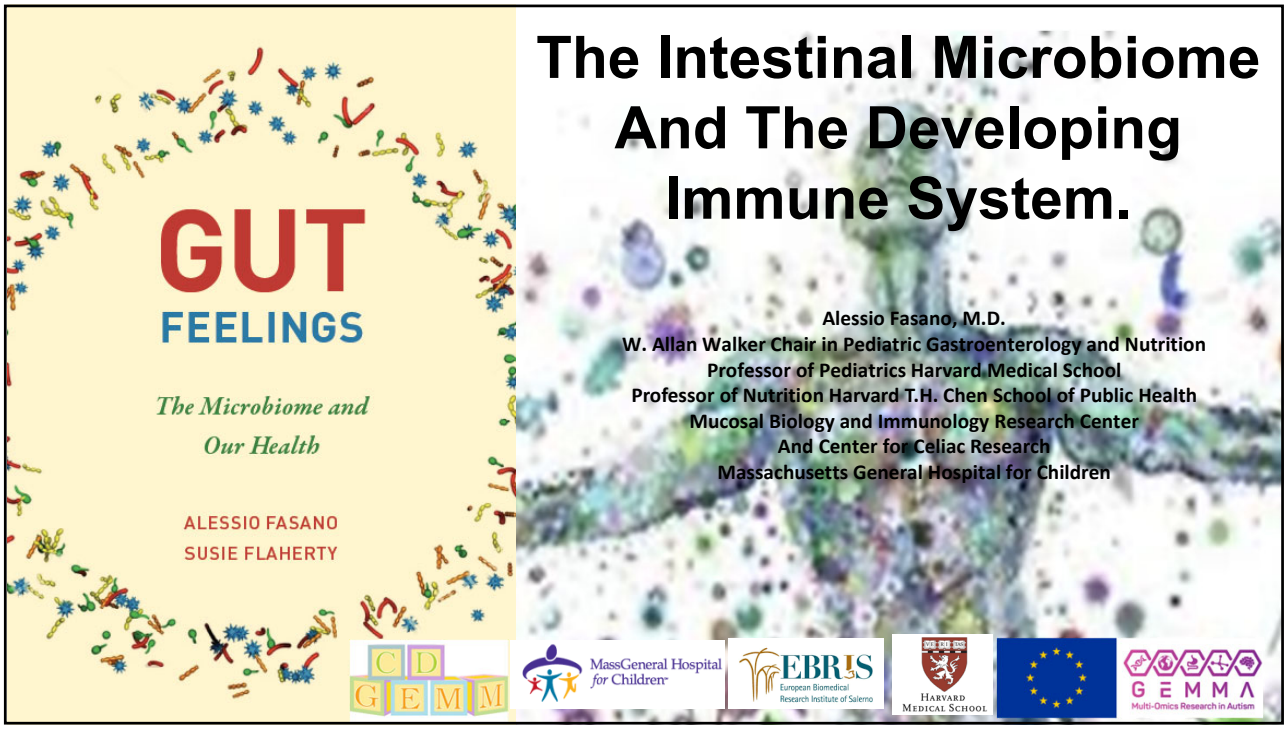
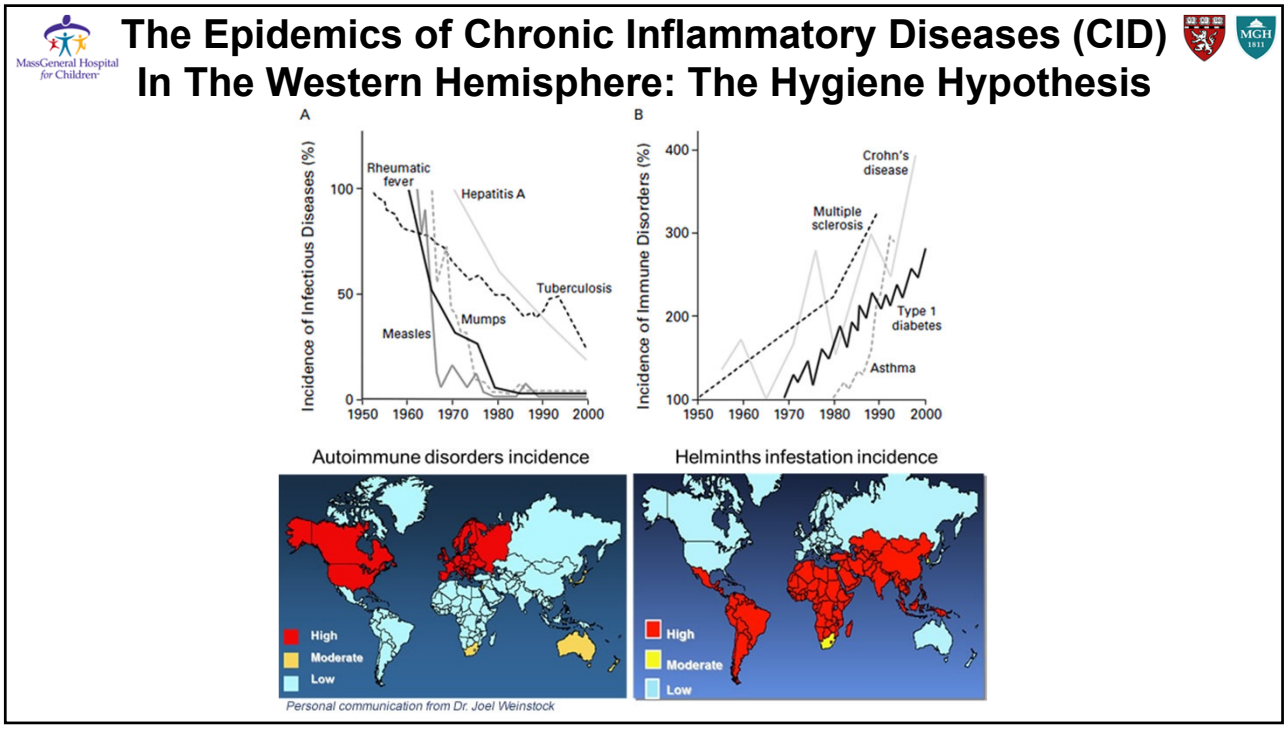

GUT
FEELINGS
The Microbiome and Our Health
ALESSIO FASANO
SUSIE FLAHERTY
The Intestinal Microbiome And The Developing Immune System.
Alessio Fasano, M.D.
W. Allan Walker Chair in Pediatric Gastroenterology and Nutrition
Professor of Pediatrics Harvard Medical School
Professor of Nutrition Harvard T.H. Chen School of Public Health
Mucosal Biology and Immunology Research Center
And Center for Celiac Research
Massachusetts General Hospital for Children
C D
G E M M
MassGeneral Hospital for Children
EBRIS
European Biomedical Research Institute of Salerno
Harvard Medical School
GEMMA
Multi-Omics Research in Autism

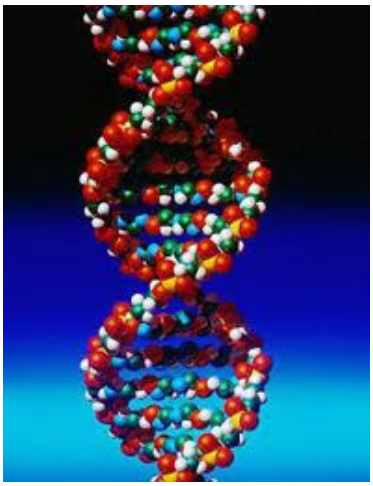
1


MassGeneral Hospital for Children
The Epidemics of Chronic Inflammatory Diseases (CID)
In The Western Hemisphere: The Hygiene Hypothesis
A
Incidence of Infectious Diseases (%)
Rheumatic fever
Hepatitis A
Tuberculosis
Mumps
Measles
100
50
0
1950 1960 1970 1980 1990 2000
B
Incidence of Immune Disorders (%)
Crohn's disease
Multiple sclerosis
Type 1 diabetes
Asthma
400
300
200
100
1950 1960 1970 1980 1990 2000
Autoimmune disorders incidence
Helminths infestation incidence
High
Moderate
Low
High
Moderate
Low
Personal communication from Dr. Joel Weinstock

2

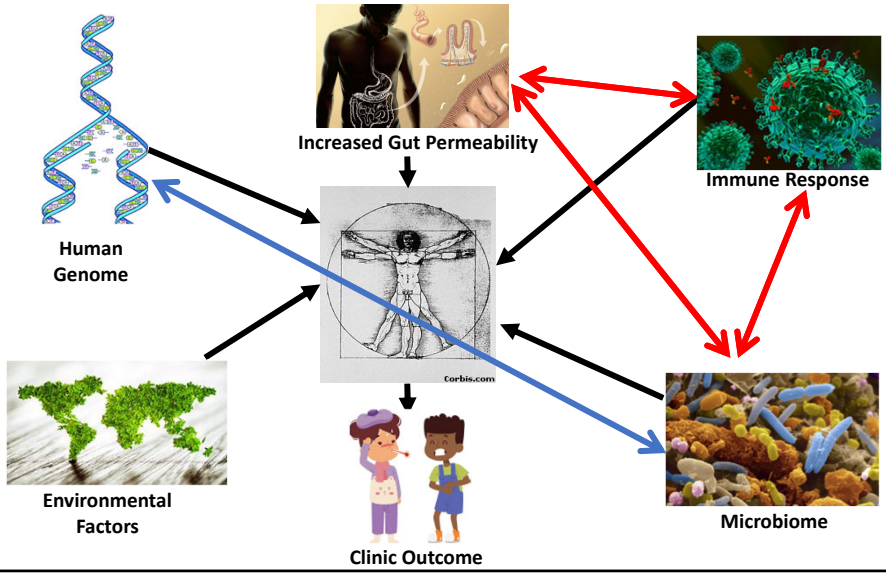
Pathogenesis CID

We May Be "*Predisposed*", but Are Not Born "*Destined*" to Develop Chronic Inflammatory Diseases



