"From N=1 to N=100: What I Have Learned From Quantifying My Superorganism Body"

Institute for Systems Biology

Seattle, WA

March 20, 2014

Dr. Larry Smarr

Director, California Institute for Telecommunications and Information Technology

Harry E. Gruber Professor,

Dept. of Computer Science and Engineering

Jacobs School of Engineering, UCSD

http://lsmarr.calit2.net

Where I Believe We are Headed: Predictive, Personalized, Preventive, & Participatory Medicine

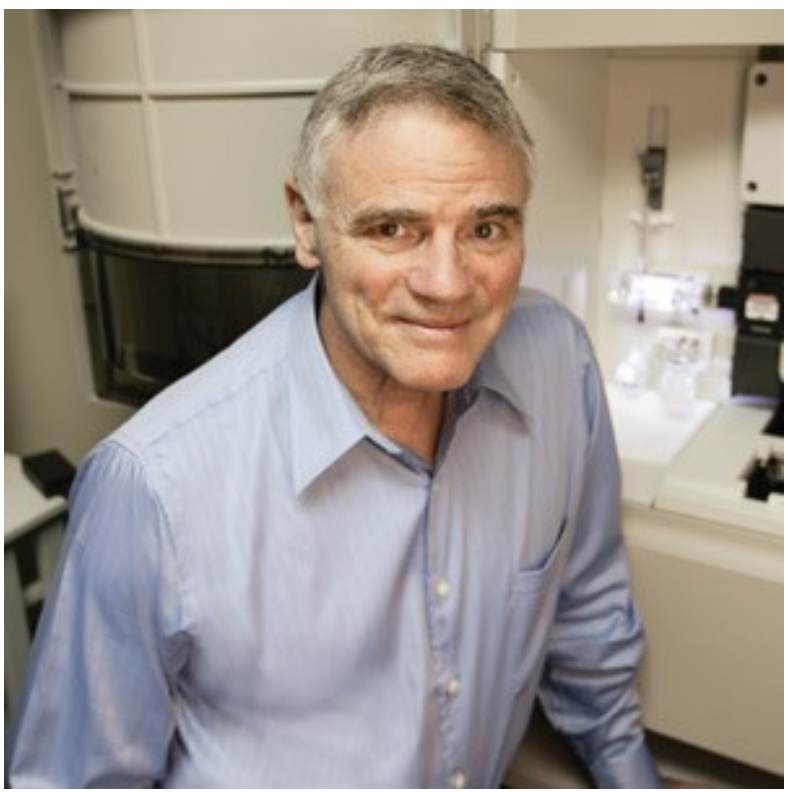
Newsweek

I am Lee Hood's Lab Rat!

A Doctor's Vision of the Future of Medicine

Leroy Hood
NEWSWEEK

From the magazine issue dated Jul 13, 2009



www.newsweek.com/2009/06/26/a-doctor-s-vision-of-the-future-of-medicine.html

I Arrived in La Jolla in 2000 After 20 Years in the Midwest and Decided to Move Against the Obesity Trend

Age 41



Age 51



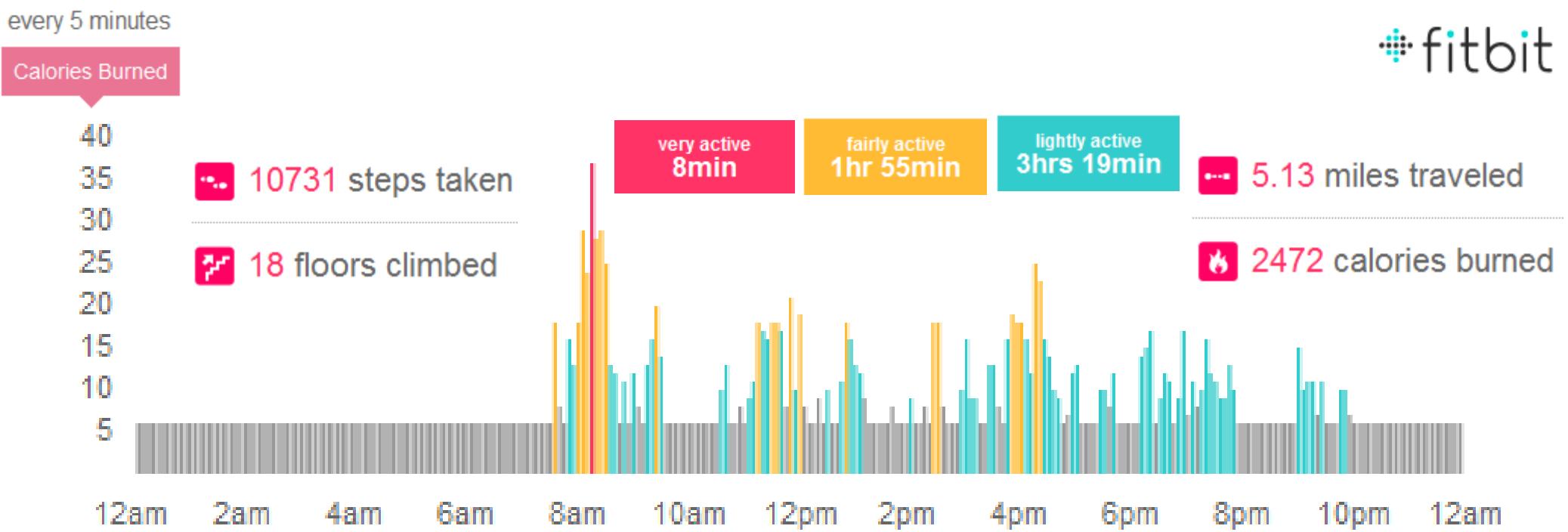
Age 61

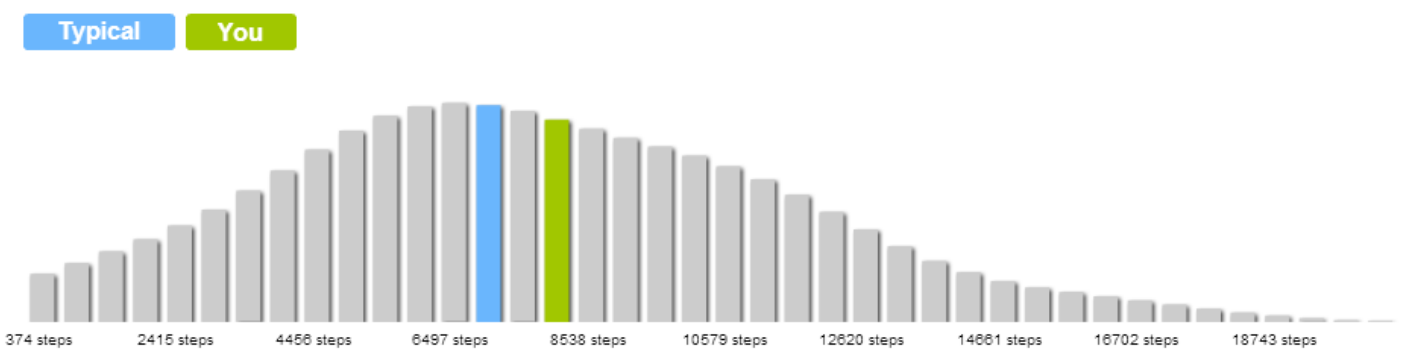


I Reversed My Body's Decline By Quantifying and Altering Nutrition and Exercise

http://lsmarr.calit2.net/repository/LS_reading_recommendations_FiRe_2011.pdf

Wireless Monitoring
Helps Drive Exercise Goals







This week you walked

8028 steps

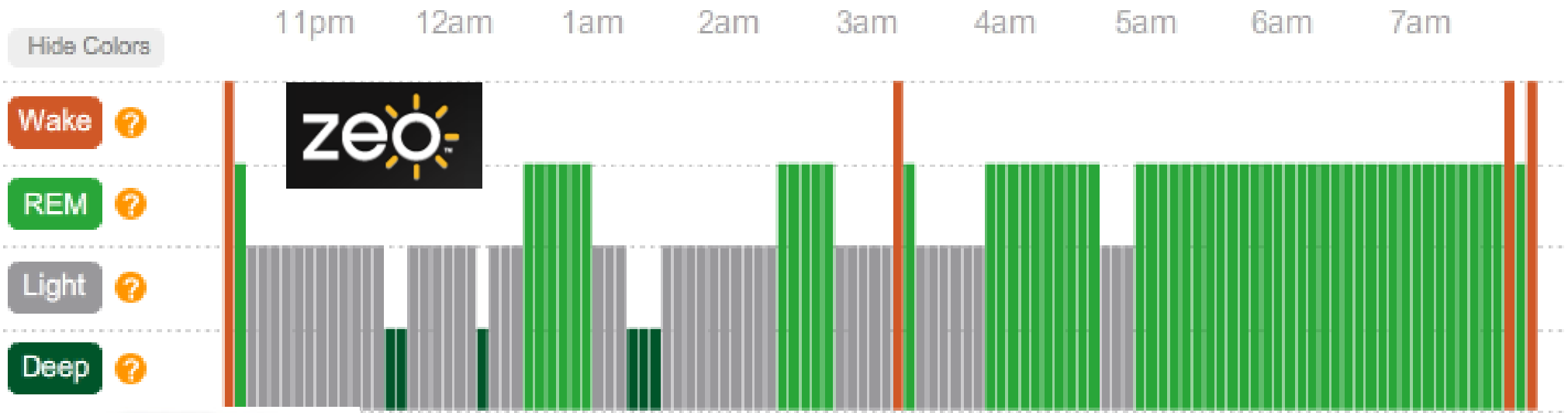
15% more than the median for men 55 to 64 yrs who are normal or underweight

You are in the

60 percentile

of all men 55 to 64 yrs who are normal or underweight

Quantifying My Sleep Pattern Using a Zeo - Increased My Average to 8 Hours/Night

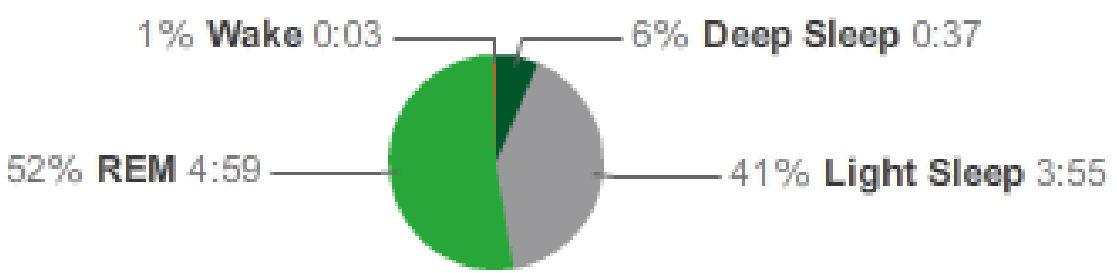




Stroke risk increased by sleeping less than six hours a night
-M. Ruiter, *Sleep* 2012

Total Z: 9 hr 31 min

Time to Z: 0 hr 2 min

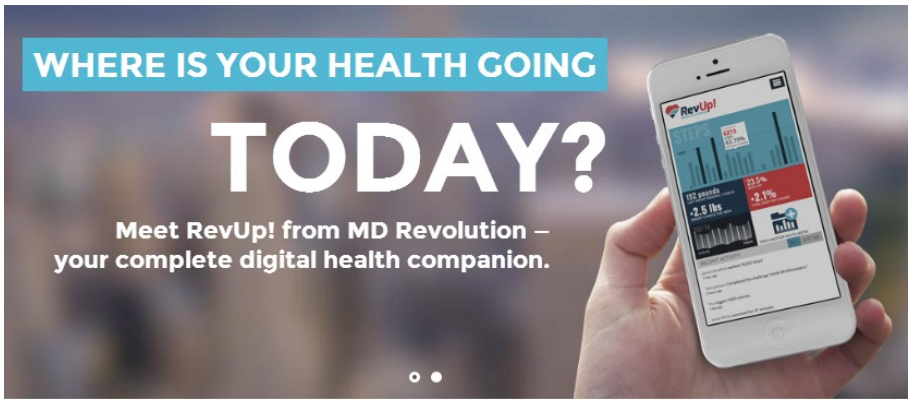


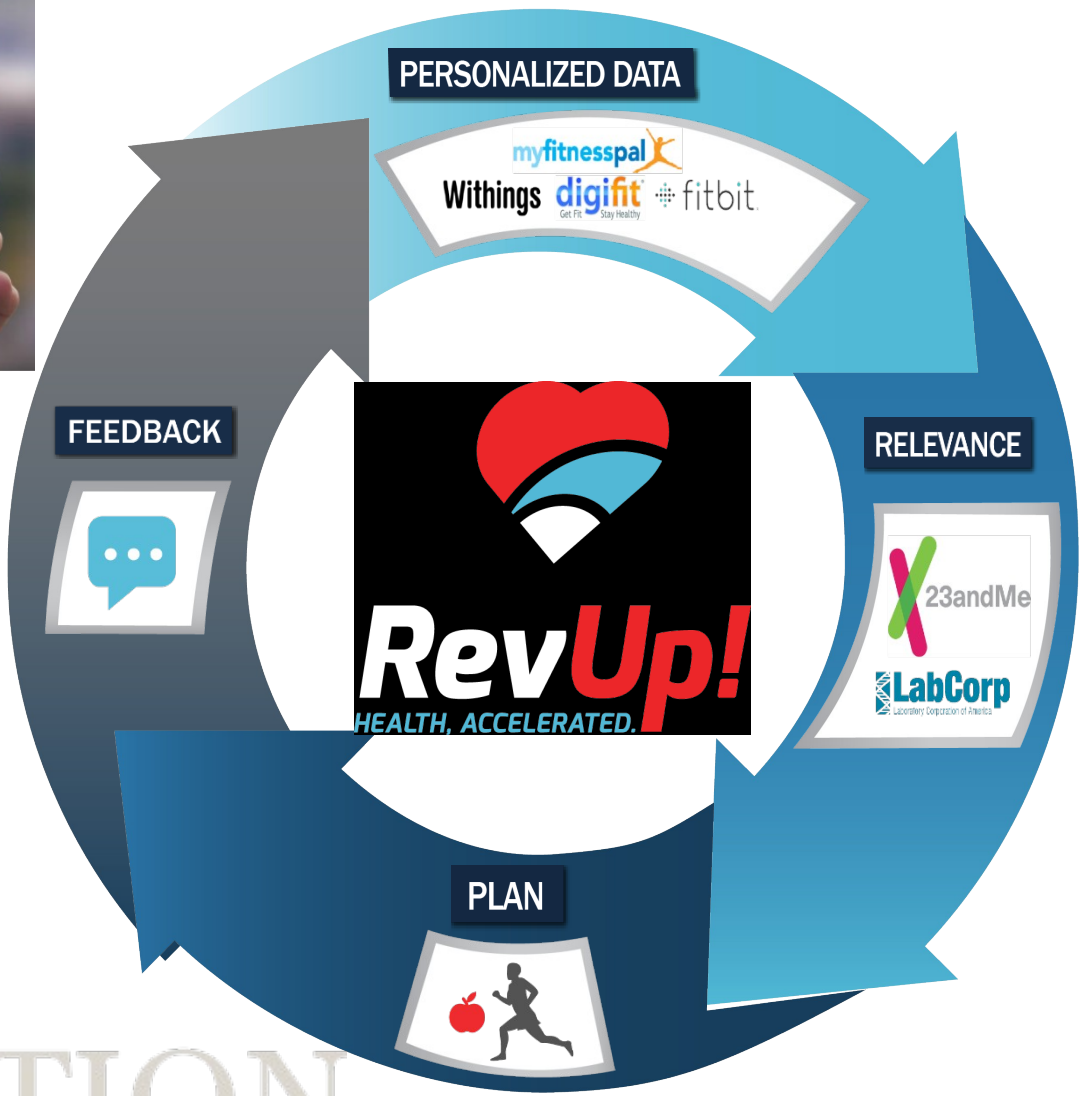
Why are these numbers different from the sleep graph above?

