Manipulationg Gut Bacteria To Prevent The Onset Of Celiac Disease

A Paradigm of Multi-omics in Autoimmune Diseases

State Of The Art Research Lecture

NASPGHAN Annual Meeting– Washington DC October 7-11 2015

Alessio Fasano, M.D.
Mucosal Immunology and Biology Research Center
And Center for Celiac Research
Massachusetts General Hospital, Boston MA – U.S.A.

inflamed epithelial

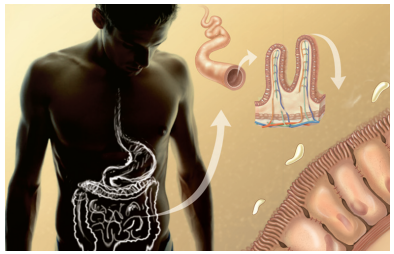
MassGeneral Hospital for Children

Harvard Medical School

DISCLOSURES

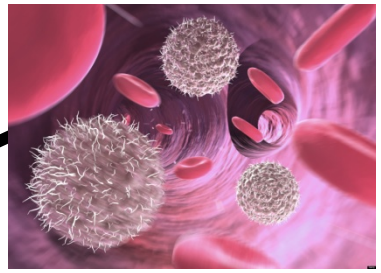
- **Alba Therapeutics: Co-founder and stock holder;**
- **Mead Johnson Nutrition: Sponsored research;**
- **Inova Diagnostics: Sponsored research;**
- **Regeneron: Sponsored research;**
- **Pfizer: Consultant**

The Yin and Yang Between Tolerance and Immune Response Leading to Autoimmune Diseases

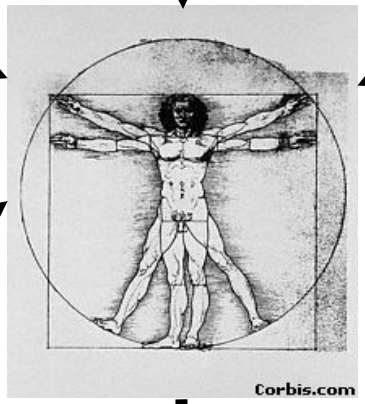


Increased Gut Permeability

Human Genome

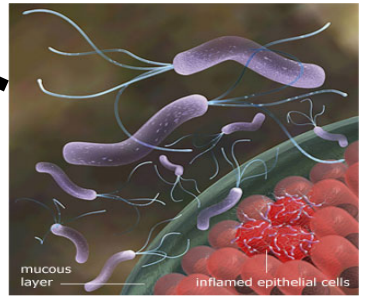


Immune Response



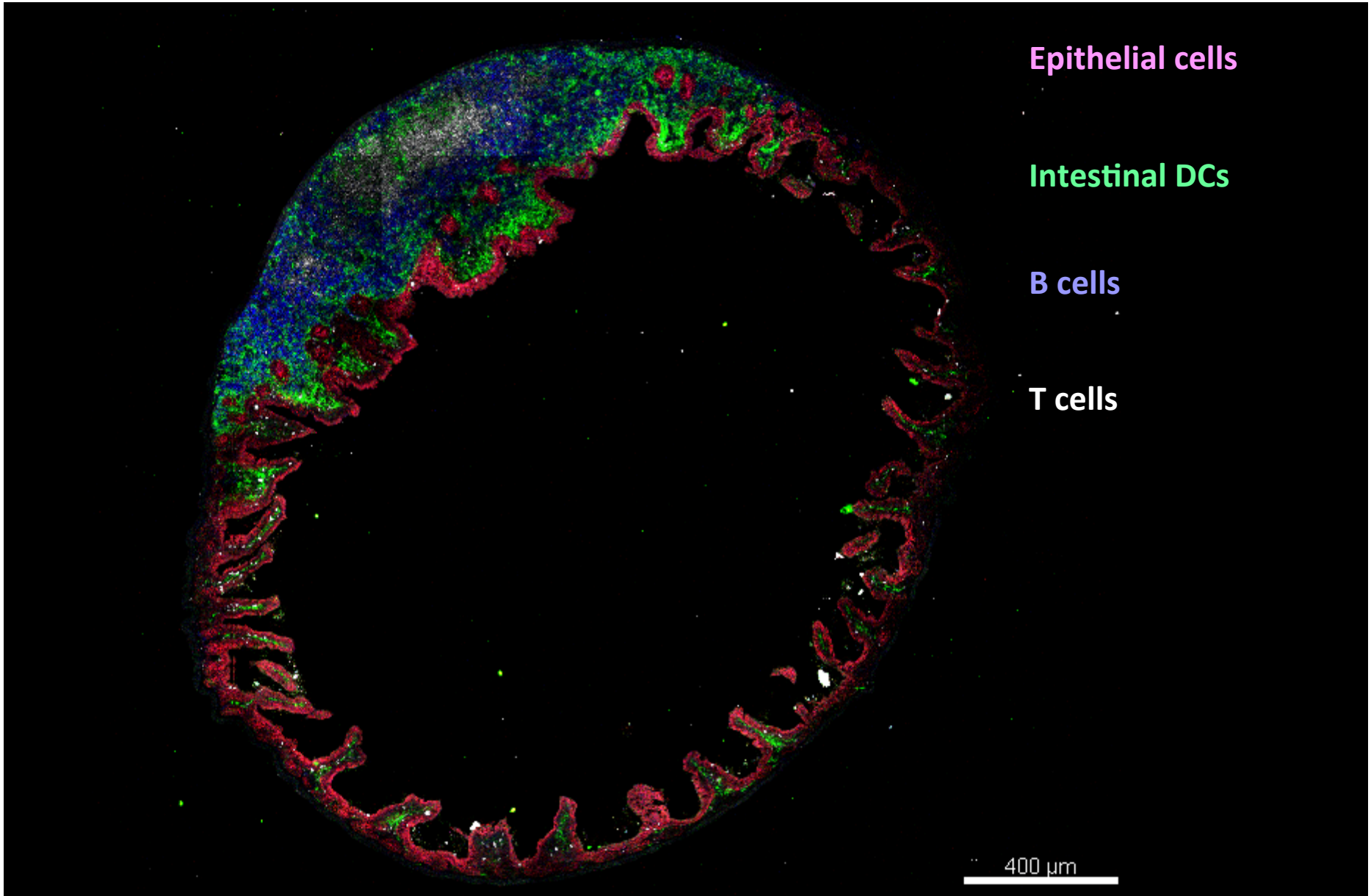
Environmental Factors

Clinic Outcome



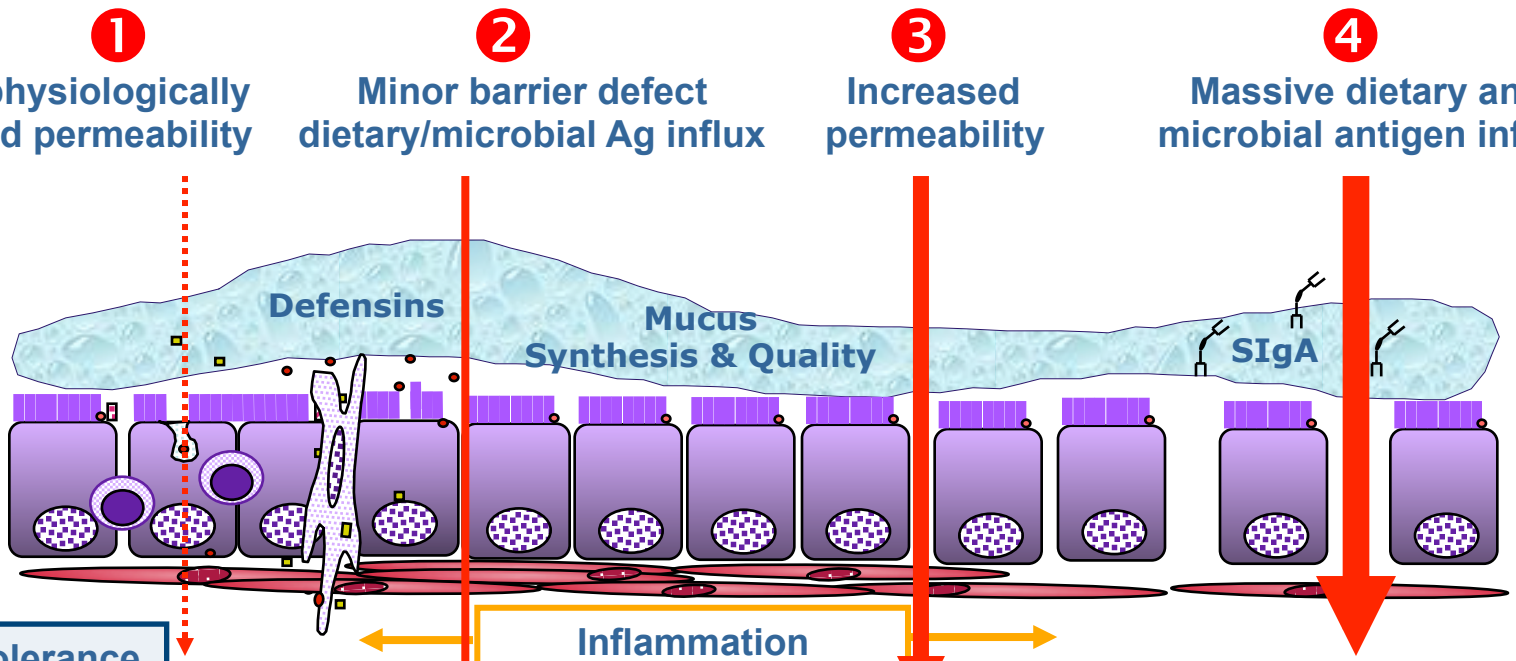
Microbiome

Several Cells Play a Role in Maintaining The Immune Homeostasis



Loss of Mucosal Immune Homeostasis

Chronic Inflammation-Autoimmunity



Mucosal Tolerance
Homeostasis
Anergy

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

Innate or immuno-regulatory defect

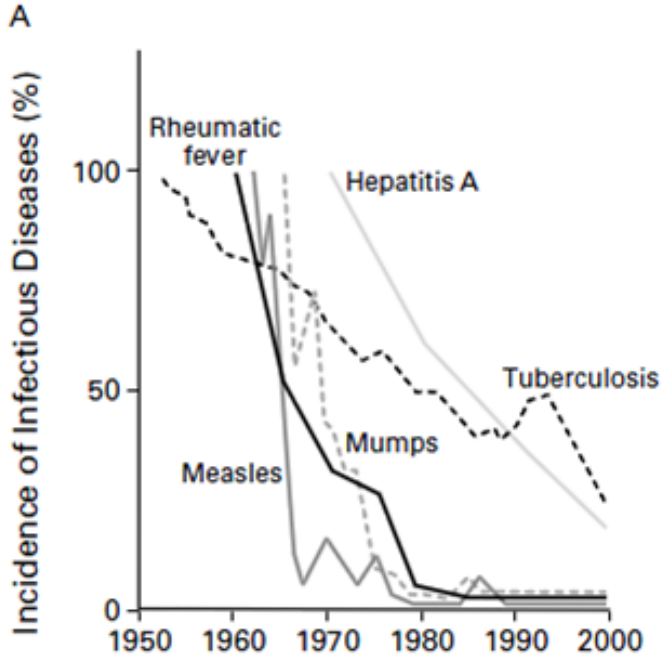
Vicious circle

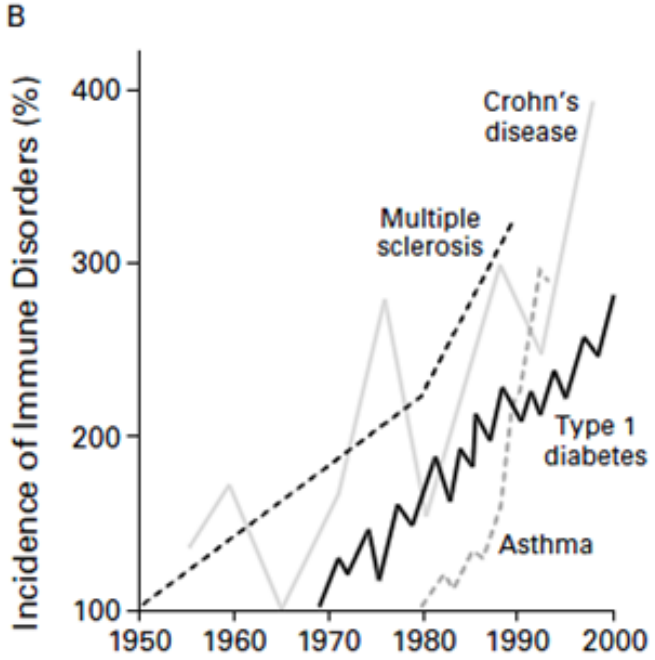
Break of Tolerance
Apoptosis resistant T cells
Tissue damage
Chronic inflammation
Autoimmune Diseases