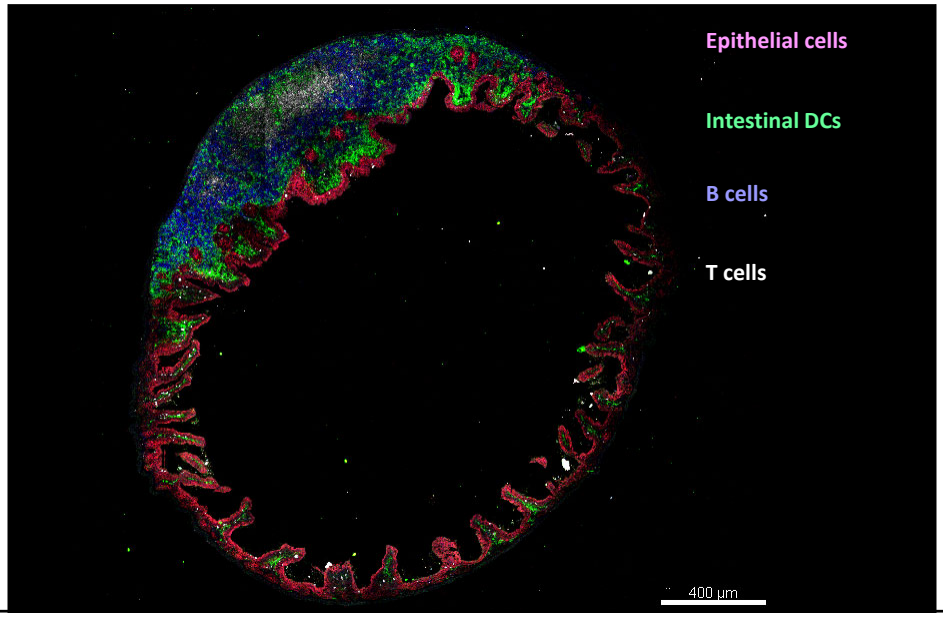
3

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



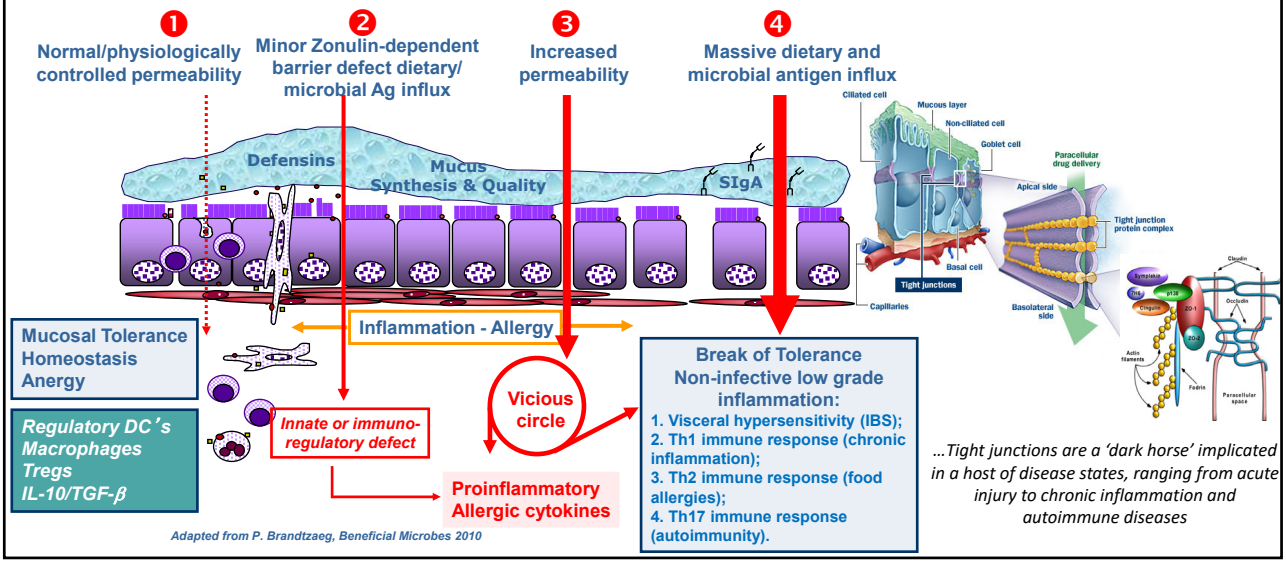
4

Several Cells Play a Role in Maintaining The Immune Homeostasis



5

Excessive and Inappropriate Inflammatory Process Associated to a Dysfunction of Intestinal Barrier:
Loss of Mucosal Immune Homeostasis



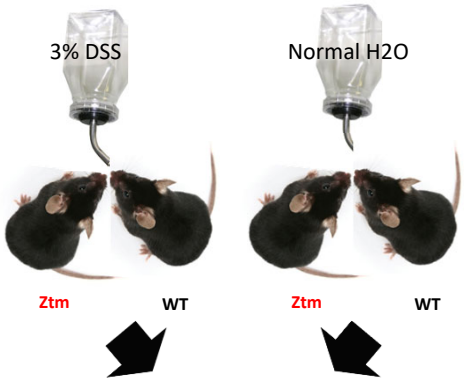
Adapted from P. Brandtzaeg, Beneficial Microbes 2010

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

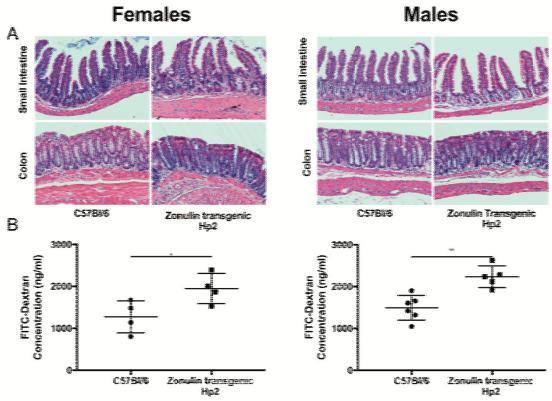
6

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



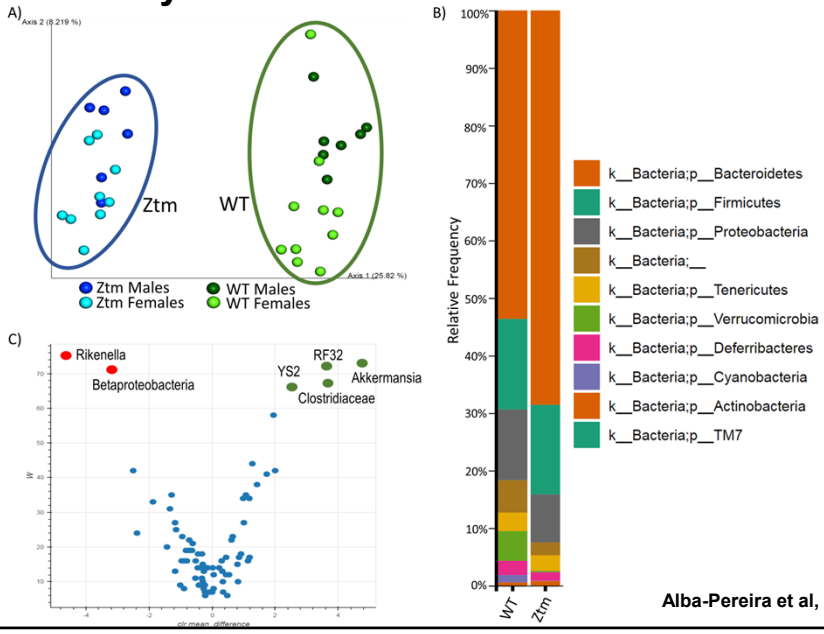
Zonulin transgenic Hp2 mice are phenotypically normal despite increased small intestinal permeability

*p<0.05

Sturgeon C et al. Ann NY Acad Sci 2017

7

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



Alba-Pereira et al, Front Immunol 2019

8

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

Ztm and Ztm$_{bWT}$ showed:

-Increase in IL7R$^+$RORγτ$^+$ innate lymphoid cells in the small intestine (no changes in the colon);

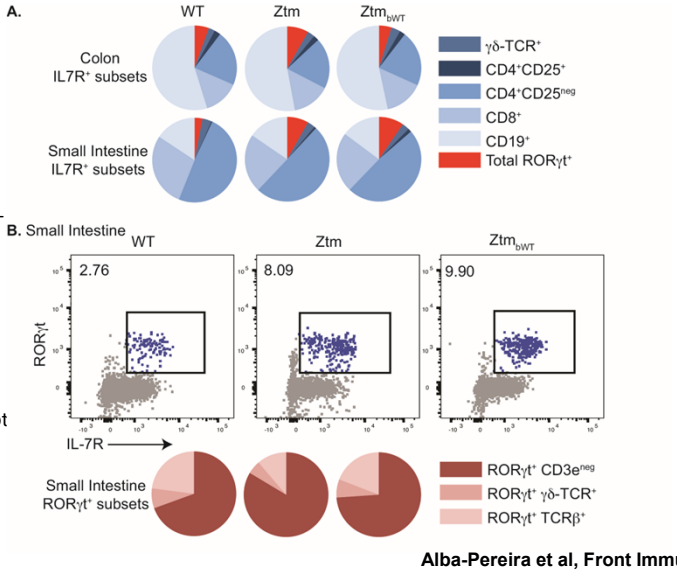
- Decreased frequency and numbers of invariant NKT (iNKT) cells (involved in mucosal immunity);

- Increased RORγτ expressing subset of iNKT cells (NKT17 cells). NKT17 cells and γδ-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.

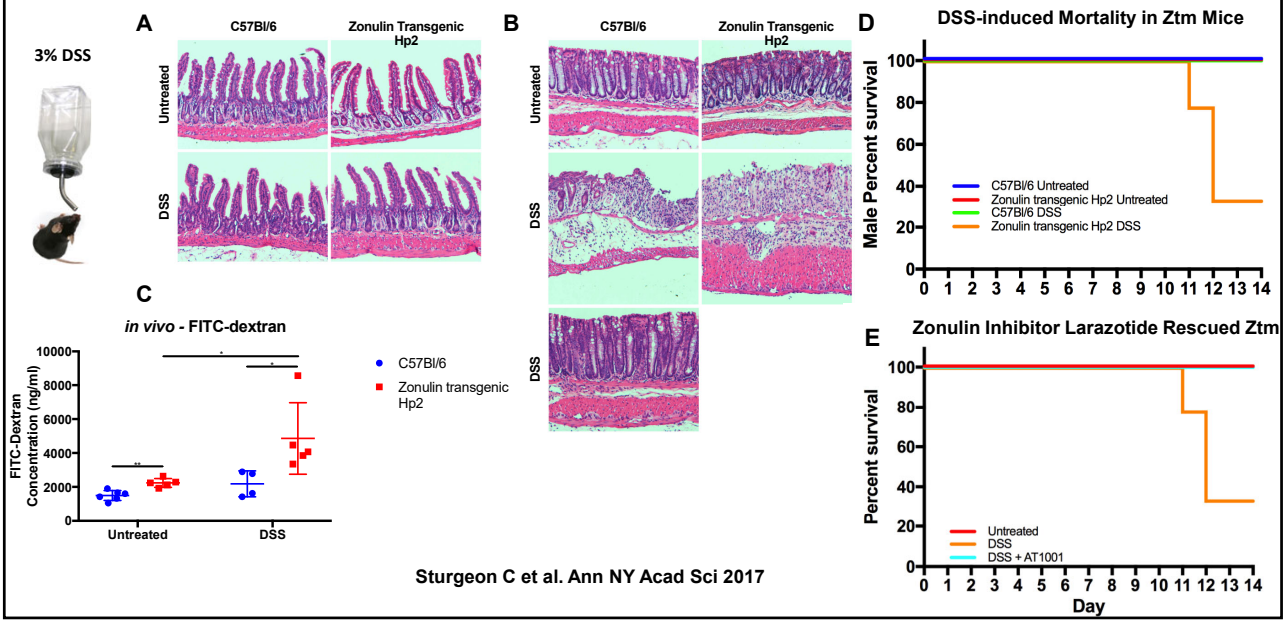
The fact that the engraftment of WT microbiota did not affect the immune phenotype in Ztm$_{bWT}$ suggests that the increased antigen trafficking through an impaired gut barrier more than the function of an imbalanced microbiota primarily imprints the development of the immune system in the Ztm.