REM is Normally 20% of Sleep
Mine is Between 45-65% of Sleep

An Infant Typically Has 50% REM

MDRevolution's RevUp! Integrates a Variety of Sensors & Then Completes the Behavior Feedback Loop

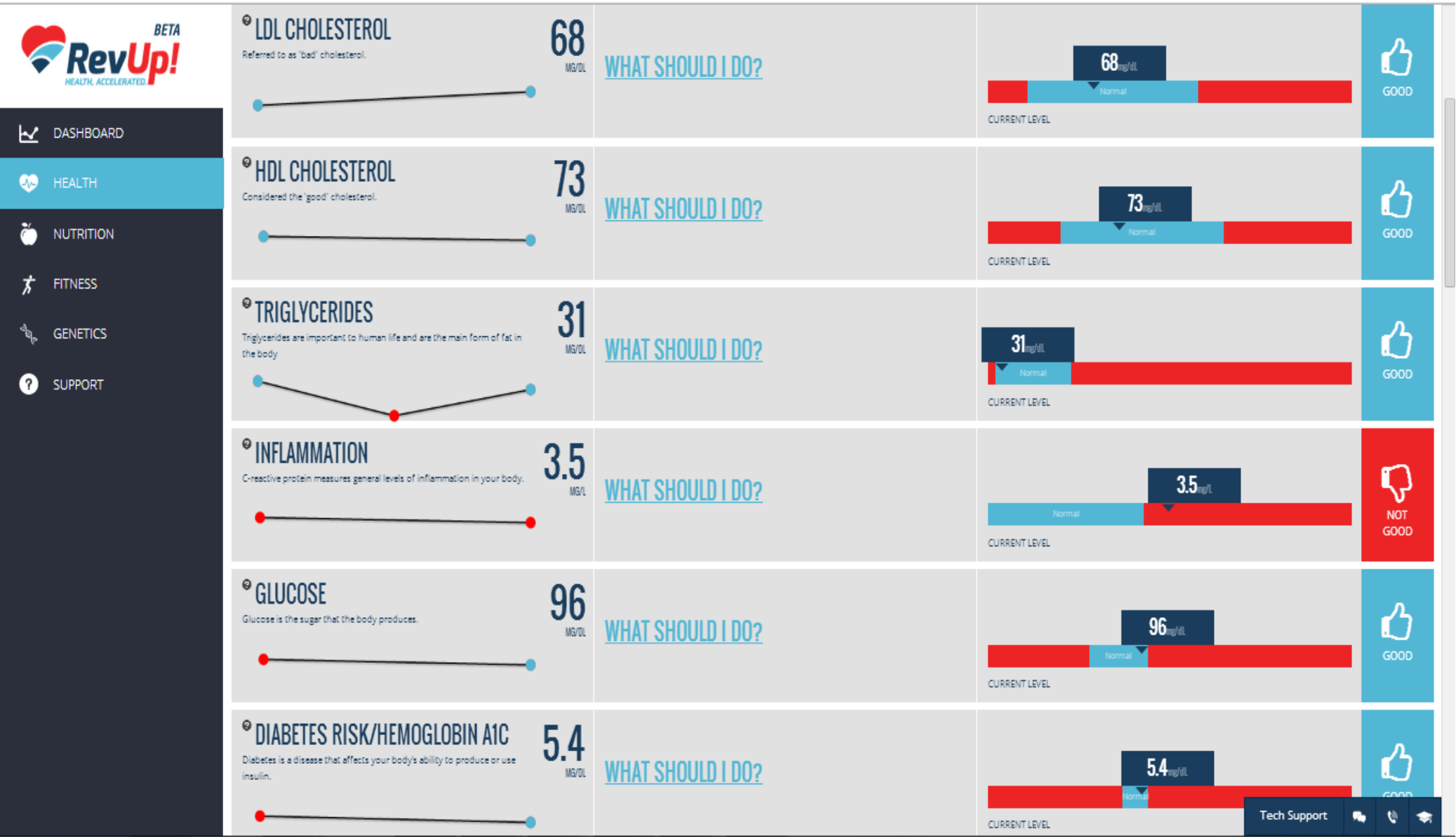




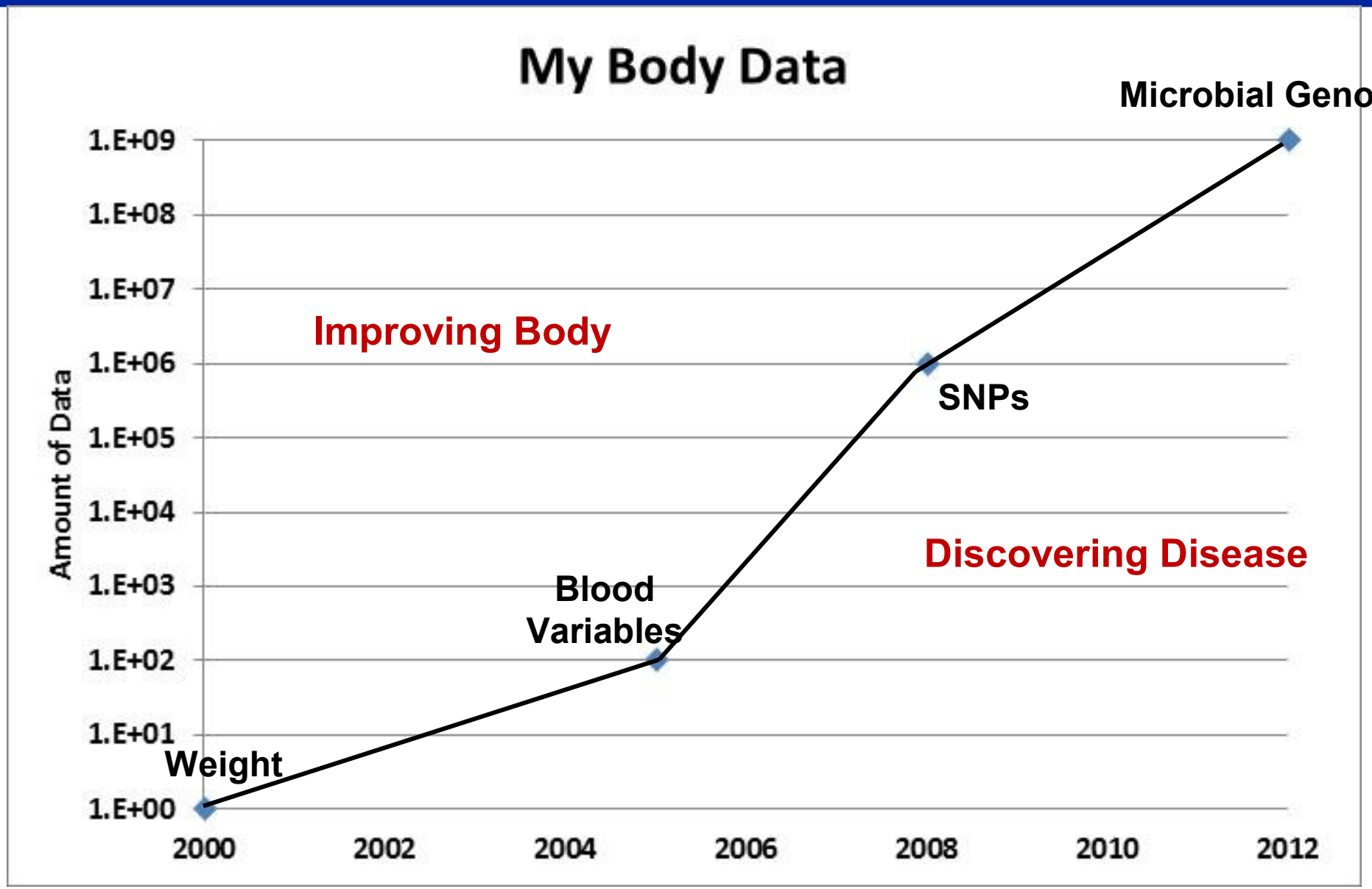
MD REVOLUTION

Source: Samir Damani, MD Revolution

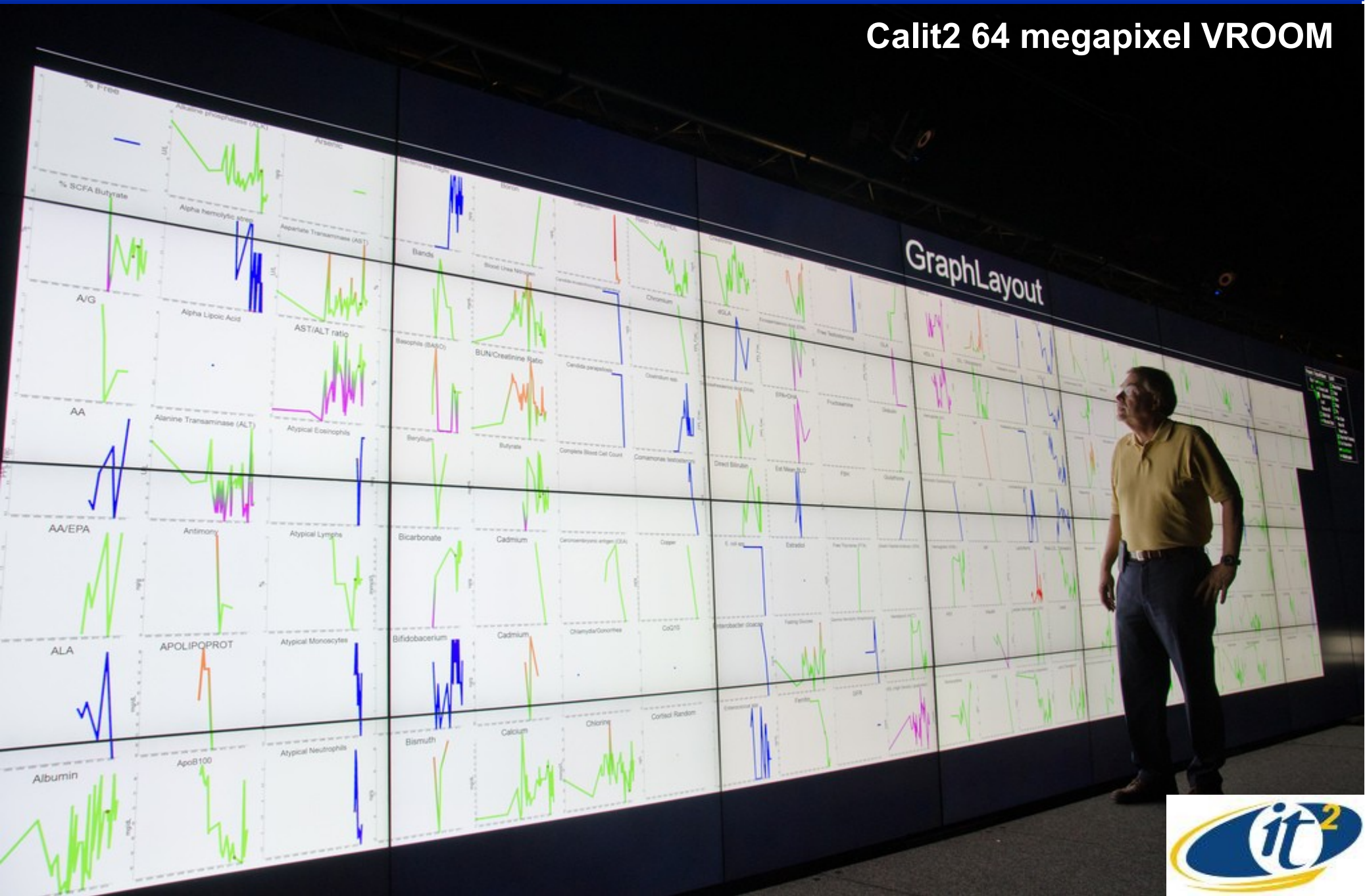
MDRevolution's RevUp!
Also Integrates Blood Variables and Genetics



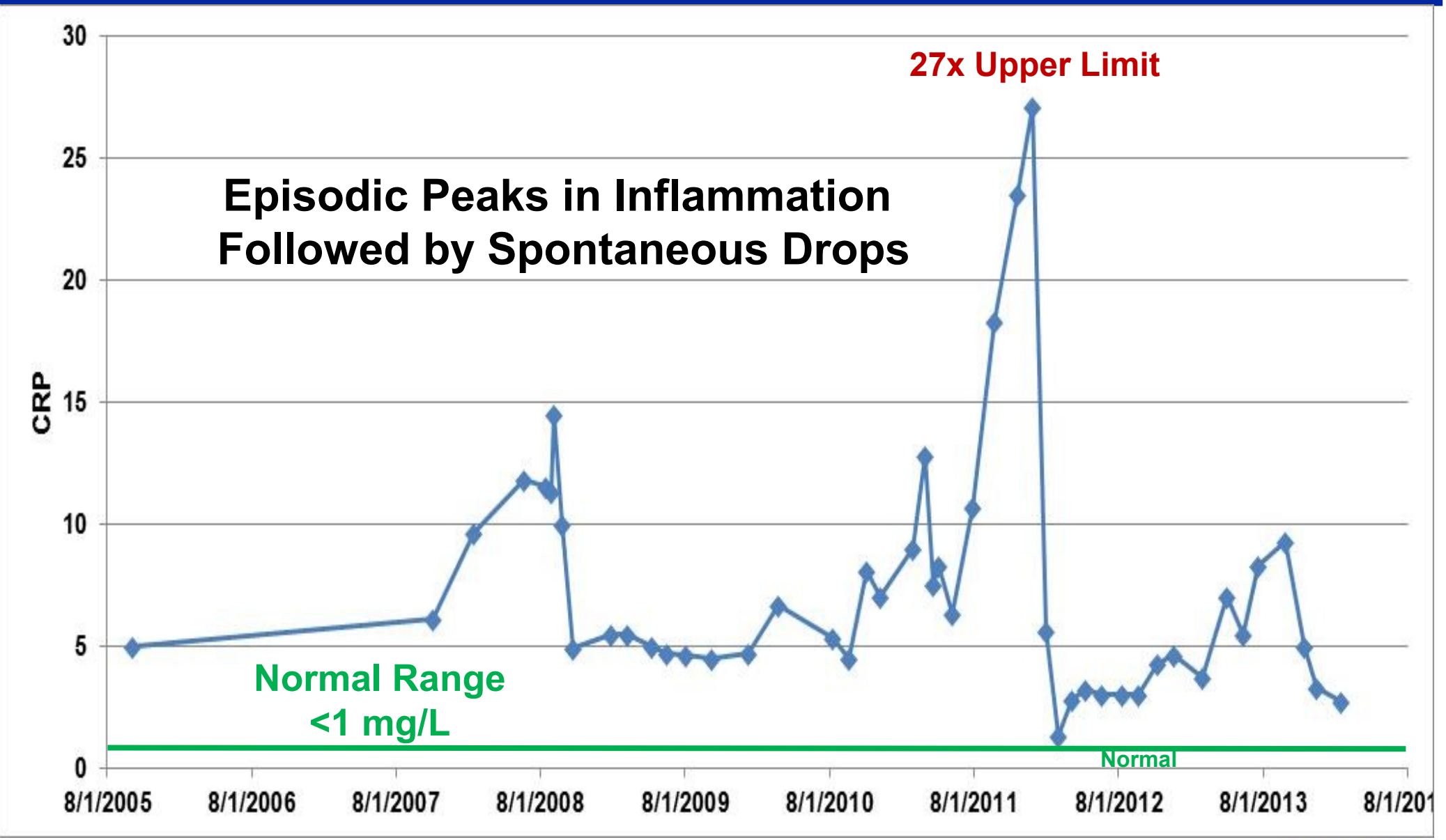
From One to a Billion Data Points Defining Me: The Exponential Rise in Body Data in Just One Decade!



