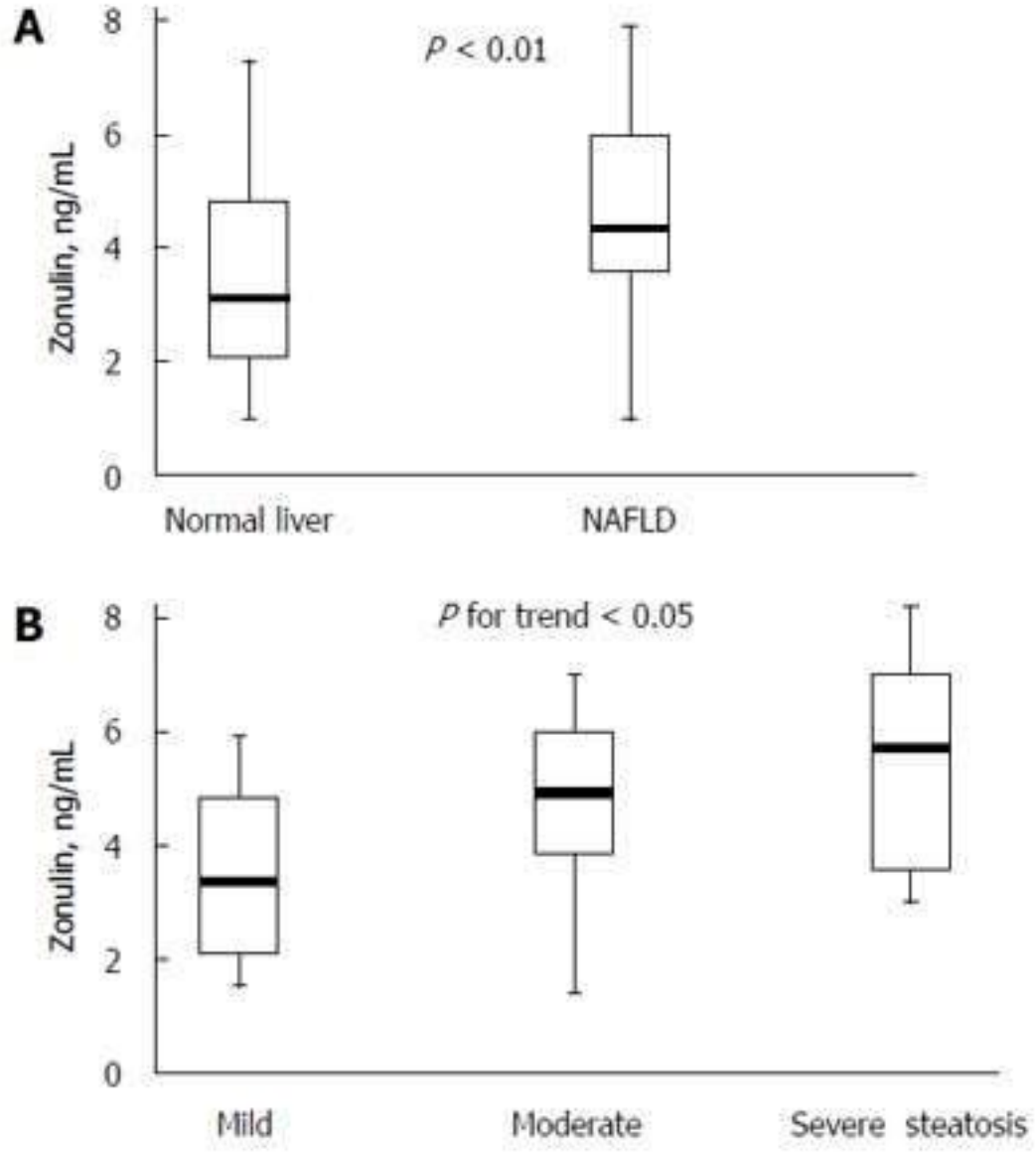
Increased Circulating Zonulin In Children With Biopsy-Proven Nonalcoholic Fatty Liver Disease



Zonulin levels for obese children.
A: Zonulin levels for obese children with and without nonalcoholic fatty liver disease (NAFLD);

B: Zonulin levels for obese children with NAFLD according to severity of steatosis.

World J Gastroenterol. 2014 Dec 7; 20(45): 17107–17114.

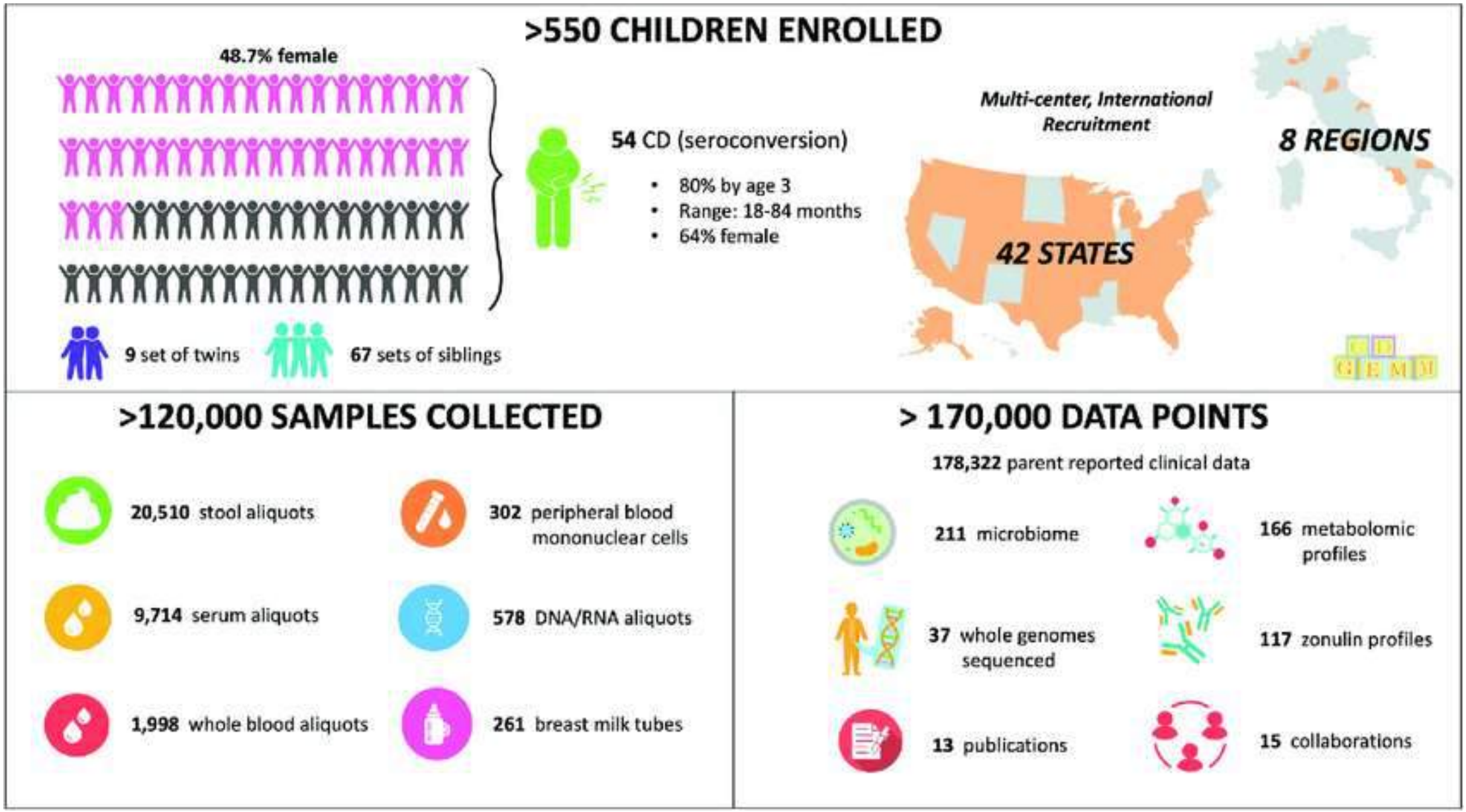
Autoimmunity:
The Celiac Disease Paradigm