Alba-Pereira et al, Front Immunol 2019

9

Ztm Showed Increased Morbidity (Colonic Mucosal Damage) And Mortality (70%) Following DSS-Induced Colitis

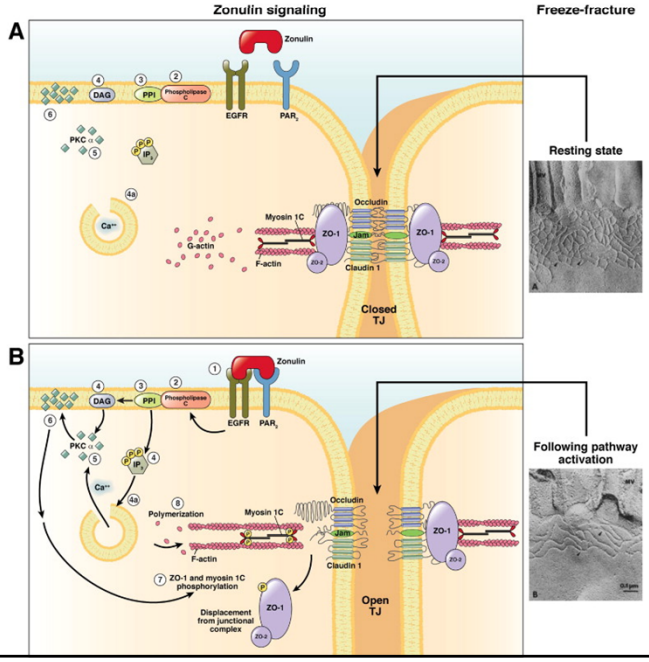


Sturgeon C et al. Ann NY Acad Sci 2017

10

Literature Report On Zonulin Association With CID

Disease	Model	Zonulin Shown to be Involved
ADHD	Human	YES
Aging	Human	YES
Ankylosis spondylitis	Human	YES
Autism	Human	YES
Bipolar Disorders	Human	YES
Celiac Disease	Human	YES
Colitis/IBD (Crohn's disease)	Human	YES
Colitis	Mouse	YES
Depressive Disorders	Human	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



11

The Changing Face Of Gut Microbes

12

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

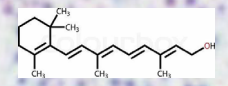
Microbial cells than human cells and the majority live in our gut

150x More

Genes than the human genome

Energy From Food

Regulates Metabolism



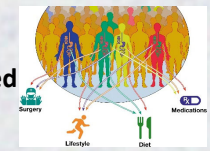
Producing Essential Vitamins

Regulate Immune System

Protection from pathogenic bacteria

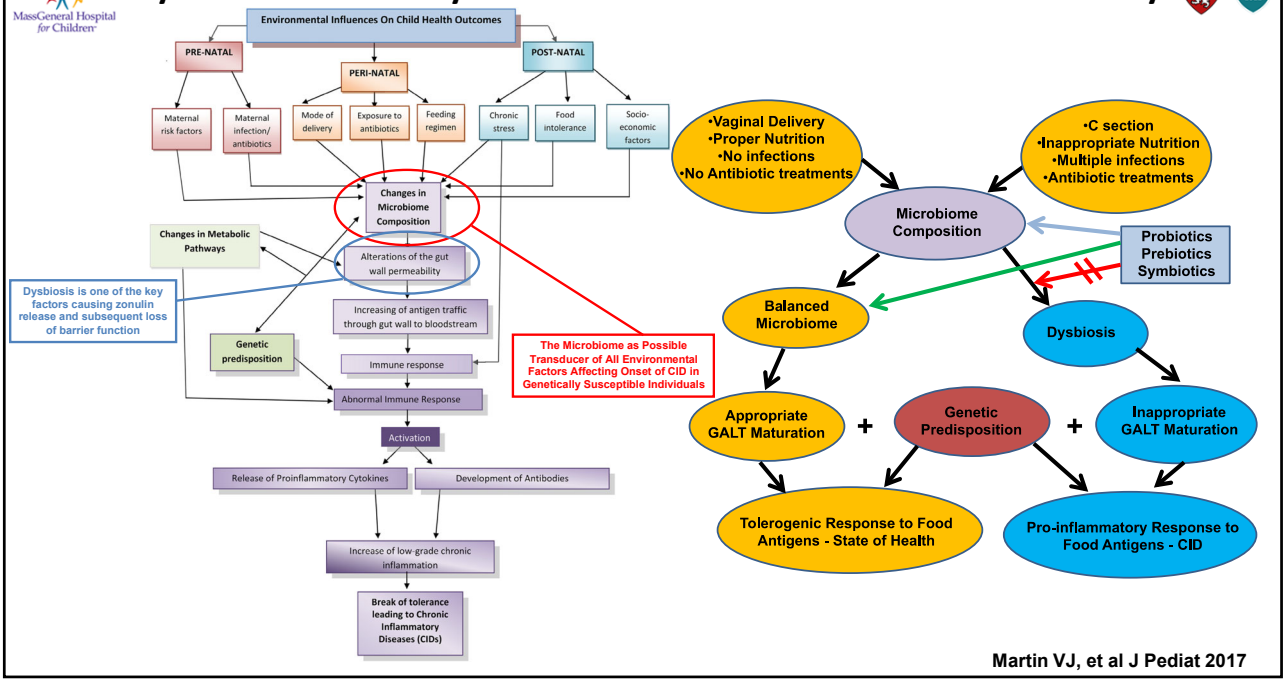
Symbiotic

Personalized



13

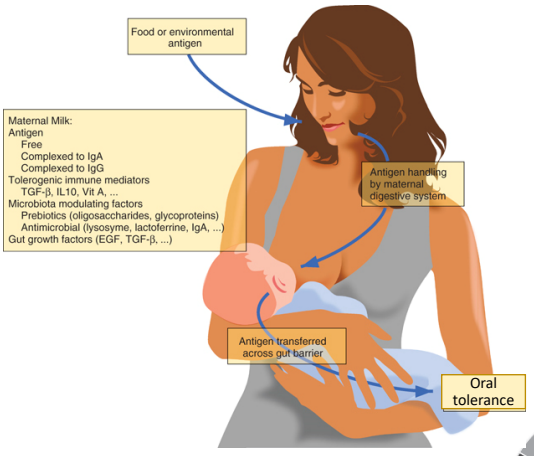
Why The First 1000 Days Of Life Are Instrumental For Clinical Destiny



Martin VJ, et al J Pediat 2017

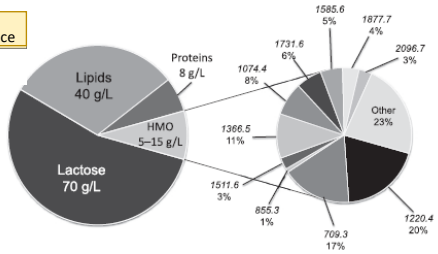
14

Role of Breastmilk



http://www.nature.com

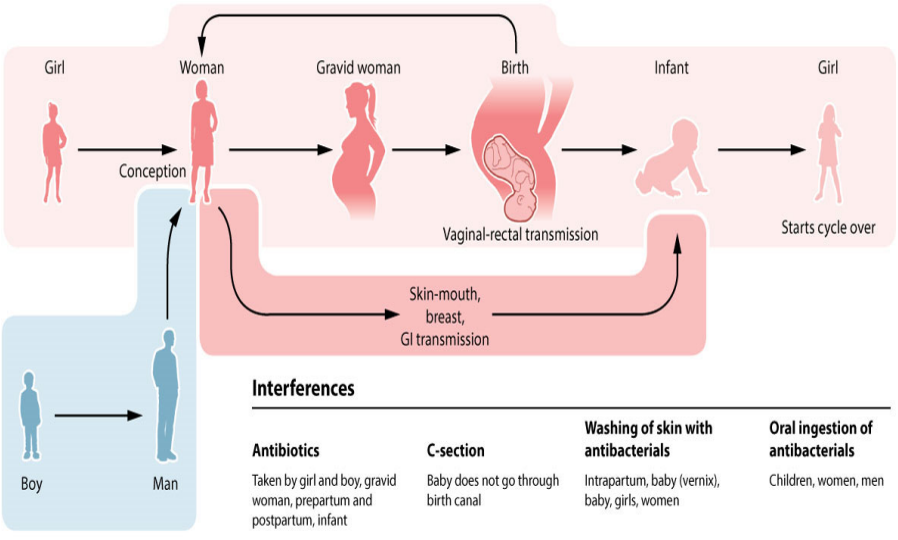
Impact of human milk glycobiome on the infant intestinal microbiota



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

15

Cycle of Microbiota Transmission



Interferences

Antibiotics	C-section	Washing of skin with antibacterials	Oral ingestion of antibacterials
Taken by girl and boy, gravid woman, prepartum and postpartum, infant	Baby does not go through birth canal	Intrapartum, baby (vernix), baby, girls, women	Children, women, men

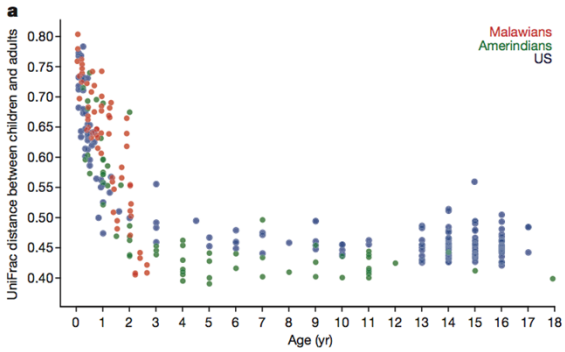
Dominguez-Bello et al Science Trans Med 2015;7:307-39
Fischbach et al Cell 2016;164:1288-1300

16

Baby's first bacteria

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

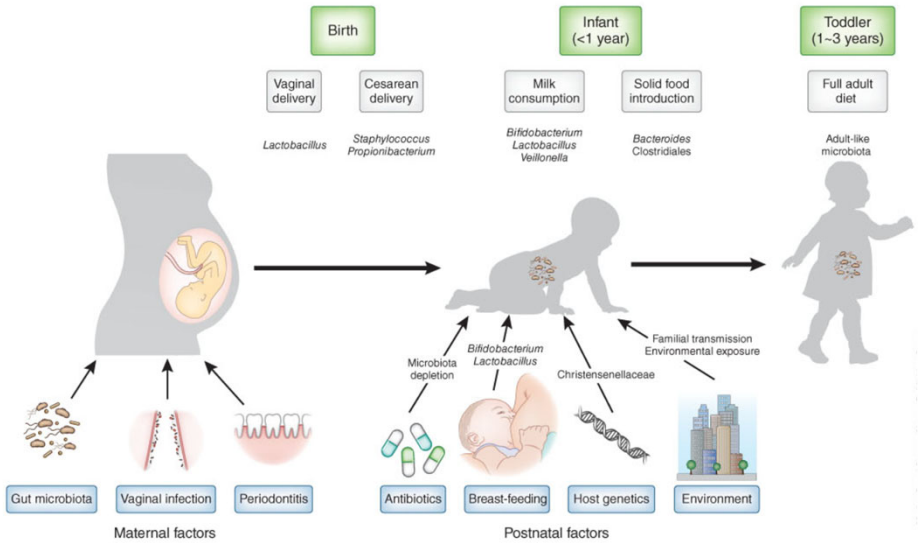
- Exactly when an infant is first exposed to microbes is still under debate
- Largest microbial transfer occurs at birth
- Microbial colonization of the newborn intestine contributes to the development of the host's immune function
- The first 1-3 years of an infant's microbiome development is characterized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