Visualizing Time Series of 150 LS Blood and Stool Variables, Each Over 5-10 Years

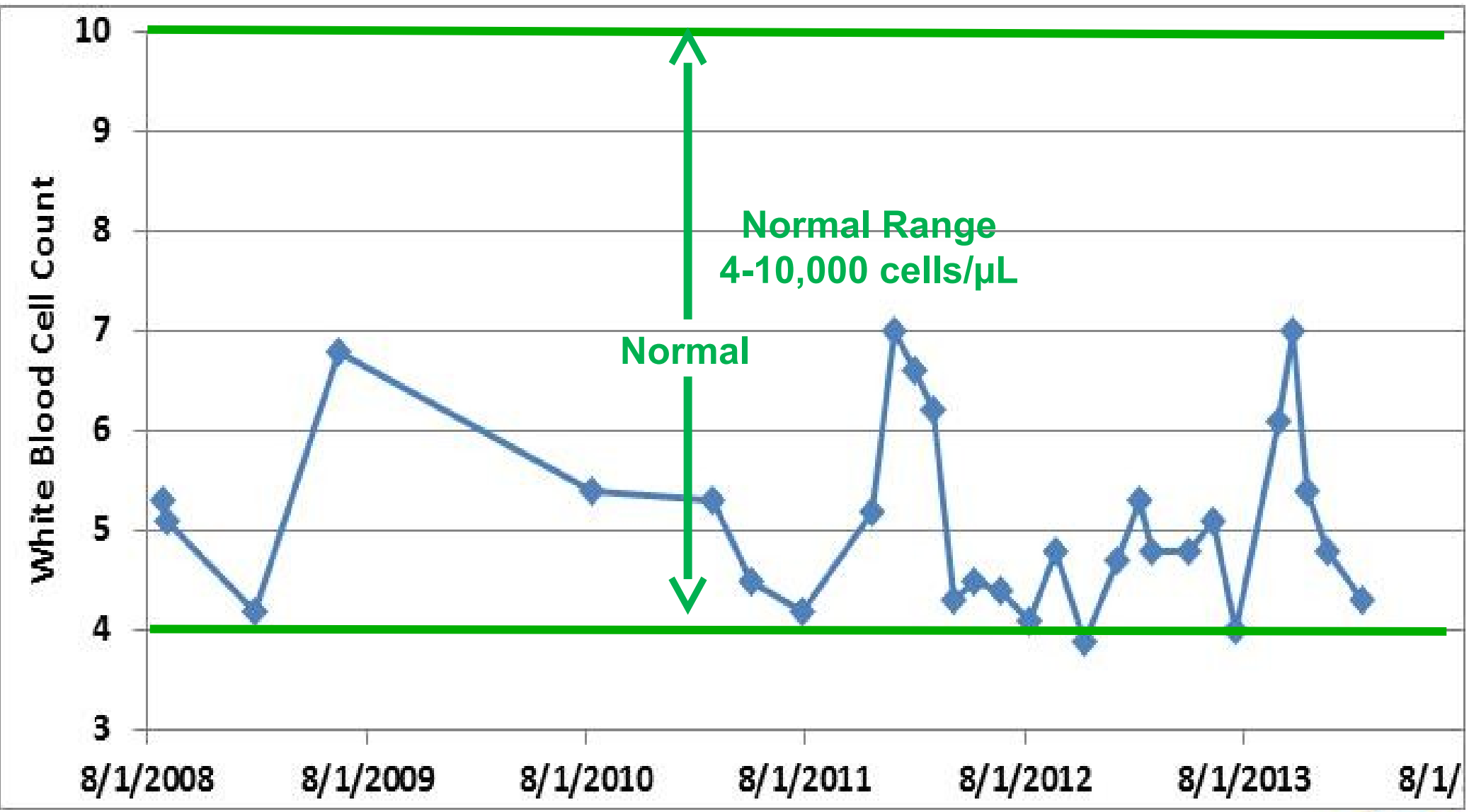


Only One of My Blood Measurements Was Far Out of Range--Indicating Chronic Inflammation

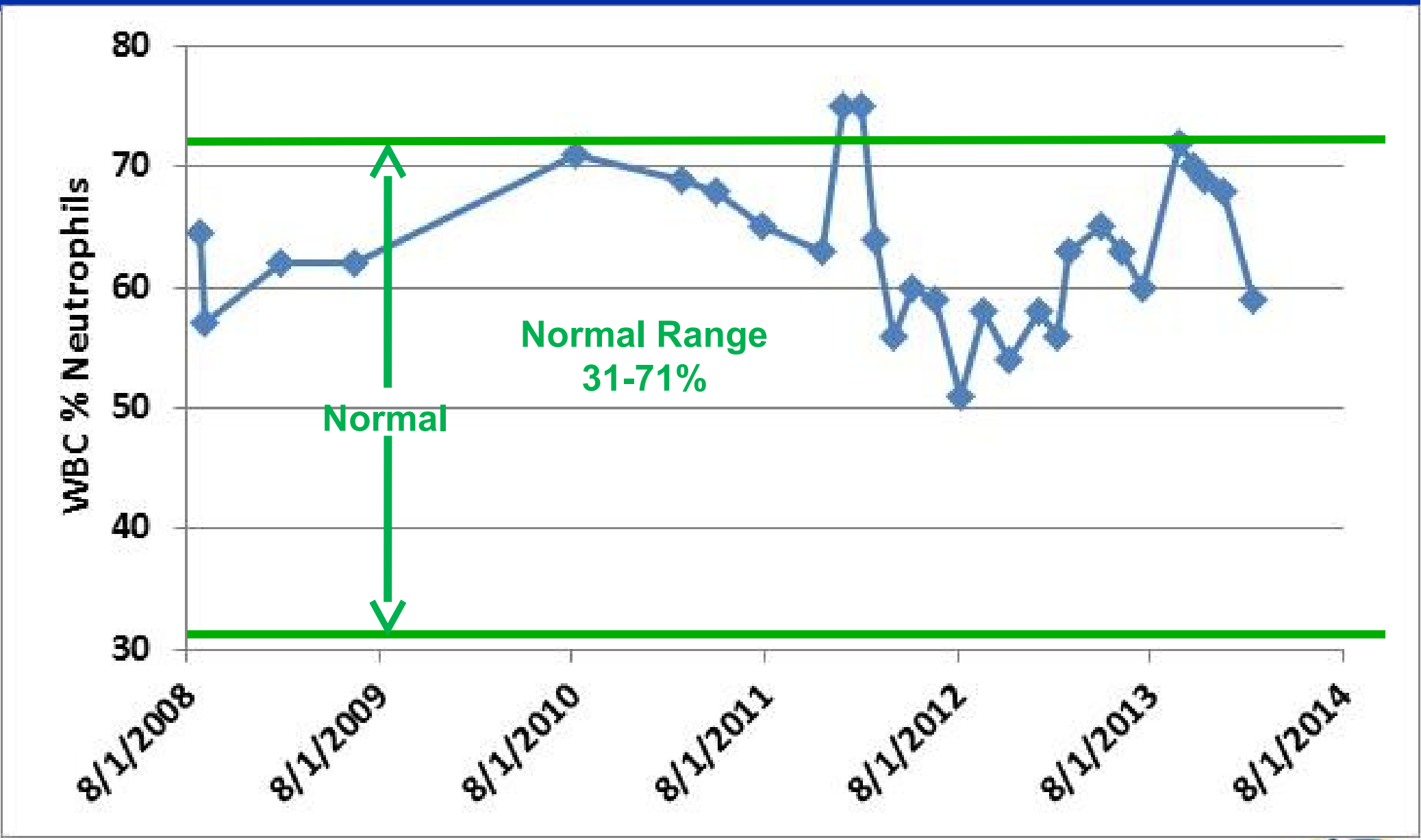


CRP is a Generic Measure of Inflammation in the Blood

White Blood Cell Count
Is Near Low End of Healthy Range

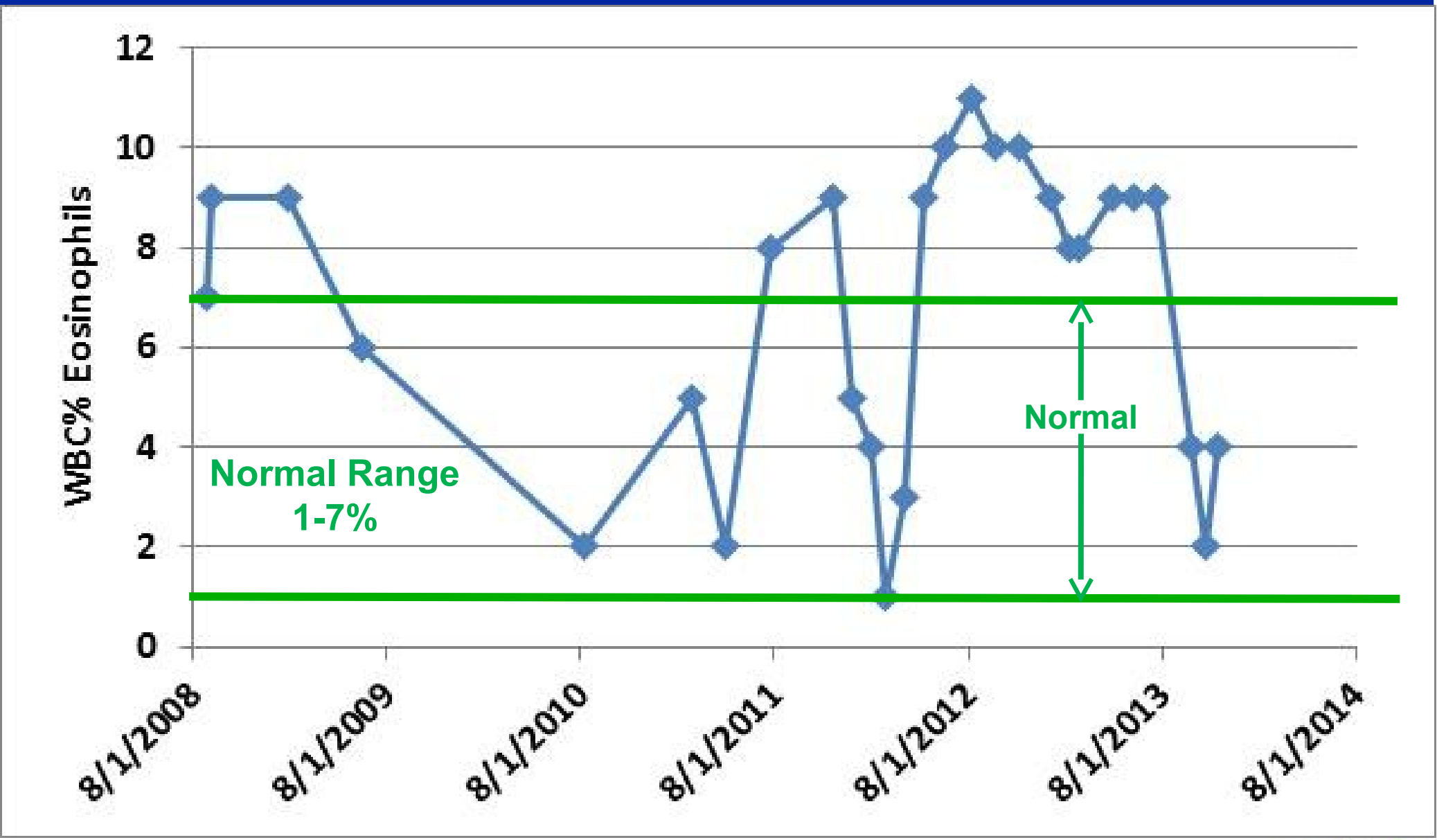


Neutrophils as % of WBCs Are Safely Inside Healthy Range



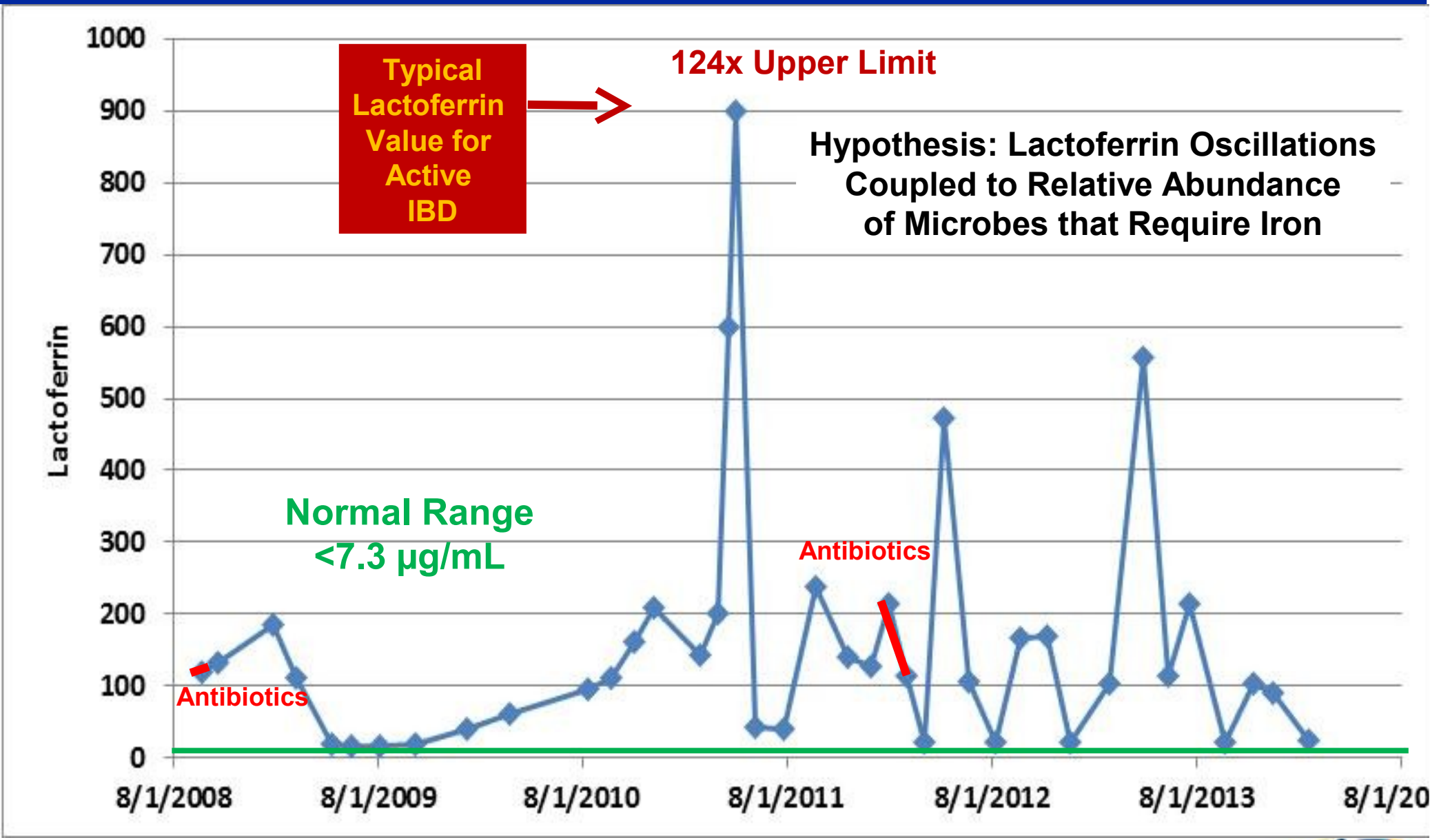
Note: current value is highest since 12/29/11

