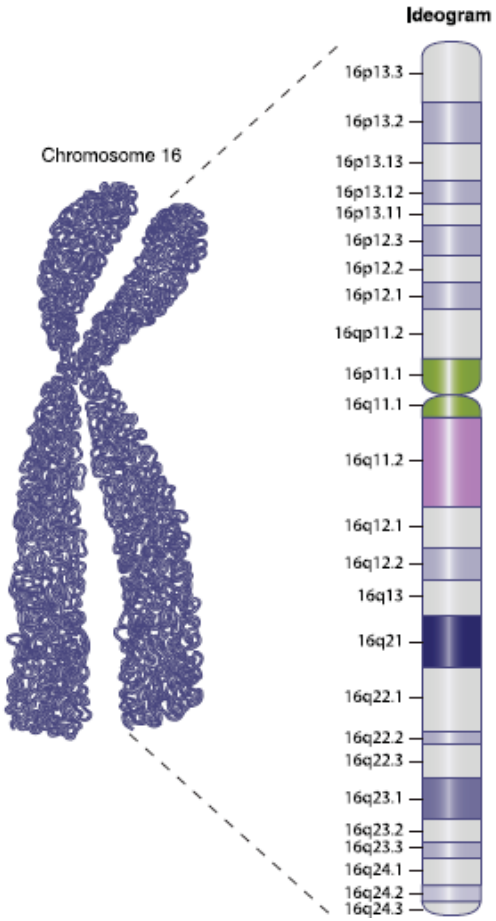
AT1001 (larazotide acetate) treatment ameliorates increased DSS induced morbidity and mortality in ztm

Presented in ICoMI 2017

Sturgeon C et al. Ann NY Acad Sci 2017 Apr 19 (Epub ahead print)

Zonulin Gene Is Located on Chromosome 16

Chromosome 16 contains about 98 million bases, or some 3% of the human genome, encoding for ~1,300 genes.



Presented in ICoMI 2017

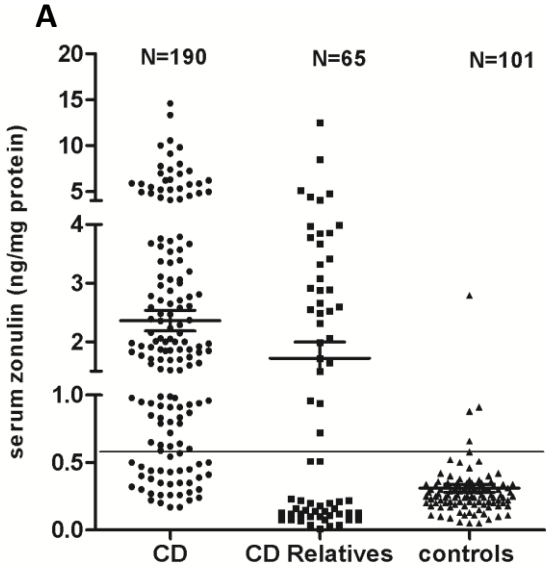
Fasano A. Physiol Rev. 2011 Jan;91(1):151-75

Literature Report On Zonulin Association With CID

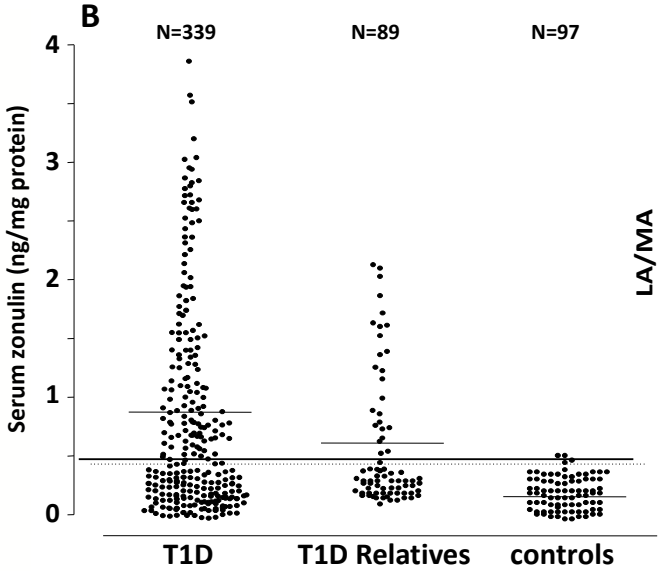
Disease	Model	Zonulin Shown to be Involved
Ankylosis spondylitis	Human	YES
Autism	Human	YES
Celiac Disease	Human	YES
Colitis/IBD (Crohn's disease)	Human	YES
Colitis	Mouse	YES
Fe metabolism in heart transplant	Human	NO
Glioma	Human	YES
Glioma	Cell	YES
Irritable bowel syndrome	Human	YES
HIV	Human	YES
Multiple sclerosis	Mouse	YES
Necrotizing Enterocolitis (NEC)	Rat	YES
Nonalcoholic fatty liver disease	Human	YES
Non-Celiac Gluten Sensitivity	Human	YES
Obesity/Insulin resistance	Human	YES
Post-surgery Sepsis	Human	YES
Post-surgery Sepsis	Mouse	YES
Psoriasis	Human	NO
Sepsis	Human	YES
Type 1 diabetes	Human	YES
Type 2 diabetes	Human	YES

Serum Zonulin Levels and Their Correlation With Intestinal Permeability In Celiac Disease and Type 1 Diabetes

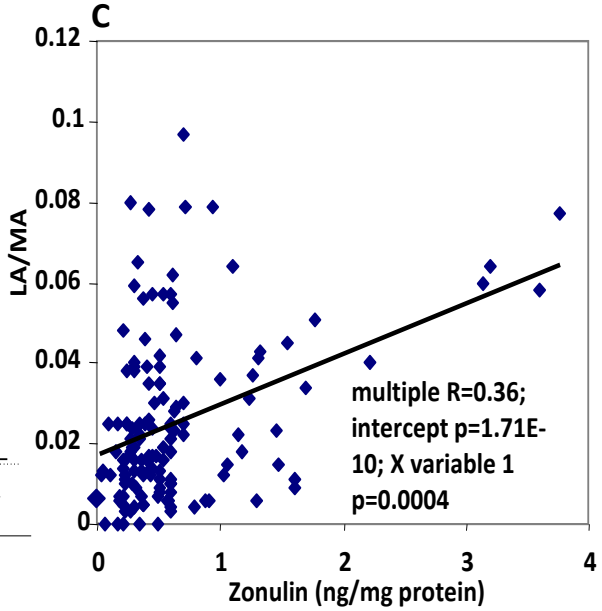
Celiac Disease



Type 1 Diabetes



Zonulin-LA/MA

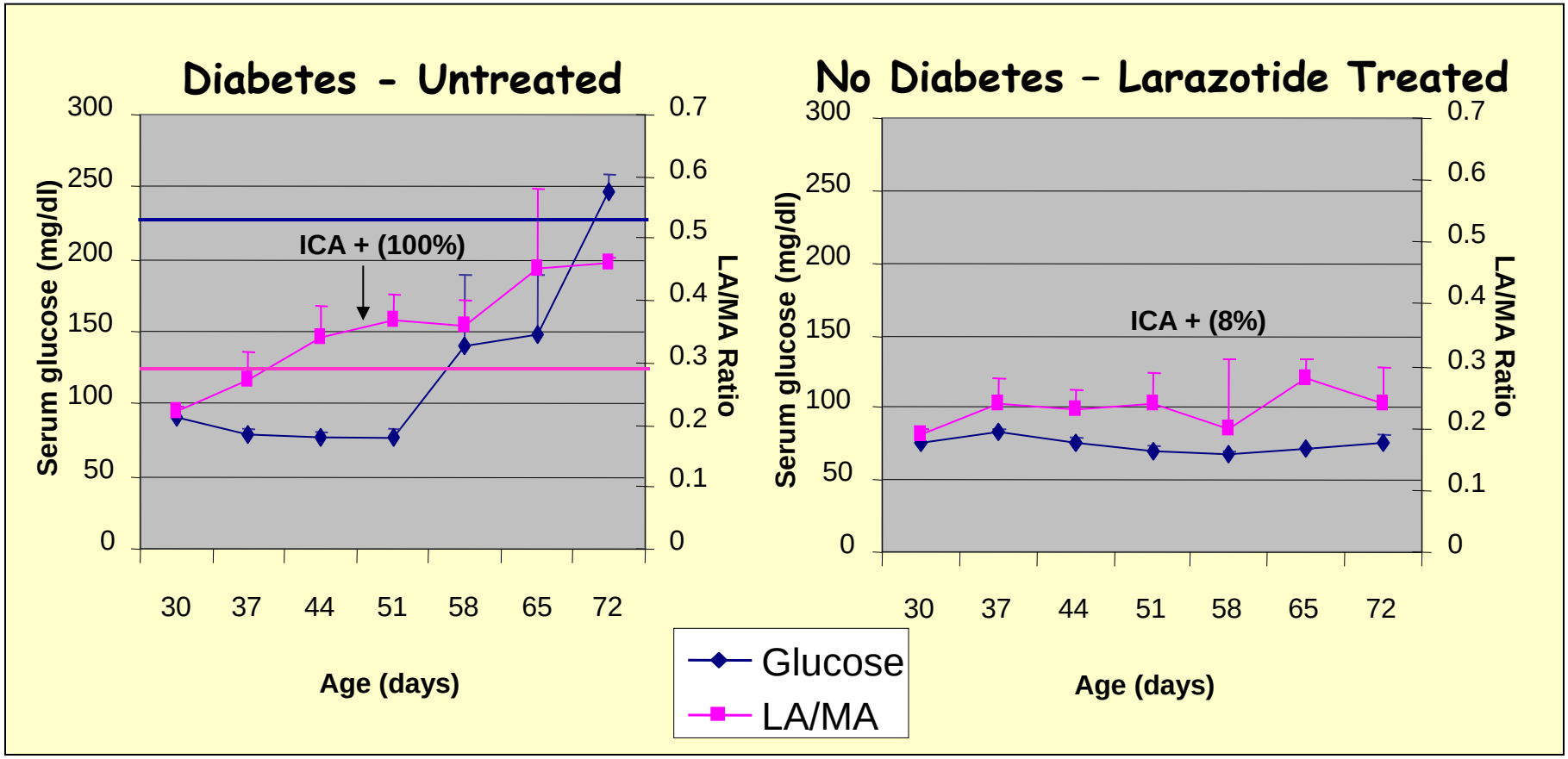


Sapone et al Diabetes 2006; 55:1443-9

Presented in ICoMI 2017

Type 1 Diabetes

Evidence for Zonulin-Dependent Increased Intestinal Permeability in the Pathogenesis of Type 1 Diabetes