Proinflammatory
Allergic cytokines

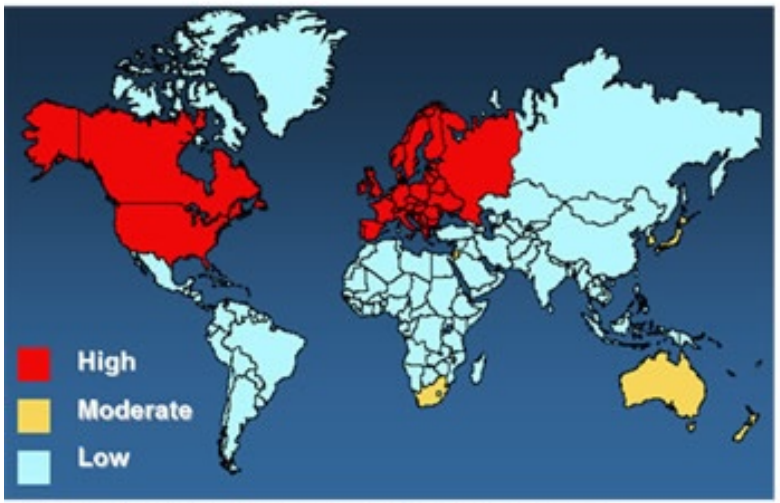
Adapted from P. Brandtzaeg, Beneficial Microbes 2010

The Hygiene Hypothesis





Autoimmune disorders incidence



Helminths infestation incidence

Personal communication from Dr. Joel Weinstock

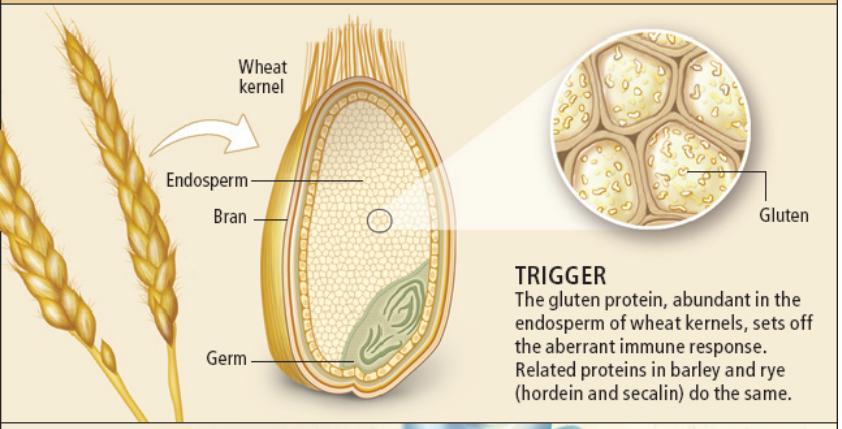
The Hygiene Hypothesis Has Been Recently Questioned

Improved Hygiene In Some Developing Countries Was Not Paralleled by Increased Autoimmune Diseases

Celiac Disease As A Unique Model of Autoimmunity

A TRIO OF CAUSES

Three factors underlie celiac disease: an environmental trigger, a genetic susceptibility and, according to the author's research, an unusually permeable gut (*below*). The author suspects that the same basic triad contributes to other autoimmune diseases, although each disorder will have its own triggers and genetic components.

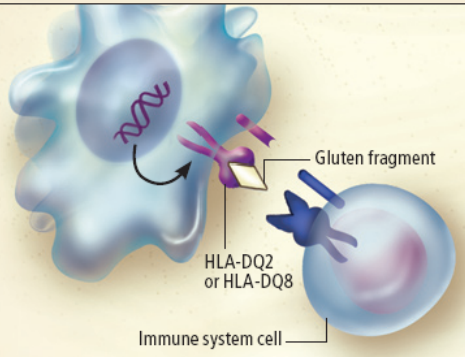


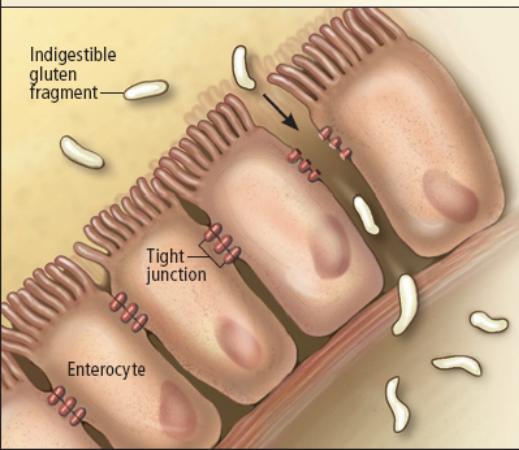
TRIGGER

The gluten protein, abundant in the endosperm of wheat kernels, sets off the aberrant immune response. Related proteins in barley and rye (hordein and secalin) do the same.

GENETIC PREDISPOSITION

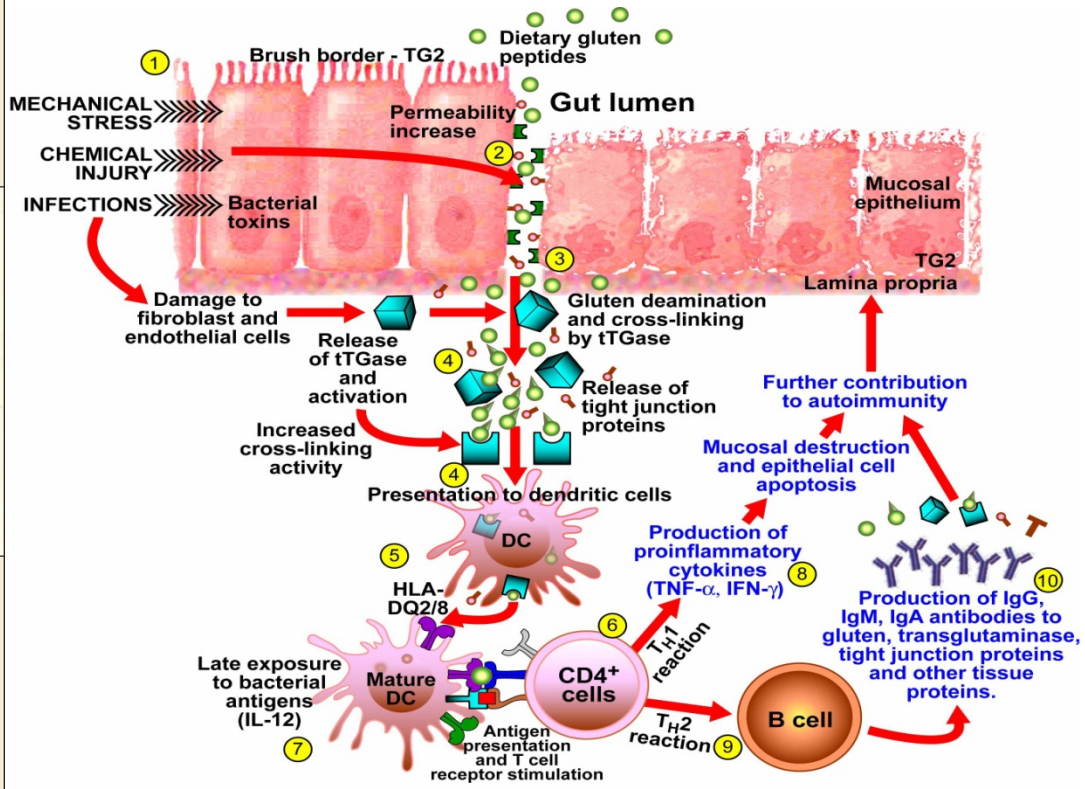
Almost all patients harbor the genes *HLA-DQ2* or *HLA-DQ8*, or both. These genes give rise to proteins of the same name that display gluten fragments to immune system cells, which then direct an attack on the intestinal lining. Other genes are likely to be involved as well, but these additional culprits may differ from person to person.





LEAKY SMALL INTESTINE

In most people, links known as tight junctions "glue" intestinal cells together. In those with celiac disease, the junctions come apart, allowing a large amount of indigestible gluten fragments to seep into the underlying tissue and incite immune system cells. Treatments that reduced leakiness could potentially ease not only celiac disease but also other autoimmune disorders involving unusually permeable intestines.

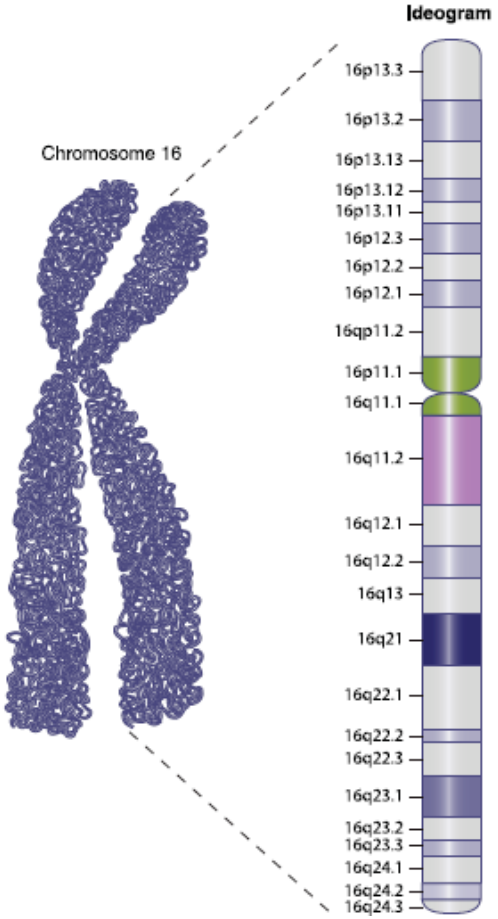


Depiction of the intestinal mucosa with emphasis on the factors involved in the development of celiac disease in individuals with HLA-DQ2/DQ8 positive

Fasano A; Scientific American Aug. 2009

Zonulin Gene Is Located on Chromosome 16

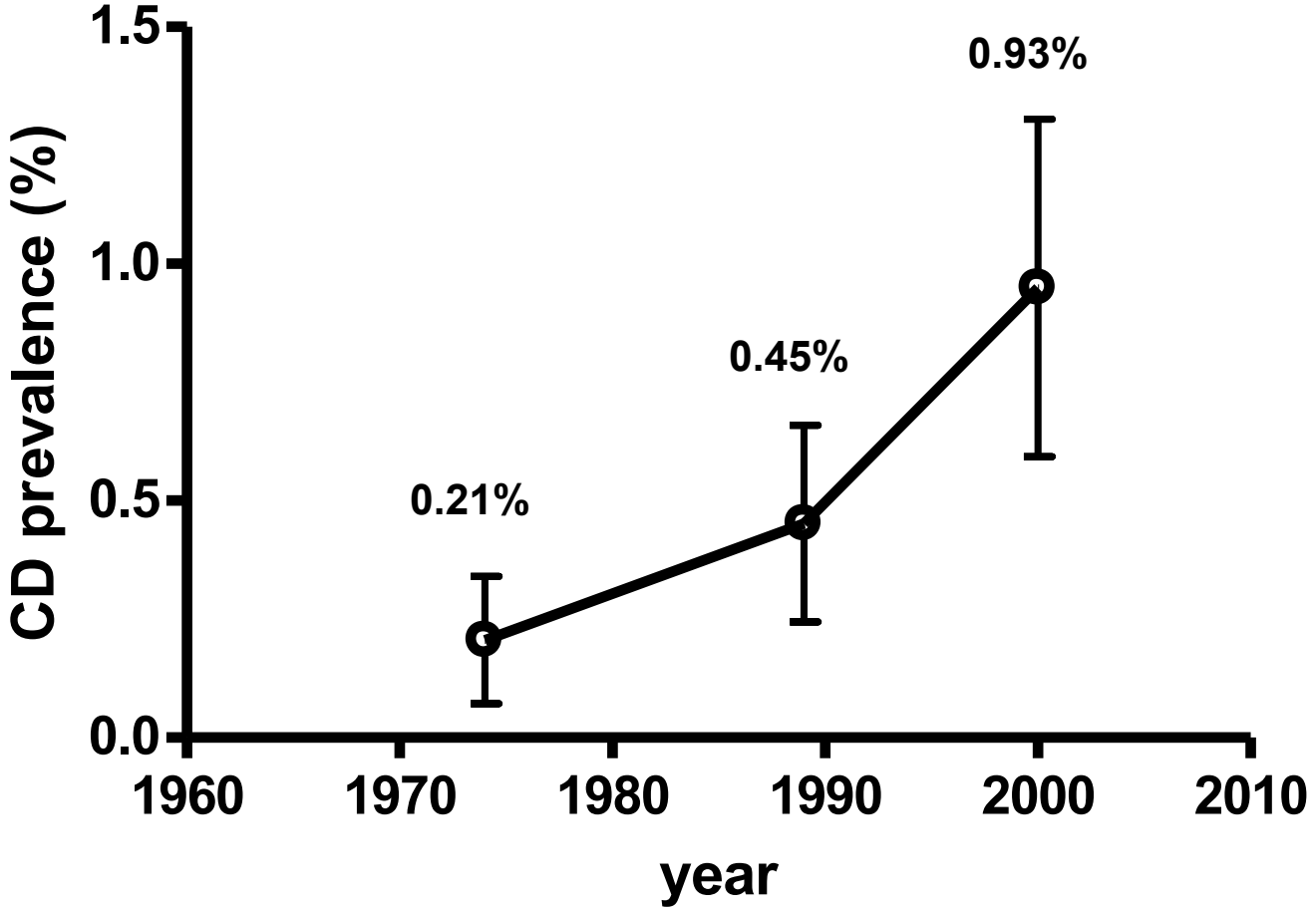
Chromosome 16 contains about 98 million bases, or some 3% of the human genome, encoding for ~1,300 genes.