Eosinophils as % of WBCs Are Staying Inside Healthy Range



Note: Finally back to normal after a year outside normal

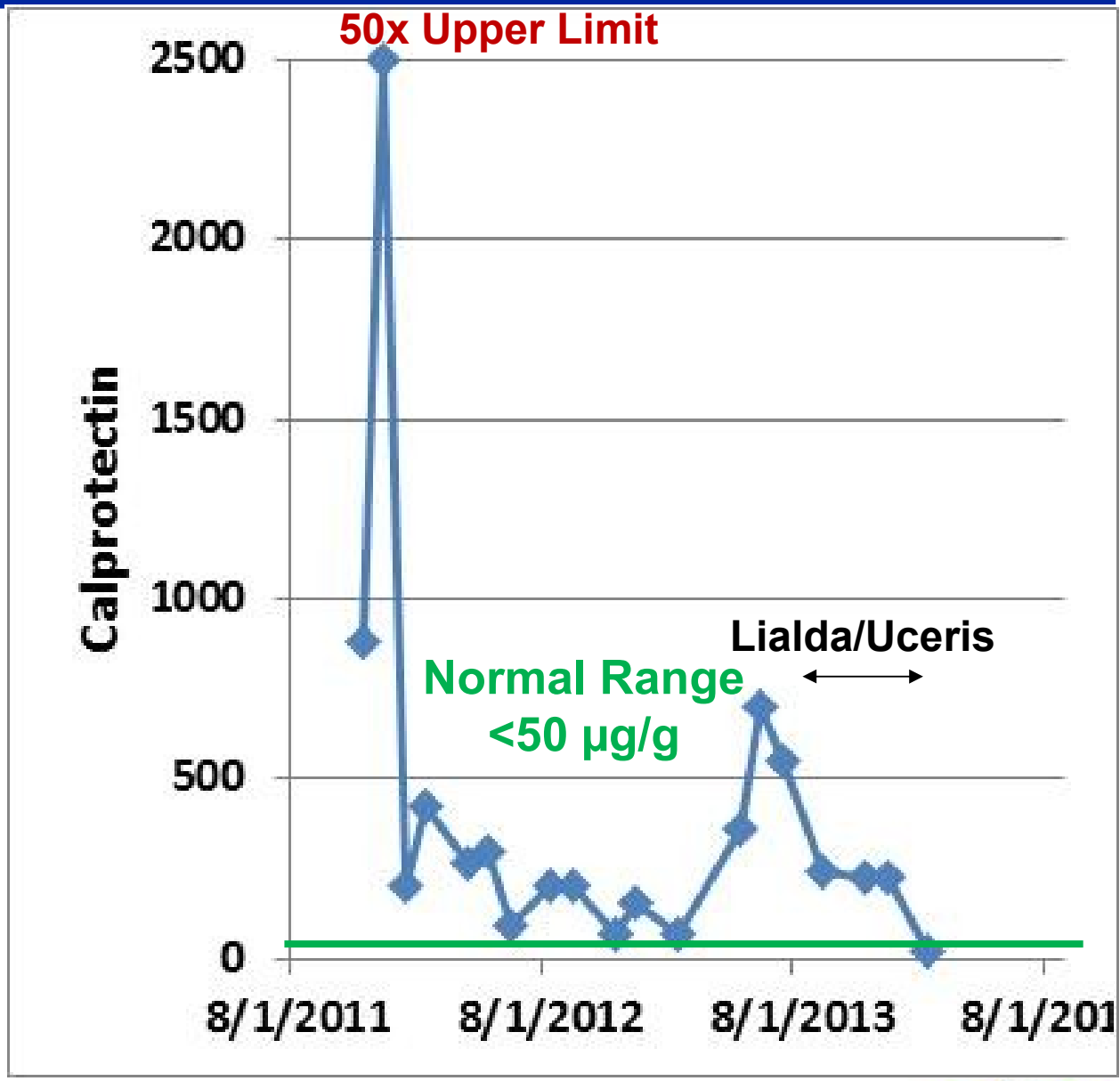
Adding Stool Tests Revealed Oscillatory Behavior in an Immune Variable



Lactoferrin is a Protein Shed from Neutrophils - An Antibacterial that Sequesters Iron

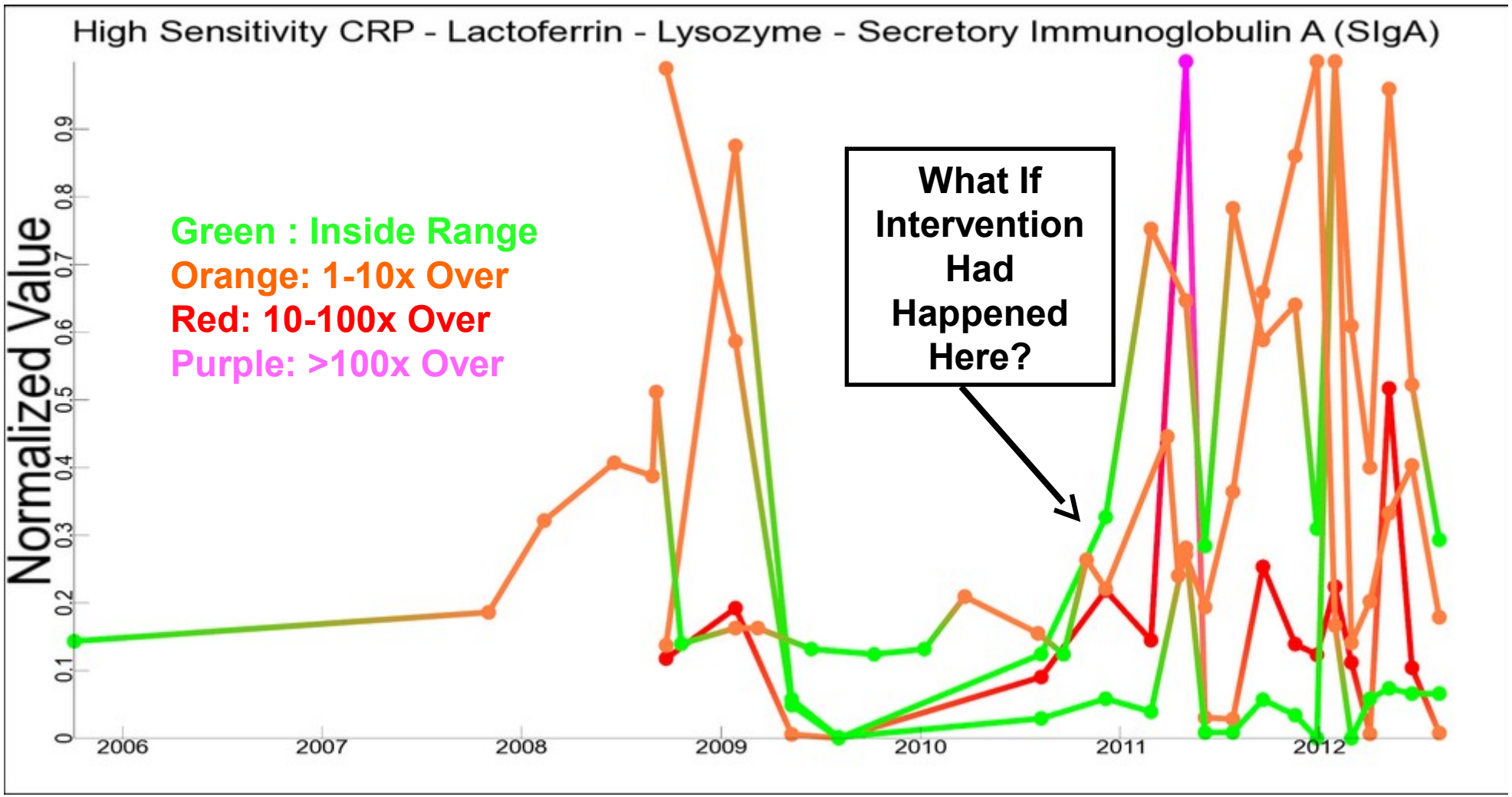
Calprotectin is Lowest Ever
First Time Inside Healthy Range

Note: Latest Calprotectin Is 1/4 of Previous Lowest Value



Calprotectin is a Protein Shed from Neutrophils -
An Antibacterial that Sequesters Zinc and Manganese

Putting Multiple Immunological Biomarker Time Series Together, Reveals Major Immune Dysfunction



Source: Calit2 Future Health Expedition Team

Four Immune Biomarkers Over Time Compared with Four Signs/Symptoms



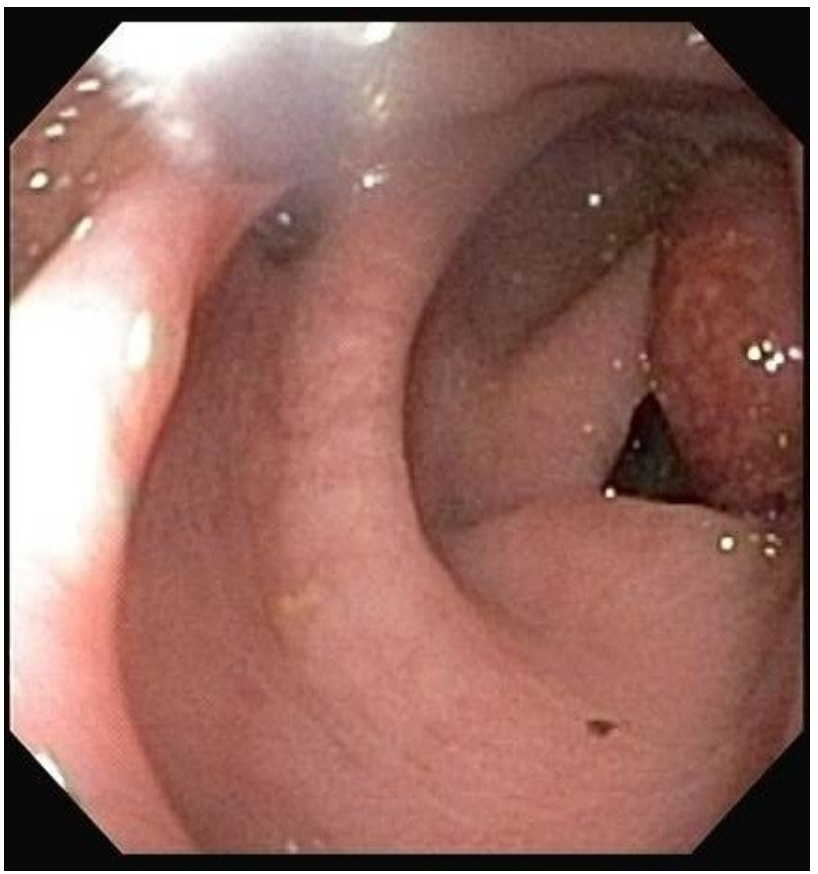
Here Immune biomarkers are normalized 0 to 1, with 1 being the highest value in five years

Source: Photo of Calit2 64-megapixel VROOM

Colonoscopy Images Show Inflamed Pseudopolyps in 6 inches of Sigmoid Colon

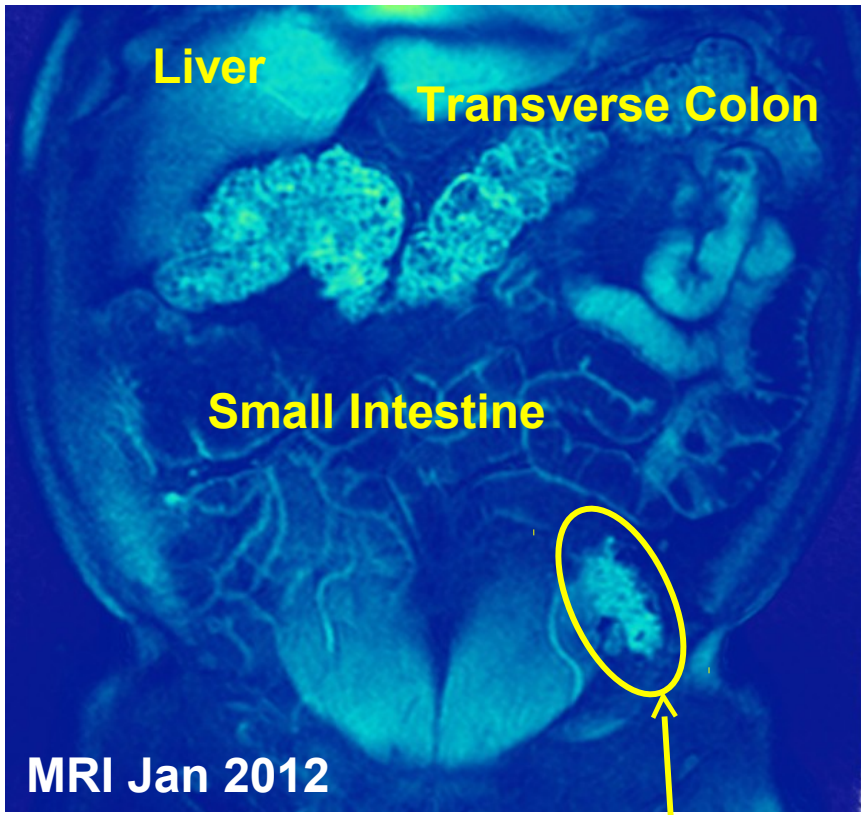


Dec 2010



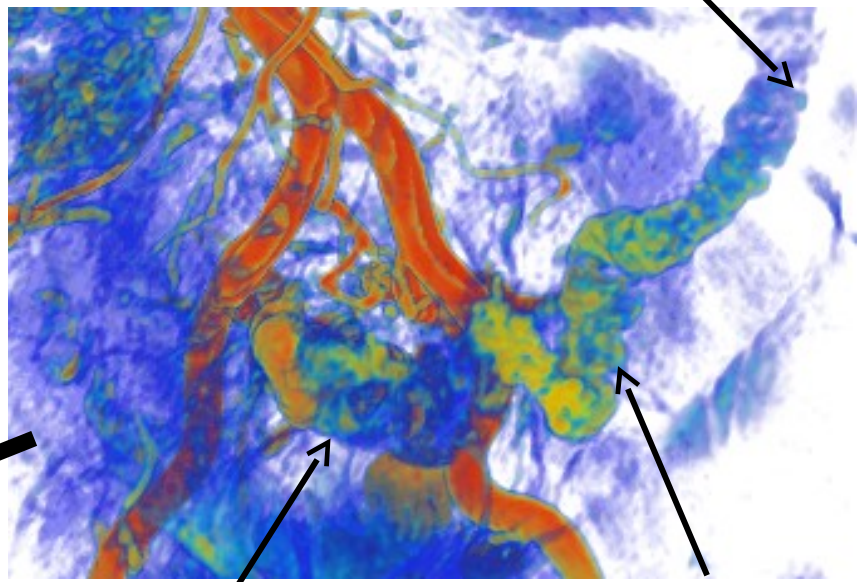
Jan 2012

Confirming the IBD (Crohn's) Hypothesis: Finding the "Smoking Gun" with MRI Imaging



I Obtained the MRI Slices From UCSD Medical Services and Converted to Interactive 3D Working With Calit2 Staff & DeskVOX Software

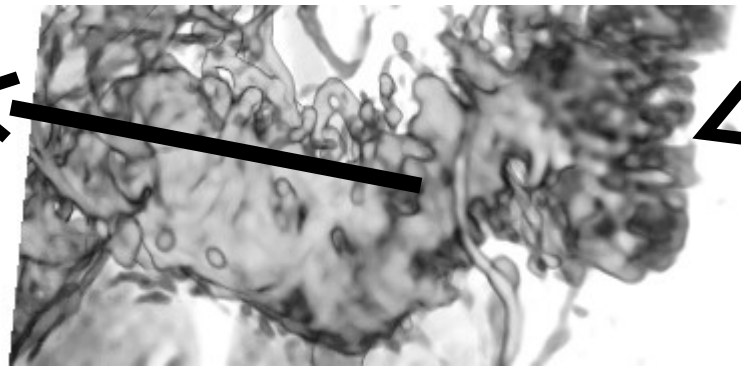
Descending Colon



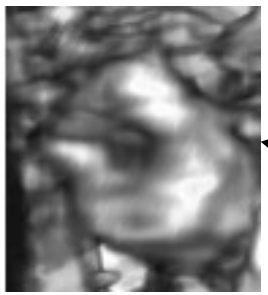
Major Kink

Sigmoid Colon Threading Iliac Arteries

Diseased Sigmoid Colon



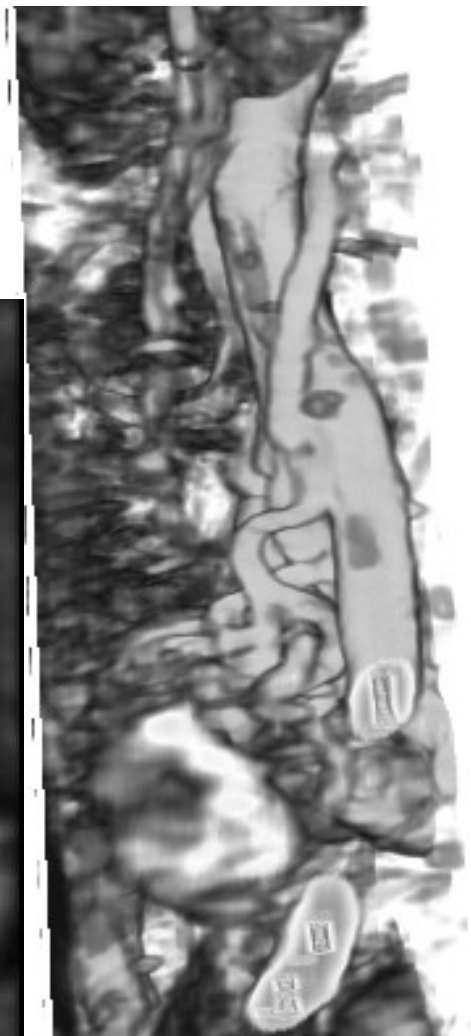
Cross Section



MRE Reveals Inflammation in 6 Inches of Sigmoid Colon
Thickness 15cm – 5x Normal Thickness