Watts et al PNAS 2005;102:2916-21

Presented in ICoMI 2017

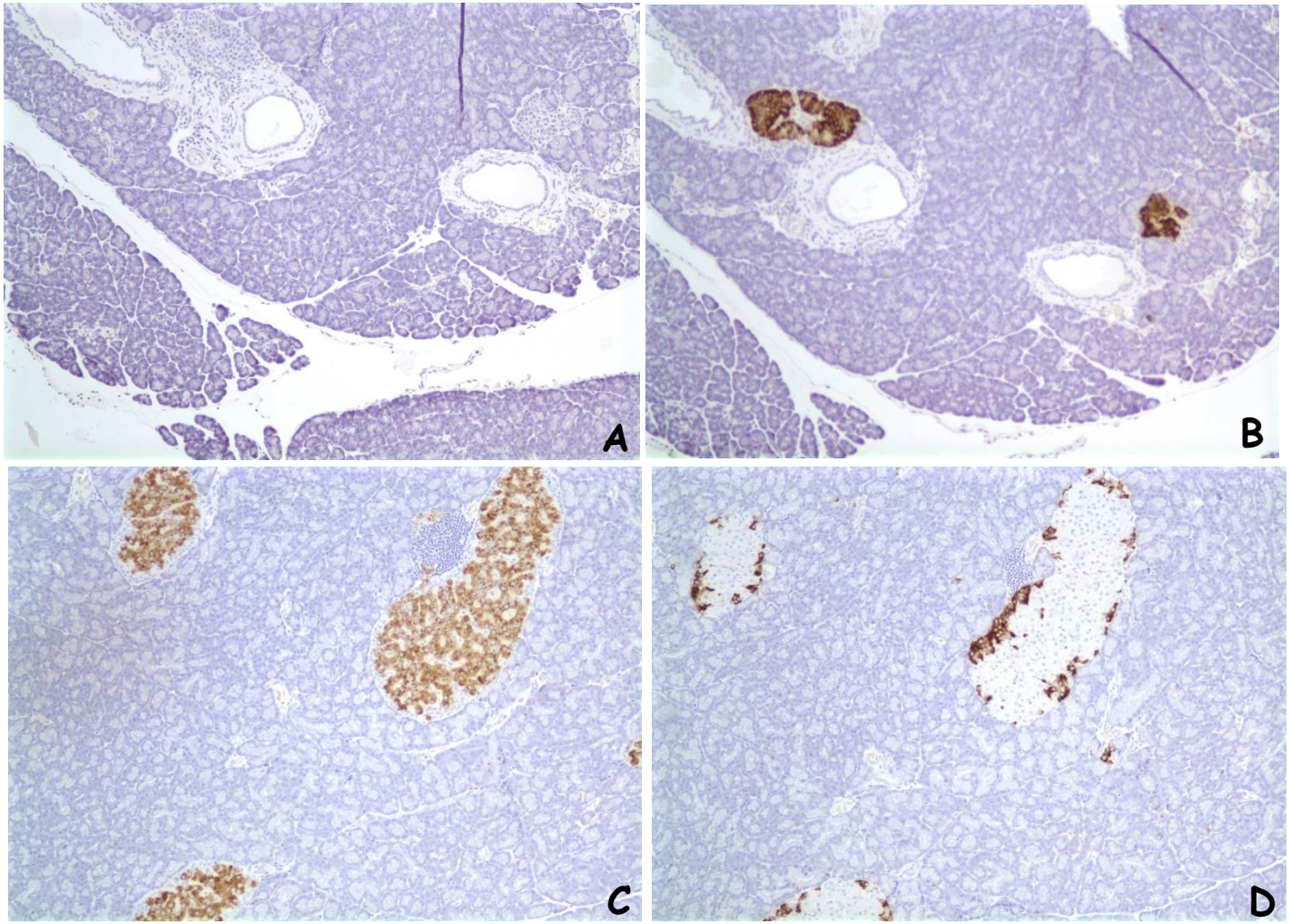
Blocking the Zonulin-Dependent Increased Intestinal Permeability Aborts The Autoimmune Process

Islet Immunohistochemistry

Insulin staining | Glucagon staining

Untreated BBDP rats that developed T1D

Larazotide-treated BBDP rats that DID NOT develop T1D: No insulitis



Celiac Disease

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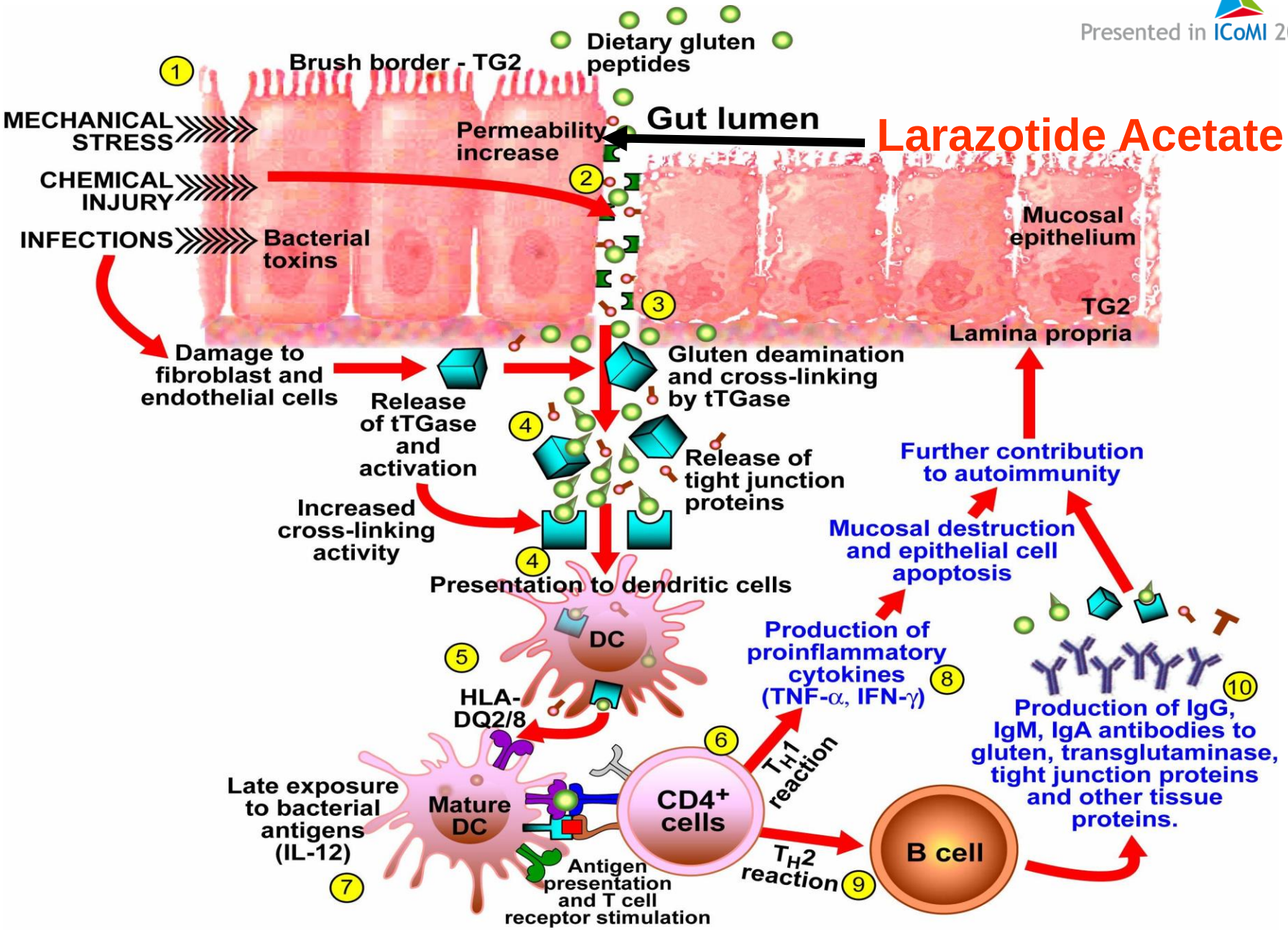
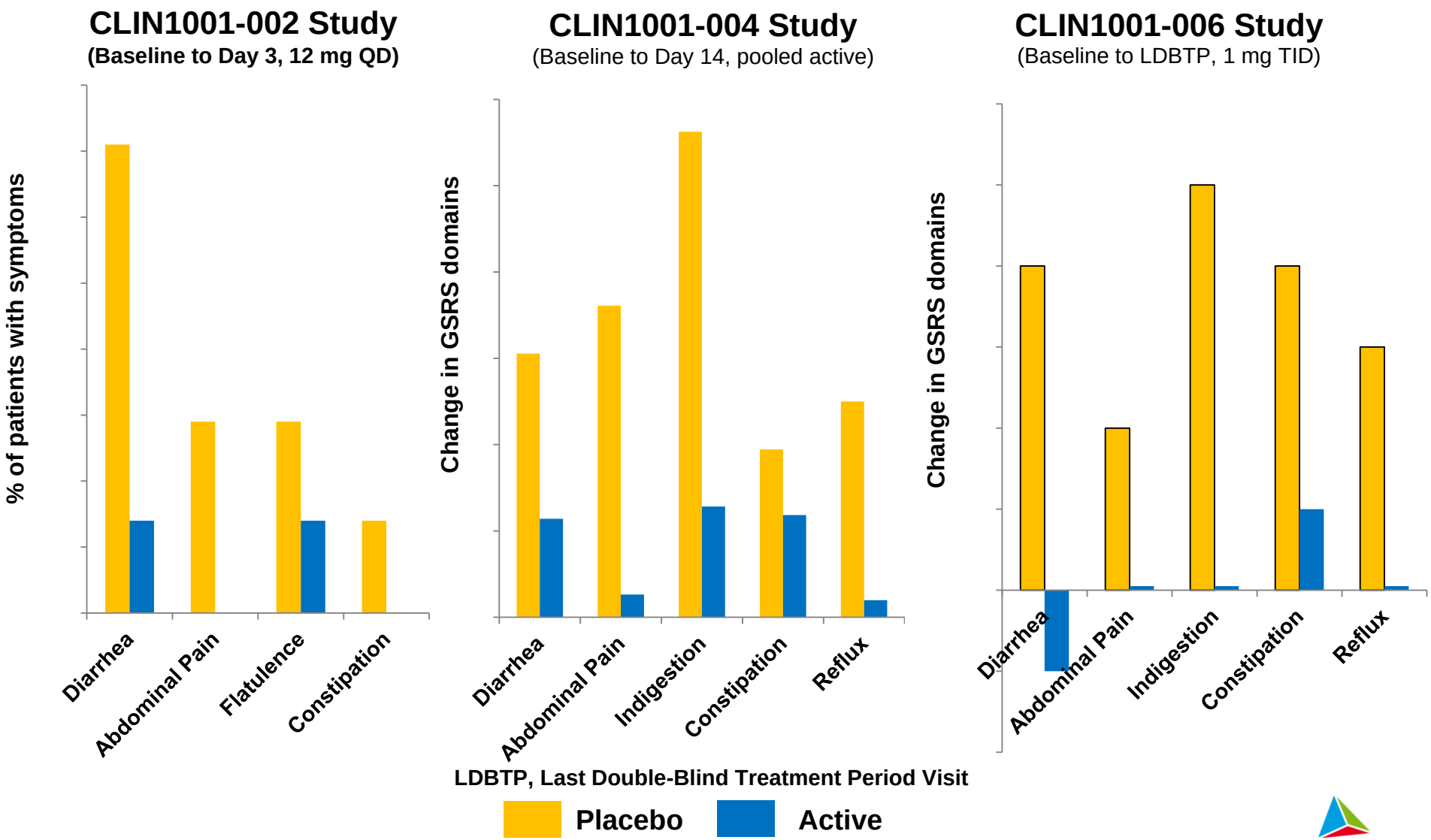