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

Increased Prevalence Over Time in U.S.A.

(in Line with Other Autoimmune Diseases)



During the past 35 years the true prevalence of CD in USA doubled every 15 years.

C. Catassi et al, Annal Med 2010;42:530-8.

The Epidemics Of Celiac Disease:
Which Additional Factors are Driving this Epidemics?

- Quality of gluten: GE grains
- Quantity of gluten;
- Breast Feeding;
- Timing of gluten introduction
- Maturity of gut functions influencing Ag trafficking and handling:
    - GALT
    - PRRs
    - Mucous production
    - Barrier function
- Changes in microbiome composition.

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Introduction of Gluten, HLA Status, and the Risk of Celiac Disease in Children

Elena Lionetti, M.D., Stefania Castellaneta, M.D., Ruggiero Francavilla, M.D., Ph.D., Alfredo Pulvirenti, Ph.D., Elio Tonutti, M.D., Sergio Amarri, M.D., Maria Barbato, M.D., Cristiana Barbera, M.D., Graziano Barera, M.D., Antonella Bellantoni, M.D., Emanuela Castellano, M.D., Graziella Guariso, M.D., Maria Giovanna Limongelli, M.D., Salvatore Pellegrino, M.D., Carlo Polloni, M.D., Claudio Ughi, M.D., Giovanna Zuin, M.D., Alessio Fasano, M.D., Ph.D., and Carlo Catassi, M.D., Ph.D., for the SIGENP (Italian Society of Pediatric Gastroenterology, Hepatology, and Nutrition) Working Group on Weaning and CD Risk

Published on October 2, 2014

Home Take Messages

- **Window of tolerance concept (4-7 months best period to introduce baby food) not supported anymore;**
- **Breast feeding in general or introduction of gluten while breast feeding showed no protective effect on CD onset in at-risk infants;**
- **Early introduction (16 weeks) of gluten traces to potentially induce tolerance did not protect against CD in at-risk infants;**
- **Delaying the introduction of gluten in at-risk infants does not prevent CD but merely postpones its onset by approximately 8 months (significant difference at 2 years FU that disappeared by 5 years FU);**
- **GI infections during the first year of life seems influential in increased the risk of CD onset;**
- **High-risk HLA profiles seems to be the most influential factor predictor of increased risk of CD onset;**
- **The high prevalence of CD among the study cohort suggests that the CD epidemics continues.**

The Epidemics Of Celiac Disease:
Which Additional Factors are Driving this Epidemics?

- Quality of gluten;
- Quantity of gluten;
- Breast Feeding;
- Timing of gluten introduction
- Maturity of gut functions influencing Ag trafficking and handling:
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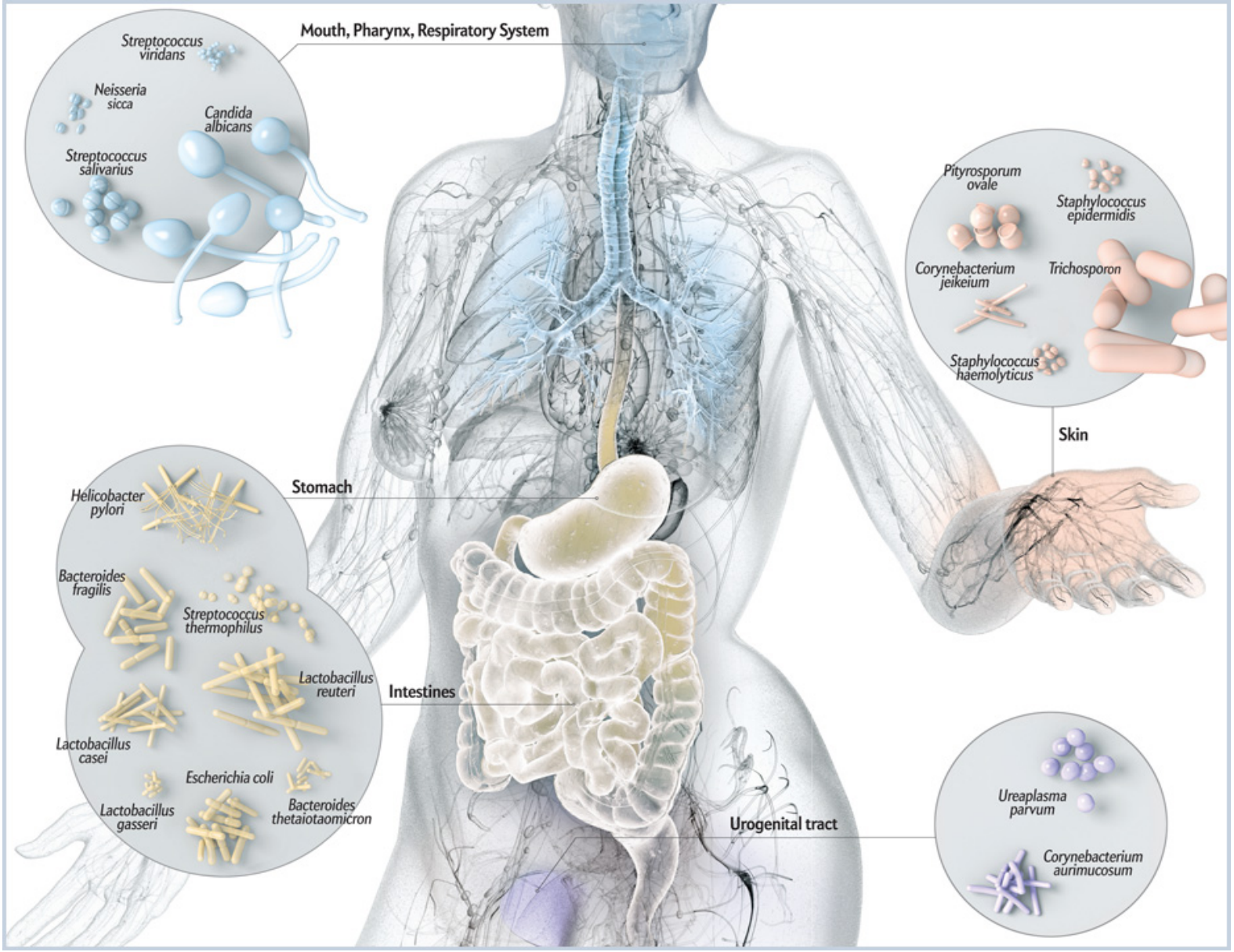
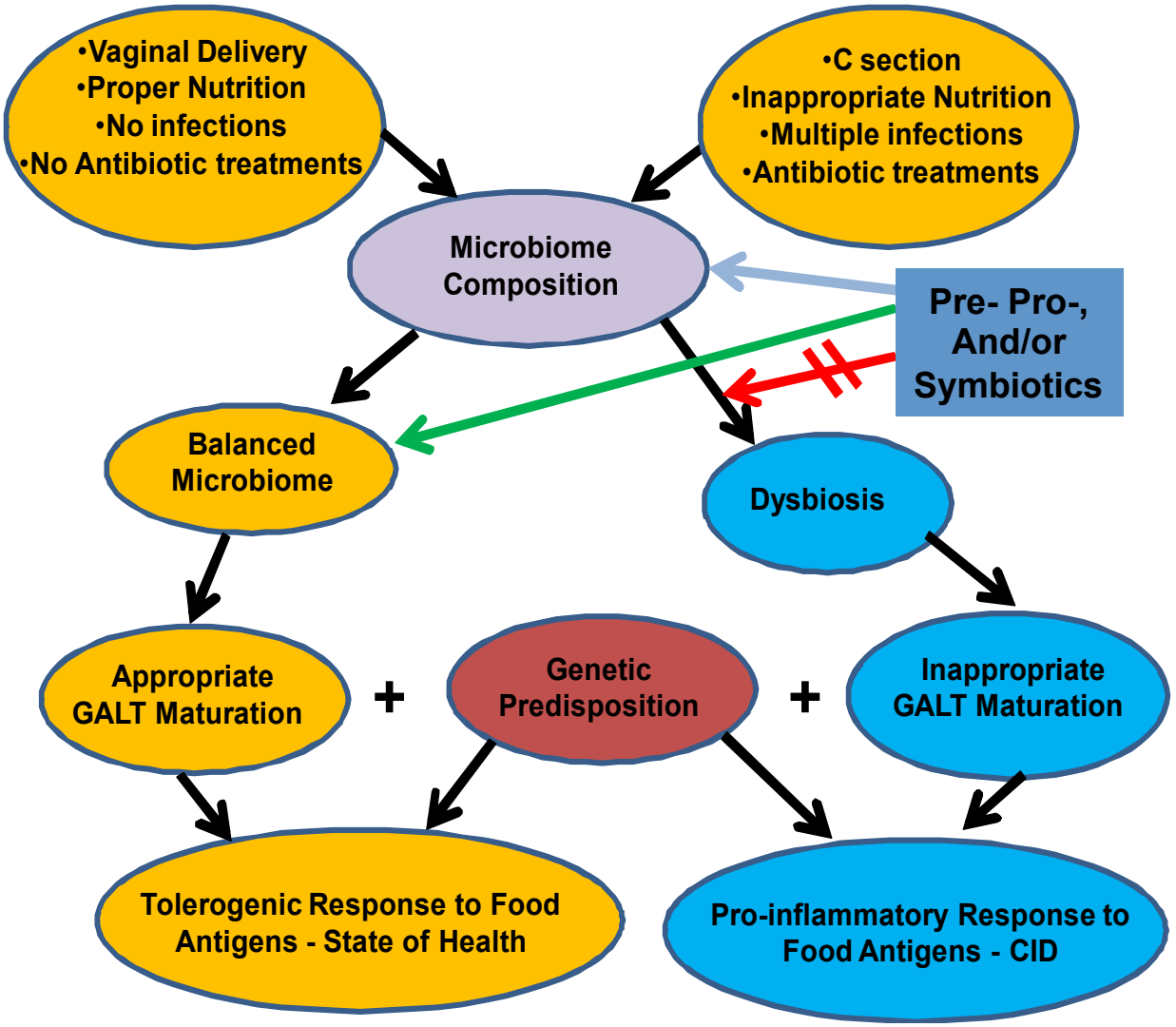
- Changes in microbiome composition.