- **Which Lesson Learned From Celiac Disease:**
- **The CD-GEMM Prospective Cohort to Link Gut Microbiome Composition and Function to Celiac Disease Pathogenesis And To Identify Early Biomarkers Of Disease Development**

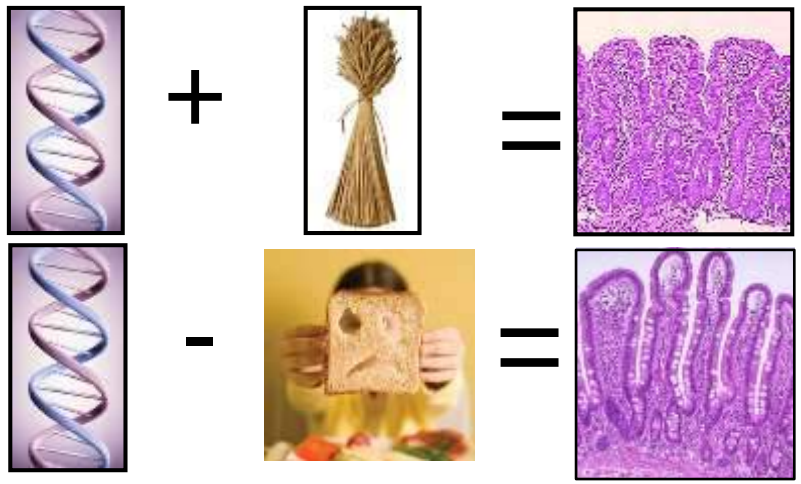
Celiac Disease Genome, Environment, Microbiome, and Metabolomic Studies