**"Long segment wall thickening
in the proximal and mid portions of the sigmoid colon,
extending over a segment of approximately 16 cm,
with suggestion of intramural sinus tracts.
Edema in the sigmoid mesentery
and engorgement of the regional vasa recta."
– MRI report**

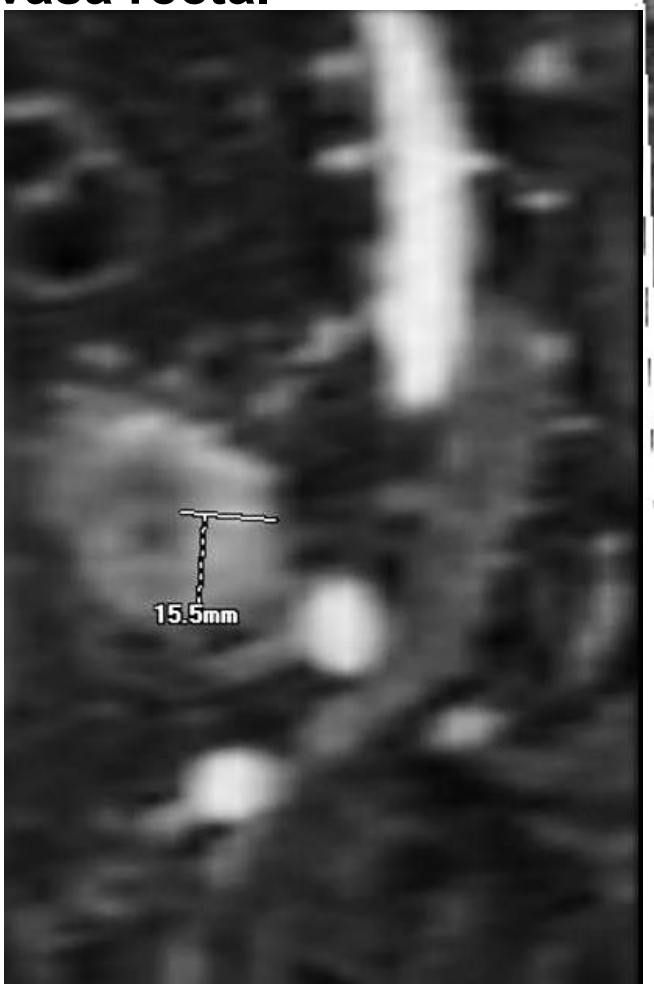
DeskVOX 3D Image



MAYO CLINIC

**Crohn's disease
affects the thickness
of the intestinal wall.**

**Having Crohn's disease
that affects your colon
increases your risk
of colon cancer.**



**Clinical MRI
Slice Program**

Why Did I Have an Autoimmune Disease like IBD?

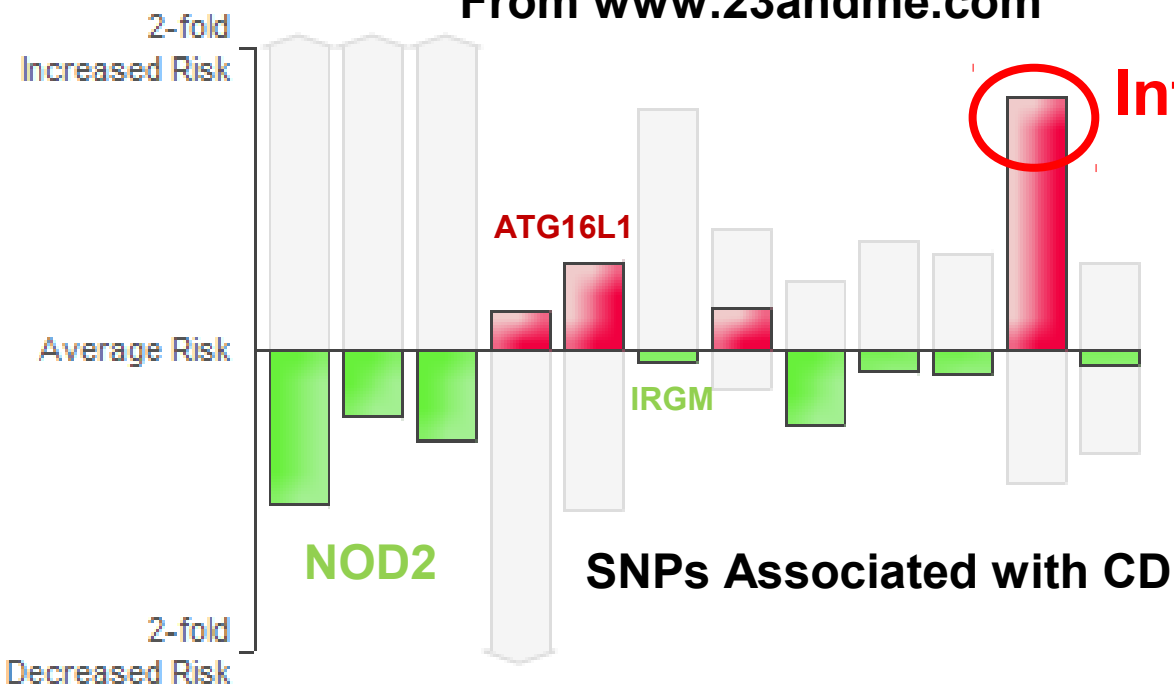
Despite decades of research,
the etiology of Crohn's disease
remains unknown.
Its pathogenesis may involve
a complex interplay between
host genetics,
immune dysfunction,
and microbial or environmental factors.
--*The Role of Microbes in Crohn's Disease*

So I Set Out to Quantify All Three!

Paul B. Eckburg & David A. Relman
Clin Infect Dis. 44:256-262 (2007)

I Found I Had One of the Earliest Known SNPs Associated with Crohn's Disease

From www.23andme.com



Polymorphism in Interleukin-23 Receptor Gene — 80% Higher Risk of Pro-inflammatory Immune Response rs1004819

A Genome-Wide Association Study Identifies *IL23R* as an Inflammatory Bowel Disease Gene

Richard H. Düerr, *et al.*
Science **314**, 1461 (2006)

News and Views

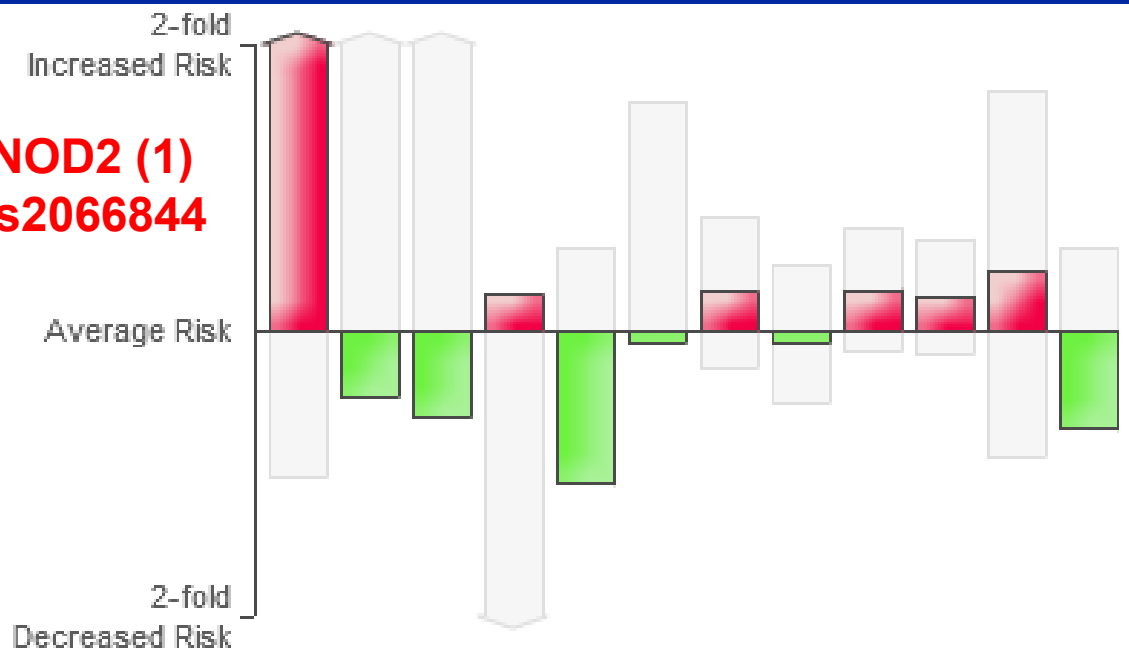
Nature Medicine **13**, 26 - 28 (2007)
doi:10.1038/nm0107-26

IL-23: a master regulator in Crohn disease

Markus F Neurath[1]

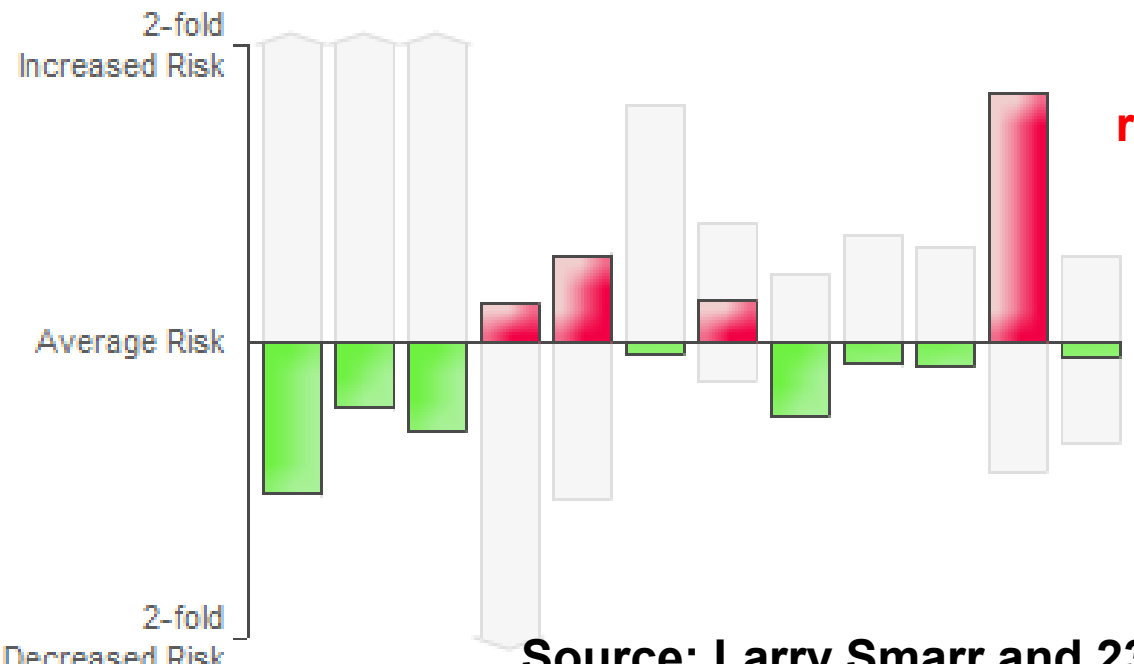
There Is Likely a Correlation Between CD SNPs and Where and When the Disease Manifests

Subject with Ileal Crohn's



Female
CD Onset
At 20-Years Old

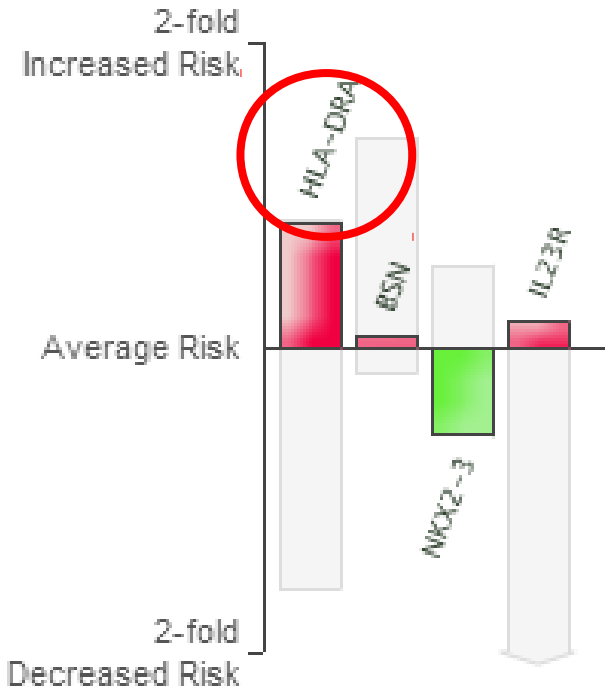
Subject with Colon Crohn's



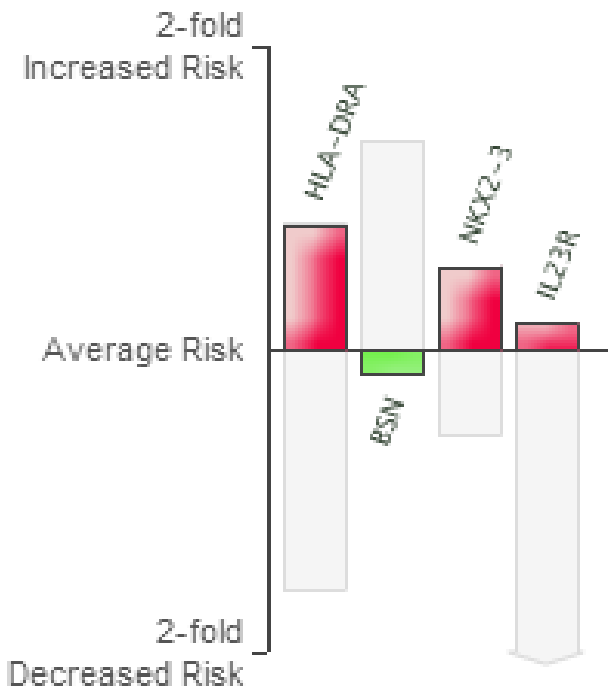
Me-Male
CD Onset
At 60-Years Old

Source: Larry Smarr and 23andme

I Also Had an Increased Risk for Ulcerative Colitis, But a SNP that is Also Associated with Colonic CD



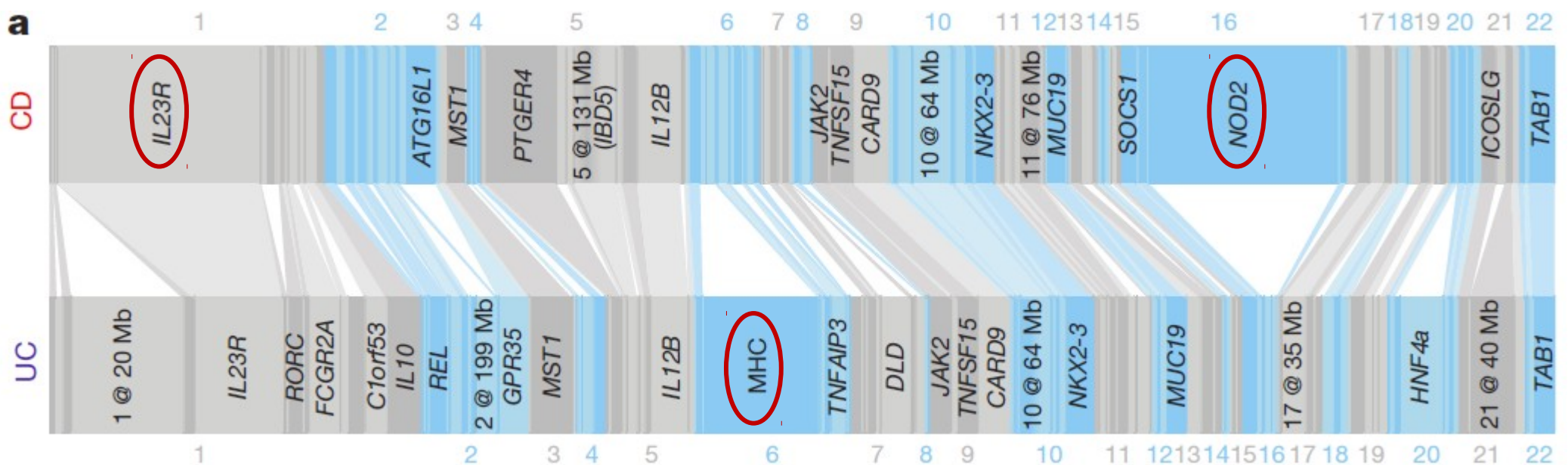
I Have a 33% Increased Risk for Ulcerative Colitis HLA-DRA (rs2395185)