Depiction of the intestinal mucosa with emphasis on the factors involved in the development of celiac disease in individuals with HLA-DQ2/DQ8 positive

Larazotide Acetate Consistently Reduced Gastro-Intestinal Symptoms in 3 Gluten-Challenge Clinical Trials



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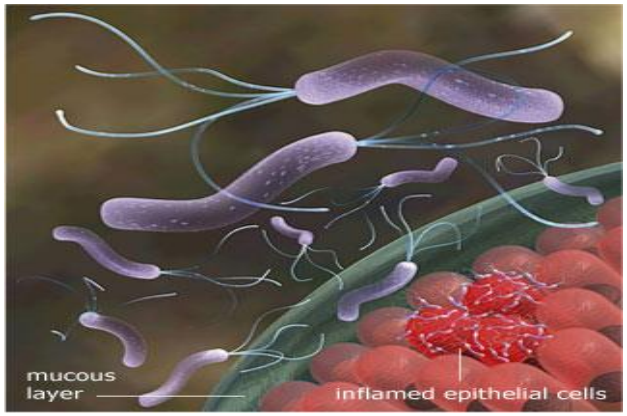
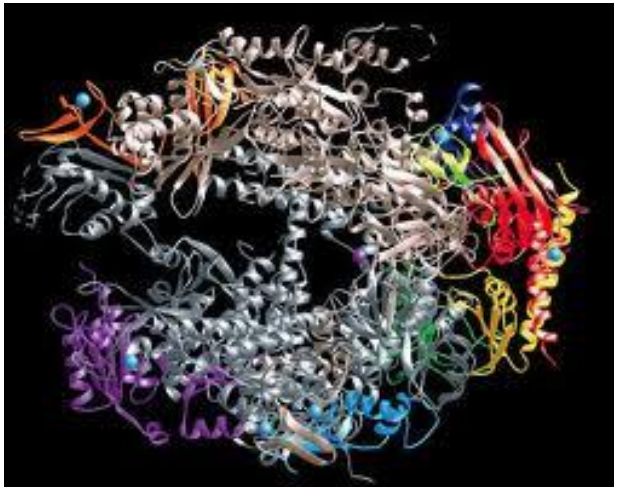
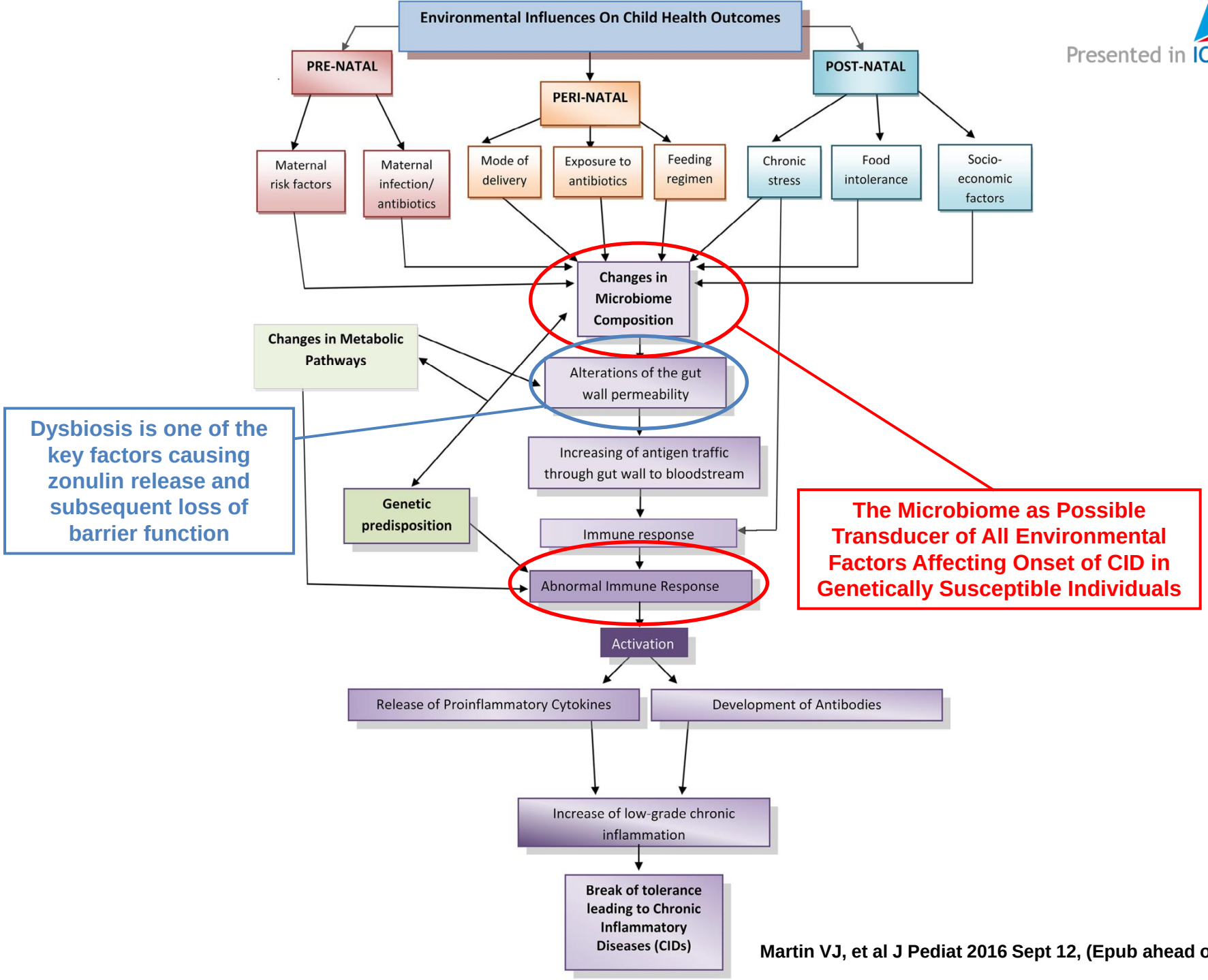
Environmental Triggers Causing Zonulin Release