www.CDGEMM.org

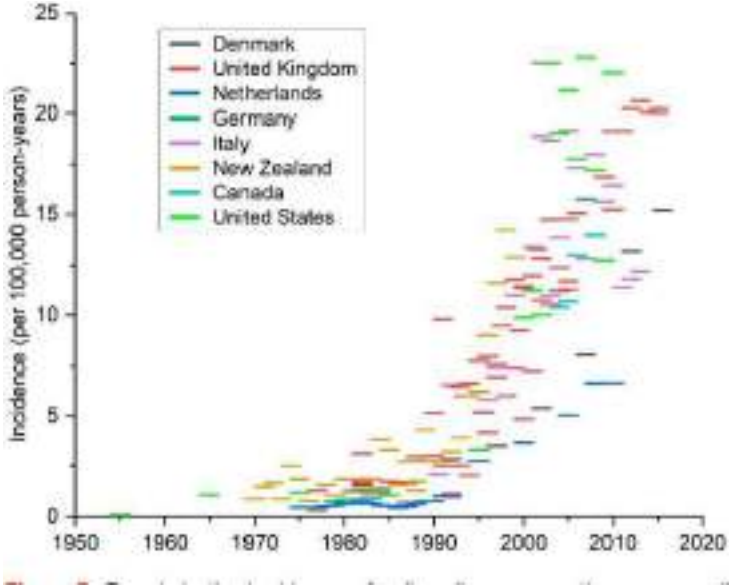
Overview of Cohort: CDGEMM study

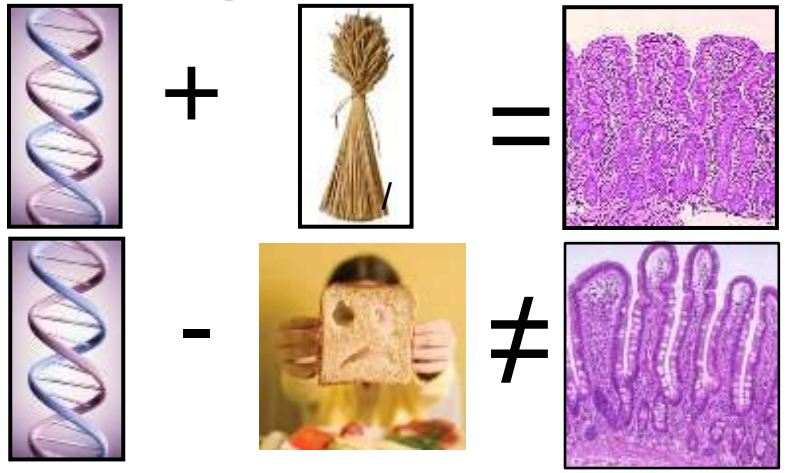


CD Pathogenesis: More Than Genes + Environment Paradigm

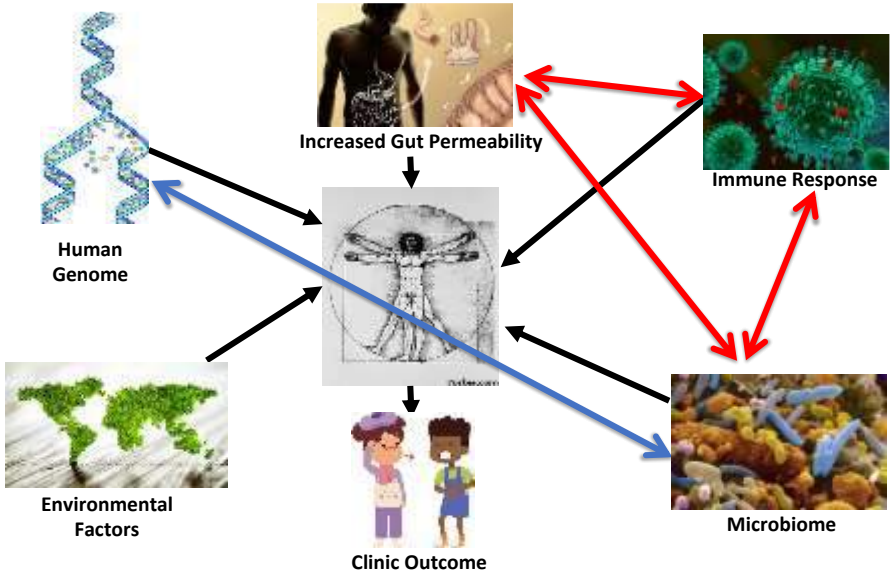


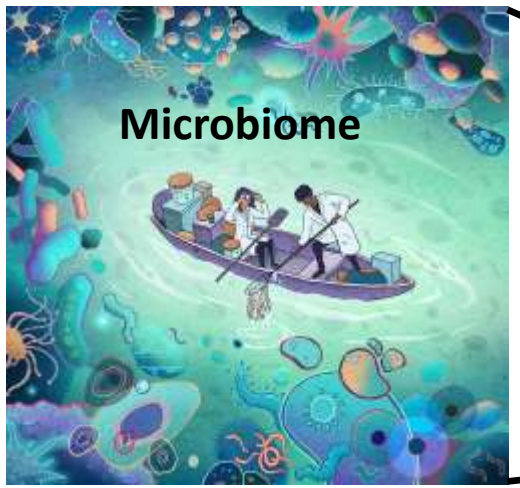
Necessary and Sufficient

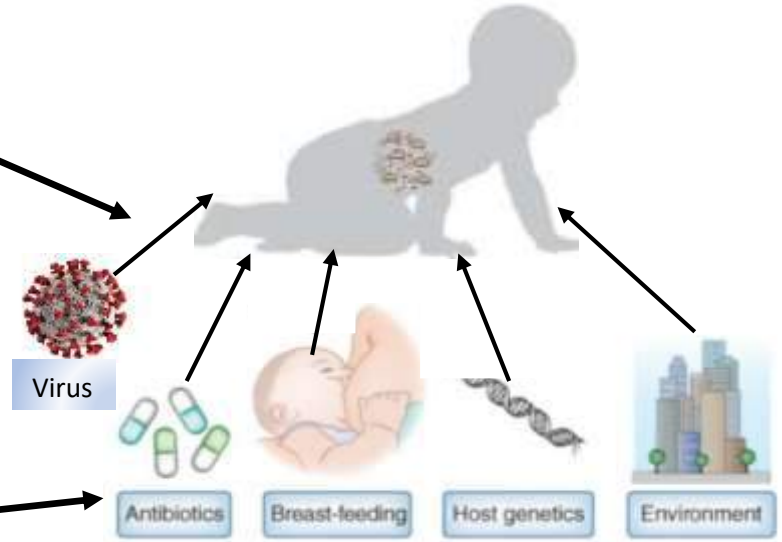




Necessary But Not Sufficient







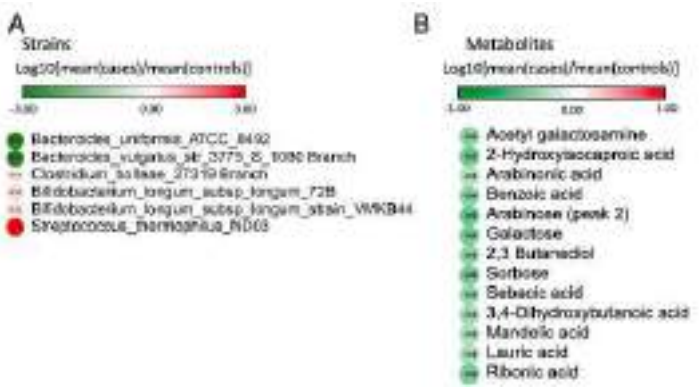
Microbiome signatures of progression toward celiac disease onset in at-risk children in a longitudinal prospective cohort study

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]

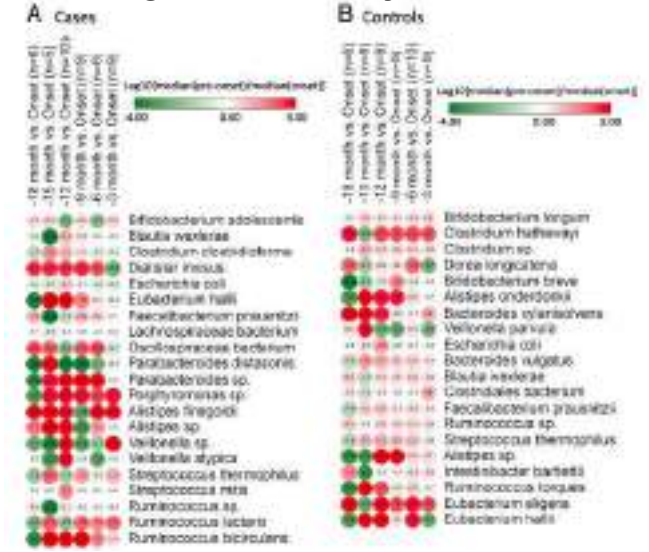
Significance

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

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

Longitudinal Metagenomic Analysis Identifies Bacterial Species, Pathways, Metabolites More Abundant In Controls Prior to Disease Onset

1. ***Bifidobacterium longum*** 
   - Increase IL-10 production and decrease the production of inflammatory cytokines
   - Associated with protection against gliadin-induced enteropathy in animal models

2. ***Bifidobacterium breve*** 
   - Linked to protection against NEC and allergic disease

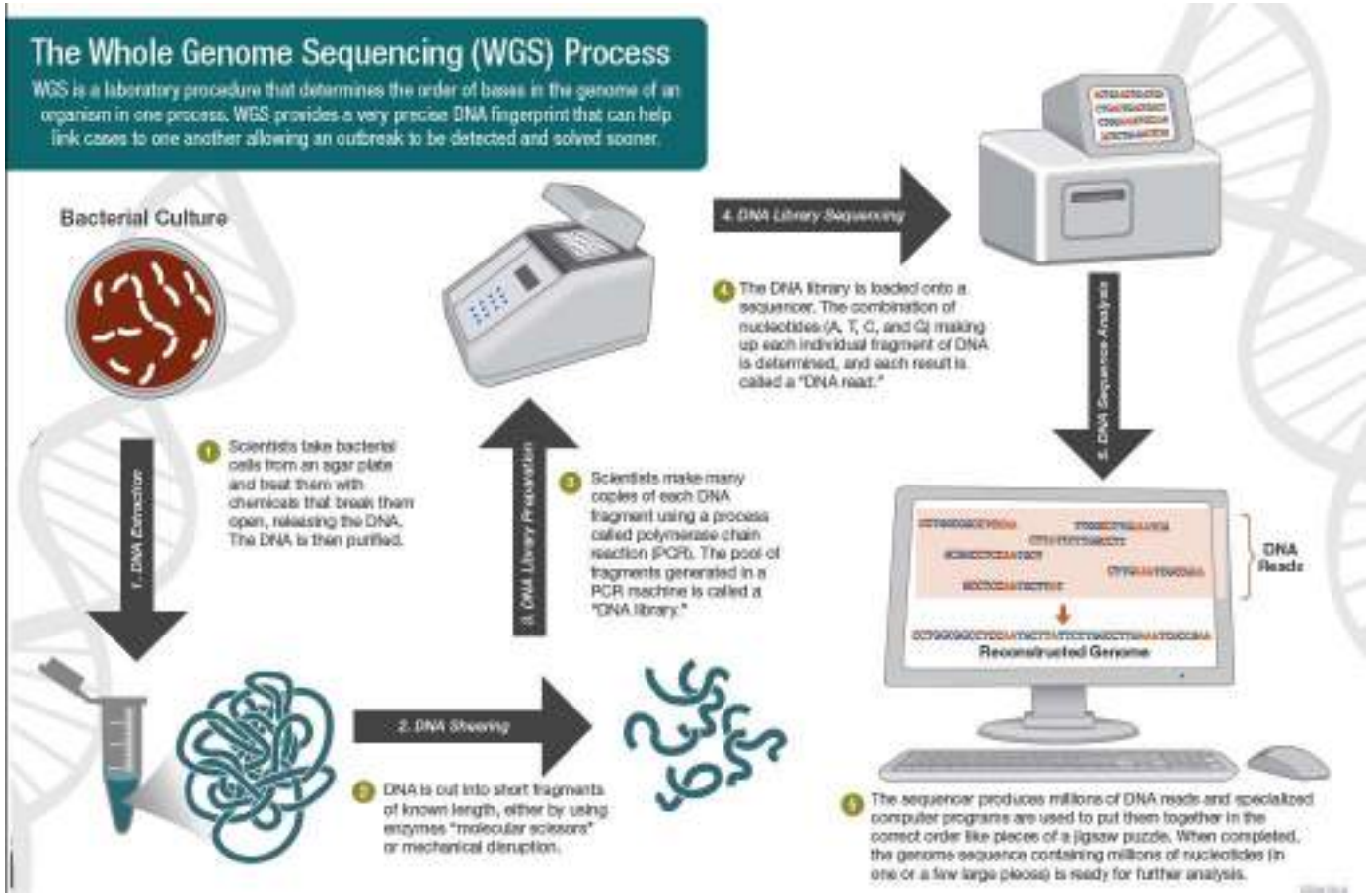
3. ***Bifidobacterium thetaiotaomicron*** 
   - Reported to be protective in a preclinical model of IBD

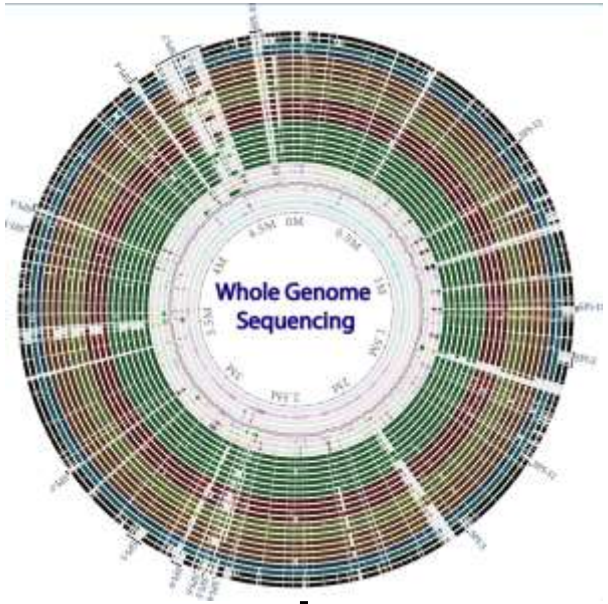
4. ***Bacteroides vulgatus*** 
   - Protects against LPS-induced inflammation
   - Mitigate inflammation in the DSS model of gut inflammation in mouse model