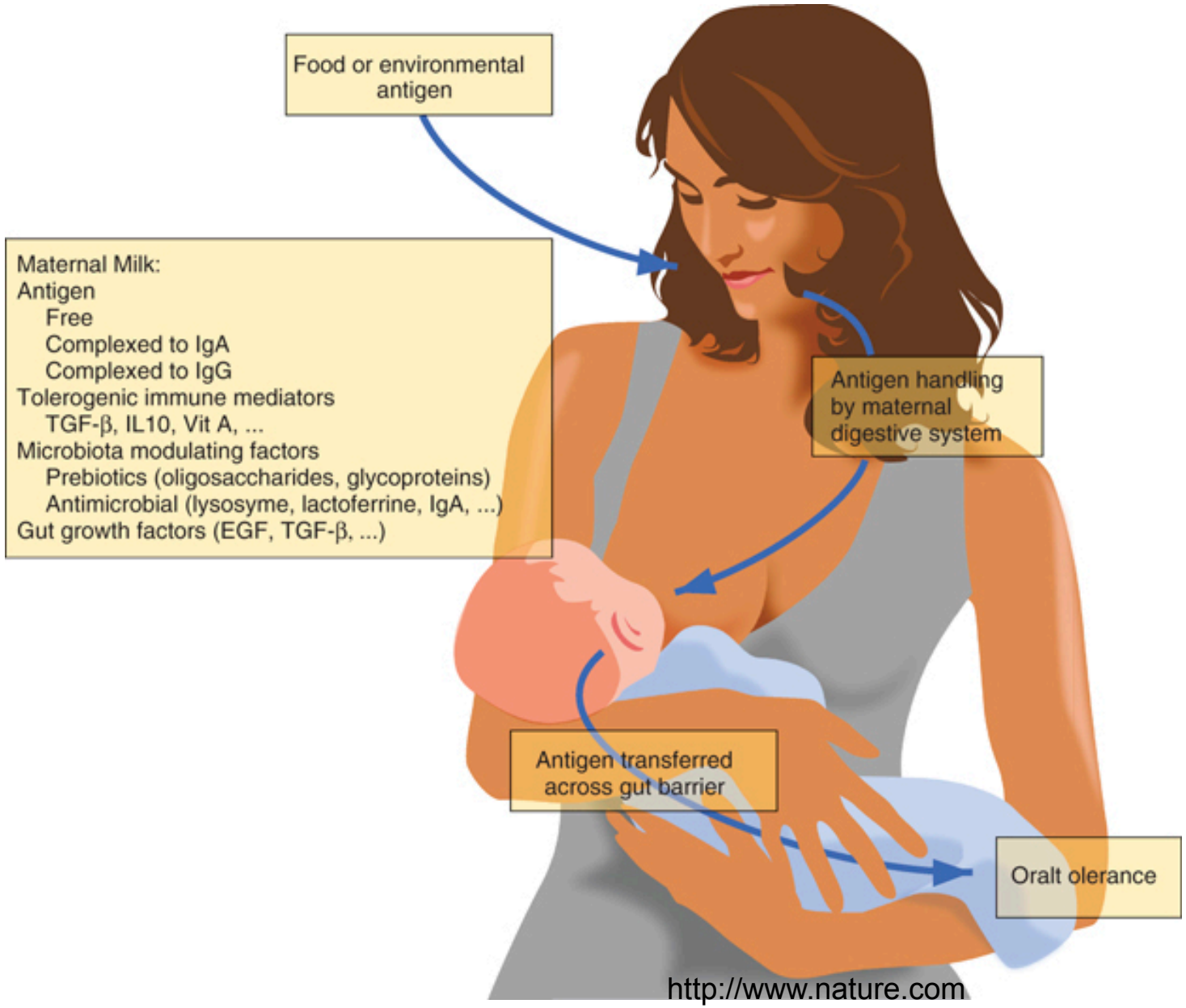


- The human gut harbors 10^{11}-10^{12} bacteria per gram colonic content (>10^{14} total bacteria)
- Total bacteria outnumber human cells 10:1
- Total bacterial genes outnumber human genes >150:1
- >10,000 different species of bacteria are resident in the human intestinal microbiota (400-500 per person)

Which Factors are Driving This Autoimmunity Epidemics?

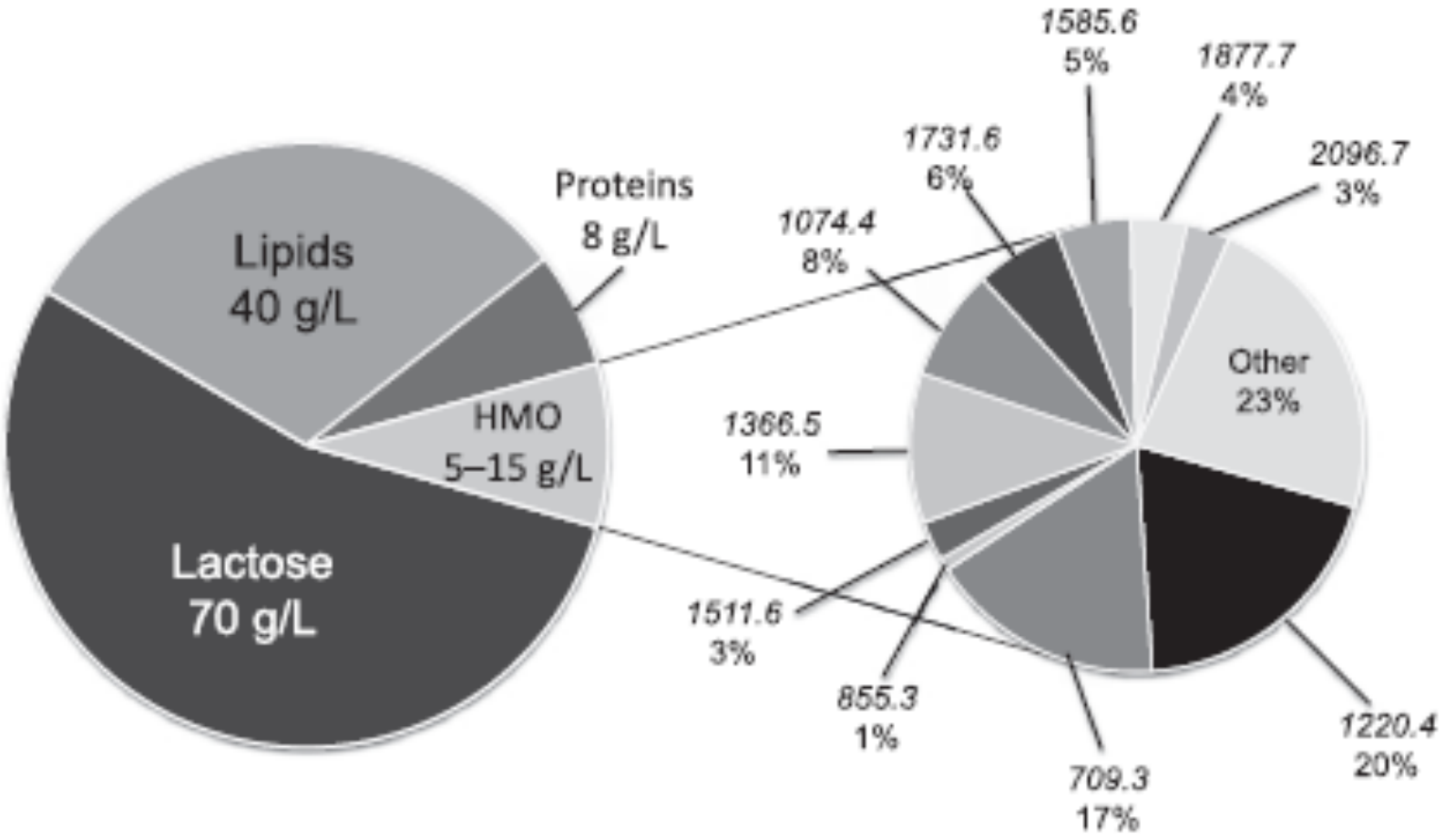


Role of Breastmilk



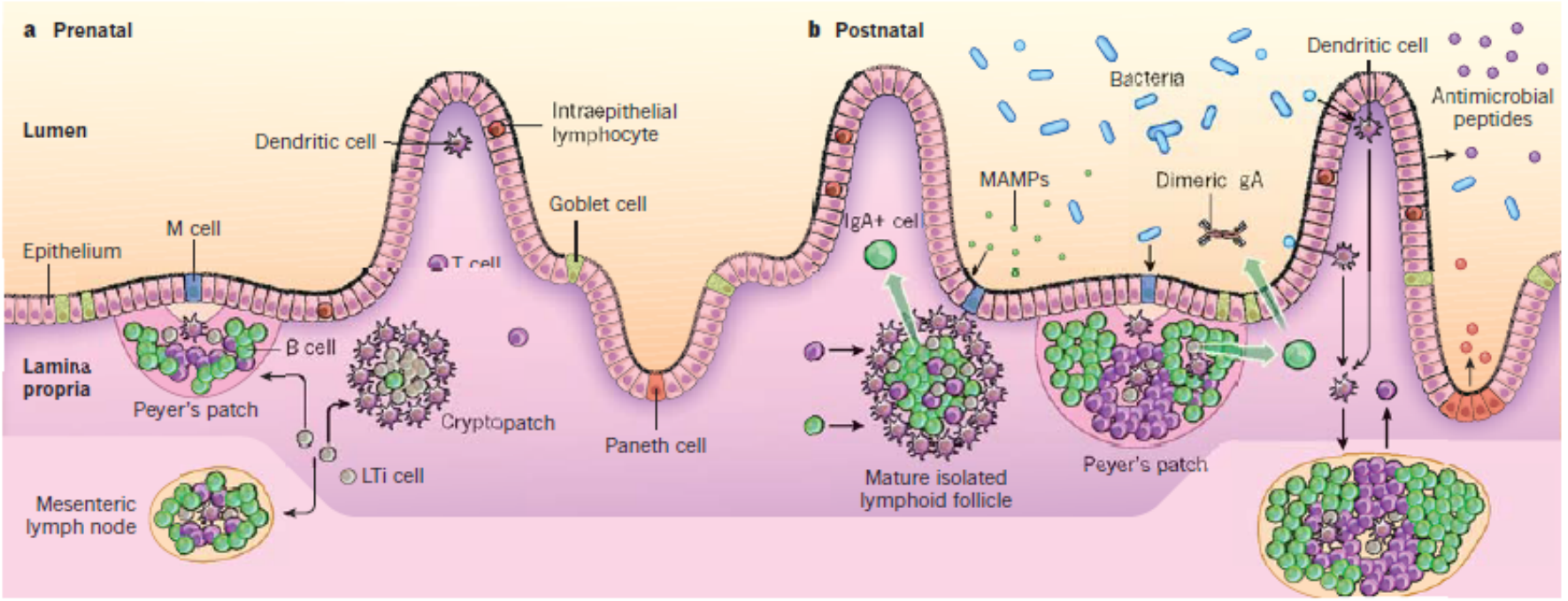
http://www.nature.com

Impact of human milk glycobiome on the infant intestinal microbiota



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

Intestinal Flora Influences Postnatal Immune System Development



Maynard, CL, et al. *Nature* 2012; 489: 231-41

PLOS BIOLOGY July 2007 | Volume 5 | Issue 7 | e177

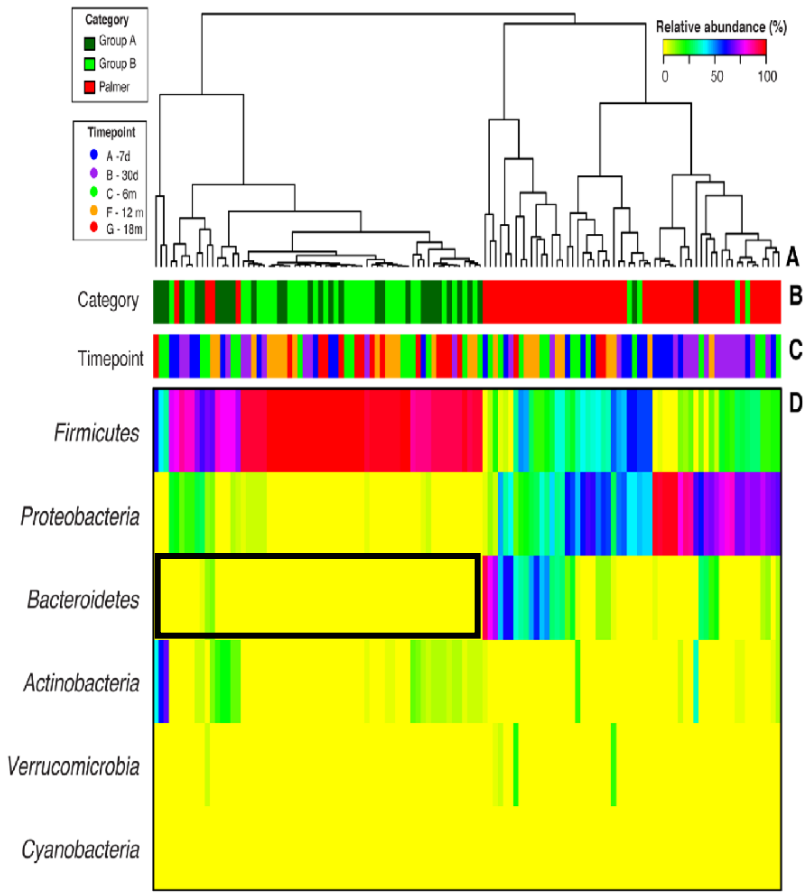
Development of the Human Infant Intestinal Microbiota