Gluten

Gut Microbiome

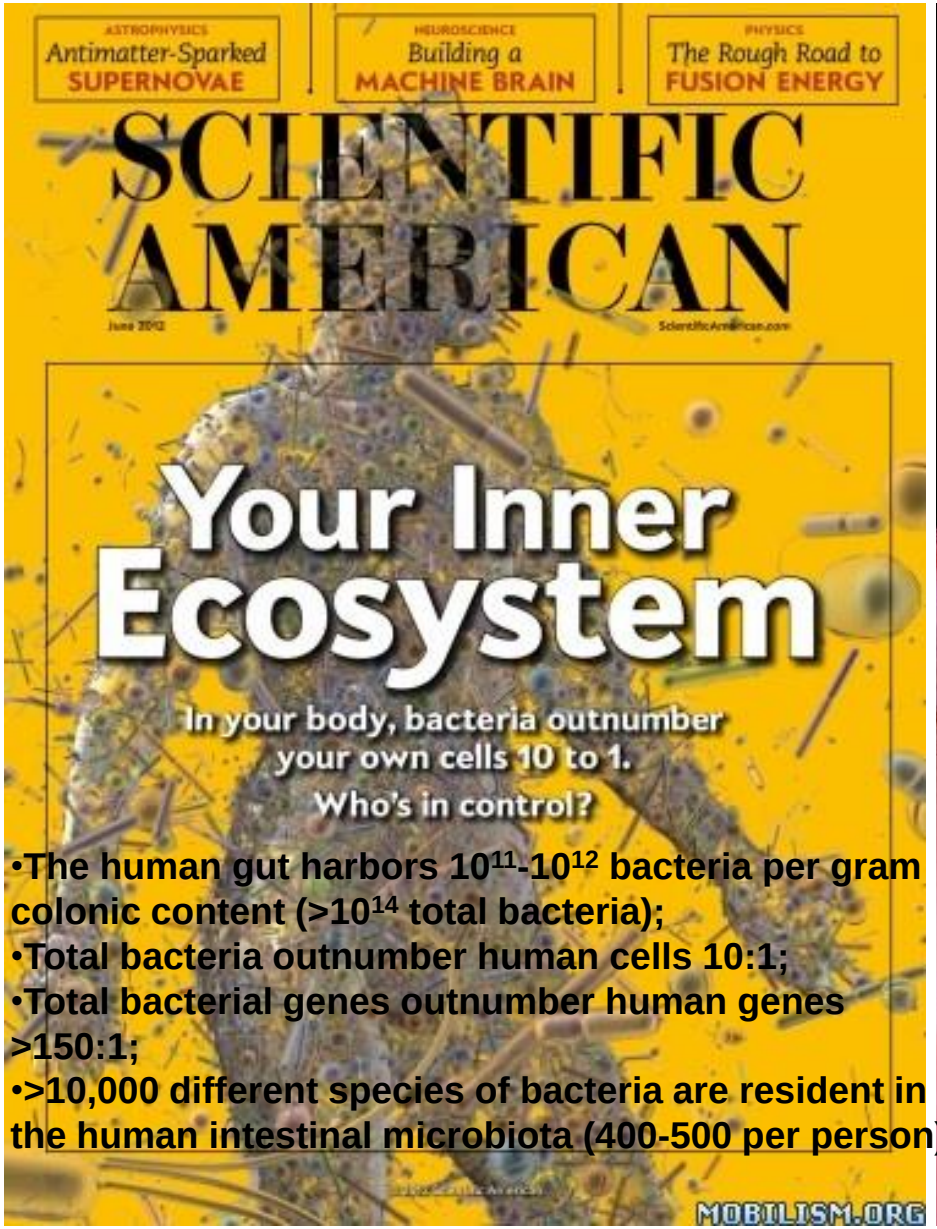




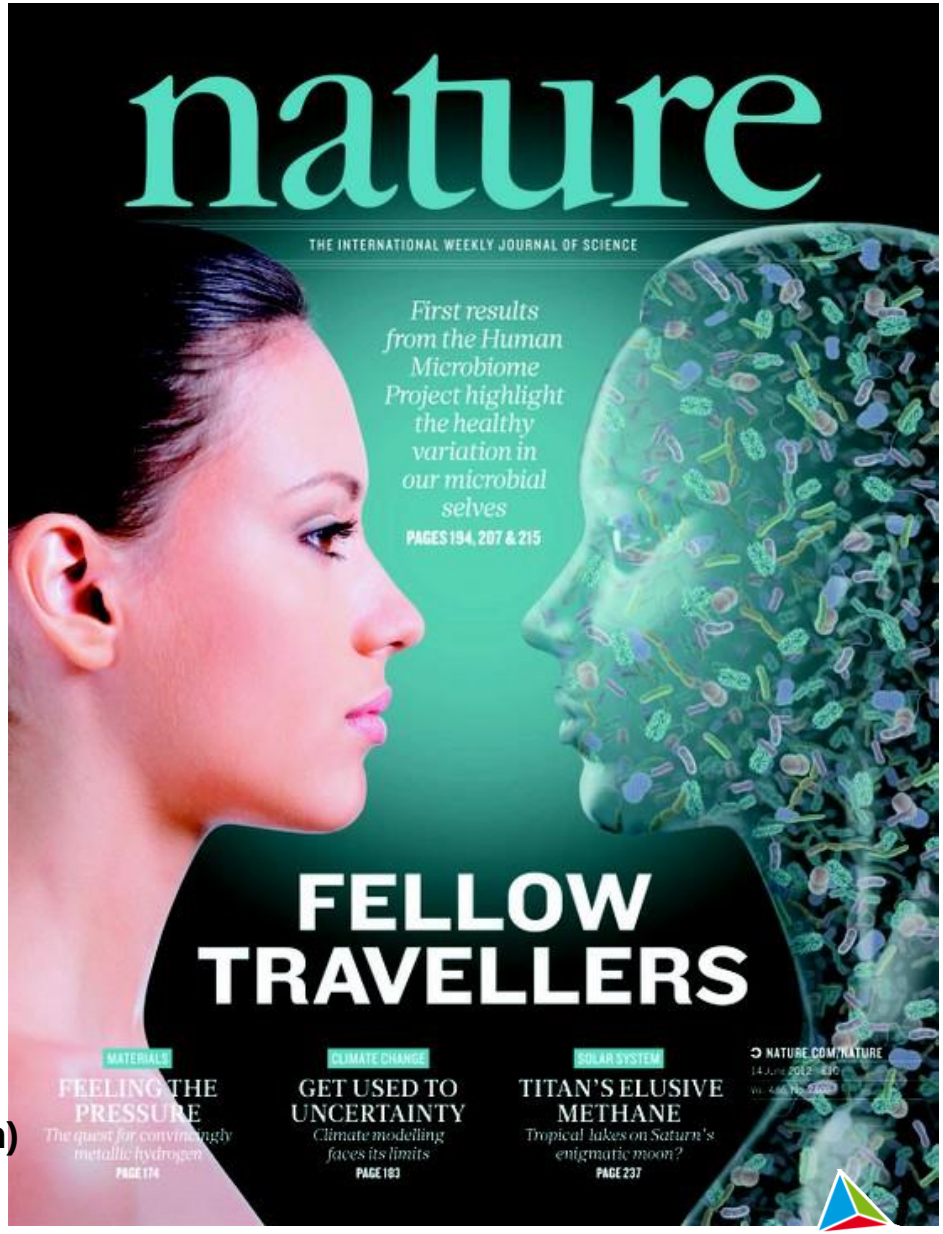


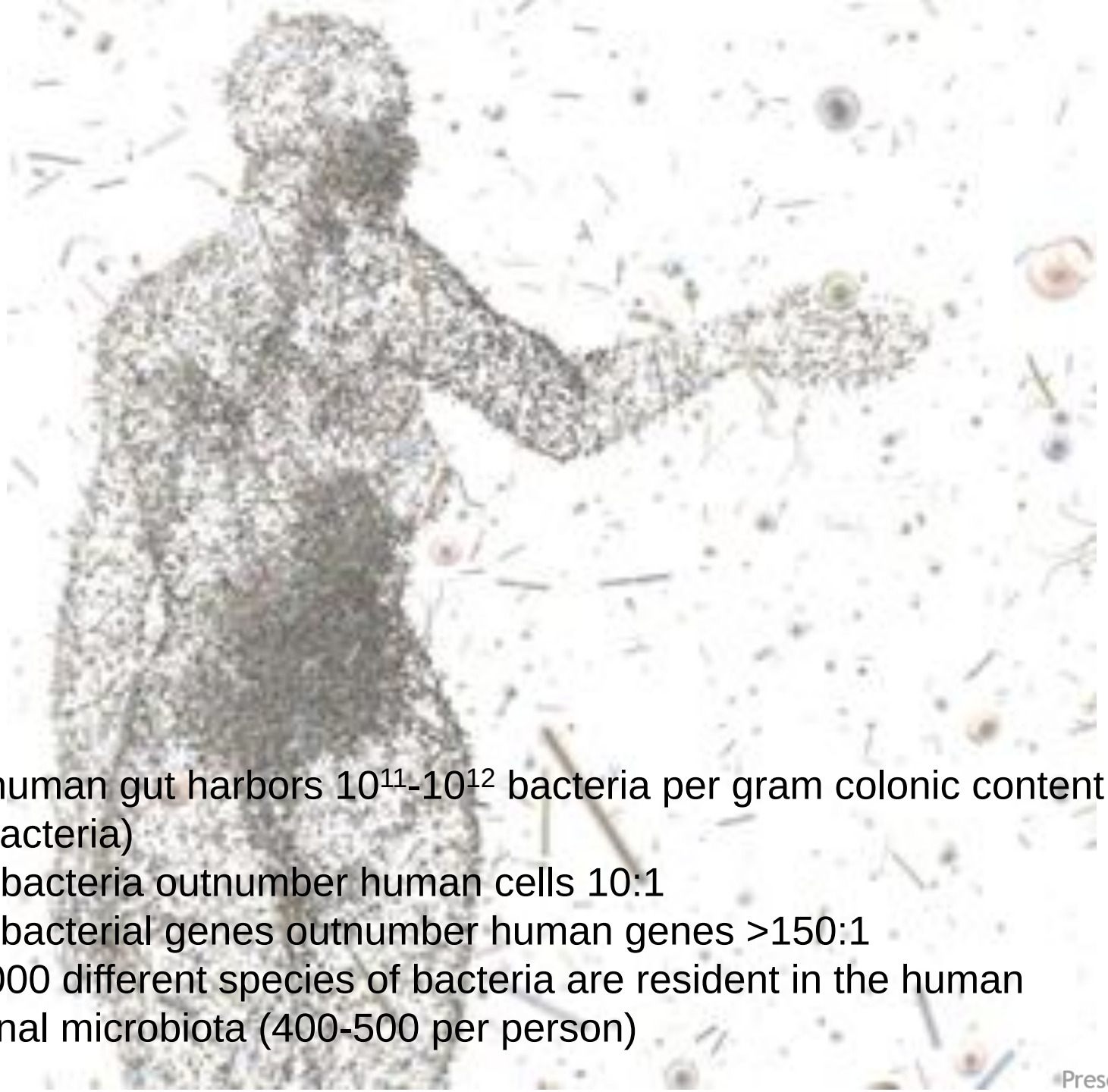
Martin VJ, et al J Pediat 2016 Sept 12, (Epub ahead of print)

The changing face of gut microbes



- The human gut harbors 10^{11}-10^{12} bacteria per gram colonic content (>10^{14} total bacteria);
- Total bacteria outnumber human cells 10:1;
- Total bacterial genes outnumber human genes >150:1;
- >10,000 different species of bacteria are resident in the human intestinal microbiota (400-500 per person)