5. ***Bacteroides uniformis*** 
   - Improve immune defense mechanisms in a preclinical model of obesity

Laparra JM, Plosone, 2012.
Wong CB et al. Nutrients 2019
Klemenak M et al. Dig Dis Sci 2015
Sijia L. et al. J Immunol Res 2021

Microbiome Bacterial Strains Enriched in Controls But Missing in CD Cases: Whole Genome Sequencing

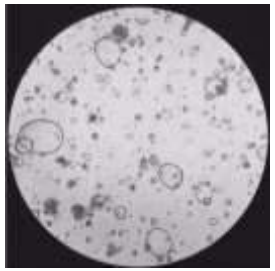




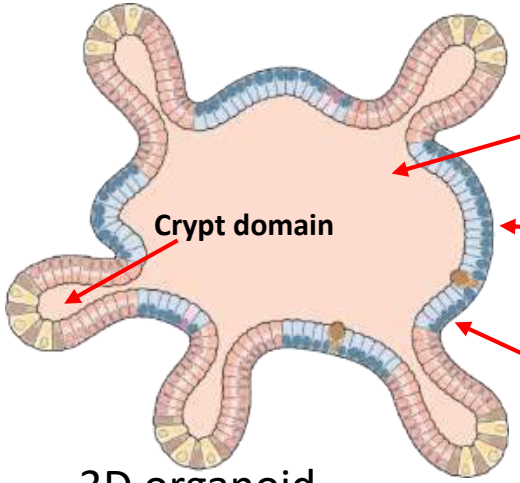
- **The 5 strains sequenced showed ~2% mutation compared to reference strains**
- **Among other genes, the mutations involved genes controlling host inflammatory pathways and barrier function**

2D Model Human Gut Organoids From Healthy Controls (HC), Potential Celiac Disease (PCD) and Active Celiac Disease (CD)

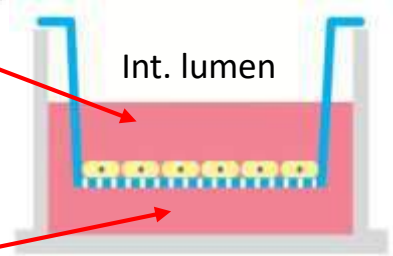
3D duodenum organoids
(7 days in culture)



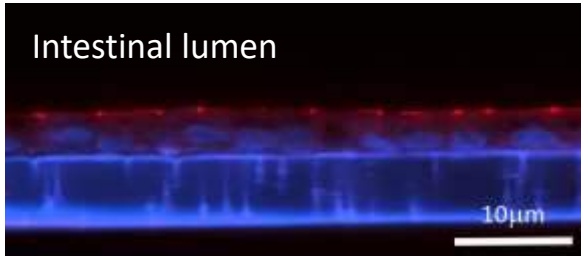
Single cells plated

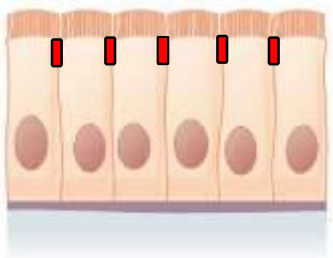


3D organoid



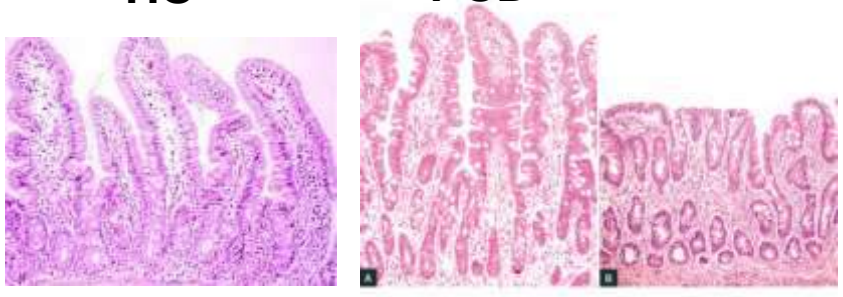
2D organoid monolayer development and differentiation (9 days culture)





ZO1 Tight Junction protein (RED) at cell-cell contact- Cell Nuclei (Blue)

HC **PCD** **CD**

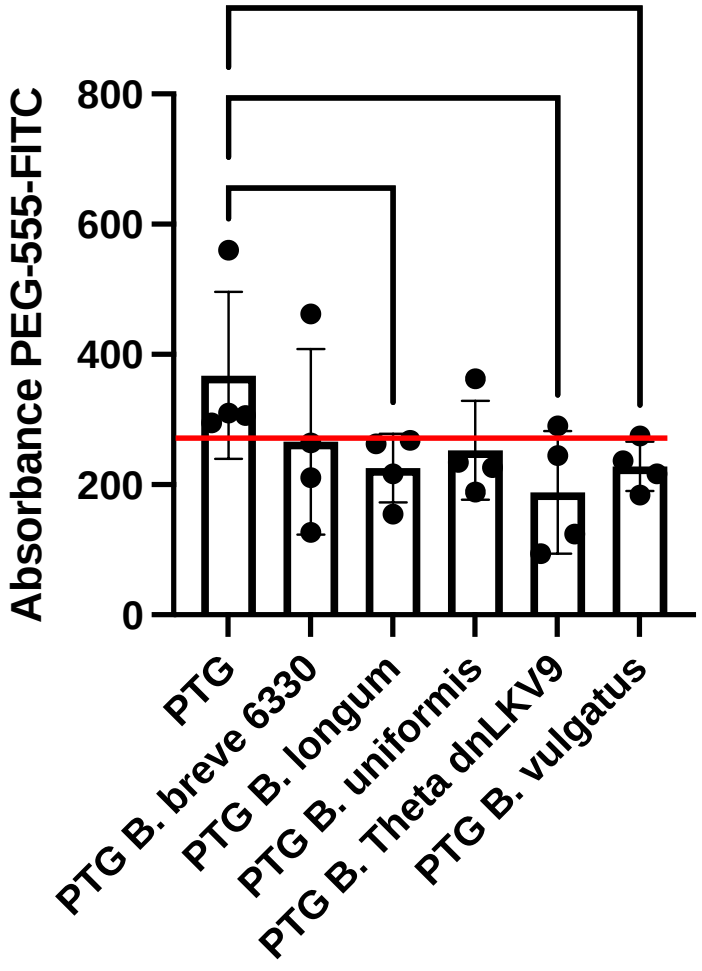


TEER
Dextran FITC } Permeability
Cytokines secretion
Gene expression and miRNA epigenetic changes
Cytotoxicity

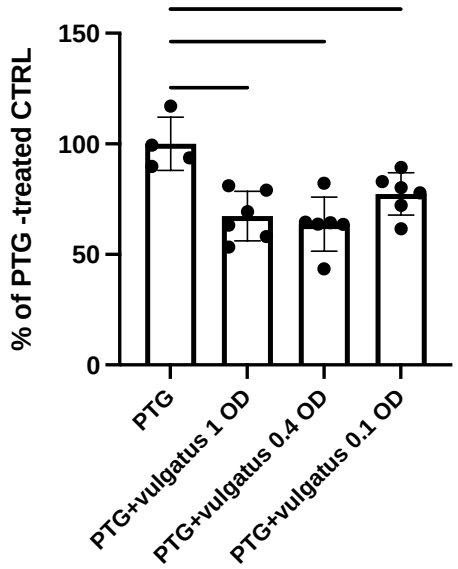
Organoid-derived monolayers developed in polarized cells, with readable TEER, form tight junctions based on ZO1 staining