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

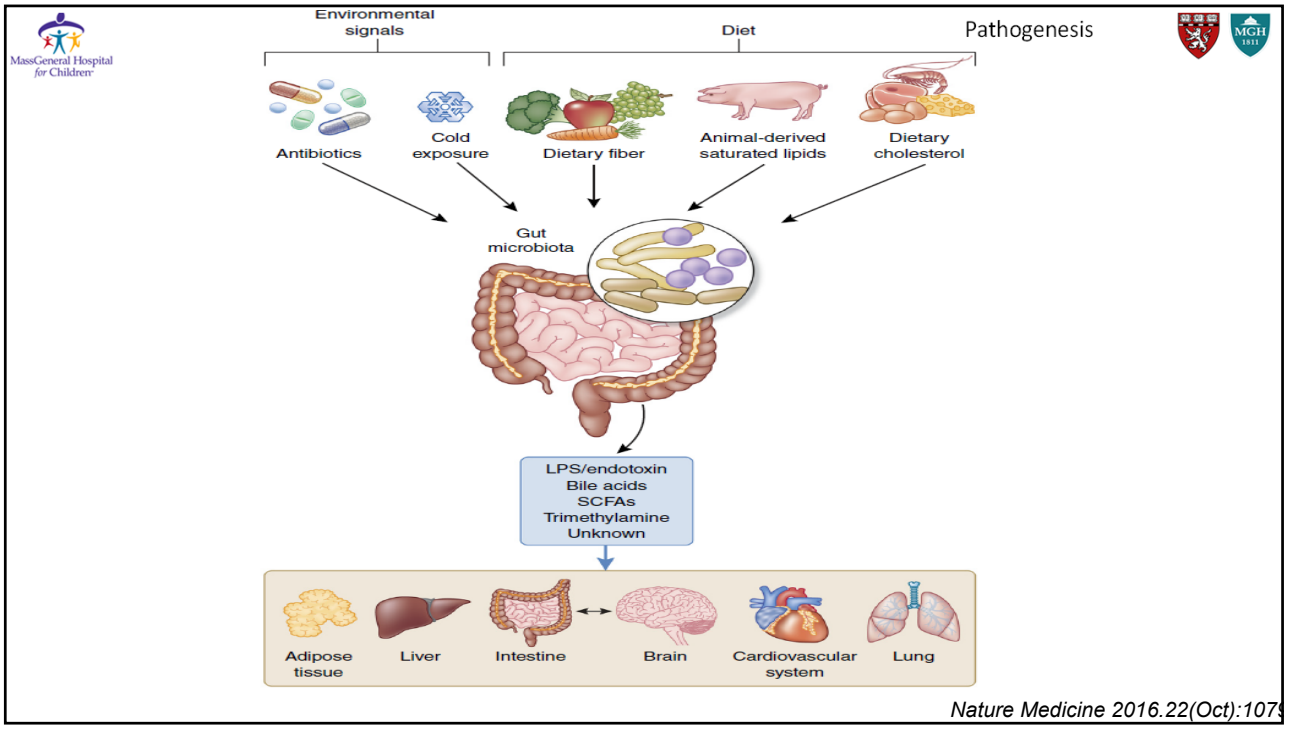
17

The Wisdom of Microscopic Species

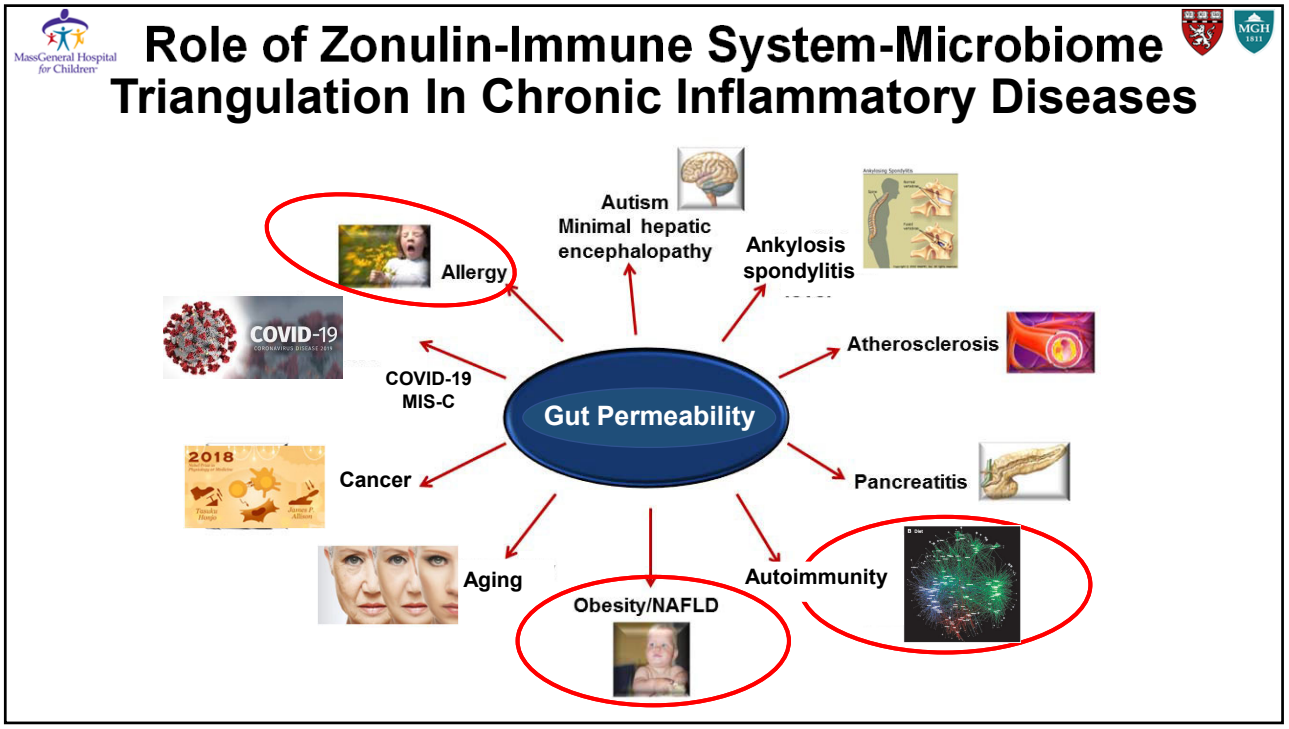


Tamburini, *Nature* 2016.

18

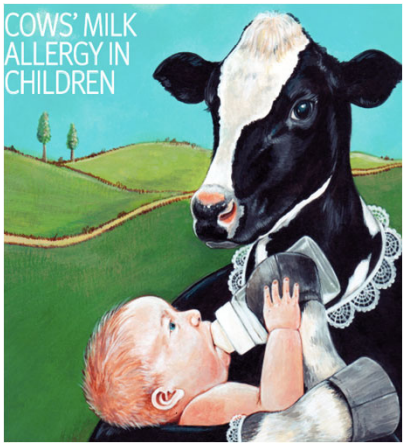

Environmental signals
Diet
Pathogenesis
Antibiotics
Cold exposure
Dietary fiber
Animal-derived saturated lipids
Dietary cholesterol
Gut microbiota
LPS/endotoxin
Bile acids
SCFAs
Trimethylamine
Unknown
Adipose tissue
Liver
Intestine
Brain
Cardiovascular system
Lung
Nature Medicine 2016.22(Oct):107

19


Role of Zonulin-Immune System-Microbiome Triangulation In Chronic Inflammatory Diseases
Autism
Minimal hepatic encephalopathy
Ankylosis spondylitis
Allergy
COVID-19
Atherosclerosis
COVID-19 MIS-C
Gut Permeability
Cancer
Pancreatitis
Aging
Autoimmunity
Obesity/NAFLD

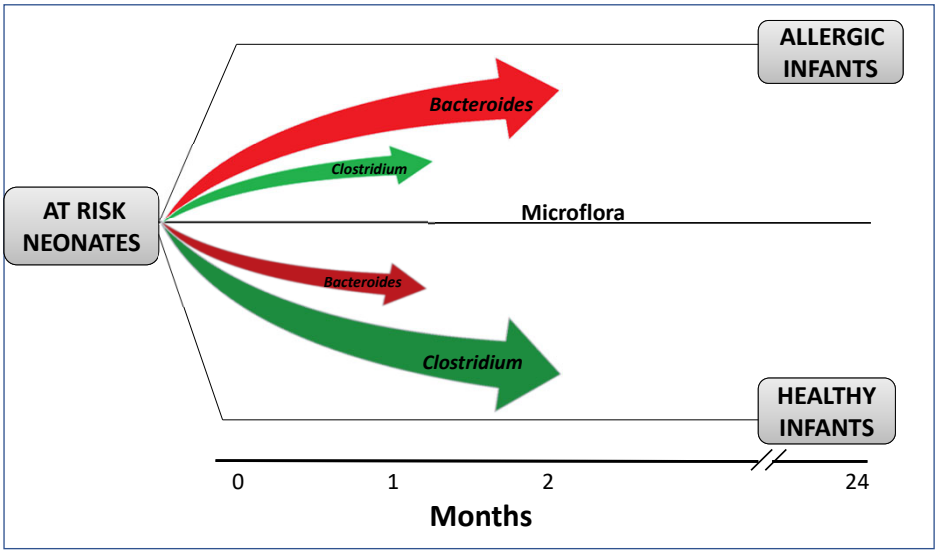
20

Food Allergies



21

Gut Dysbiosis Correlates With Allergy Development



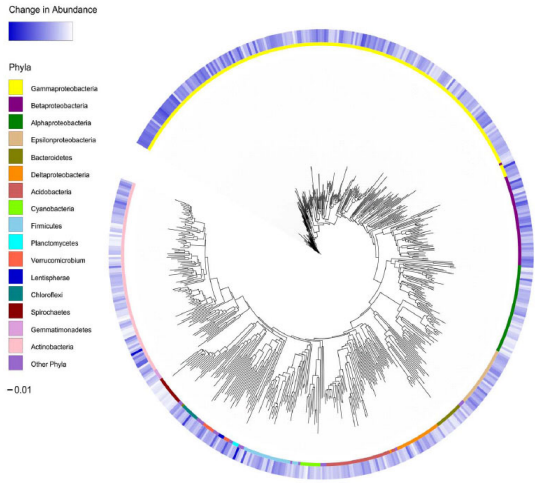
Nakayama OC, et al. FEMS Immunol Med Microbiol 2011

22

OPEN ACCESS Freely available online PLoS one

Lactobacillus casei Abundance Is Associated with Profound Shifts in the Infant Gut Microbiome

Michael J. Cox[1,9], Yvonne J. Huang[2,9], Kei E. Fujimura[1], Jane T. Liu[2], Michelle McKean[3], Homer A. Boushey[2], Mark R. Segal[4], Eoin L. Brodie[5], Michael D. Cabana[2,3], Susan V. Lynch[1]*



16S rRNA PhyloChip
High-density, culture-independent microarray that can identify ~8,500 bacterial taxa

Restoring Microbial Health

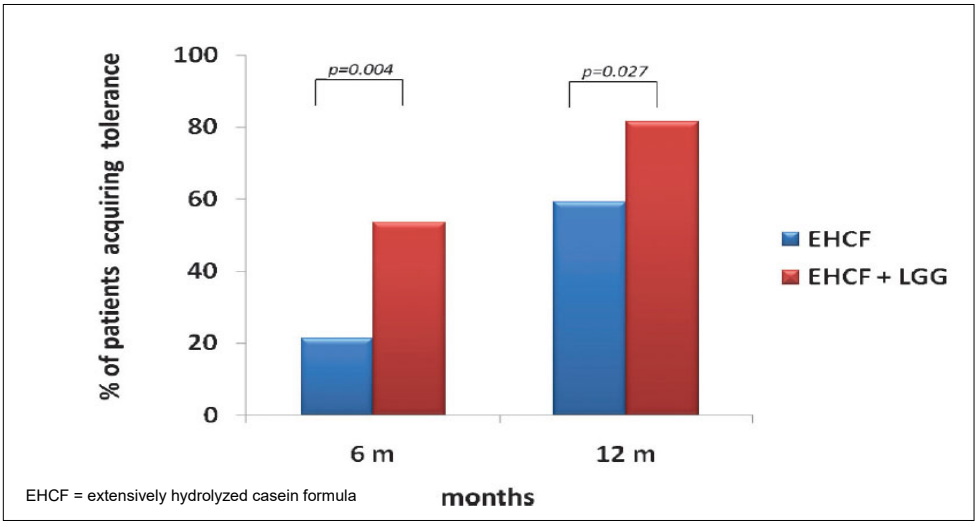
***Lactobacillus* GG (LGG) restores the normal microflora composition in infants with CMA**

January 2010 | Volume 5 | Issue 1 | e8745

23

LGG Accelerates CMA Recovery

THE JOURNAL OF Allergy AND Clinical Immunology



EHCF = extensively hydrolyzed casein formula

Berni Canani R, et al. 2012

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Slide 23

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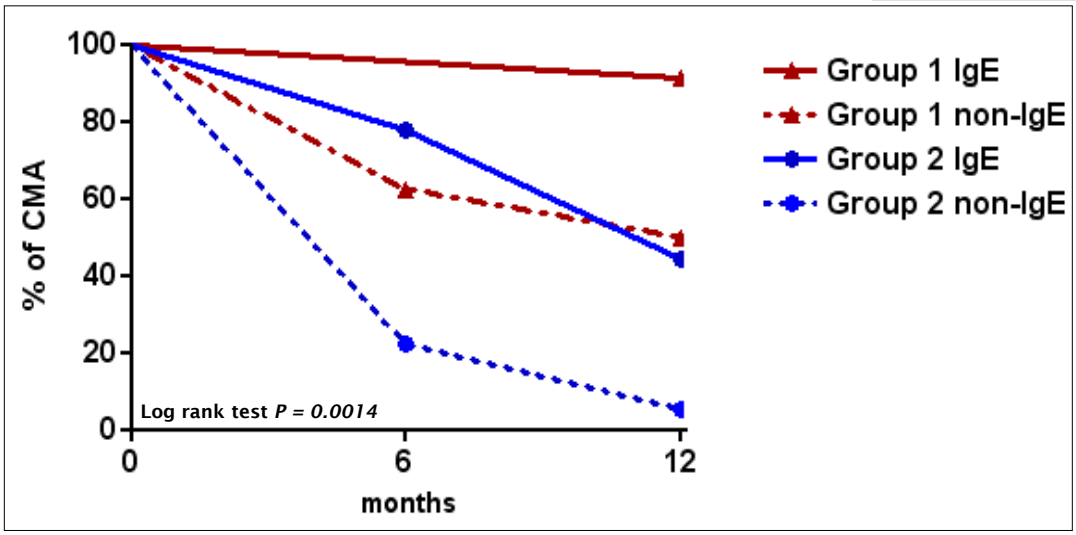
2 Fixed spelling of independent
Owner, 7/13/2012