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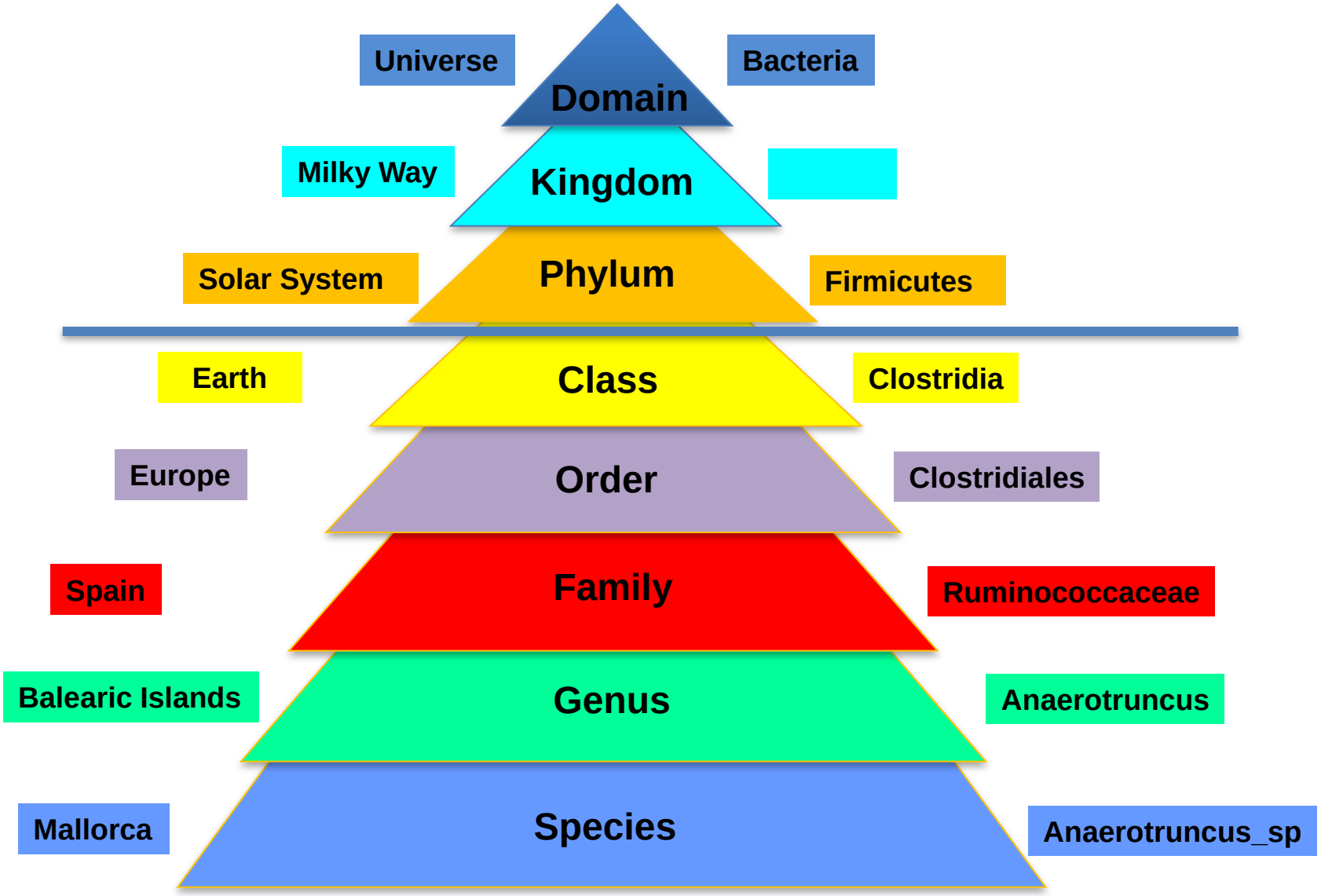
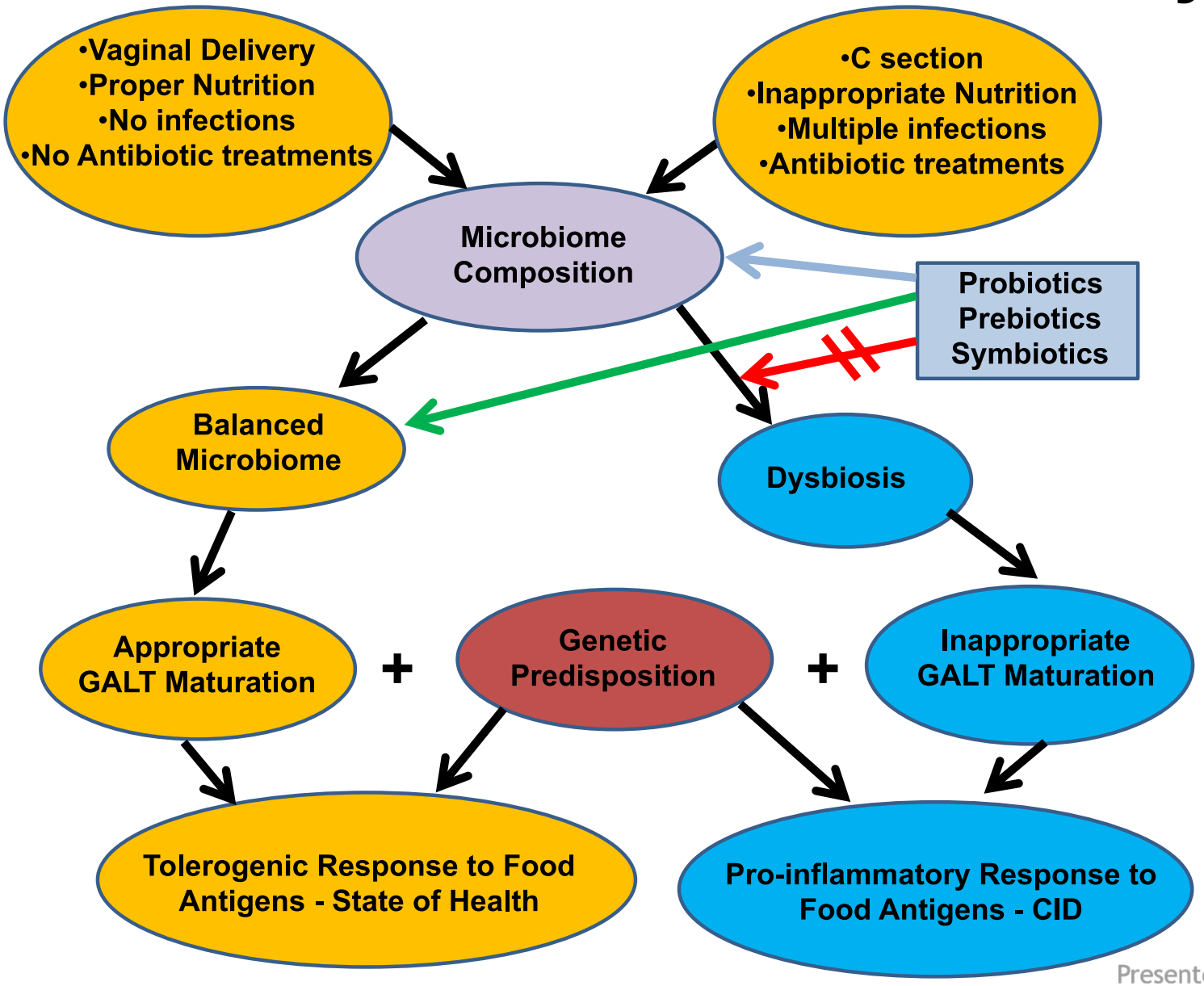
Is Microbiome Science Ready For Primetime Clinical Applicability?

Classification of Organisms



Adapted from http://biology-medicine.blogspot.com/2012/09/scientific-classification-of-organisms.html

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



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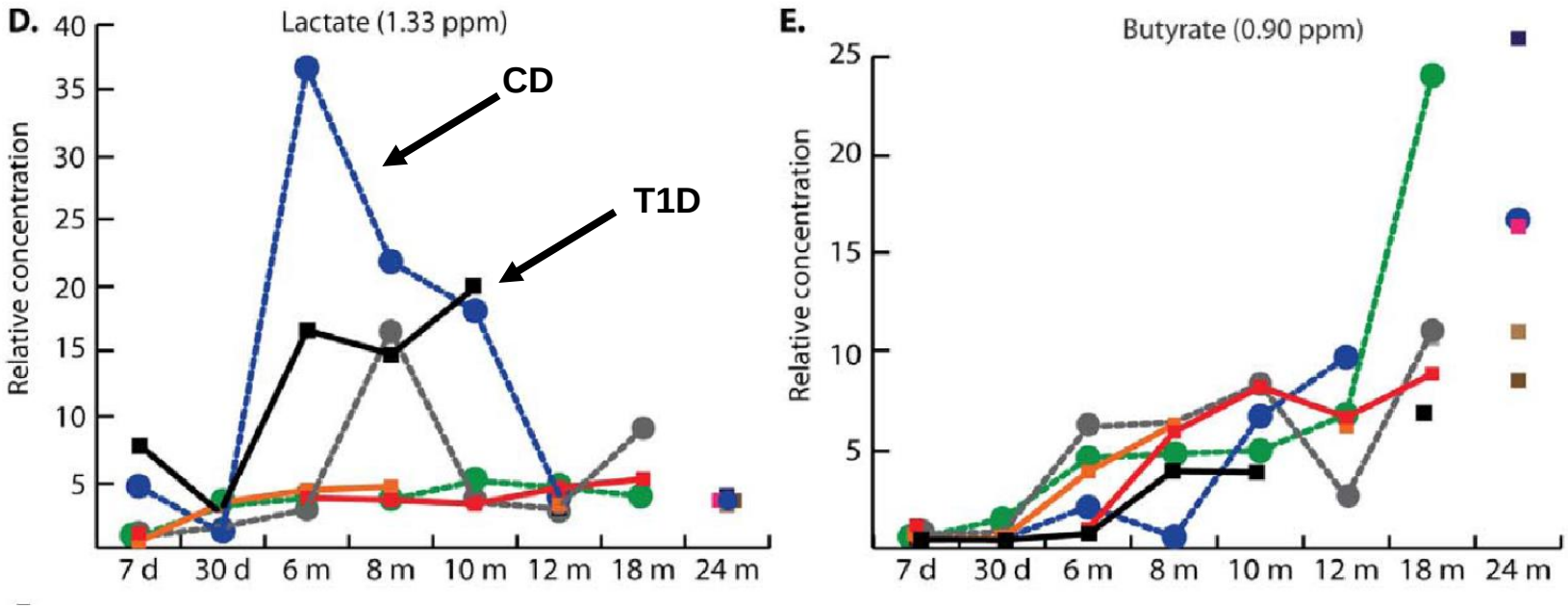
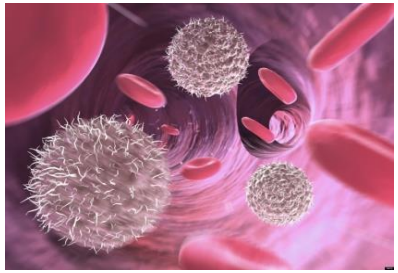
Metabolome Analysis In At-Risk Infants Developing CD



- **Infants who developed autoimmune diseases during the time of the study had high levels of lactate combined with low levels of butyrate before the onset of the disease;**
- **Just before developing the disease, lactate decreased while butyrate increased;**
- **These changes paralleled changes in microbiome composition, with decreased of Lactobacillaceae and increased of butyrate-producing Firmicutes.**

T REGULATORY CELLS IN CD



Immune Response

- Minor subpopulation of $CD4^+$ T cells (5-10%).
- Traditionally described as **$CD4^+CD25^{++}FOXP3^+$**

➢ Maintenance of self-tolerance and immune homeostasis.

➢ Induction of oral tolerance.

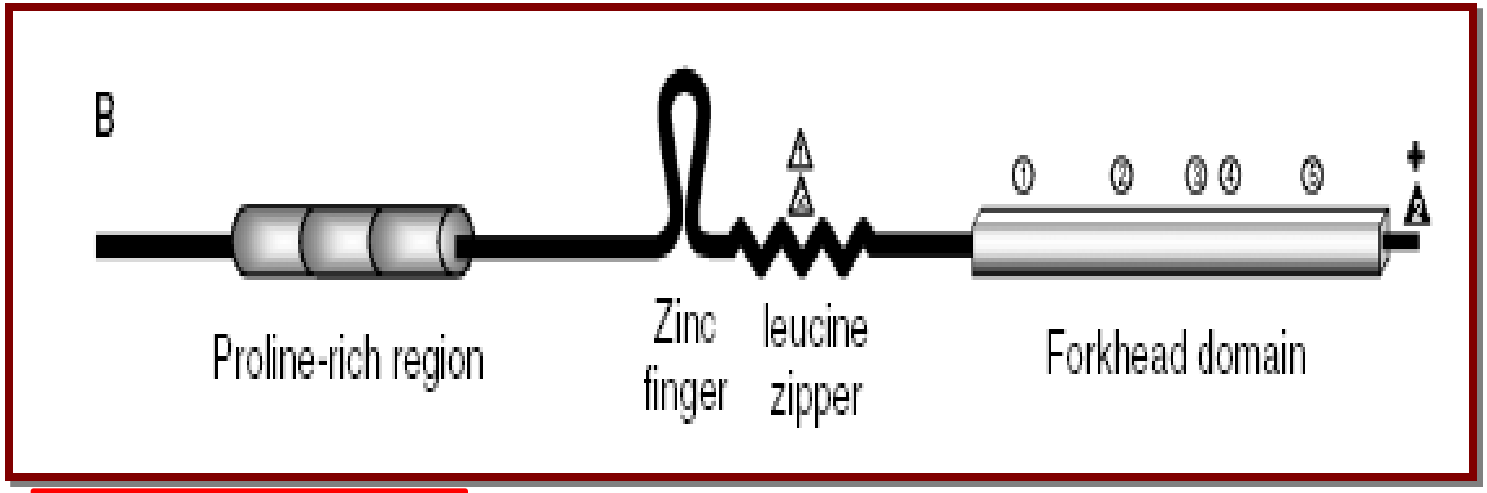
- Low frequency of $CD4^+CD25^+$ Treg cells in

1. Systemic Lupus Erythematosus patients
2. Multiple Sclerosis patients
3. in Type 1 diabetes

- Number of Treg cells is <u>increased</u> in active celiac patients.
- <u>Suppressive function</u> of Treg cells is significantly <u>impaired</u> in celiac patients.