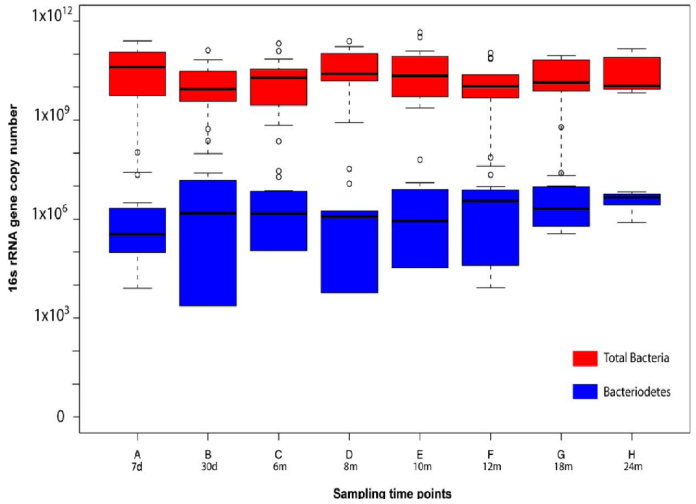
Chana Palmer[1], Elisabeth M. Bik[2], Daniel B. DiGiulio[3,4], David A. Relman[2,3,4], Patrick O. Brown[5,6*]

- The earliest colonizers were often organisms predicted to be aerobes (e.g., Staphylococcus, Streptococcus, and Enterobacteria), whereas the later colonizers tended to be strict anaerobes (Eubacteria and Clostridia).
- The Bacteroides varied greatly from baby to baby in the timing of their first appearance, but were **consistently** present in nearly all babies by 1 y.
- Several other taxa, including Prevotella, Acinetobacter, Desulfovibrio, Veillonella, and Clostridium perfringens, tended to appear only transiently, sometimes appearing and disappearing repeatedly within a baby's first year of life.
- By the end of the first year of life, the microbial ecosystems in each baby had converged toward a profile characteristic of the adult GI tract.
- **All these changes are mainly driven by nutritional variables**

Proof of Concept of Microbiome-Metabolome Analysis and Delayed Gluten Exposure on Celiac Disease Autoimmunity in Genetically At-Risk Infants

Maria Sellitto[1¤], Guoyun Bai[2], Gloria Serena[1], W. Florian Fricke[2], Craig Sturgeon[1], Pawel Gajer[2], James R. White[2], Sara S. K. Koenig[2], Joyce Sakamoto[2], Dustin Boothe[1], Rachel Gicquelais[1], Deborah Kryszak[1], Elaine Puppa[1], Carlo Catassi[1,3], Jacques Ravel[2]*, Alessio Fasano[1]*





Infants genetically predisposed to CD were characterized by a low abundance of Bacteroidetes (undetectable to 1%) combined with abundance of Firmicutes.

The Real Story of Our Genetic Complexity:

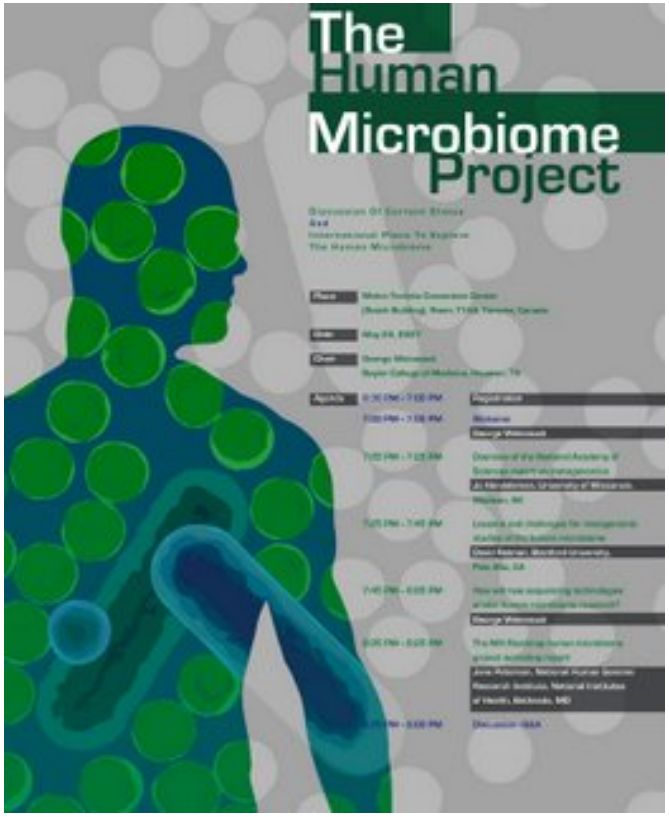
We Inherit two Parallel Genomes

Human Genome:

Inherited from both parents, stable, never change in its composition

Microbiome:

Inherited from the mother, extremely dynamic, changes from individual to individual and in the same individual over time



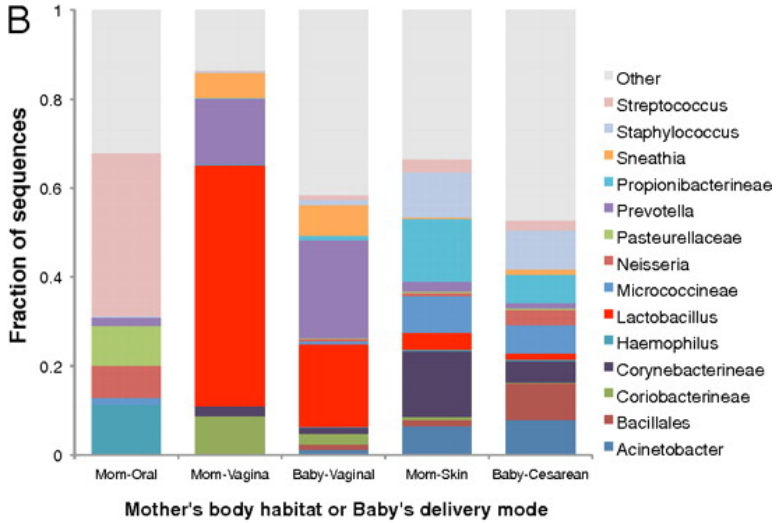
Higher Risk of Celiac Disease After Elective Cesarean Delivery

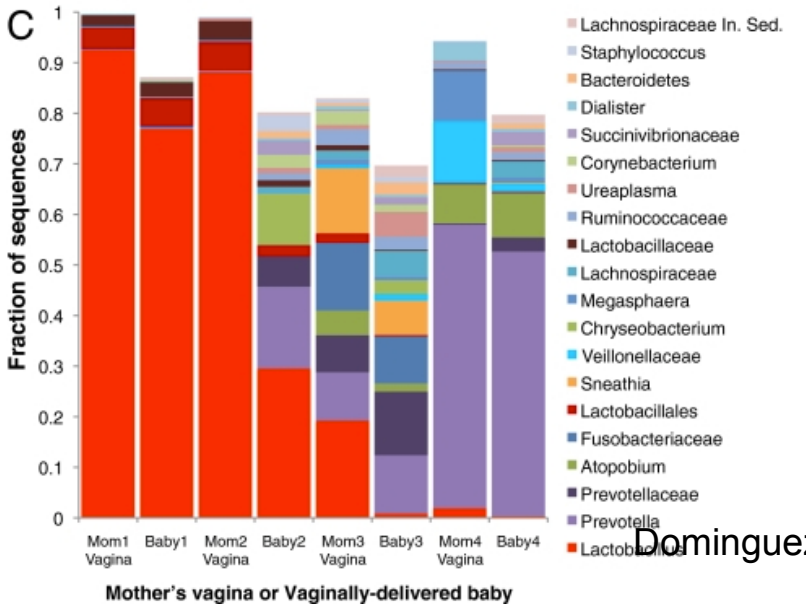
Risk of celiac disease after cesarean delivery.

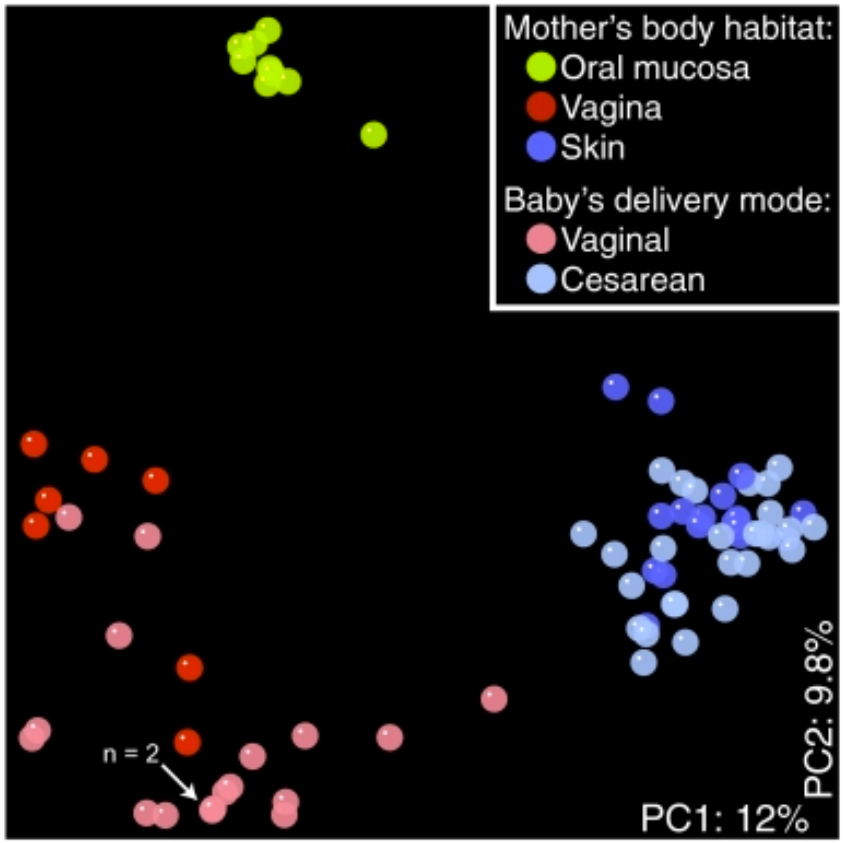
	Matched controls (%)	Celiac disease (%)	Odds ratio; 95% CI OR	P-value	Adjusted odds ratio*, 95% CI AOR	P-value
Cesarean delivery	5,766/53,887 (10.7)	1,299/11,749 (11.1)	1.04; 0.98-1.10	0.232	1.06; 0.99-1.13	0.074
Number of participants			65,636		65,493	
Emergency cesarean delivery †	2,136/41,699 (5.1)	444/8,827 (5.0)	0.99; 0.90-1.10	0.857	1.02; 0.92-1.13	0.749
Number of participants			50,526		50,415	
Elective cesarean delivery †	2,125/41,688 (5.1)	508/8,891 (5.7)	1.11; 1.01-1.22	0.027	1.15; 1.04-1.26	0.005
Number of participants			50,579		50,471	