1 Added abbreviation LGG for Lactobacillus GG since he uses it later.
Owner, 7/13/2012

LGG Stimulates Tolerance Acquisition in Infants With IgE or non-IgE Mediated CMA

THE JOURNAL OF Allergy AND Clinical Immunology



Berni Canani R, et al. 2012

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SCIENTIFIC REPORTS

Corrected: Publisher Correction

OPEN

Randomized controlled trial on the influence of dietary intervention on epigenetic mechanisms in children with cow's milk allergy: the EPICMA study

Received: 28 September 2018
Accepted: 9 January 2019
Published online: 26 February 2019

Lorella Paparo[1,4], Rita Nocerino[1,4], Cristina Bruno[1,4], Carmen Di Scala[1,4], Linda Cosenza[1], Giorgio Bedogni[2], Margherita Di Costanzo[1], Maurizio Mennini[3], Valeria D'Argenio[4,5,7], Francesco Salvatore[4,5,7] & Roberto Berni Canani[1,4,6,7]

Epigenetic mechanisms could drive the disease course of cow's milk allergy (CMA) and formula choice could modulate these pathways. We compared the effect of two different dietary approaches on epigenetic mechanisms in CMA children. Randomized controlled trial on IgE-mediated CMA children receiving a 12-month treatment with extensively hydrolyzed casein formula containing the probiotic *L.rhamnosus* GG (EHCF + LGG) or with soy formula (SF). At the baseline, after 6 and 12 months of treatment *FoxP3* methylation rate and its expression in $CD4^+$ T cells were assessed. At same study points IL-4, IL-5, IL-10, and IFN-γ methylation rate, expression and serum concentration, miRNAs expression were also investigated. 20 children (10/group) were evaluated. Baseline demographic, clinical and epigenetic features were similar in the two study groups. At 6 and 12 months, EHCF + LGG group showed a significant increase in *FoxP3* demethylation rate compared to SF group. At the same study points, EHCF + LGG group presented a higher increase in IL-4 and IL-5 and a higher reduction in IL-10 and IFN-γ DNA methylation rate compared to SF group. A different modulation of miR-155, -146a, -128 and -193a expression was observed in EHCF + LGG vs. SF. Dietary intervention could exert a different epigenetic modulation on the immune system in CMA children.

Key message:
EHCF+LGG induces a stronger modulation of epigenetic mechanisms associated with a trend toward higher rate of immune tolerance acquisition in children

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Obesity

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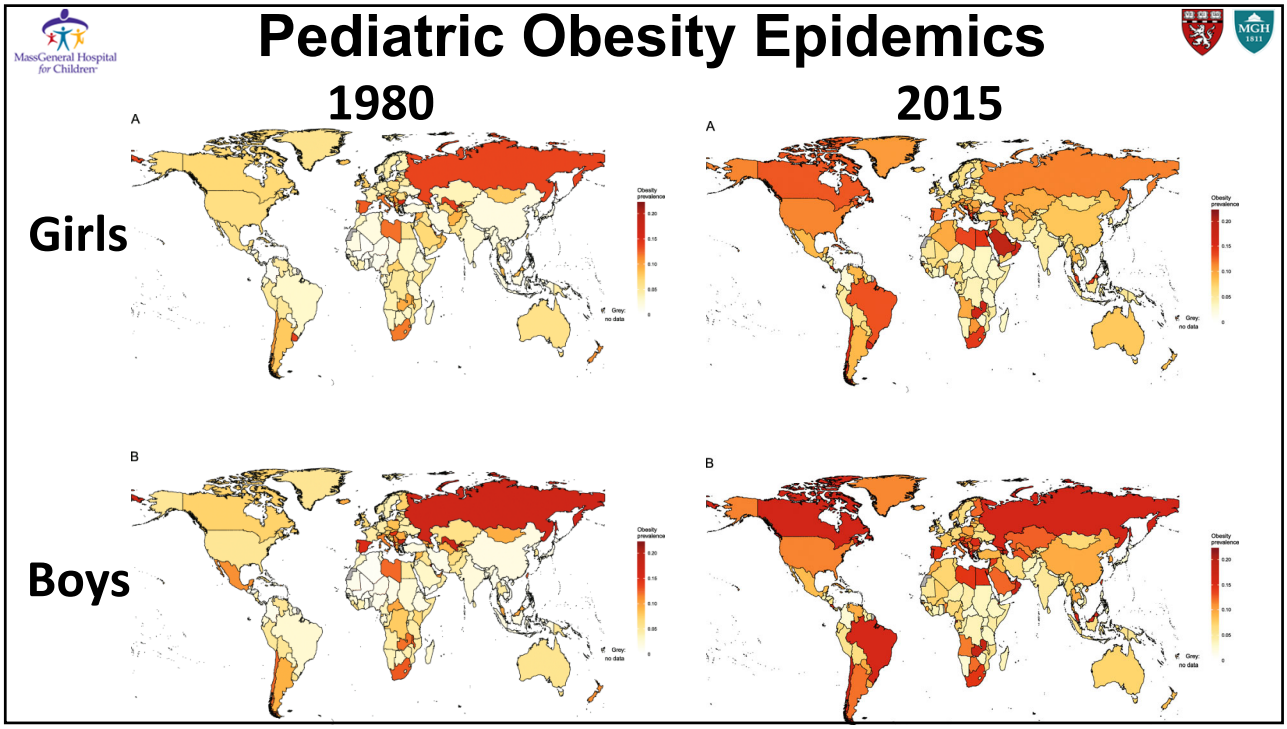
Pediatric Obesity Epidemics



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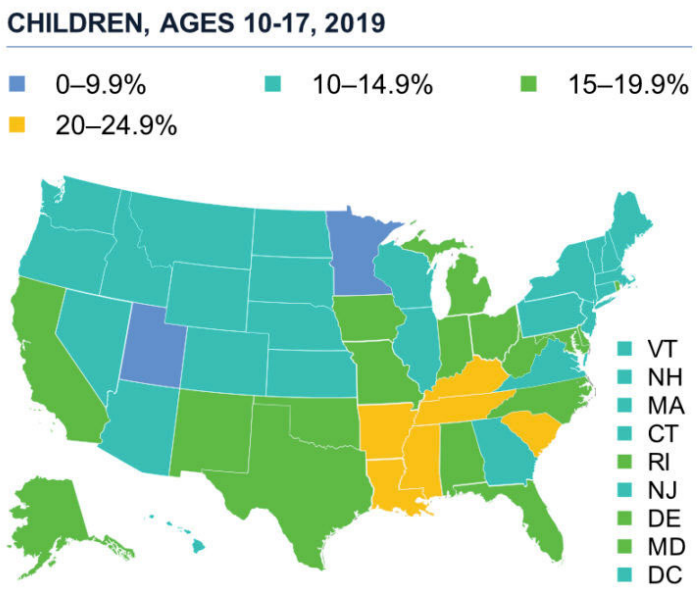
State by state obesity rates by age group



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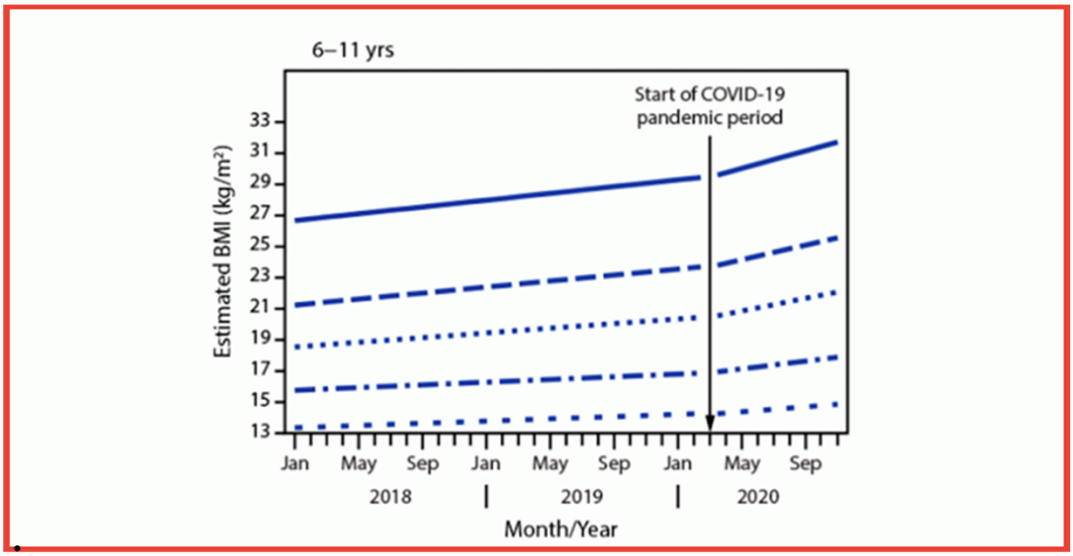
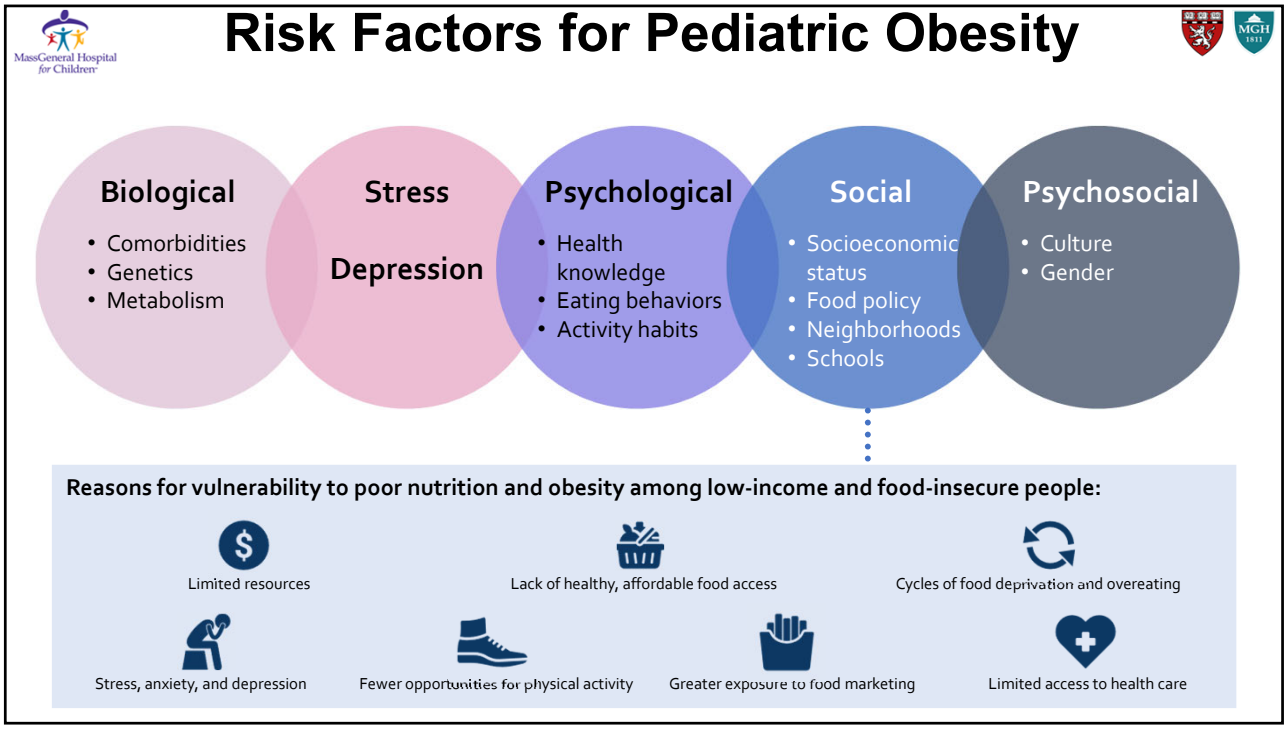
Pediatric Obesity During COVID-19 Pandemic