FOXP3

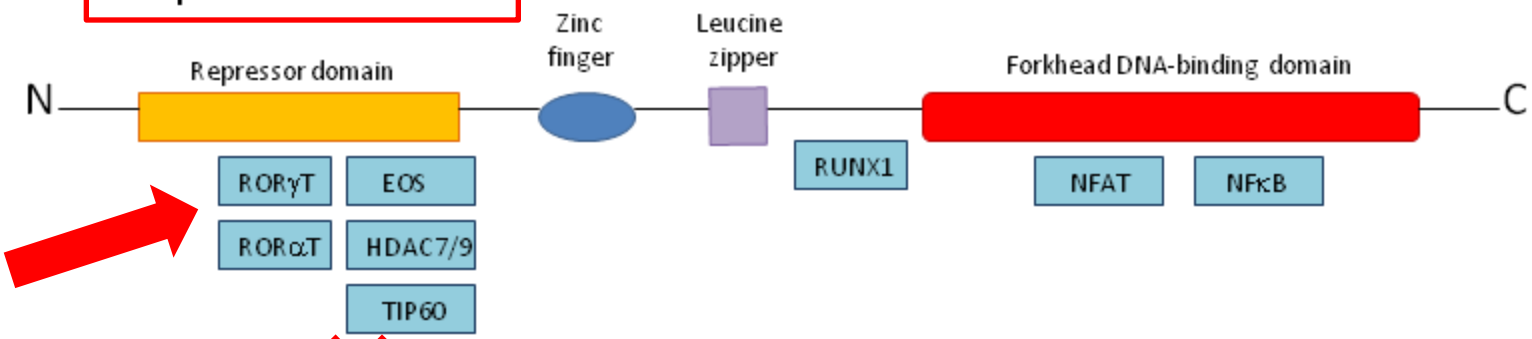
- Important for differentiation and function of Treg cells.

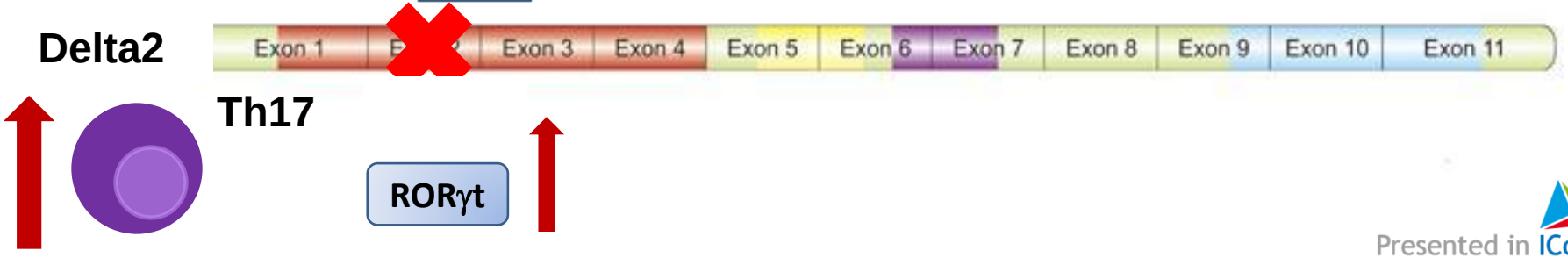


Transcriptional repression

Homodimerization

DNA binding, nuclear location





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Different Foxp3 FL/Δ2 Ratio Between Gut Mucosa and PBMC

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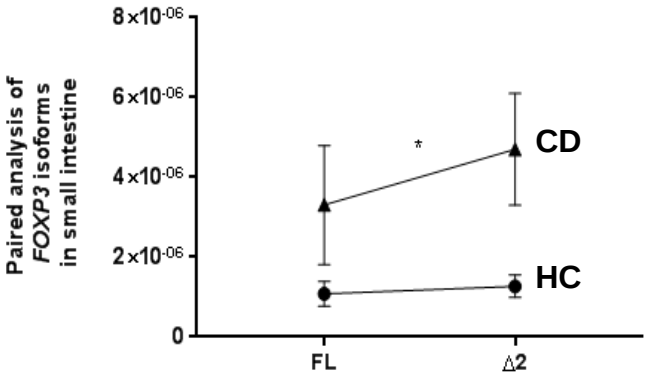
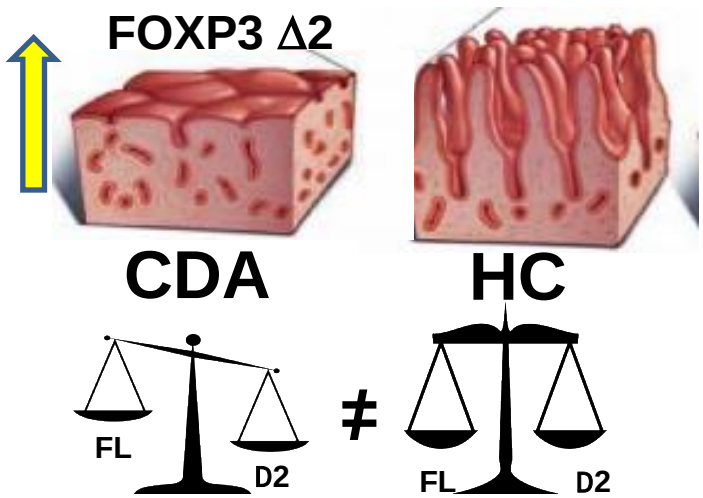
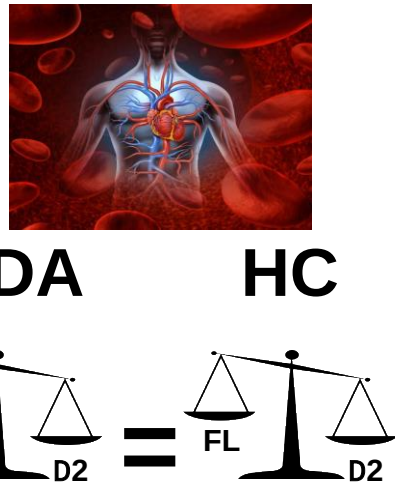
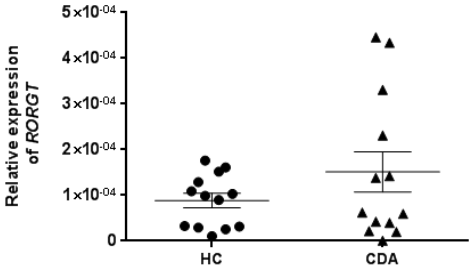
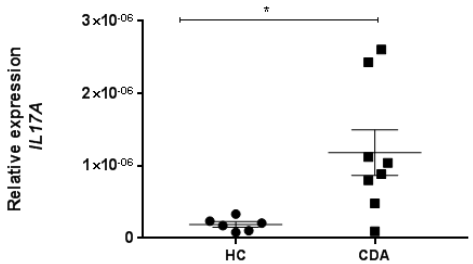


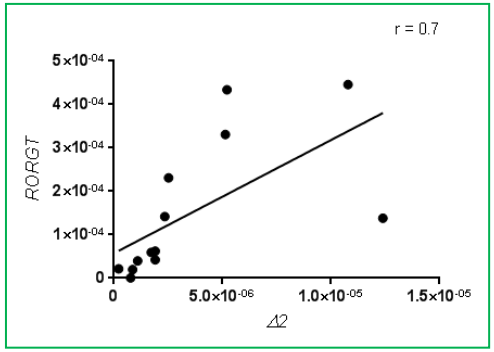


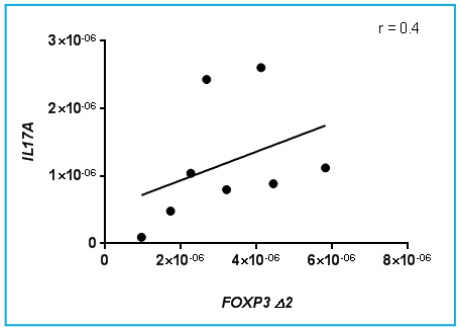






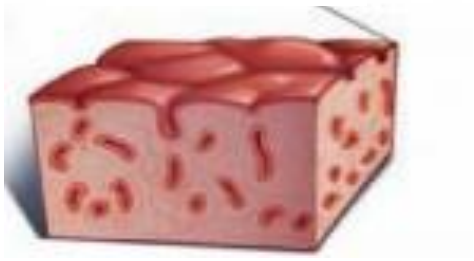
Increased Expression of FOXP3 Δ2 is Correlated With Increased Expression of *RORGT* and *IL17A* in CD Patients