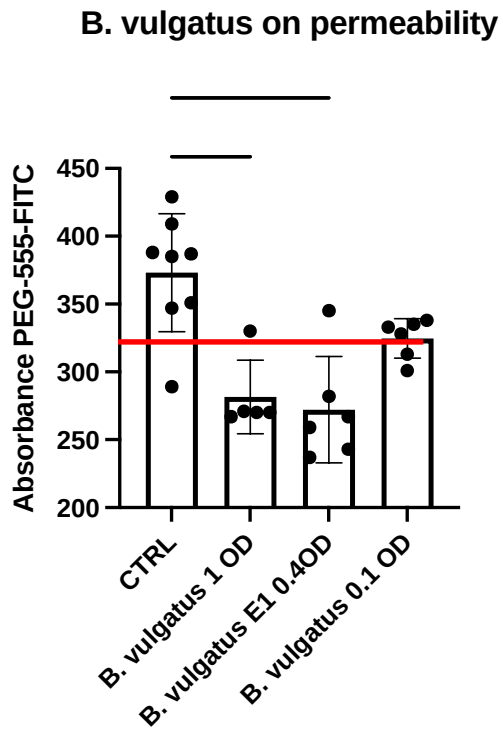
Probiotic Strains Isolated From Healthy Controls Protect Against PTG-Induced Cytotoxicity and Increased Permeability In PCD Human Gut Organoids

Paracellular permeability evaluated by passage of FITC-PEG550 across monolayers in PCD coculture upon 10% CFS treatment before being exposed to PTG. ANOVA (*) P<.05, (**) P<.01



Dose dependent effect of *B. vulgatus* CFS on coculture cell viability exposed to PTG and on epithelial paracellular permeability. ANOVA test (*) P<.05, (**)P<.01, (***)P<.001, (****) P< .0001.





Effect Of *B. Vulgatus* on PCD Gut Organoids miRNA Epigenetic Changes

Target Prediction: TargetScan Human 8.0

Gene set enrichment analysis: EnrichR – KEGG 2021 Human database

Signaling pathways regulating pluripotency of stem cells

miRNAs	Target genes
Let-7-5p	HOXD1;ONECUT1;ACVR1B;IGF1R;NRAS;ACVR1C;AKT2;SMARCAD1;DVL3;HOXA1;WNT1;SKIL;SMAD2;APC2;FZD3;FZD4;PCGF3;STAT3;WNT9B;IGF1;WNT9A;DUSP9;ACVR2B;ACVR2A;MEIS1;PIK3CA;HAND1;HOXB1;BMPR1A
miR-15-5p	GSK3B;WNT2B;PIK3R1;FGF2;IGF1R;AKT3;OTX1;JARID2;SKIL;WNT4;WNT10B;MAP2K1;ZFHX3;SMAD3;FZD4;WNT3A;PCGF5;FZD6;WNT7A;IGF1;AXIN2;SMAD5;ACVR2B;ACVR2A;APC;KRAS;TCF3;RAF1;BMPR1A;FGFR1
miR-128-3p	GSK3B;BMPR2;PIK3R1;BMI1;ACVR1C;DVL2;MAPK1;SMAD2;FZD3;ZFHX3;WNT3A;PCGF3;AXIN1;SMAD9;LIFR;PAX6;IGF1;KLF4;MAPK14;ISL1;SMAD5;ACVR2A;PIK3CA;KAT6A;COMMD3-BMI1;ID3;GRB2
miR-223-3p	ZFHX3;APC;FZD4;KAT6A;LIF;PAX6;IL6ST;FGFR2;ACVR2A;IGF1R
miR-145-5p	FLT1;CACNA1D;CRKL;IGF1R;ELK4;FGF5;RPS6KA3;PPP3CA;RPS6KA6;NRAS;ERBB3;ERBB4;PDGFD;AKT3;FLNB;MAP4K4;MAP3K5;MAP2K6;MAP4K2;MAP3K3;TGFB2;MAP3K1;ANGPT2;PLA2G4A;DUSP6;TGFBR2;EFNA1;CACNB2;EFNA3;TAOK1;RASA1;RASA2;RAPGEF2;CRK;SOS2;MAP3K11;FGF10

IGF1R: 4/5 (no: miR-128-3p)
IGF1: 4/5 (no: miR-145-5p)
ACVR2A: 4/5 (no: miR-145-5p)

Epithelial Cell Turnover And Pluripotency

MAPK signaling pathway

miRNAs	Target genes
Let-7-5p	PDGFB;FASLG;NLK;CACNA1E;RASGRP1;DUSP16;IGF1R;ELK4;FGF5;RPS6KA3;PPP3CA;CACNA1I;NRAS;PAK1;MAPK8;AKT2;CASP3;MAP4K3;CACNG4;MAP4K4;DUSP4;MAP3K2;MAP3K3;MEF2C;MAP3K1;CHUK;DUSP1;INSR;IGF1;NGF;DUSP9;TGFBR1;DUSP7;CACNB4;TAOK1;FAS;TAB2;MAP3K13;TP53;CRK
miR-15-5p	PTPRR;CSF1;FLT3;FGF1;FGF2;CACNA1E;CRKL;IGF1R;ELK4;IKBKB;RPS6KA3;CACNA1I;RPS6KA6;FGF7;MAPK8;FGF9;MKNK1;AKT3;GNA12;KDR;PAK2;MAP3K4;MAP2K6;MAP2K3;MAP3K3;MAP2K1;BDNF;CACNA2D1;INSR;NFATC3;IGF1;GADD45G;VEGFA;CDC25B;CACNB1;PPM1A;MRAS;TAOK1;FGF18;NF1;RAP
miR-128-3p	GEF2;KRAS;RAF1;LAMTOR3;CRK;SOS2;FGFR1
	CSF1;CACNA1A;CRKL;RAP1B;RPS6KA5;RRAS;MKNK2;MAPK1;MAP2K7;CACNG2;MAP3K4;MAP3K2;NGFR;DUSP5;PDGFRA;INSR;HGF;CACNA2D3;VEGFC;IGF1;GNG12;MAPK14;MAPK8IP3;TGFBR1;TGFBR2;MAPK10;KITLG;EFNA2;MAPKAPK3;TAOK1;RASA2;NF1;RAPGEF2;GRB2;SOS1;MET;PTPN5

MAPK8: 3/3
CRK: 3/3
TAOK1: 3/3
INSR: 3/3
IGF1: 3/3

Barrier Function

mTOR signaling pathway

miRNAs	Target genes
Let-7-5p	PRKAA2;IGF1R;RPS6KA3;NRAS;AKT2;DVL3;SLC38A9;RICTOR;WNT1;FNIP1;RNF152;FNIP2;ATP6V1C1;FZD3;CHUK;MIOS;FZD4;INSR;WNT9B;TSC1;IGF1;WNT9A;PIK3CA;RRAGD;ULK2
miR-15-5p	GSK3B;SEH1L;WNT2B;CAB39;IRS1;PIK3R1;IGF1R;LRP6;IKBKB;RPS6KA3;RPS6KA6;DEPTOR;AKT3;SESN2;GRB10;RICTOR;RNF152;EIF4E;FNIP2;EIF4B;WNT4;WNT10B;MAP2K1;FZD4;WNT3A;INSR;STRADB;FZD6;WNT7A;TSC1;IGF1;RRAGA;ATP6V1B2;KRAS;RAF1;SGK1;SOS2;LAMTOR3

RPS6KA3: 2/2
RICTOR: 2/2
TSC1: 2/2
RNF152: 2/2
FNIP2: 2/2
IGF1R: 2/2
IGF1: 2/2
INSR: 2/2

Innate and Adaptive Immune Response

TGF-beta signaling pathway

miRNAs	Target genes
miR-145-5p	SMAD2;SMAD1;SMAD4;TGFB2;SMAD3;ROCK1;ZFYVE9;INHBB;ACVR1B;SMAD5;ACVR2A;TGFBR2;RPS6KB1;SP1;SKP1
miR-128-3p	SMAD2;BMPR2;TGIF2;SMURF2;SMAD9;GDF6;SMAD5;TGFBR1;ACVR2A;TGFBR2;GREM1;ACVR1C;RPS6KB1;SP1;MAPK1;ID3;NEO1
miR-152-3p	ACVR1;SMAD2;TGFB2;TGIF2;ROCK1;SMURF2;NOG;INHBB;GDF6;LTBP1;ACVR2B;SMAD5;RGMA;SKP1;FBN1

SMAD2: 3/3
SMAD5: 3/3
TGFB2: 2/3 (no: miR-128-3p)

Garrote JA 2008 Drug Target Insights 2008:3 1-11.
CD vs CTRL; intestinal biopsy, adult patients.