I Have the Same Level of HLA-DRA Increased Risk as Another Male Who Has Had Ulcerative Colitis for 20 Years

"Our results suggest that at least for the SNPs investigated [including HLA-DRA], colonic CD and UC have common genetic basis."
-Waterman, et al., IBD 17, 1936-42 (2011)

I Compared my 23andme SNPs With the 163 Known SNPs Associated with IBD

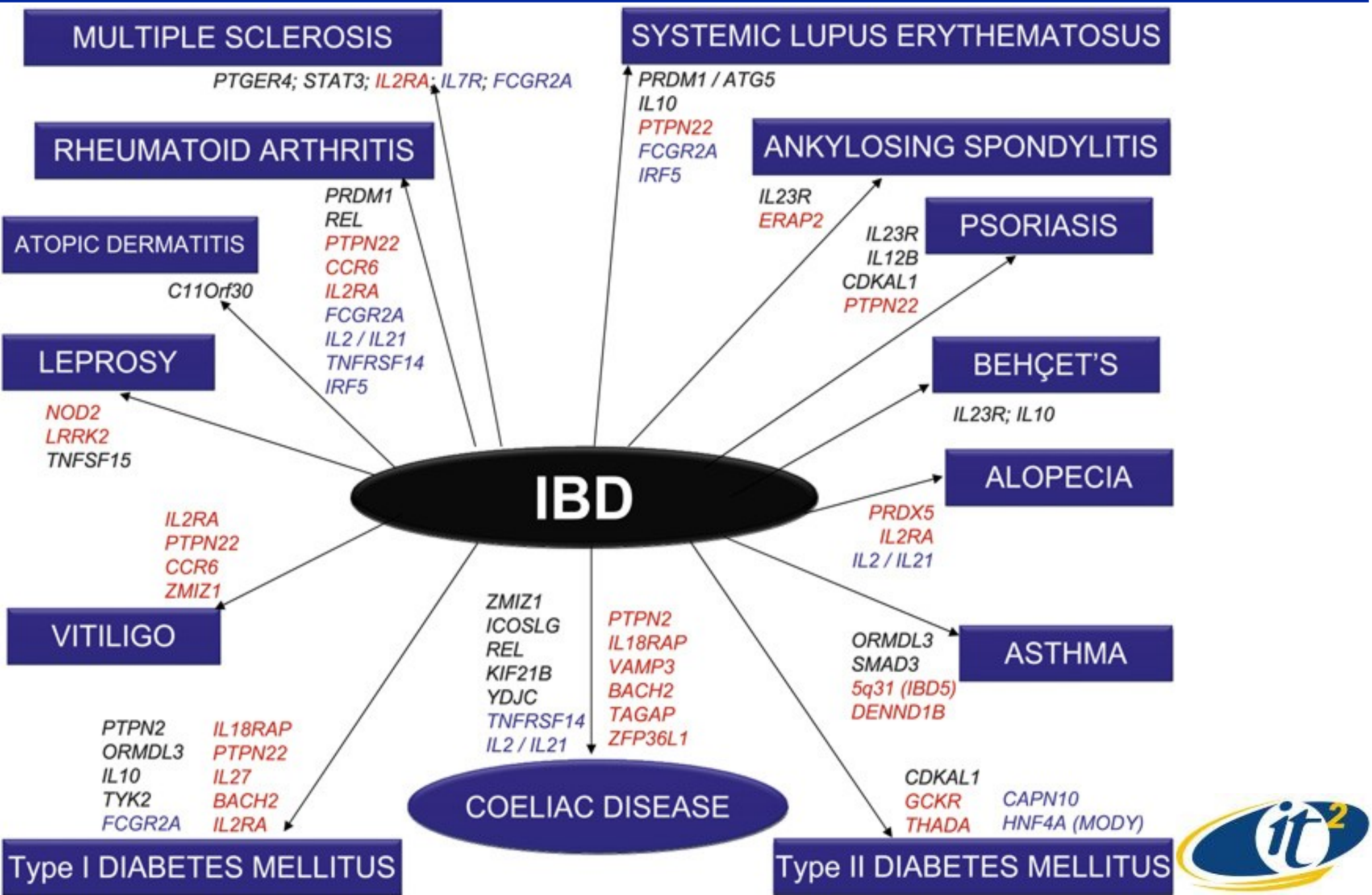


- The width of the bar is proportional to the variance explained by that locus
- Bars are connected together if they are identified as being associated with both phenotypes
- Loci are labelled if they explain more than 1% of the total variance explained by all loci

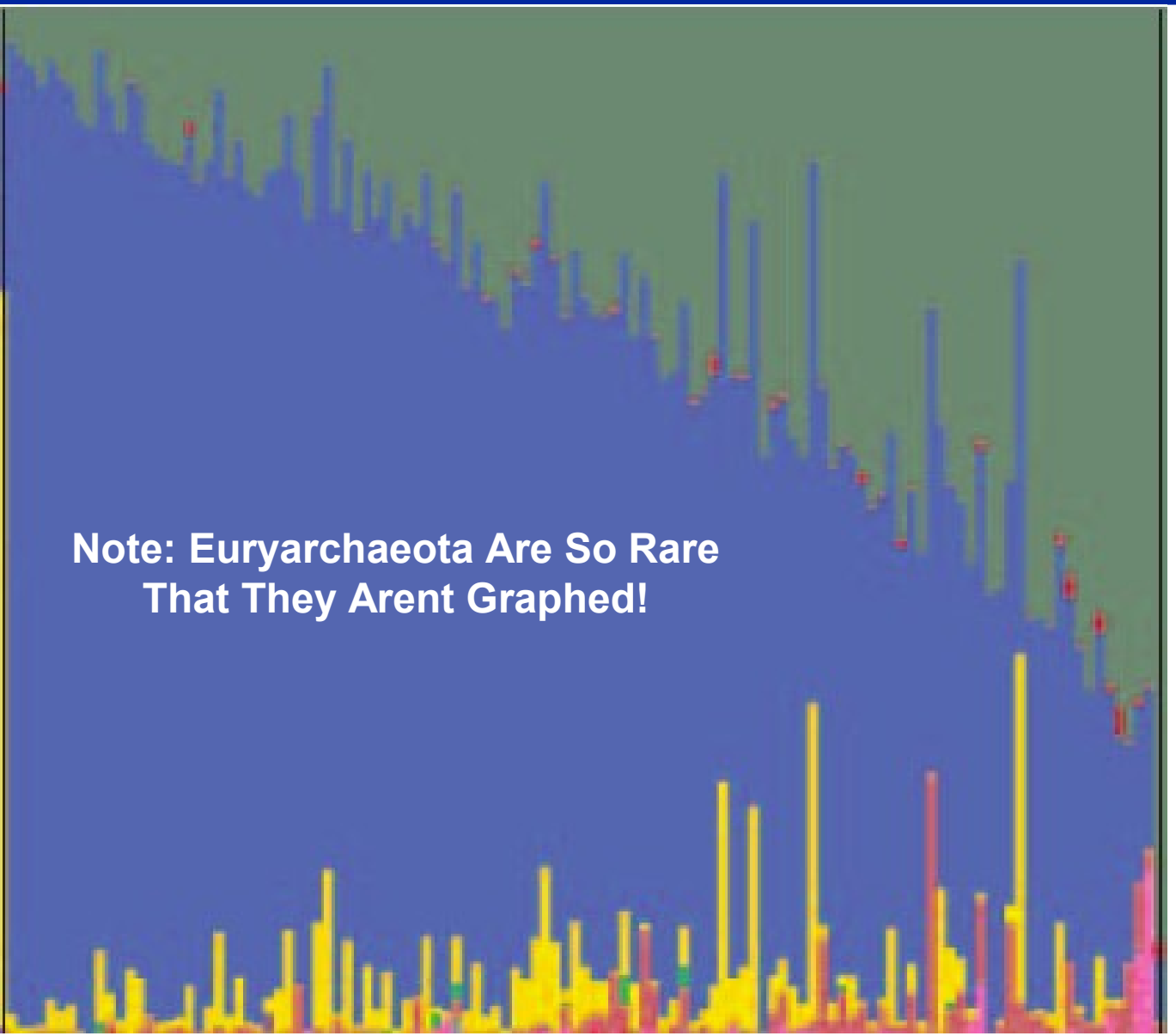
"Host–microbe interactions have shaped the genetic architecture of inflammatory bowel disease," Jostins, et al. Nature 491, 119-124 (2012)

Autoimmune Disease Overlap from SNP GWAS

Gut Lees, et al. 60:1739-1753 (2011)



What is a "Healthy" Gut Microbiome? Considerable Phyla Variation Found in HMP



Based on 16S

- Firmicutes
- Actinobacteria
- Bacteroidetes
- Proteobacteria
- Fusobacteria
- Tenericutes
- Spirochaetes
- Cyanobacteria
- Verrucomicrobia
- TM7

Source: "Structure, function and diversity of the healthy human microbiome," HMP Consortium, Nature, 486, 207-212 (2012)

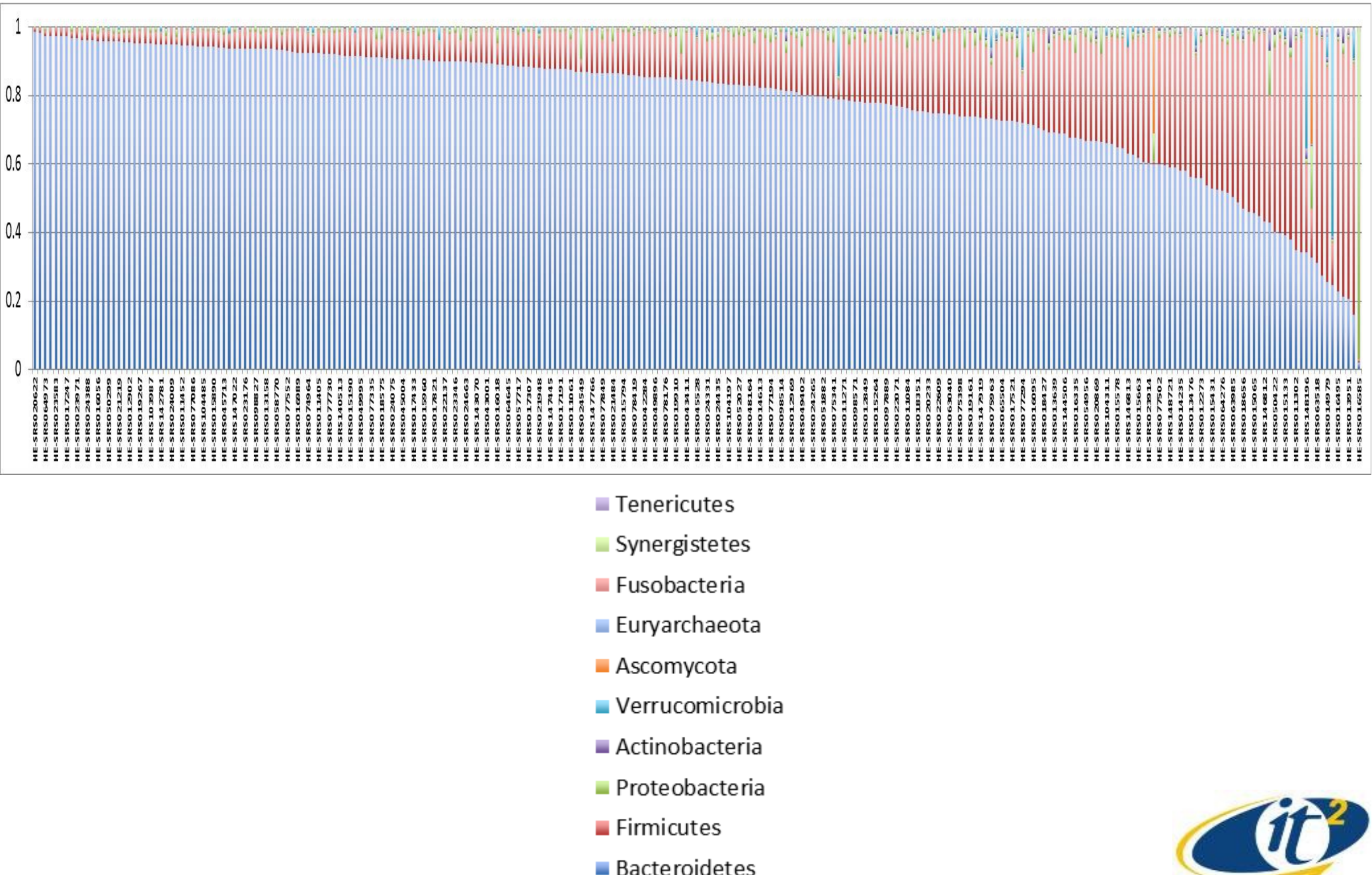
We Used Dell's Supercomputer (Sanger) to Analyze additional 219 HMP and 110 MetaHIT samples

- **Dell's Sanger cluster**
  - **32 nodes, 512 cores,**
  - **48GB RAM per node**
  - **50GB SSD local drive, 390TB Lustre file system**
- **We used faster but less sensitive method with a smaller reference DB (duo to available 48GB RAM)**
- **Only processed to taxonomy mapping**
  - **~35,000 Core-Hrs on Dell's Sanger**
  - **30 TB data**

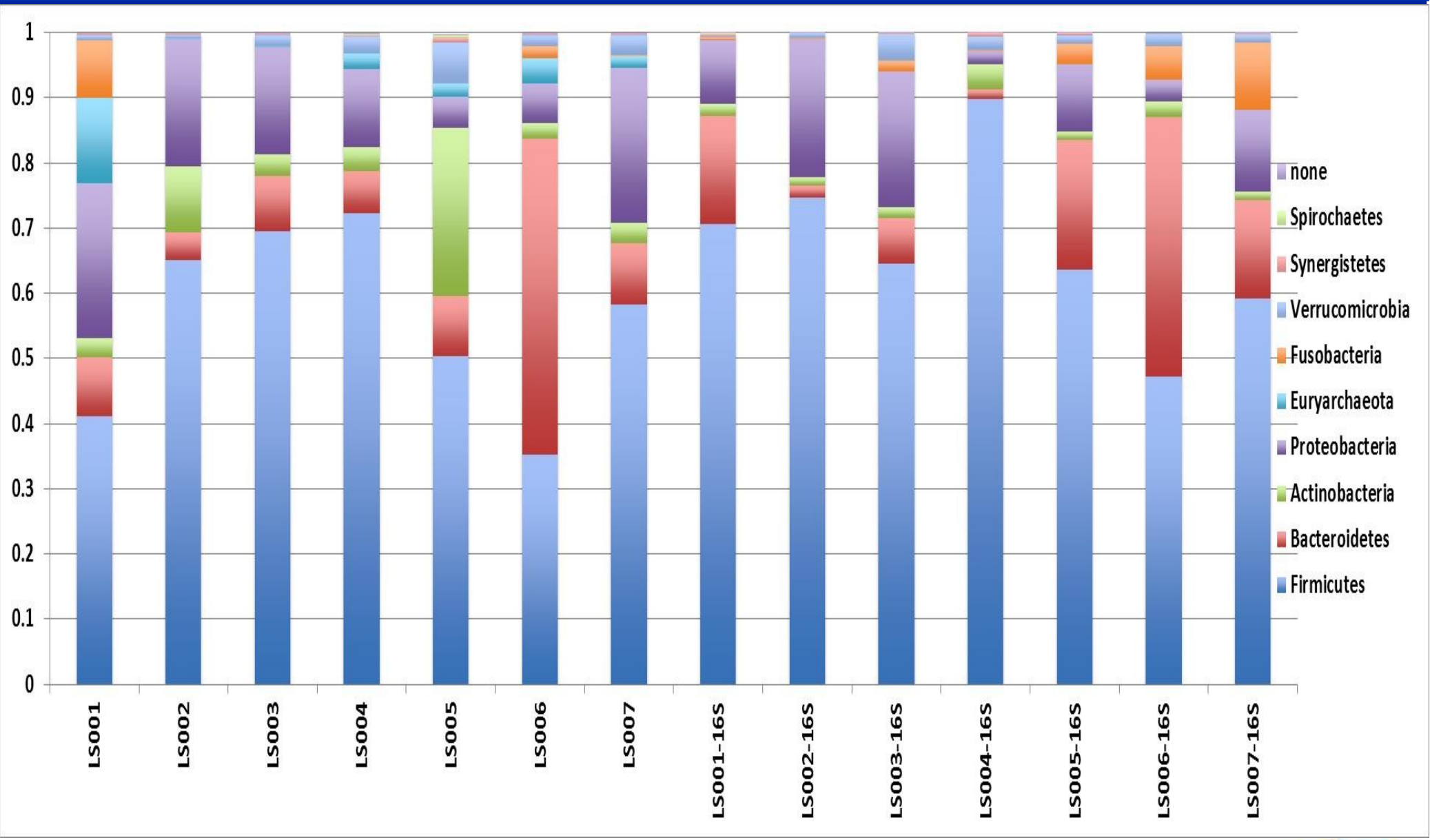


Dell Solutions Center
Industry Solutions Lab
SANGER DSU

Our Metagenomic Analysis Finds a Wide Variation of Firmicutes/Bacteroidetes in Healthy People