New CDC research finds that American children's rate of BMI increase nearly doubled during the early months of the pandemic

30


Risk Factors for Pediatric Obesity
Biological
• Comorbidities
• Genetics
• Metabolism
Stress
Depression
Psychological
• Health knowledge
• Eating behaviors
• Activity habits
Social
• Socioeconomic status
• Food policy
• Neighborhoods
• Schools
Psychosocial
• Culture
• Gender
Reasons for vulnerability to poor nutrition and obesity among low-income and food-insecure people:
Limited resources
Lack of healthy, affordable food access
Cycles of food deprivation and overeating
Stress, anxiety, and depression
Fewer opportunities for physical activity
Greater exposure to food marketing
Limited access to health care

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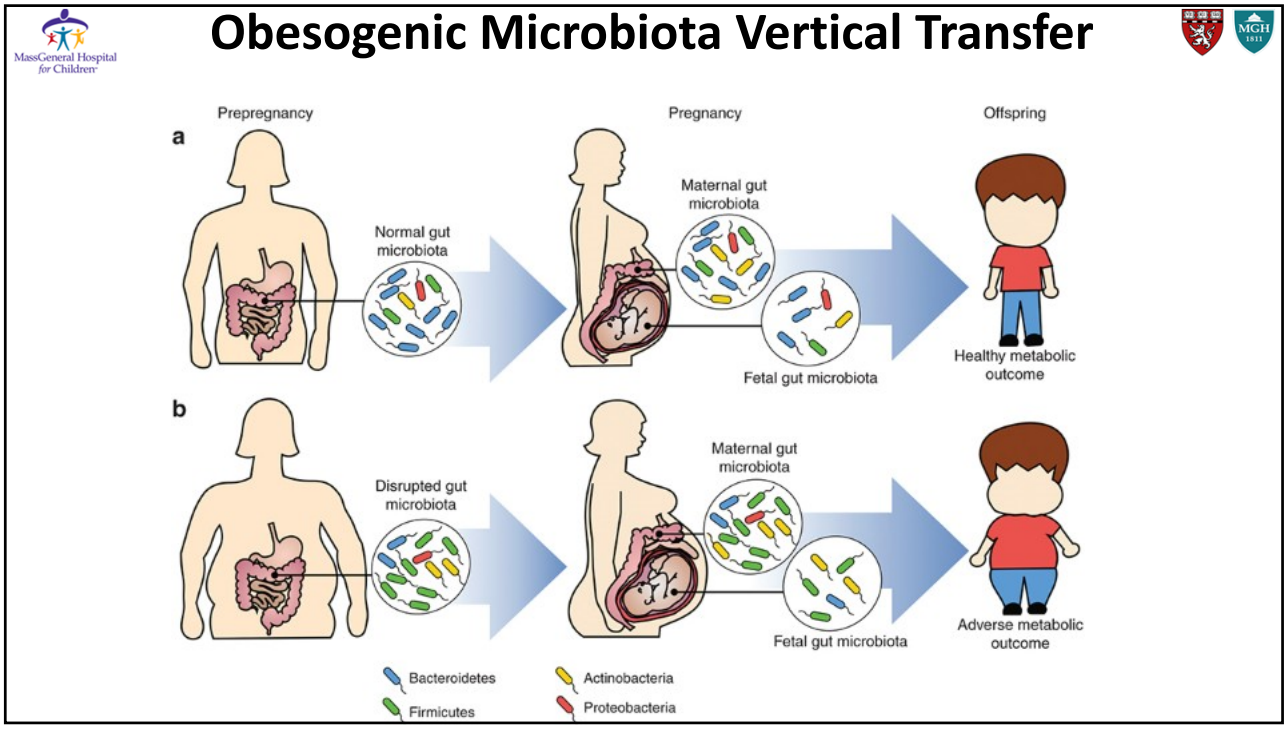
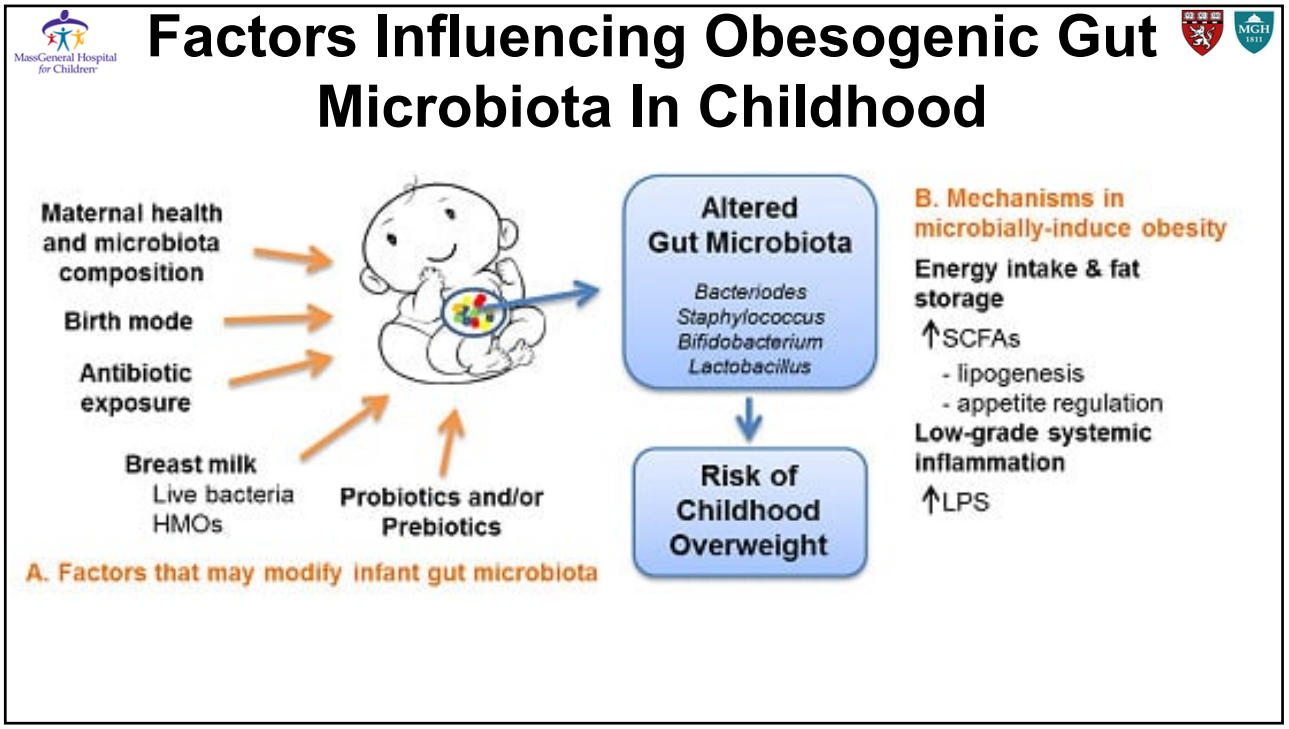

Obesogenic Microbiota Vertical Transfer
Prepregnancy
Pregnancy
Offspring
a
Normal gut microbiota
Maternal gut microbiota
Fetal gut microbiota
Healthy metabolic outcome
b
Disrupted gut microbiota
Maternal gut microbiota
Fetal gut microbiota
Adverse metabolic outcome
Bacteroidetes
Firmicutes
Actinobacteria
Proteobacteria

32


Factors Influencing Obesogenic Gut Microbiota In Childhood
Maternal health and microbiota composition
Birth mode
Antibiotic exposure
Breast milk
Live bacteria
HMOs
Probiotics and/or Prebiotics
A. Factors that may modify infant gut microbiota
Altered Gut Microbiota
Bacteriodes
Staphylococcus
Bifidobacterium
Lactobacillus
Risk of Childhood Overweight
B. Mechanisms in microbially-induce obesity
Energy intake & fat storage
↑SCFAs
- lipogenesis
- appetite regulation
Low-grade systemic inflammation
↑LPS

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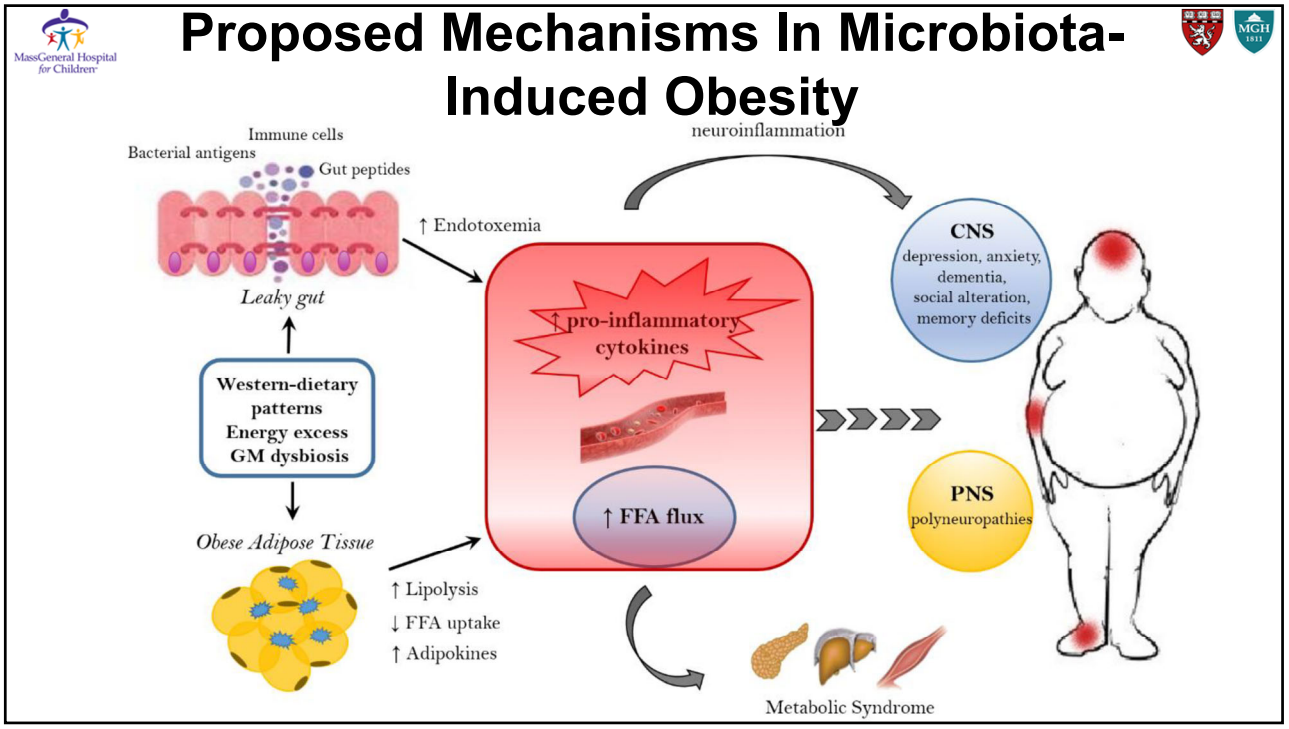
Proposed Mechanisms In Microbiota-Induced Obesity
Immune cells
Bacterial antigens
Gut peptides
Leaky gut
↑ Endotoxemia
neuroinflammation
CNS
depression, anxiety, dementia, social alteration, memory deficits
↑pro-inflammatory cytokines
↑ FFA flux
Western-dietary patterns
Energy excess
GM dysbiosis
Obese Adipose Tissue
↑ Lipolysis
↓ FFA uptake
↑ Adipokines
PNS
polyneuropathies
Metabolic Syndrome