Mårild et al *Gastroenterology* 2012;*142*(1):39

Infants intestinal microbiome is influenced by mode of delivery

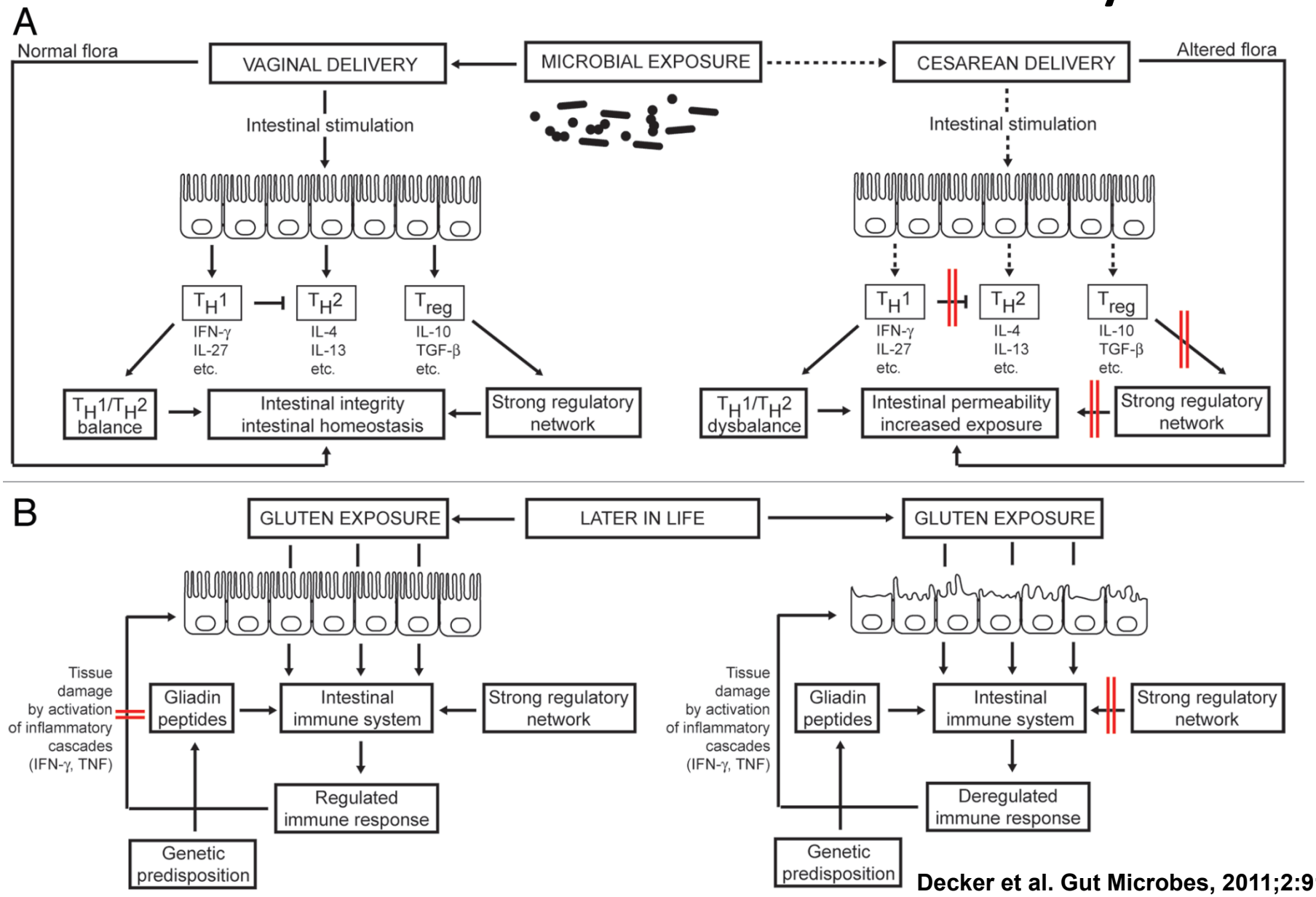






Dominguez-Bello et al *PNAS* 2010;107(26):11971-5

Bacterial dysbiosis as possible mechanism responsible of increased risk for celiac disease in children born by C-Section



Decker et al. Gut Microbes, 2011;2:91-8

Microbiome
(140-fold Human Genome)
Dynamic

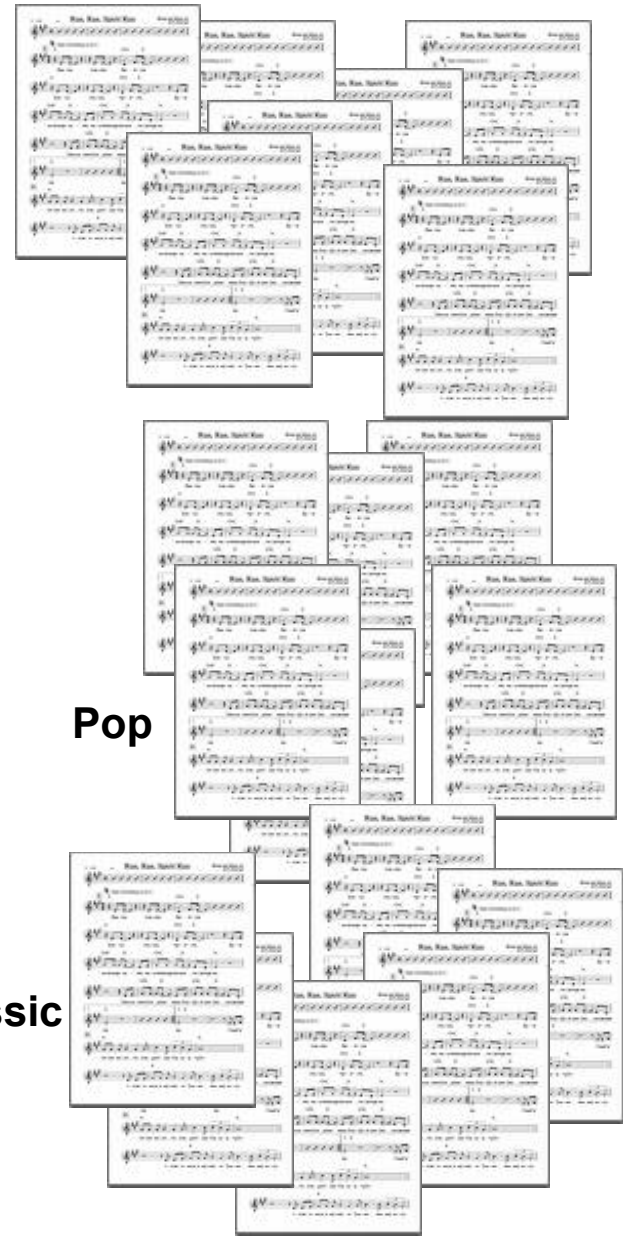
Human Genome
(~30,000 genes)
Stable

Metabonome

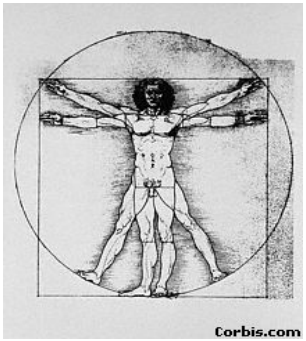
Jazz

Pop

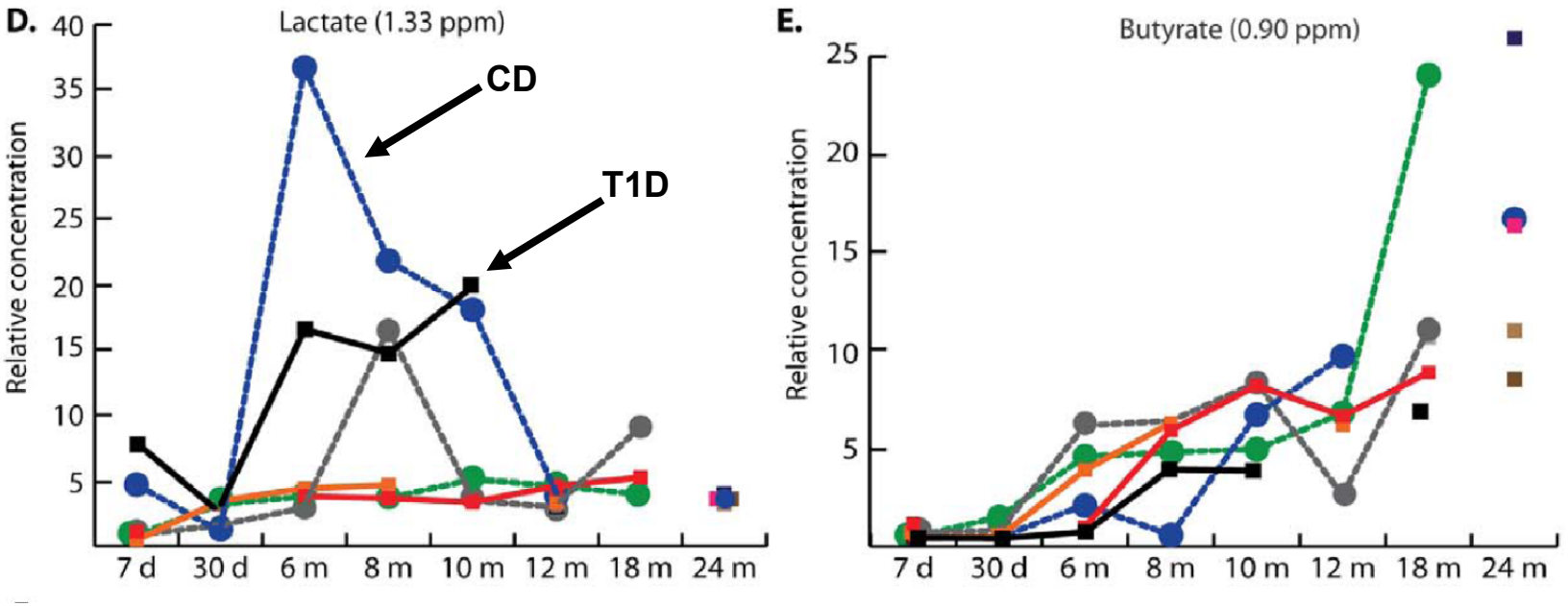
Classic



Clinical Outcome



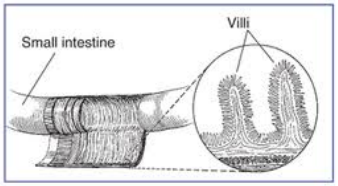
NMR Analysis

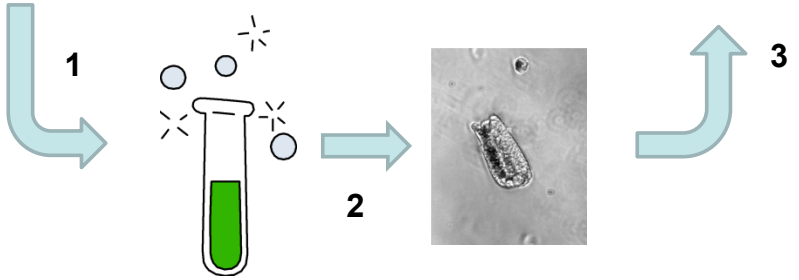


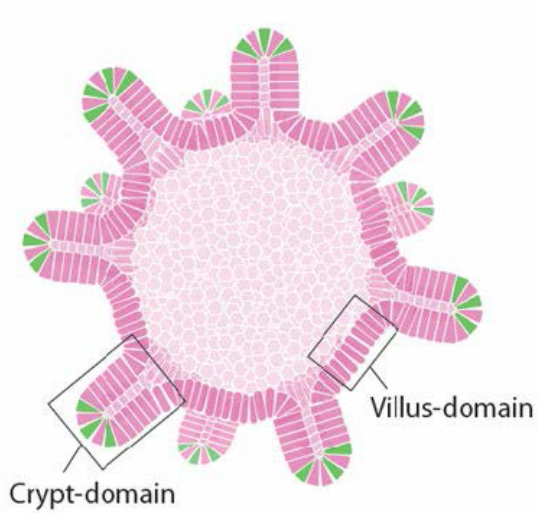
- **Infants who developed autoimmune diseases during the time of the study had high levels of lactate combined with low levels of butyrate before the onset of the disease.**

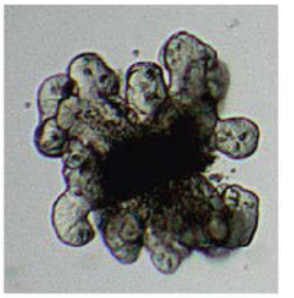
- **During the active state of the disease (24 months) the same subjects showed an increase in butyrate production and a decrease in lactate, therefore suggesting that the acute phase of CD is characterized by a different metabolomic profile.**