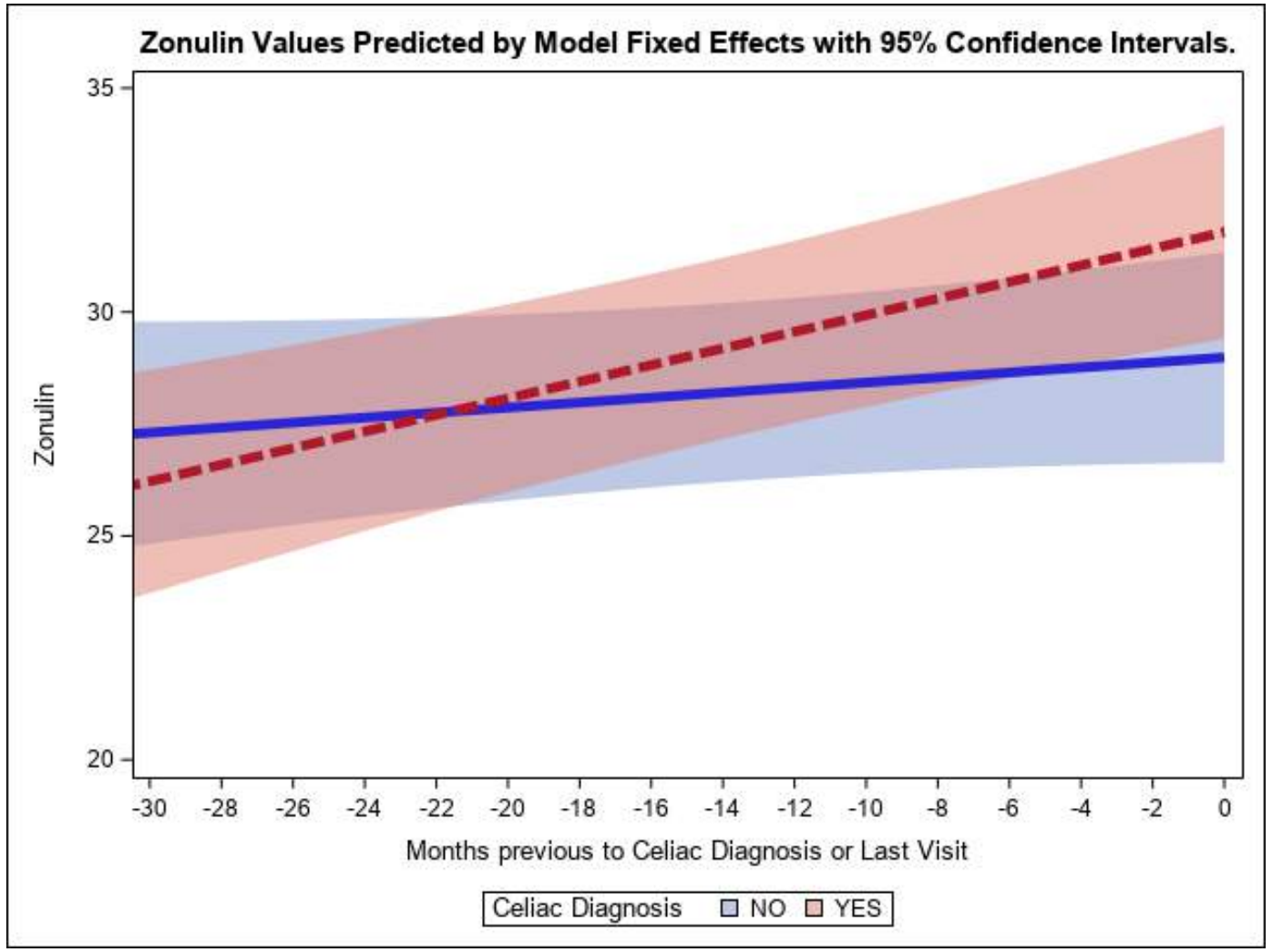
miR-146a-5p	**Target genes**
Autophagy	NRAS;PRKAA2;TRAF6;RRAGD;PIK3CB;ZFYVE1;ATG7
NF-kappa B signaling pathway	CARD10;IRAK1;TRAF6;XIAP;LTB
T cell receptor signaling pathway	NRAS;NFATC2;LCP2;PIK3CB;CD3D
Neurotrophin signaling pathway	NRAS;IRAK1;SORT1;TRAF6;PIK3CB

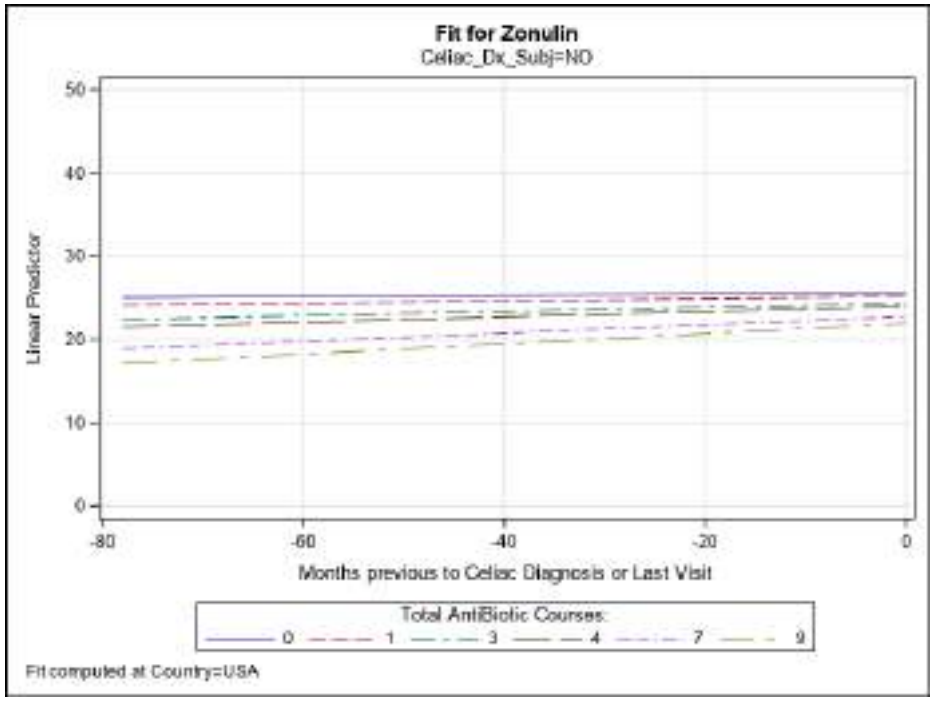
NRAS 3/4 (no in T cell Rec. pathway)
PIK3CB 3/4 (no in NFKb pathway)