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Autoimmunity: Celiac Disease

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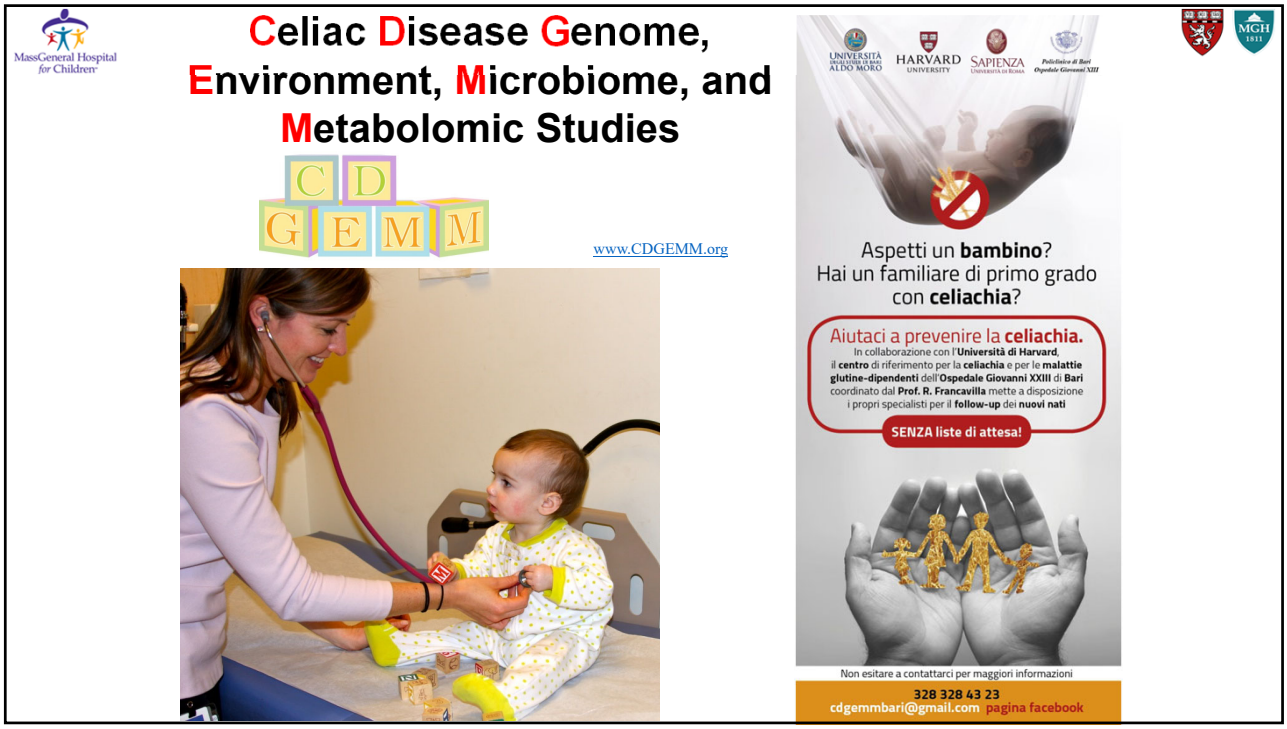
Celiac Disease Genome, Environment, Microbiome, and Metabolomic Studies
C D
G E M M
www.CDGEMM.org
HARVARD UNIVERSITY
SAPIENZA
Aspetti un bambino?
Hai un familiare di primo grado con celiachia?
Aiutaci a prevenire la celiachia.
SENZA liste di attesa!
Non esitare a contattarci per maggiori informazioni
328 328 43 23
cdgemmbari@gmail.com pagina facebook

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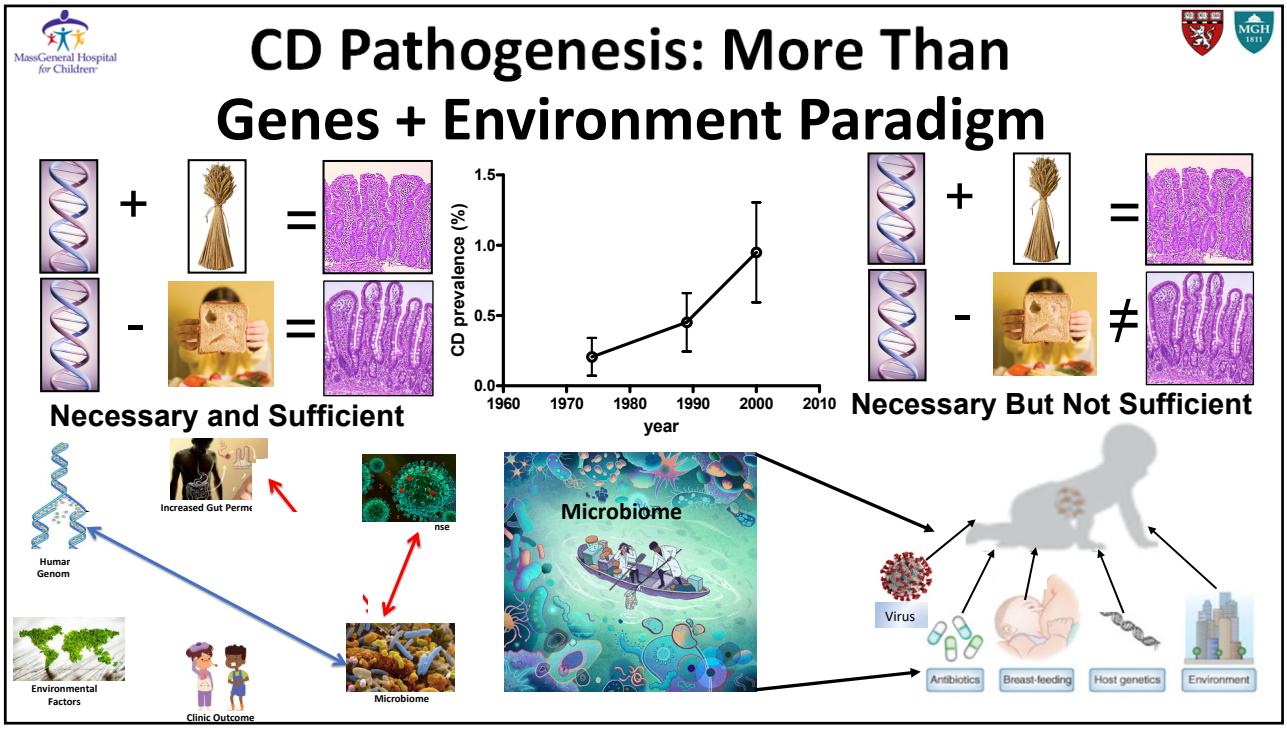
CD Pathogenesis: More Than Genes + Environment Paradigm
Necessary and Sufficient
CD prevalence (%)
1.5
1.0
0.5
0.0
1960
1970
1980
1990
2000
2010
year
Necessary But Not Sufficient
Humar Genom
Increased Gut Perme
nse
Microbiome
Environmental Factors
Clinic Outcome
Microbiome
Virus
Antibiotics
Breast-feeding
Host genetics
Environment

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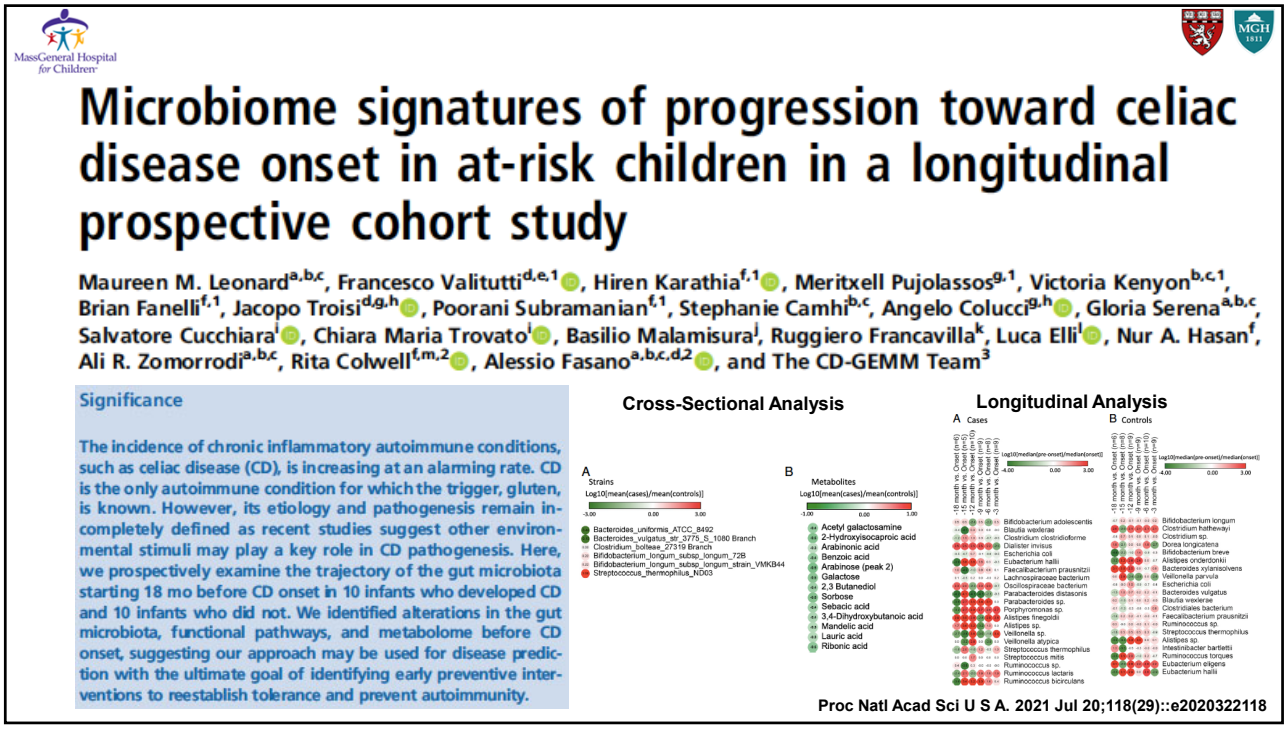
Microbiome signatures of progression toward celiac disease onset in at-risk children in a longitudinal prospective cohort study
Maureen M. Leonard[a,b,c], Francesco Valitutti[d,e,1], Hiren Karathia[f,1], Meritxell Pujolassos[g,1], Victoria Kenyon[b,c,1], Brian Fanelli[f,1], Jacopo Troisi[d,g,h], Poorani Subramanian[f,1], Stephanie Camhi[b,c], Angelo Colucci[g,h], Gloria Serena[a,b,c], Salvatore Cucchiara[i], Chiara Maria Trovato[i], Basilio Malamisura[j], Ruggiero Francavilla[k], Luca Elli[l], Nur A. Hasan[f], Ali R. Zomorrodi[a,b,c], Rita Colwell[f,m,2], Alessio Fasano[a,b,c,d,2], and The CD-GEMM Team[3]
Significance
The incidence of chronic inflammatory autoimmune conditions, such as celiac disease (CD), is increasing at an alarming rate. CD is the only autoimmune condition for which the trigger, gluten, is known. However, its etiology and pathogenesis remain incompletely defined as recent studies suggest other environmental stimuli may play a key role in CD pathogenesis. Here, we prospectively examine the trajectory of the gut microbiota starting 18 mo before CD onset in 10 infants who developed CD and 10 infants who did not. We identified alterations in the gut microbiota, functional pathways, and metabolome before CD onset, suggesting our approach may be used for disease prediction with the ultimate goal of identifying early preventive interventions to reestablish tolerance and prevent autoimmunity.
Cross-Sectional Analysis
Longitudinal Analysis
Proc Natl Acad Sci U S A. 2021 Jul 20;118(29)::e2020322118