Intestinal Organoids

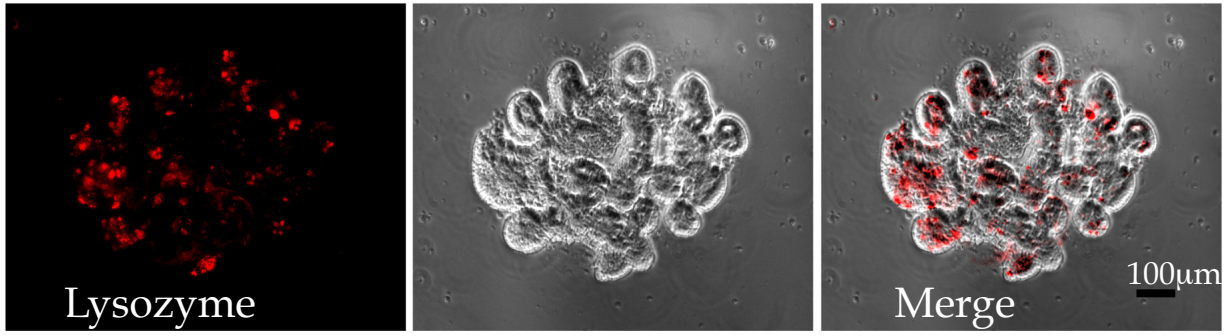


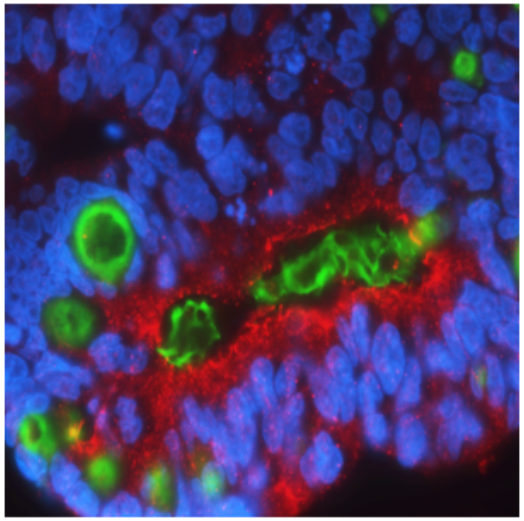






Small intestinal organoids

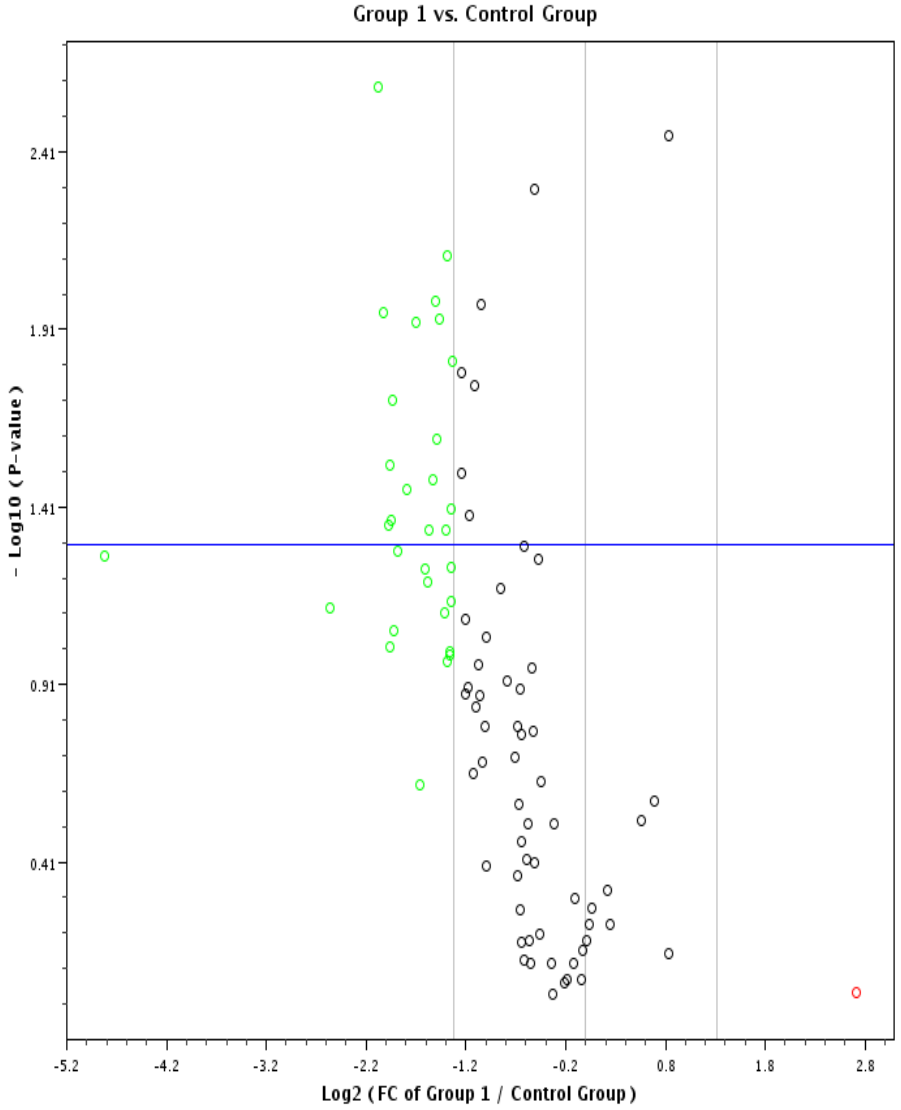




Organoids differentiate lysozyme positive cells (Paneth's cells)

DAPI MUC2 ZO1

Volcano Plot Representing 80 Stem Cell Related Genes Expression Profile Macro Array Expressed In Crypts of Acute CD vs. Healthy Subjects.

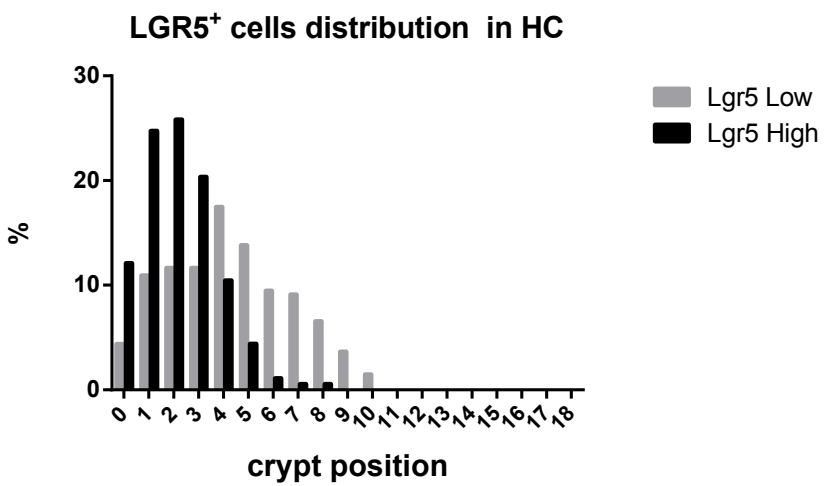


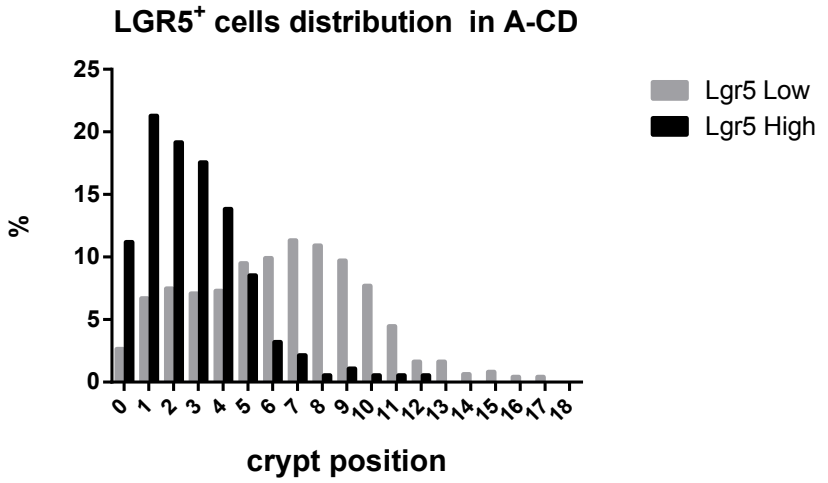
GENE	Fold Regulation	P-Value
BCL9	-3.8523	0.042667
ENG	-2.9705	0.045796
EP300	-2.8446	0.010392
FZD5	-3.4419	0.034942
GLI1	-3.8191	0.019727
GLI3	-2.7957	0.025196
LIFR	-4.201	0.002577
NFAT5	-2.6085	0.007741
NOTCH4	-3.8858	0.029935
PTCH1	-2.8943	0.032864
SMAD3	-2.5416	0.039593
SMAD4	-2.5196	0.015226
SMAD9	-3.2394	0.011844
SMO	-2.6312	0.045707
TCF7L1	-4.0579	0.011192
TGFBR3	-2.7477	0.011667
ZEB2	-3.9196	0.044099

How Mechanistically Link Microbiome/Metametabolome Profiles to Clinical Outcome

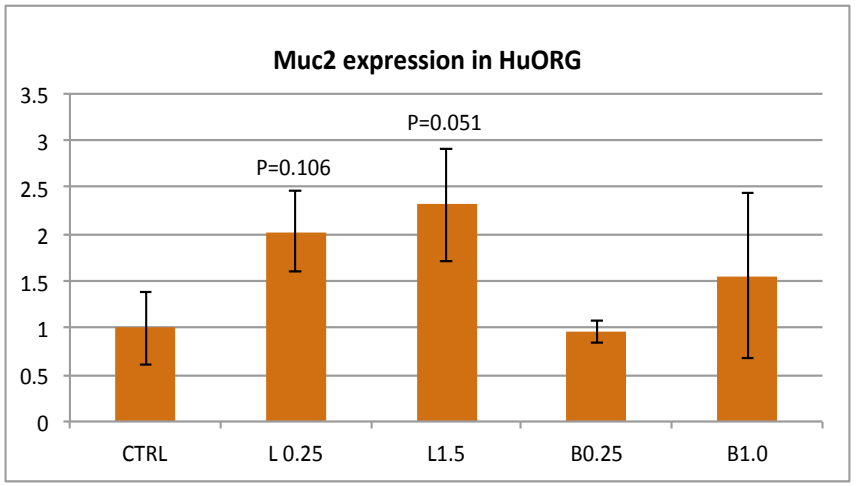
Stem Cell Niche

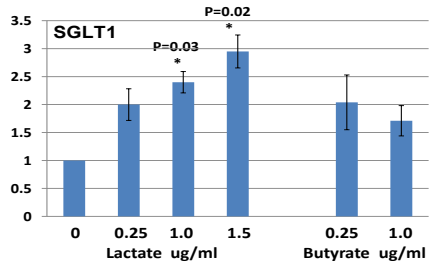
Distribution of LGR5 positive cells in the intestinal crypt.

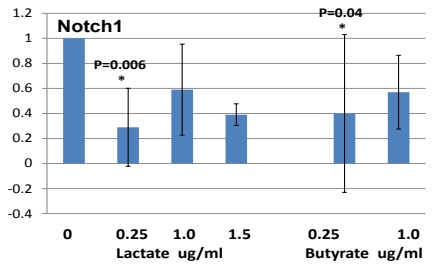


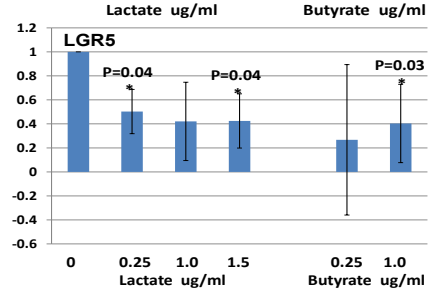


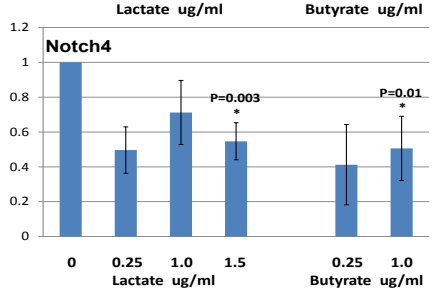
Gene Expression In Stem Cell Niche Using Gut Organoids