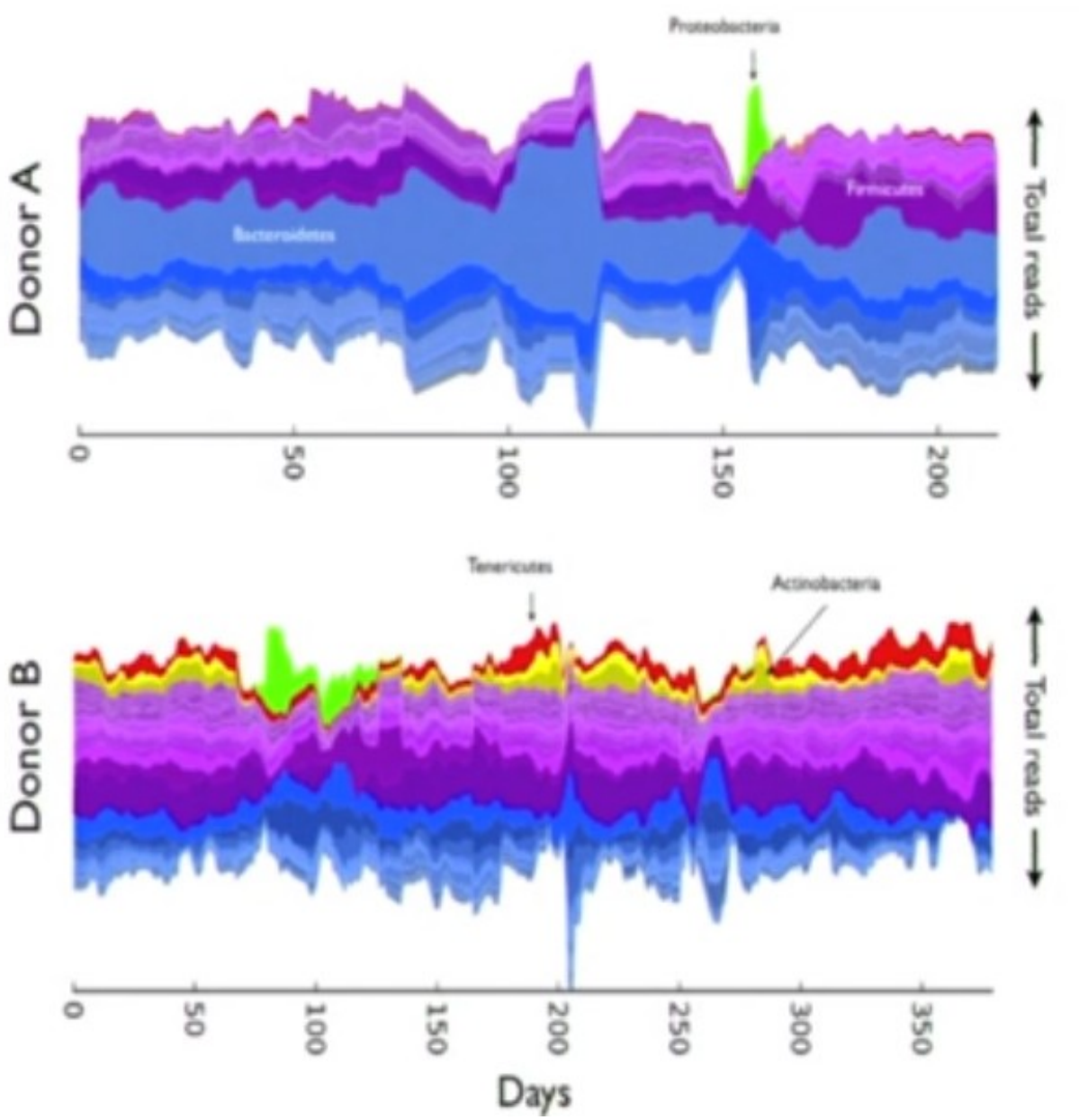
Problem: You Can't Assume 16S Will Agree in Detail With Metagenomics on Same DNA Extraction



Source: Weizhong Li, UCSD; Sequencing JCVI

The Adult Healthy Gut Microbiome Is Remarkably Stable Over Time

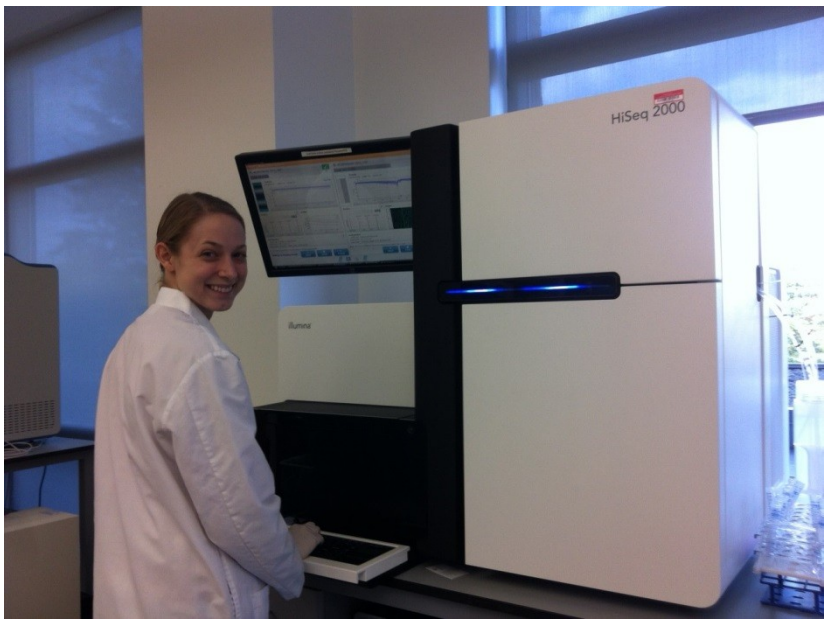
Gut communities over time



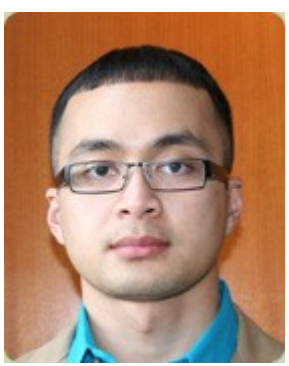
Source: Eric Alm, MIT

To Map Out the Dynamics of My Microbiome Ecology I Partnered with the J. Craig Venter Institute

- JCVI Did Metagenomic Sequencing on Six of My Stool Samples Over 1.5 Years
- Sequencing on Illumina HiSeq 2000
  - Generates 100bp Reads
  - Run Takes ~14 Days
  - My 6 Samples Produced
    - 190.2 Gbp of Data
- JCVI Lab Manager, Genomic Medicine
  - Manolito Torralba
- IRB PI Karen Nelson
  - President JCVI



Illumina HiSeq 2000 at JCVI


Manolito Torralba, JCVI


Karen Nelson, JCVI

J. Craig Venter INSTITUTE

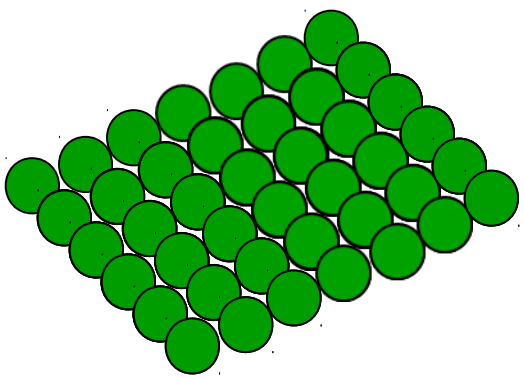
it2

We Downloaded Additional Phenotypes from NIH HMP For Comparative Analysis

Download Raw Reads
~100M Per Person

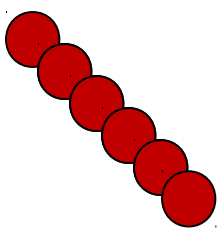
"Healthy" Individuals

35 Subjects
1 Point in Time



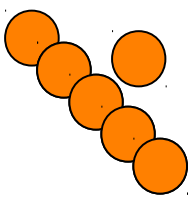
Larry Smarr

6 Points in Time

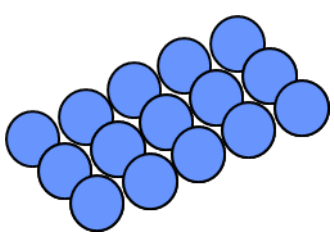


IBD Patients

2 Ulcerative Colitis Patients,
6 Points in Time



5 Ileal Crohn's Patients,
3 Points in Time



NIH

HMP

Total of 5 Billion Reads

Source: Jerry Sheehan, Calit2
Weizhong Li, Sitao Wu, CRBS, UCSD

it²

We Created a Reference Database Of Known Gut Genomes

- **NCBI April 2013**
  - **2471 Complete + 5543 Draft Bacteria & Archaea Genomes**
  - **2399 Complete Virus Genomes**
  - **26 Complete Fungi Genomes**
  - **309 HMP Eukaryote Reference Genomes**
- **Total 10,741 genomes, ~30 GB of sequences**

NCBI National Center for Biotechnology Information

HMP

Now to Align Our 5 Billion Reads Against the Reference Database

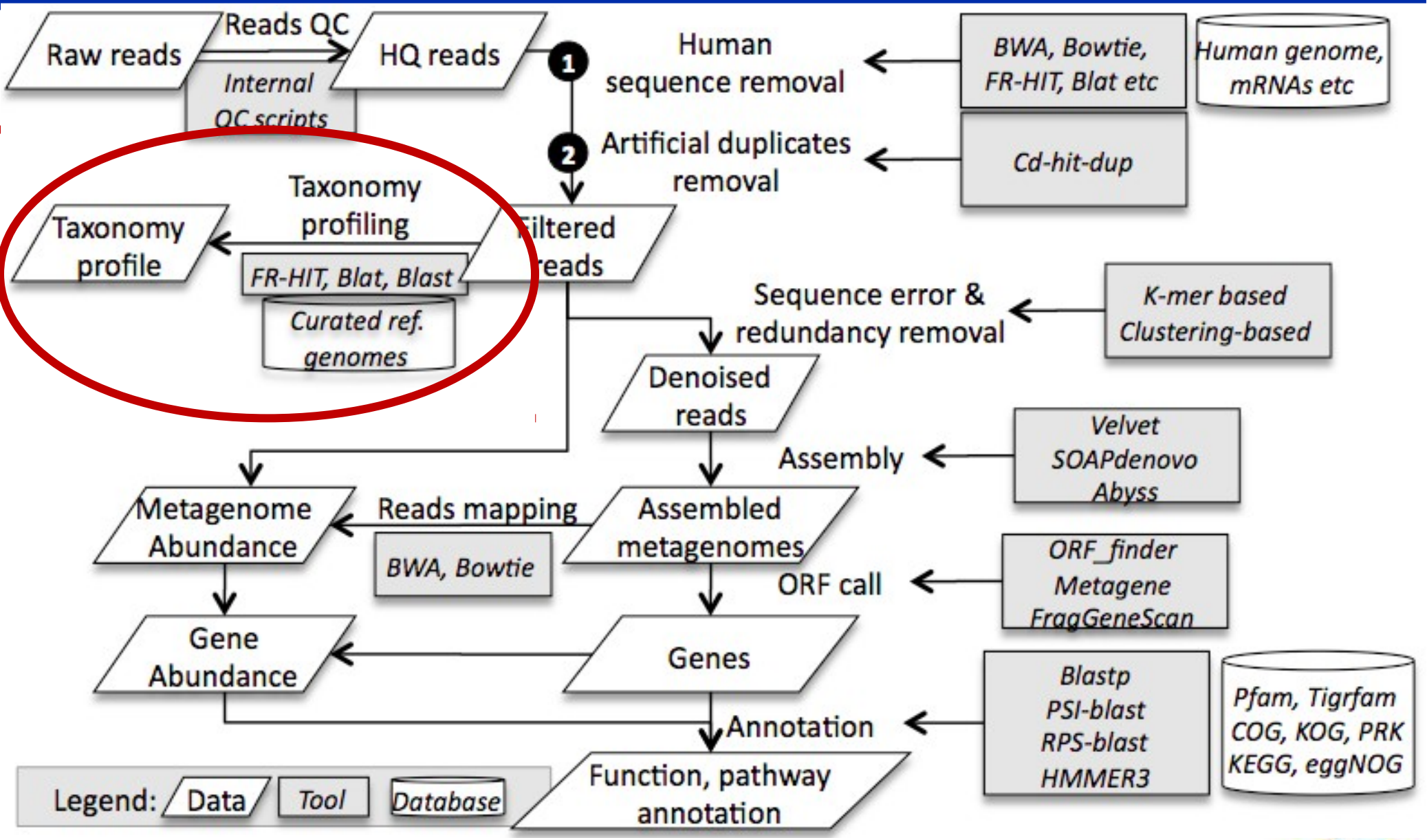
NIH

J. Craig Venter INSTITUTE

Source: Weizhong Li, Sitao Wu, CRBS, UCSD

it²

Computational NextGen Sequencing Pipeline: From "Big Equations" to "Big Data" Computing



National Human Genome Research Institute

HMP

PI: (Weizhong Li, CRBS, UCSD):
NIH R01HG005978 (2010-2013, $1.1M)

CRBS

it²

We Used SDSC's Gordon Data-Intensive Supercomputer to Analyze a Wide Range of Gut Microbiomes

- ~180,000 Core-Hrs on Gordon
  - KEGG function annotation: 90,000 hrs
  - Mapping: 36,000 hrs
    - Used 16 Cores/Node and up to 50 nodes
  - Duplicates removal: 18,000 hrs
  - Assembly: 18,000 hrs
  - Other: 18,000 hrs
- Gordon RAM Required
  - 64GB RAM for Reference DB
  - 192GB RAM for Assembly
- Gordon Disk Required
  - Ultra-Fast Disk Holds Ref DB for All Nodes
  - 8TB for All Subjects