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Longitudinal analysis provided more in-depth data by identifying microbes, pathways and metabolites with differential abundance CD onset

Cases

- ↑ Abundance of microbes/pathways/metabolites
  - Previously linked to autoimmune and inflammatory conditions
- ↓ Abundance of microbes/pathways/metabolites
  - Previously reported as probiotics or having anti-inflammatory properties
- Previously unreported microbes/pathways/metabolites that may serve as CD-specific biomarkers

Controls

- ↑ Abundance of microbes/pathways/metabolites
  - Previously linked to protection against allergic, autoimmune and inflammatory conditions

Laparra JM, Plosone, 2012.
Wong CB et al. Nutrients 2019
Klemenak M et al. Dig Dis Sci 2015
Stewart, Nature, 2018.
Ye, Microbiome 2018.
Pianta, J Clin Invest, 2017
Menard S et al, Gut, 2004.
Chien Mw et al, Int j of Mol sci, 2018

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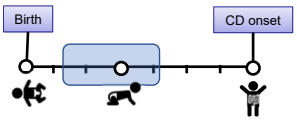
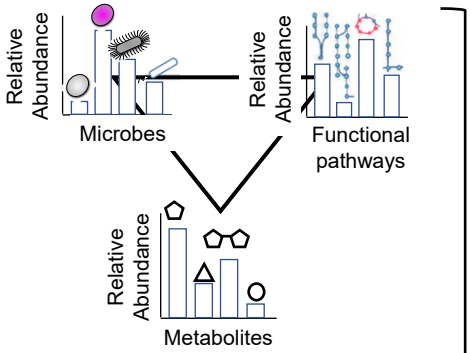
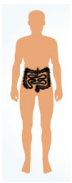
In silico modeling of host-microbiota interactions to study celiac disease pathogenesis and progression

Prospective birth cohort study





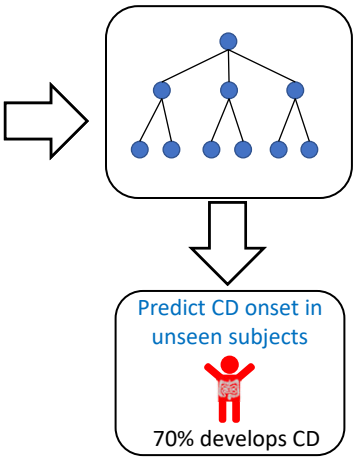


Host-derived Omics data

Clinical metadata

Early prediction of CD development using various data types and machine learning

Machine learning



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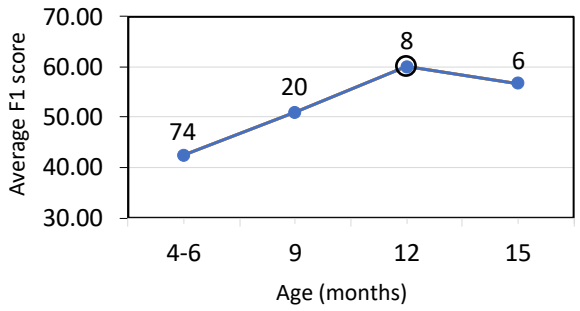
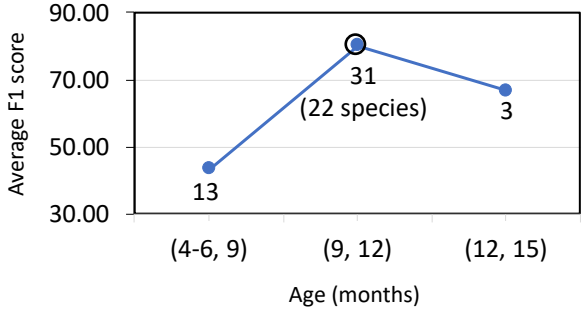
Random Forests using 10 cases and 10 controls and species abundance data identifies top microbial species discriminating cases vs. controls

One absolute time point



Two absolute time points



Microbial biomarkers and early-warning indicators of CD

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Manipulating The Microbiome To Treat CID

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Microbiome Disruption by Condition Summary

CONDITION	RELEVANT FINDINGS
Prematurity	↑ Proteobacteria ↓ Microbial diversity
Necrotizing enterocolitis	Blooms of *Proteobacteria* prior to disease onset
Sepsis	Altered microbiota structure and composition prior to disease onset has been reported, but specific microbiota reported is inconsistent across studies
Colic	Decreased microbial diversity and increased anaerobic bacteria
Malnutrition	Anaerobic depletion, early dysbiosis, and intestinal pathogenic overabundance with decreased bacterial diversity
Eczema	Early colonization with opportunistic species may be important in disease initiation
Allergies	↓ Species diversity
Asthma	No clear pattern
Inflammatory bowel disease	Data is sparse, no consistent pattern
Type I diabetes	↑ *Bacteroidetes:Firmicutes* ratios, ↑ *Clostridia* species ↓ Butyrate-producing bacteria ↓ Bacterial diversity ↓ Community stability Alterations in the microbiome seem to precede disease onset
Type II diabetes and obesity	↑ *Firmicutes:Bacteroidetes* ratio ↑ SCFAs
Autism spectrum disorder	↑ *Clostridial* species ↑ *Suttetrella* and *Desulfovibrio* species

Limitations of microbiome studies are related to unknowns if microbiota changes occur prior to disease onset, prodromal periods of disease, active disease processes. For the most part it is unknown if microbiota changes are causal to disease or are merely associated with most diseases.

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EVIDENCE FOR USE OF PROBIOTICS IN PEDIATRICS

What the evidence tells us about probiotic use for these conditions in children

Condition	Prevention*	Treatment*	Probiotic species	Typical daily dose (CFU/d)
Colic (breastfed)[6-8]	0	+	*Lactobacillus reuteri*	100 million
Atopic eczema[13-17]	+	0	*Lactobacillus rhamnosus, Lactobacillus paracasei, Bifidobacterium lactis*	3-6 billion
URI[9-12]	+	+	*Lactobacillus* and *Bifidobacterium* spp	2-10 billion
IBS[18-20]	Not studied	+	*L rhamnosus* VSL#3†	6 billion 450-900 billion
AAD[21-24]	+	+	*L rhamnosus, Saccharomyces boulardii*	20 billion
AID[23-29]	Not studied	+	*L rhamnosus, S boulardii, Bifidobacterium bifidum, Bifidobacterium infantis*	10 billion

AAD, antibiotic-associated diarrhea; AID, acute infectious diarrhea; CFU/d, colony forming units per day; IBS, irritable bowel syndrome; URI, upper respiratory infection.

* 0=no effect; +=positive effect.

†*Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium infantis, Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus paracasei, Lactobacillus bulgaricus,* and *Streptococcus hermophiles.*

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PRACTICE RECOMMENDATIONS FOR THE USE OF PROBIOTICS

› Recommend a trial of Lactobacillus reuteri for breastfed infants with colic. **A**

› Consider Lactobacillus and Bifidobacterium species for the prevention of upper respiratory infections (URIs) and to shorten the course of URI illness. **B**

› Do not recommend probiotics for the prevention of respiratory or gastrointestinal allergies. **A**

› Consider probiotics for the reduction of abdominal pain in pediatric irritable bowel syndrome, as well as to reduce diarrhea associated with antibiotic use and acute gastroenteritis. **A**

Strength of recommendation (SOR)

A Good-quality patient-oriented evidence
B Inconsistent or limited-quality patient-oriented evidence
C Consensus, usual practice, opinion, disease-oriented evidence, case series

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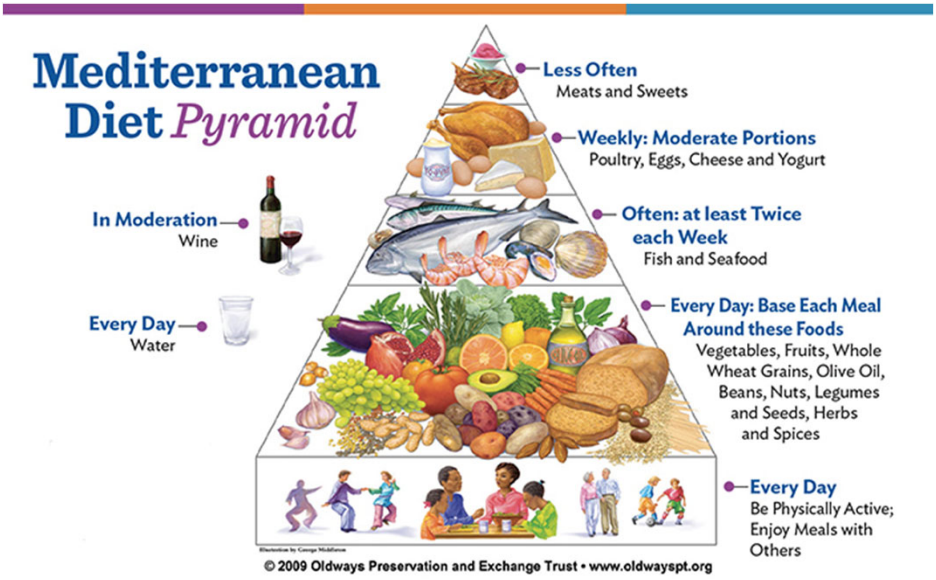
The Mediterranean Lifestyle



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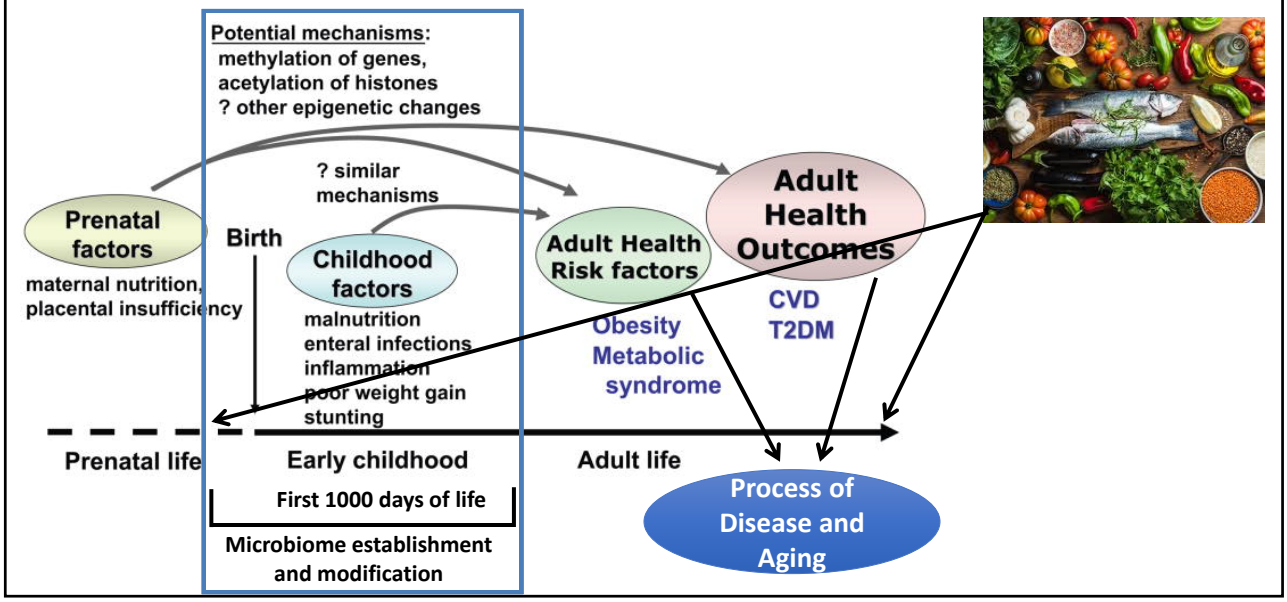
Modeling the Effects of The Mediterranean Diet On Human Health Through The Entire Lifespan



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Acknowledgments

The MIBRC Crew

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Medical research grants to improve the lives of children

Mead Johnson Nutrition

HORIZON 2020

Nutrition Obesity Research Center at Harvard

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