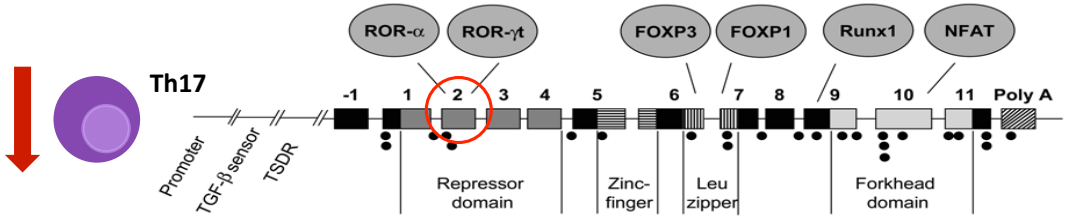




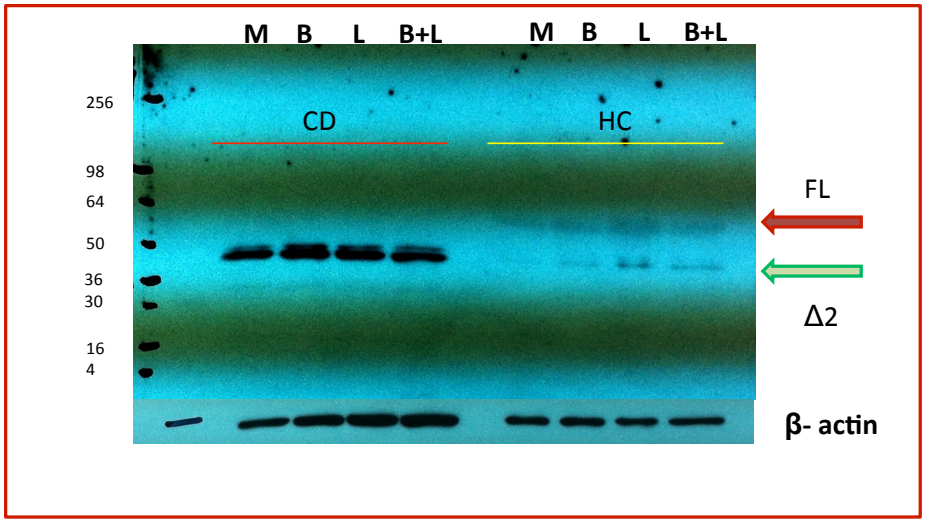


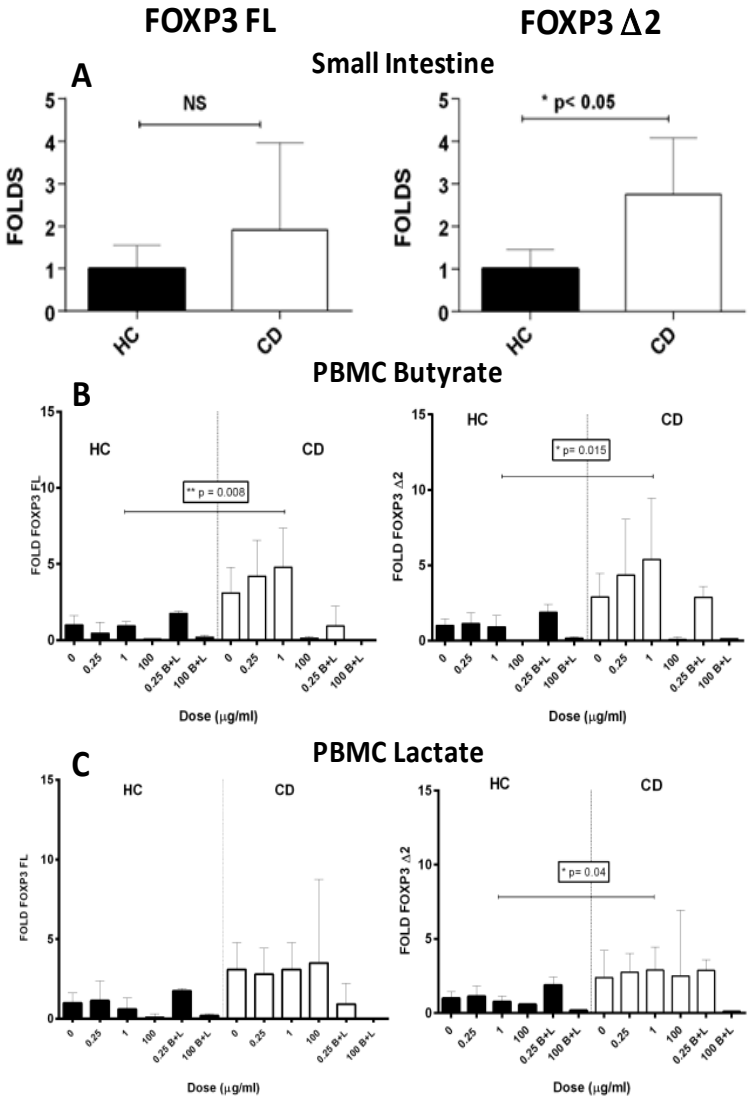
How Mechanistically Link Microbiome/Metametabolome Profiles to Clinical Outcome

Mucosal and Systemic Immune Functions



PROTEIN LEVEL IN Treg cells STIMULATED WITH METABOLITES





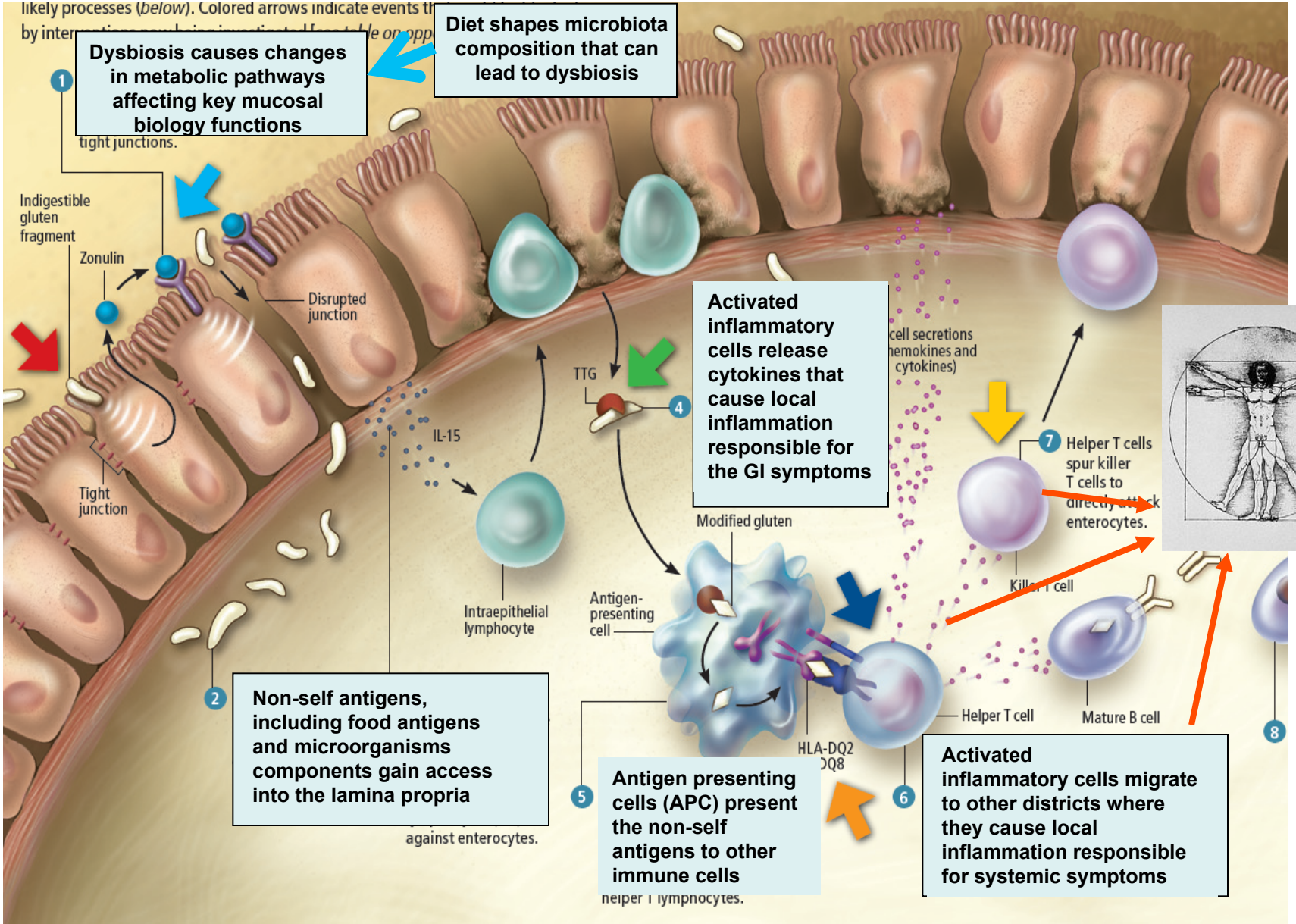
Celiac **D**isease **G**enomic **E**nvironmental **M**icrobiome and **M**etabolomic Study

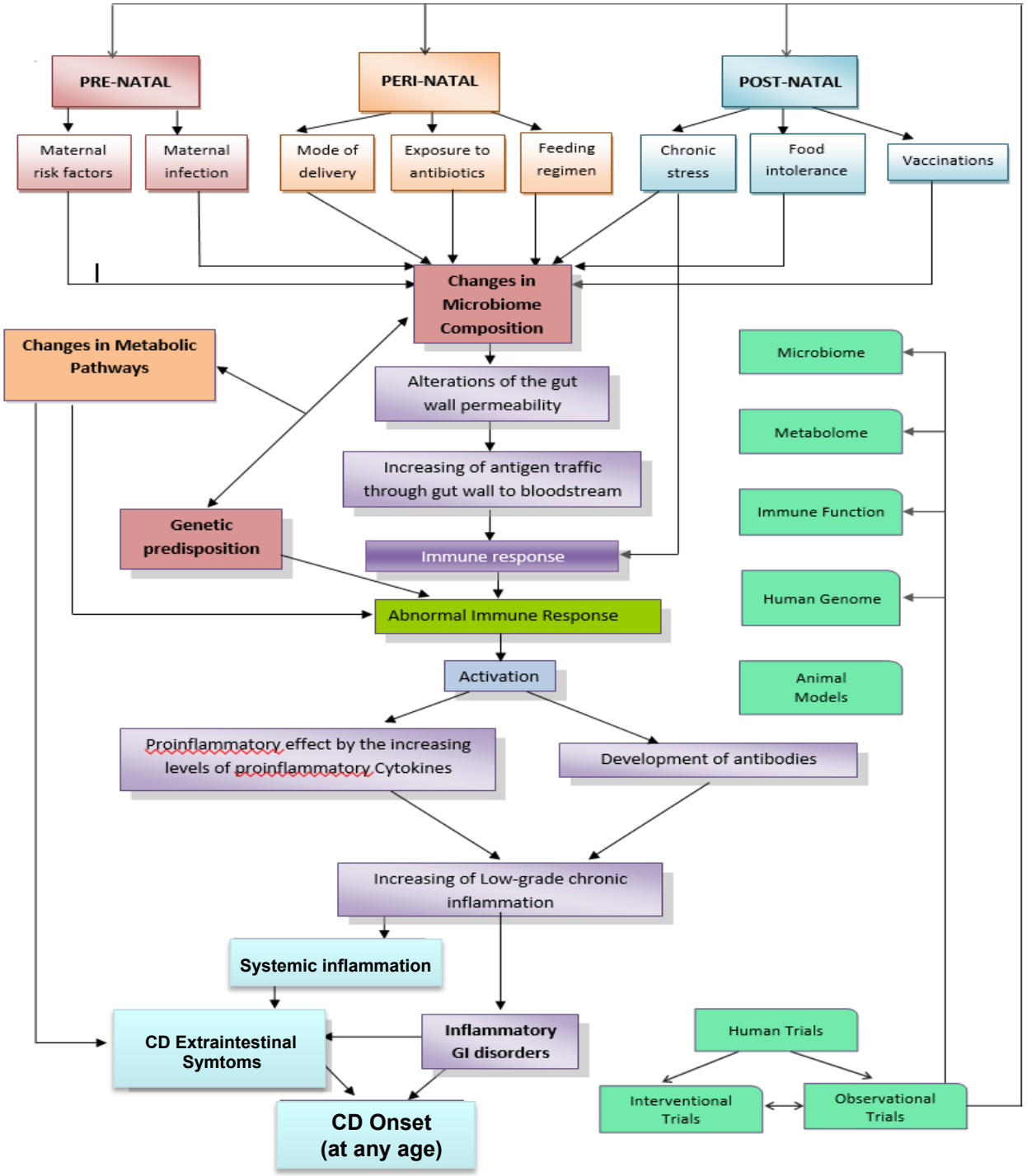
www.CDGEMM.org

Hypothesis

Combination of introduction of gluten into the diet and particular microbiota composition of infants genetically at risk for CD activates specific metabolic pathways that can contribute to the loss of tolerance to gluten and to the onset of autoimmunity, as reflected by specific metabolomic phenotypes

Working Hypothesis of the CD-GEMM Project




PRE-NATAL
PERI-NATAL
POST-NATAL
Maternal risk factors
Maternal infection
Mode of delivery
Exposure to antibiotics
Feeding regimen
Chronic stress
Food intolerance
Vaccinations
Changes in Microbiome Composition
Changes in Metabolic Pathways
Alterations of the gut wall permeability
Increasing of antigen traffic through gut wall to bloodstream
Genetic predisposition
Immune response
Abnormal Immune Response
Activation
Proinflammatory effect by the increasing levels of proinflammatory Cytokines
Development of antibodies
Increasing of Low-grade chronic inflammation
Systemic inflammation
CD Extraintestinal Symtoms
Inflammatory GI disorders
CD Onset (at any age)
Microbiome
Metabolome
Immune Function
Human Genome
Animal Models
Human Trials
Interventional Trials
Observational Trials

Acknowledgments

NIH DK078699
NIH DK048373

The MIBRC Crew