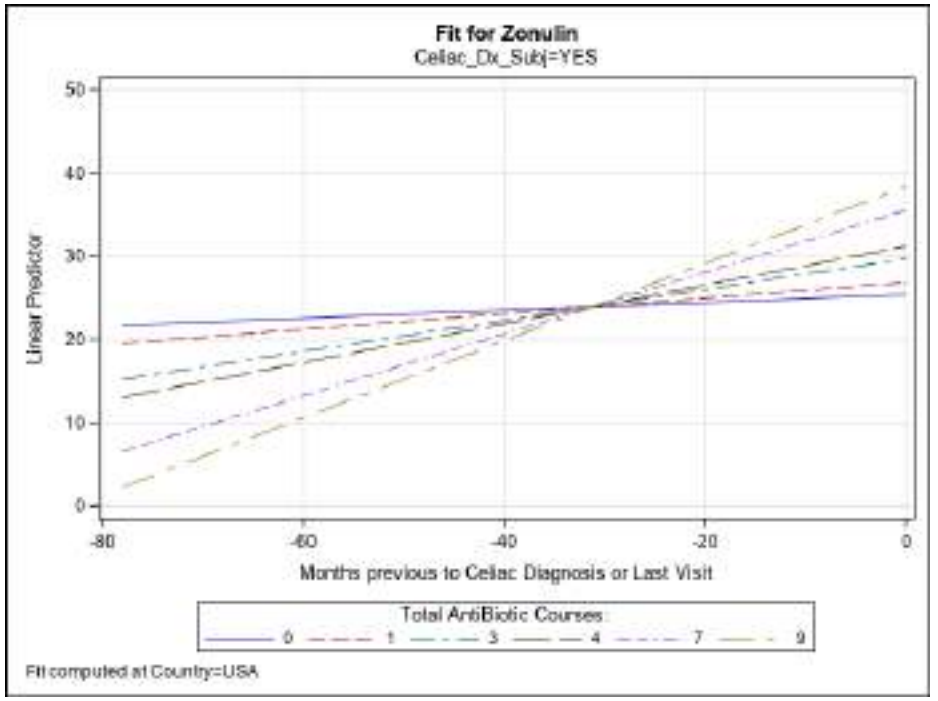
Autophagy
NF-kappa B signaling pathway
T cell receptor signaling pathway
Neurotrophin signaling pathway

maayanlab.cloud

Raising of Serum Zonulin Preceded CD Onset by 18 Months And Was Influenced By Antibiotic Exposure







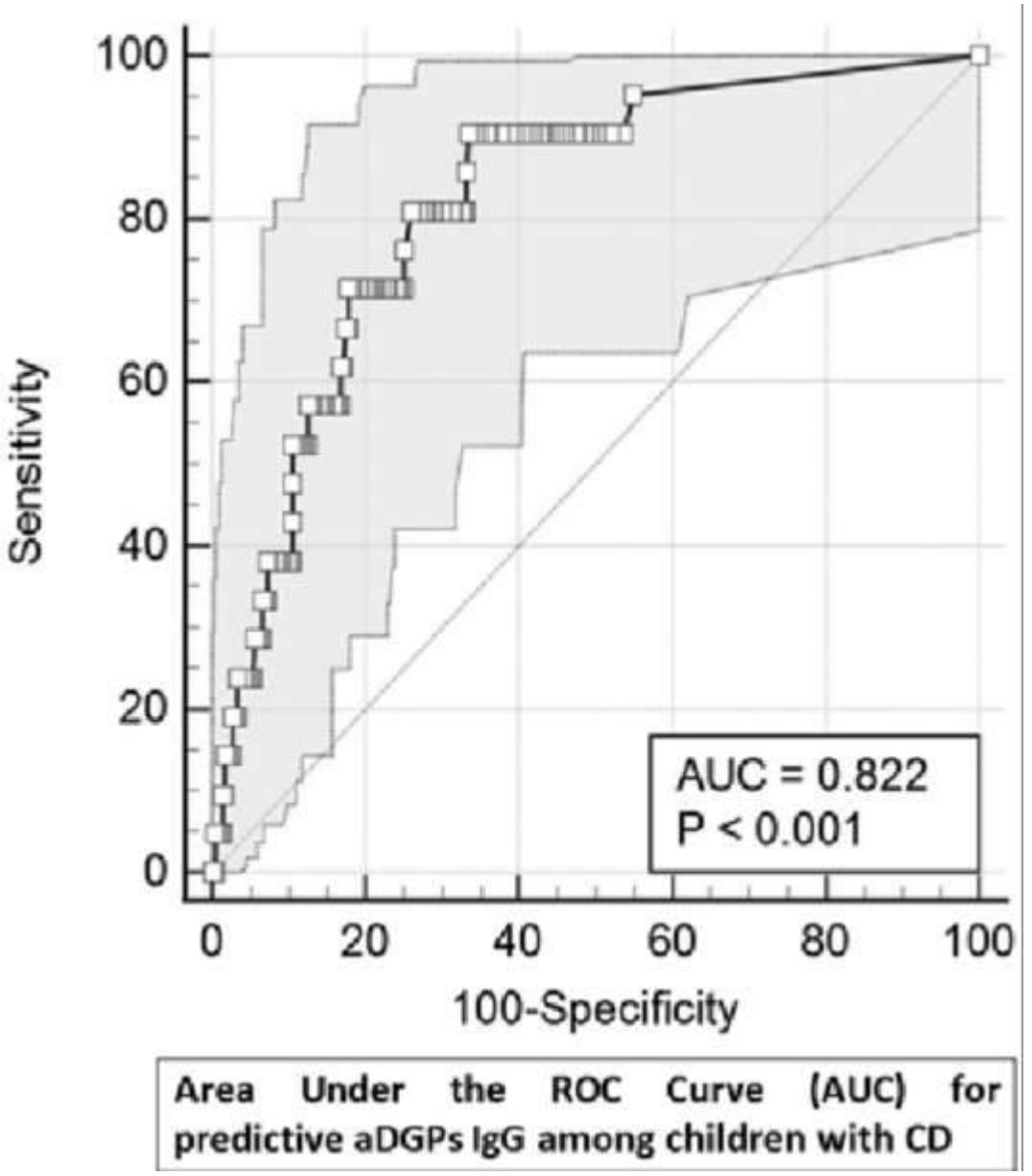
Raising of Serum Zonulin Was Followed By Breaking Tolerance To Gluten As Testified By DGP IgG Appearance 9 Months Before CD Onset

> Am J Gastroenterol. 2023 Mar 1;118(3):574-577. doi: 10.14309/ajg.0000000000002192. Epub 2023 Jan 20.

Early Antibody Dynamics in a Prospective Cohort of Children At Risk of Celiac Disease