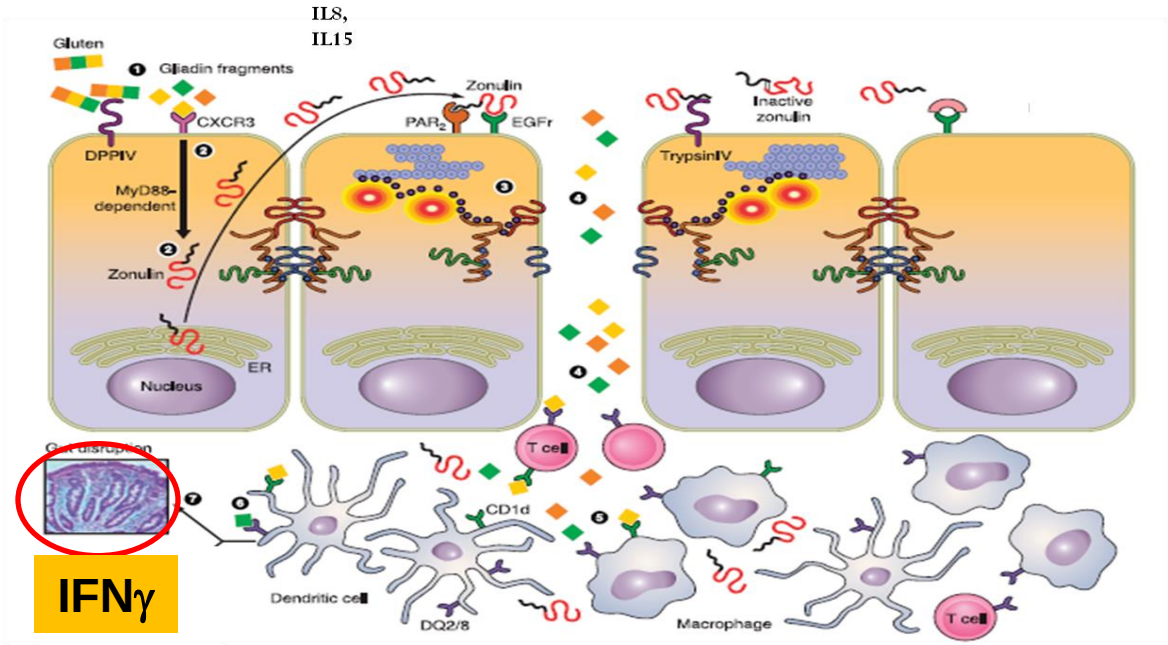
Serena G et al, Clin Exp Immunol. 2017

CDA VS HC



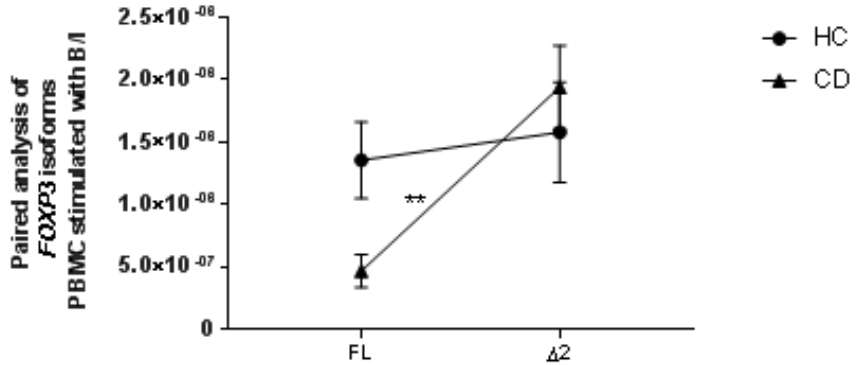
Gut pro-inflammatory microenvironment

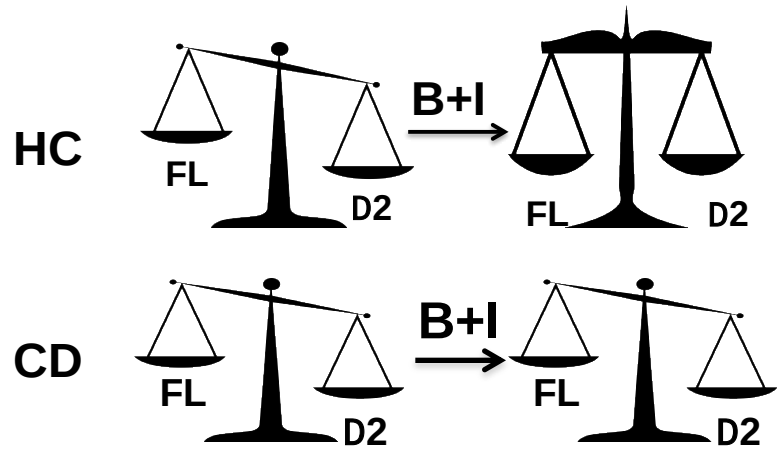
Microbiome composition

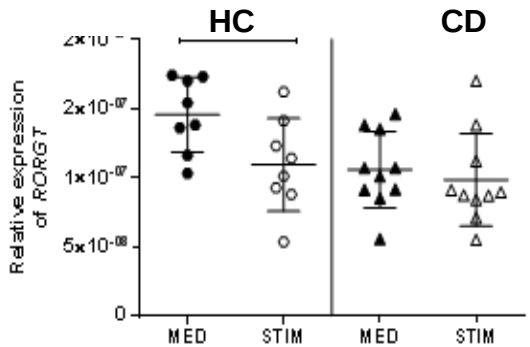


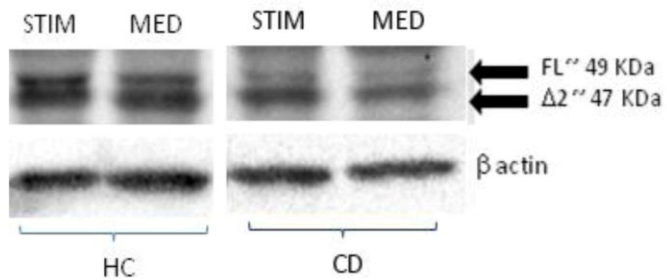
Serena G et al, Clin Exp Immunol. 2017

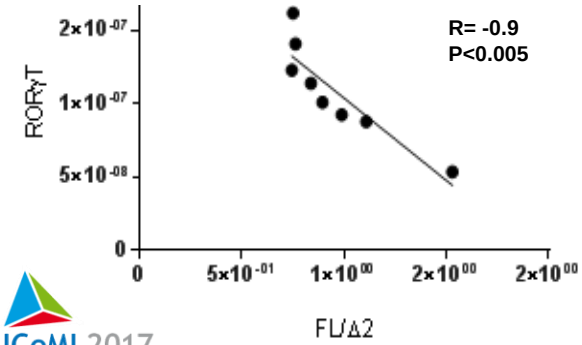
IFNγ+Butyrate Combination Leads to FOXP3 Isoforms Equilibrium in PBMC From HC BUT NOT CD subjects