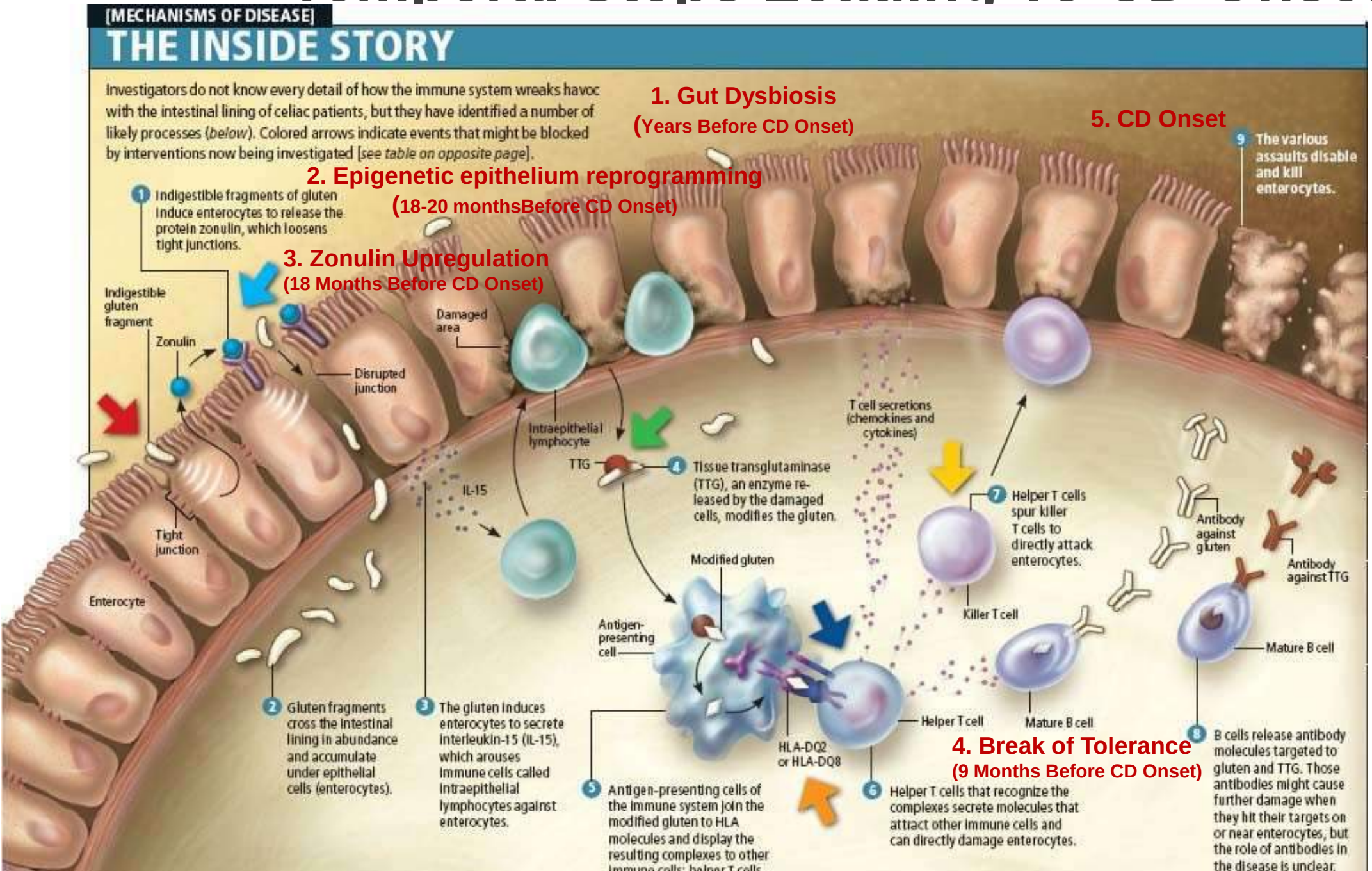
Francesco Valitutti [1 2], Maureen M Leonard [3 4], Victoria Kenyon [3 4], Monica Montuori [5], Pasqua Piemontese [6], Ruggiero Francavilla [7], Basilio Malamisura [8], Lorenzo Norsa [9], Angela Calvi [10], Maria Elena Lionetti [11], Mariella Baldassarre [12], Chiara Maria Trovato [13], Michela Perrone [6], Tiziana Passaro [8], Naire Sansotta [9], Marco Crocco [10], Annalisa Morelli [14], Lidia Celeste Raguseo [7], Federica Malerba [10], Luca Elli [15], Fernanda Cristofori [7], Carlo Catassi [11], Alessio Fasano [2 3 4]; CD-GEMM Team

Affiliations + expand



Area Under the ROC Curve (AUC) for predictive aDGPs IgG among children with CD

Temporal Steps Leading To CD Onset



Fasano A, Sci Am, 2009

Aging

Inflamm(Aging)

- **Research shows that even late in life, potential exists for physical, mental, and social growth and development.**

- **Recent scientific successes in rejuvenation and extending a lifespan of model animals give hope to achieve negligible senescence, reverse aging or at least significantly delay it.**

Theories of Aging

1. **The free radical theory (oxidative stress):** Free radicals can damage nucleic acids, proteins or lipids. For biological systems, oxygen free radicals are the most important, in particular superoxide ($\cdot O_2^-$), nitric oxide ($\cdot NO$) and the hydroxyl radical ($\cdot OH$).

2. **Cellular senescence and apoptosis theory:** The relationship between cellular aging and the aging of the whole organism is complex. Cellular "immortality" is essential for stem cells, but an "immortal" somatic cell is cancerous. Apoptosis is programmed cell suicide — a genetically ***controlled cell death*** that causes cells to shrink and be eliminated without the tissue traumas associated with inflammation that accompanies ***uncontrolled cell death*** (necrosis).

3. **The immune system theory of aging**: According to this theory, many aging effects are due to the declining ability of the immune system to differentiate "foreign" from "self" proteins. There is evidence that histocompatability genes, genes affecting DNA repair and genes for SOD production — all of which affect longevity — are located close together on human chromosome 6.

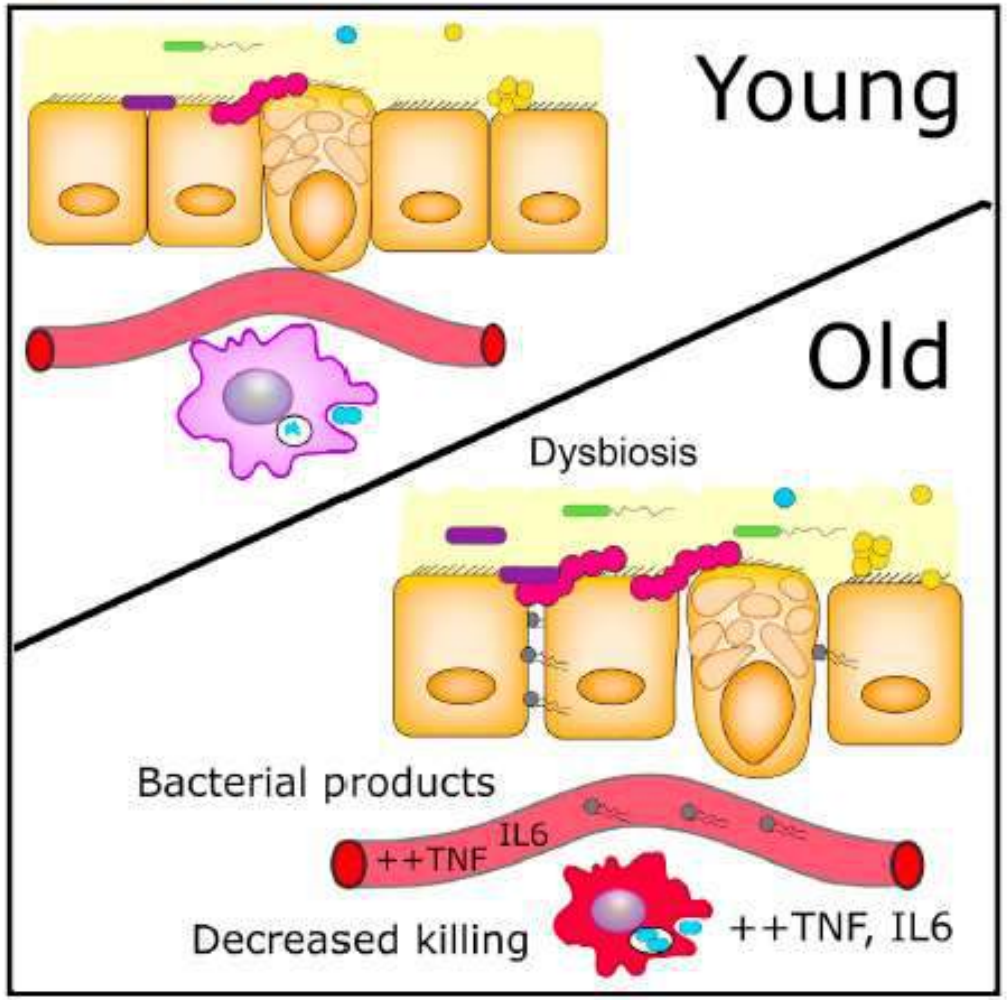
4. **Inflammation and aging:** With aging the body contains increasing quantities of pro-inflammatory cytokines. Aging is associated with increasing activity of the pro-inflammatory transcription factor NF-kB

5. **Intestinal permeability and aging:** Several reports both in animal models and humans link gut permeability to non-infective chronic inflammation leading to senescence. In fruit fly the increase in intestinal permeability is the best predictor of imminent death, even more than the actual age of the insect.

Cell Host & Microbe

Age-Associated Microbial Dysbiosis Promotes Intestinal Permeability, Systemic Inflammation, and Macrophage Dysfunction

Graphical Abstract



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In Brief

Systemic inflammation increases with age, but the underlying causes are debated. Using young and old germ-free and conventional mice, Thevaranjan et al. demonstrate that age-related microbiota changes drive intestinal permeability, age-associated inflammation, and decreased macrophage function. Reducing TNF levels rescues microbiota changes and protects old mice from intestinal permeability.

Changes In Microbiome Composition And Function With Aging