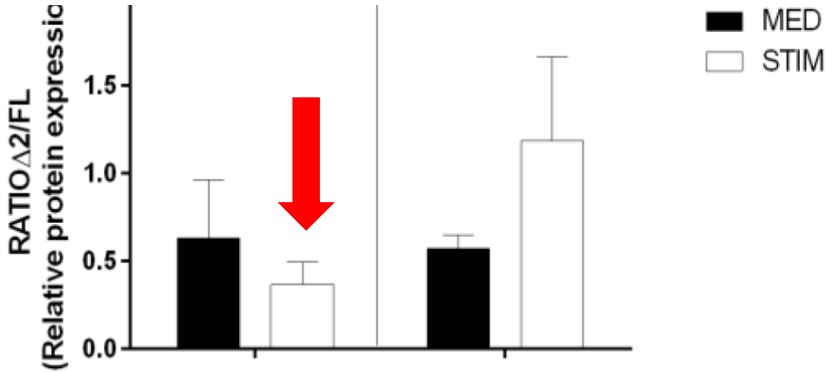






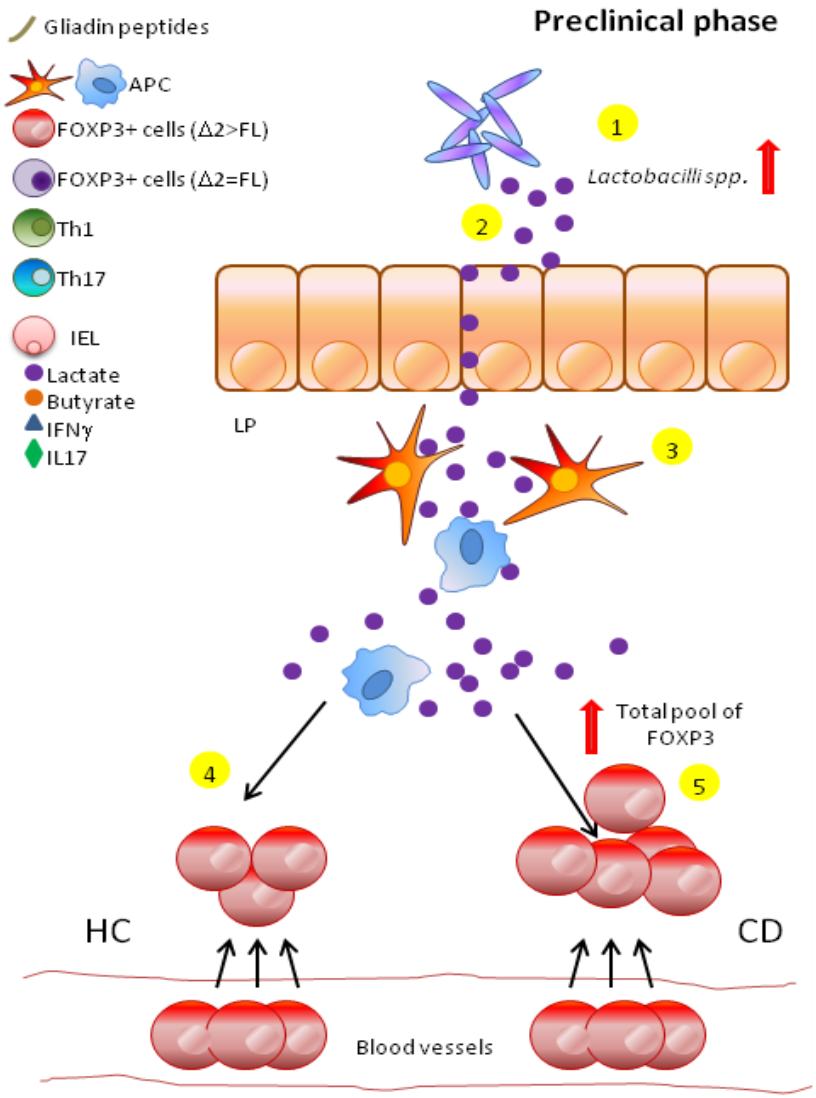






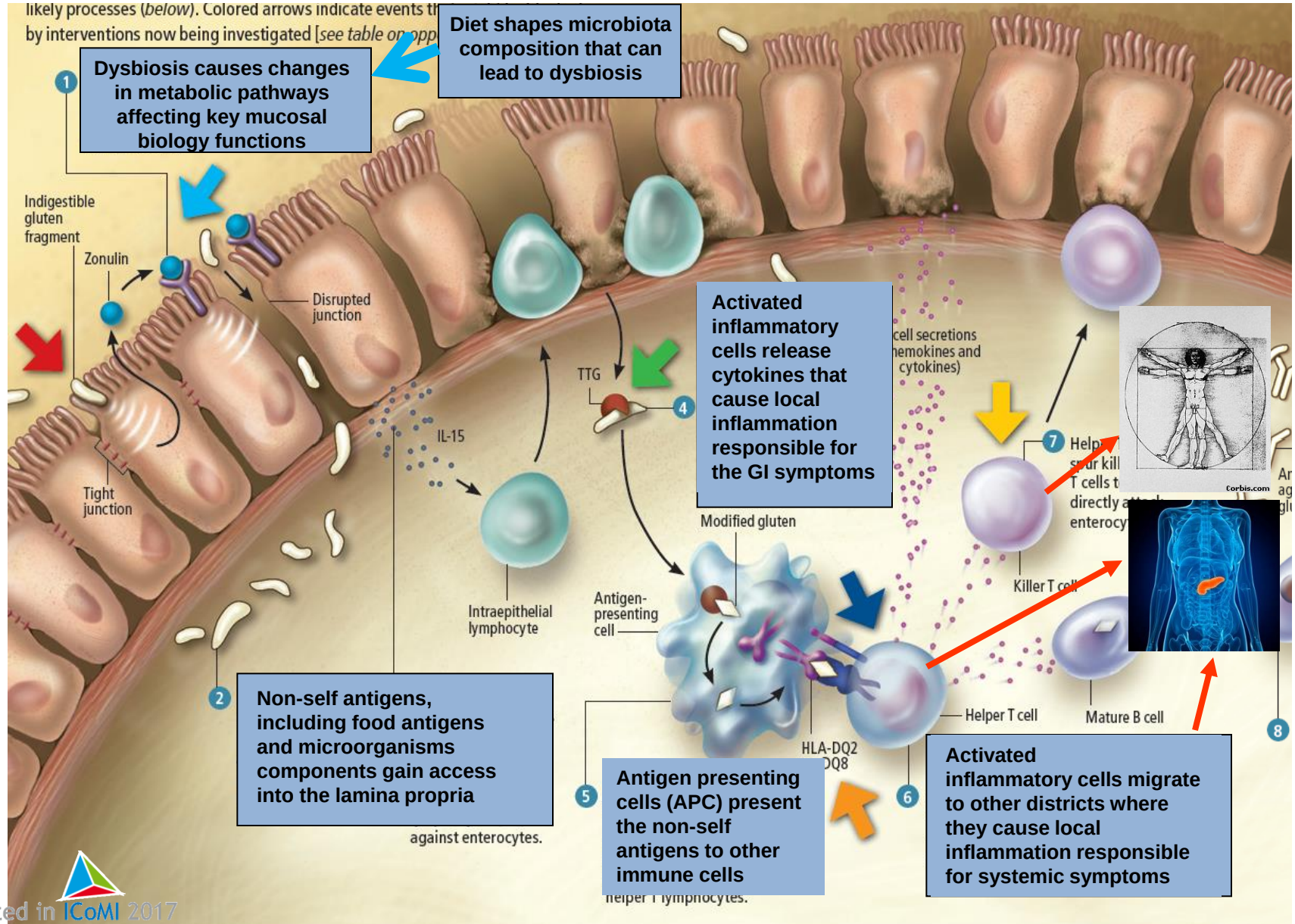
Serena G et al, Clin Exp Immunol. 2017

Working Hypothesis Linking Gut Microbiome Composition, Metabolomic Profile, and Immune Functions Leading To CD



Serena G et al, Clin Exp Immunol. 2017

Working Hypothesis: The Gut Microbiome/Permeability Link in the Pathogenesis of Autoimmune Diseases



Celiac **D**isease **G**enomic **E**nvironmental **M**icrobiome and **M**etabolomic Study

www.CDGEMM.org

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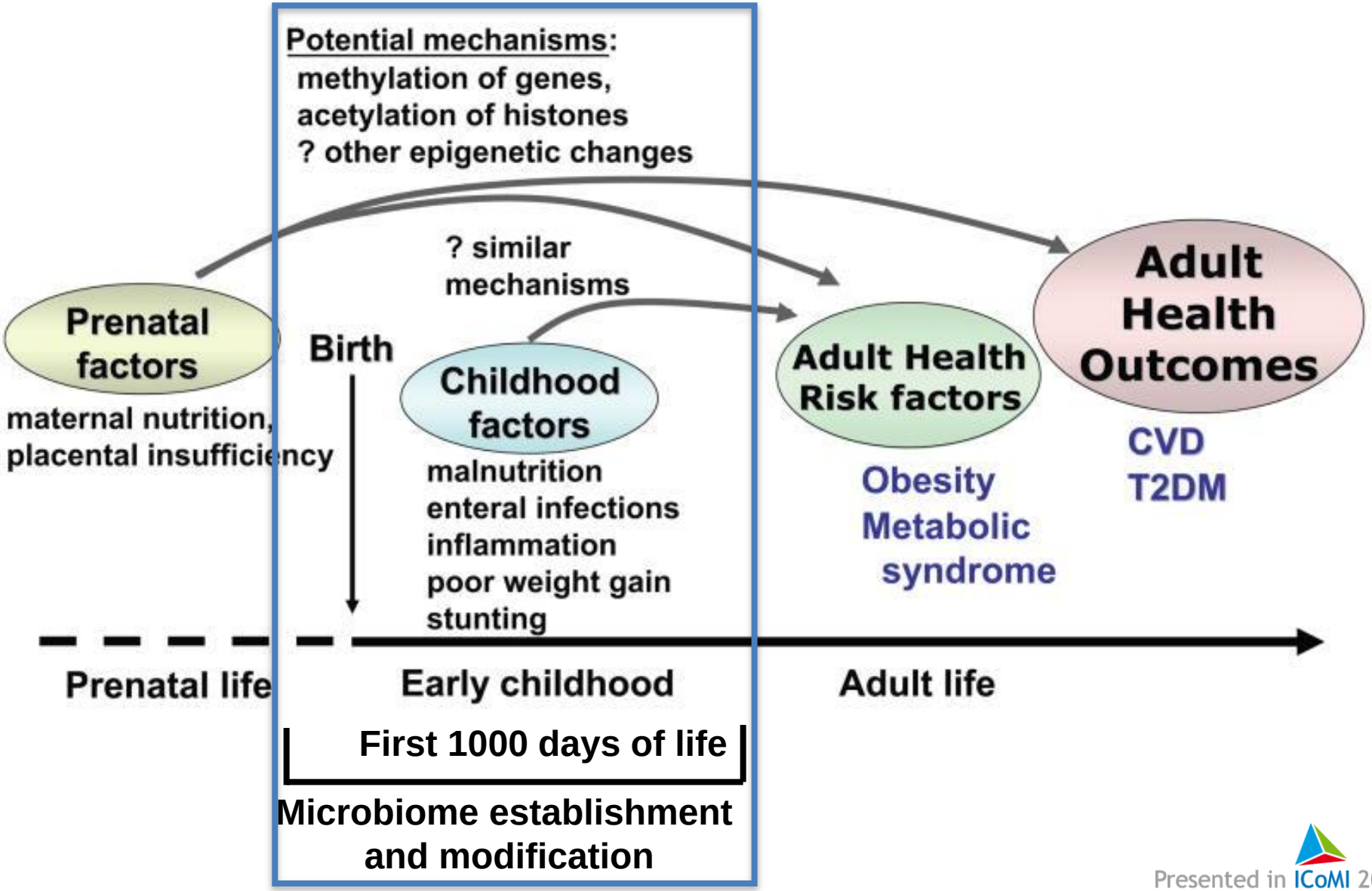
Modeling the Effects of Early Childhood Microbiome Composition On Entire Lifespan



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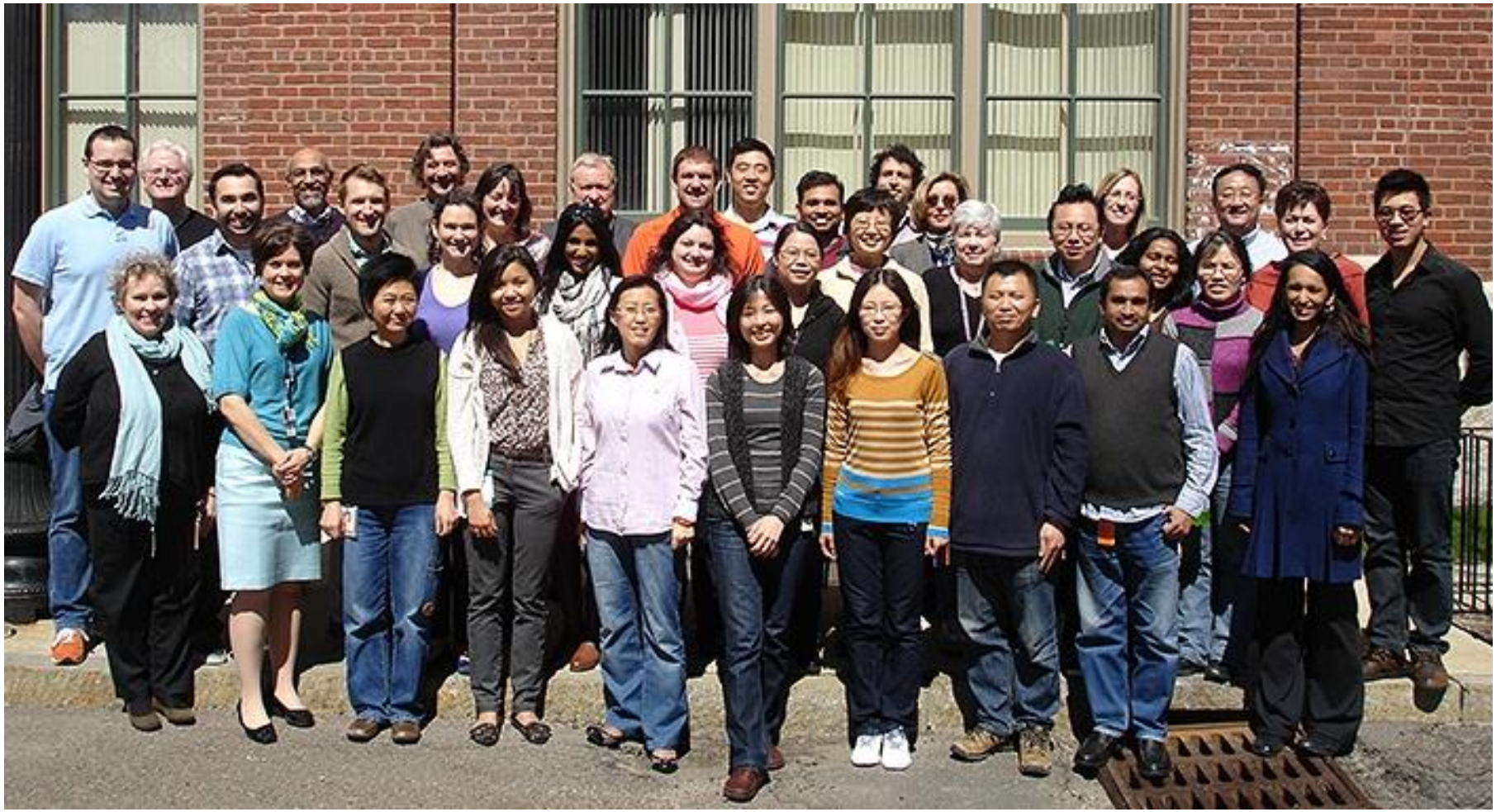
Acknowledgments



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