SDSC

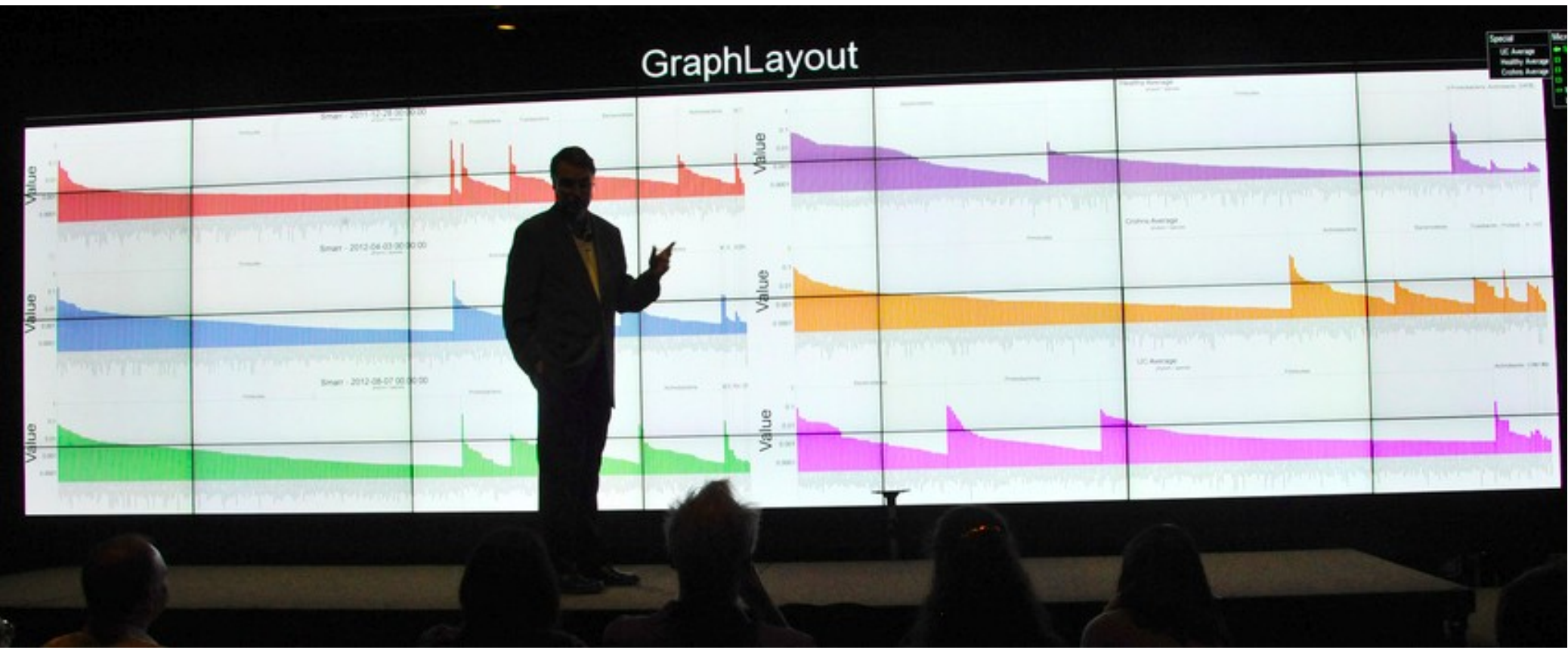
Enabled by
a Grant of Time
on Gordon from SDSC
Director Mike Norman

NSF NIH HMP

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CRBS it²

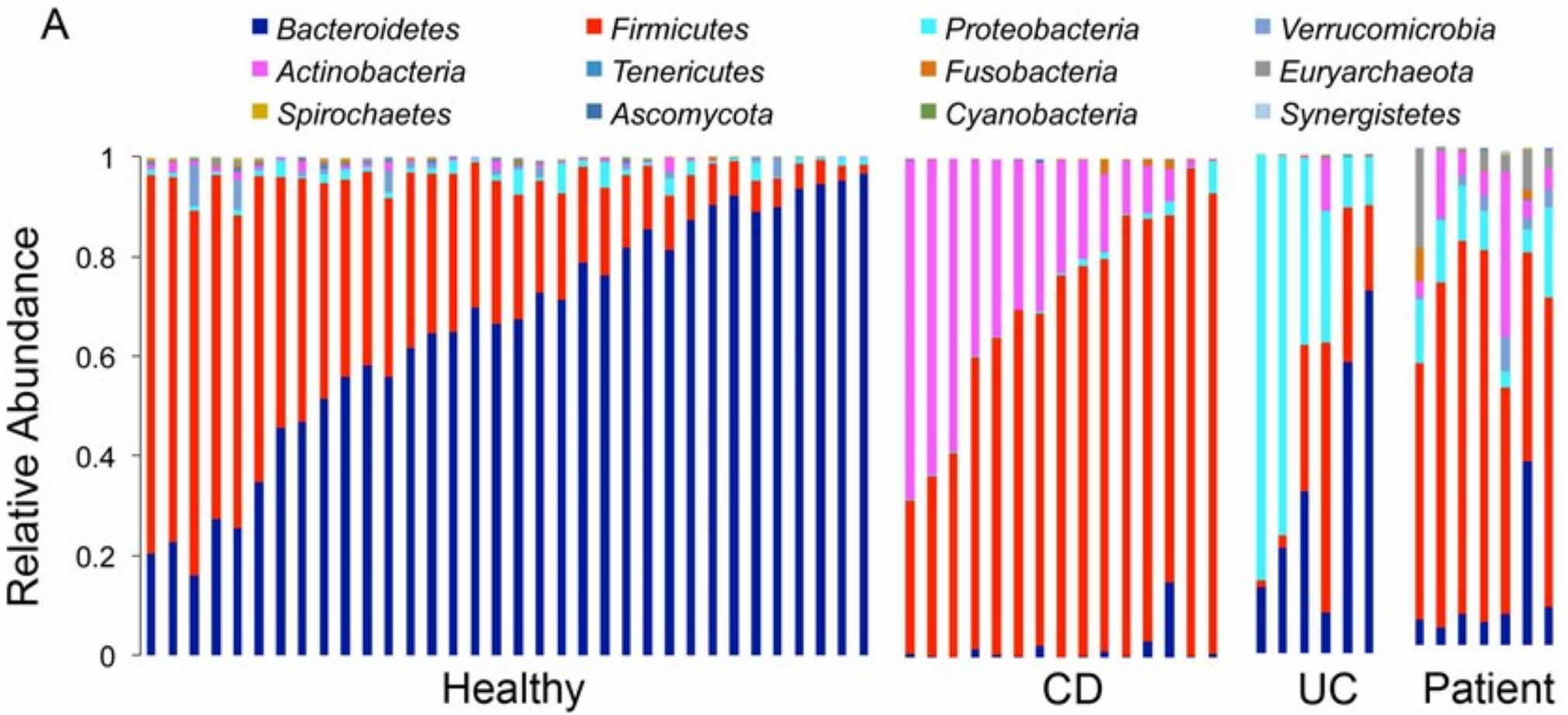
Using Scalable Visualization Allows Comparison of the Relative Abundance of 200 Microbe Species



Comparing 3 LS Time Snapshots (Left)
with Healthy, Crohn's, UC (Right Top to Bottom)

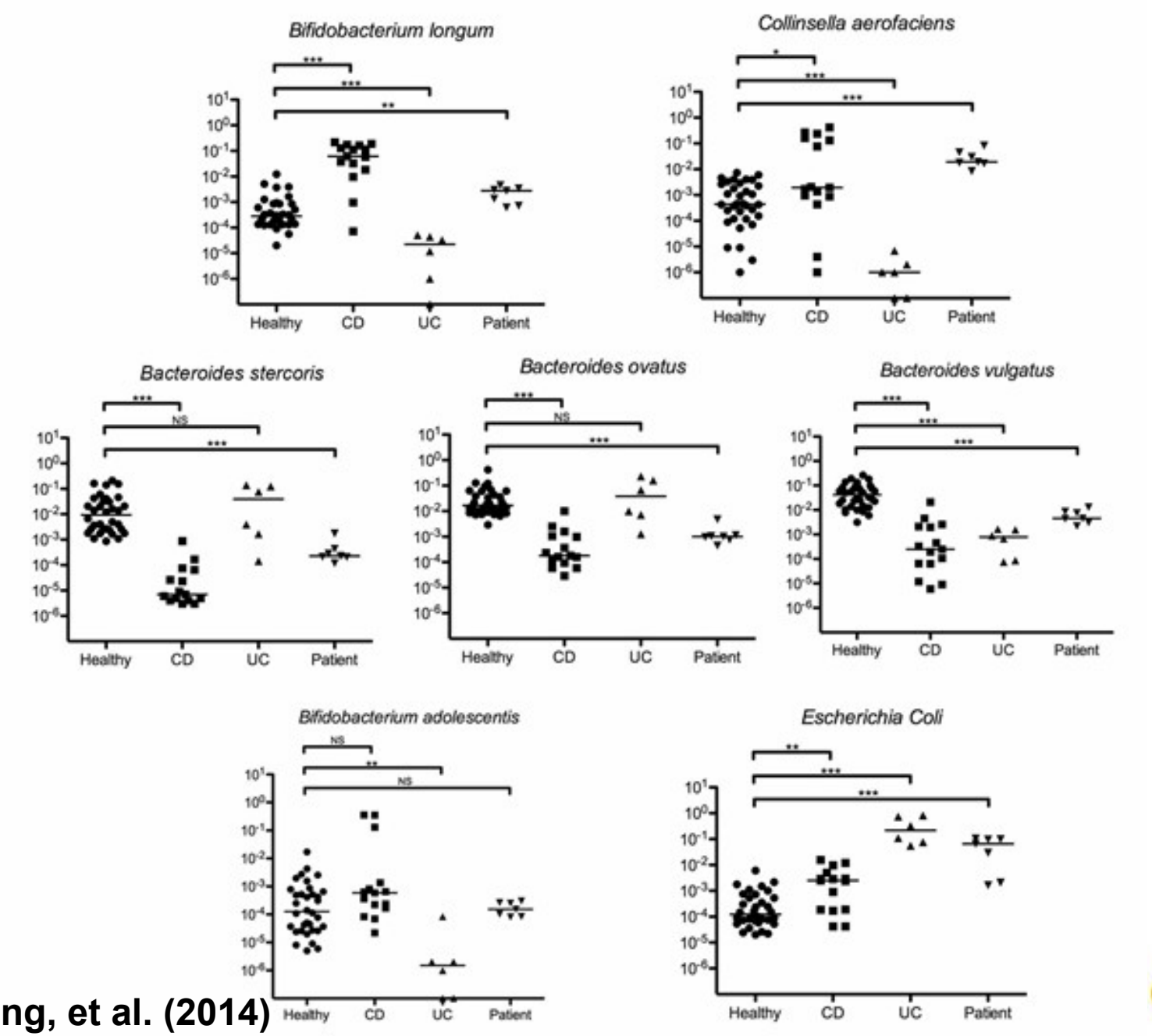
Calit2 VROOM-FuturePatient Expedition

The Emergence of Microbial Genomics Diagnostics



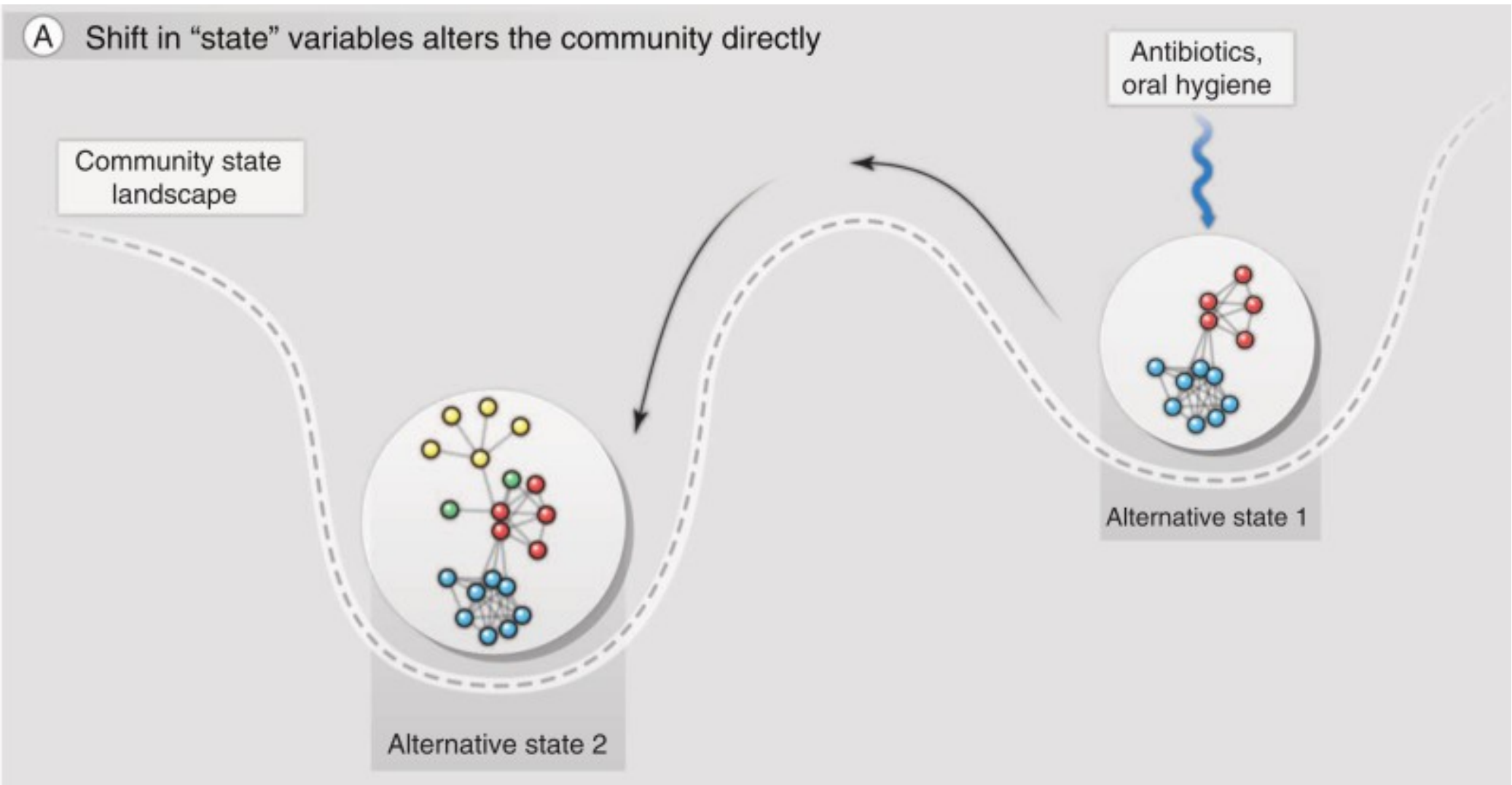
Source: Chang, et al. (2014)

Bacterial Species Which PCA Indicates Best Separate the Four States



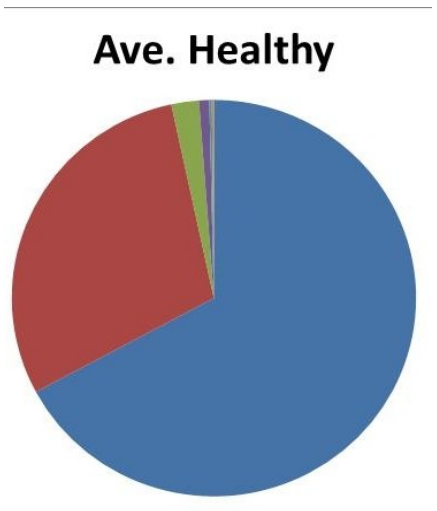
Source: Chang, et al. (2014)

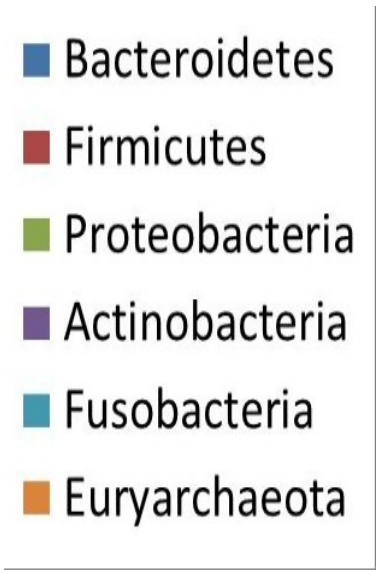
Lessons from Ecological Dynamics I: Gut Microbiome Has Multiple Relatively Stable Equilibria



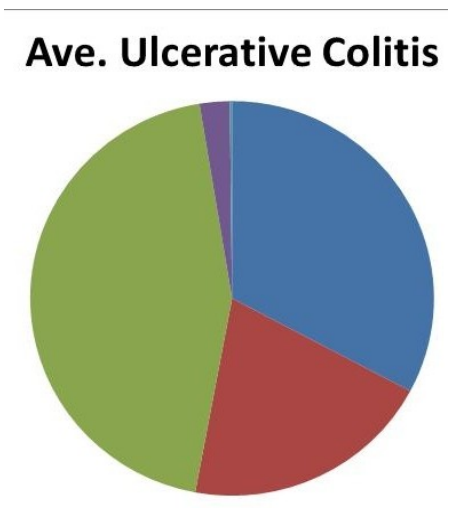
"The Application of Ecological Theory Toward an Understanding of the Human Microbiome,"
Elizabeth Costello, Keaton Stagaman, Les Dethlefsen, Brendan Bohannan, David Relman
Science 336, 1255-62 (2012)

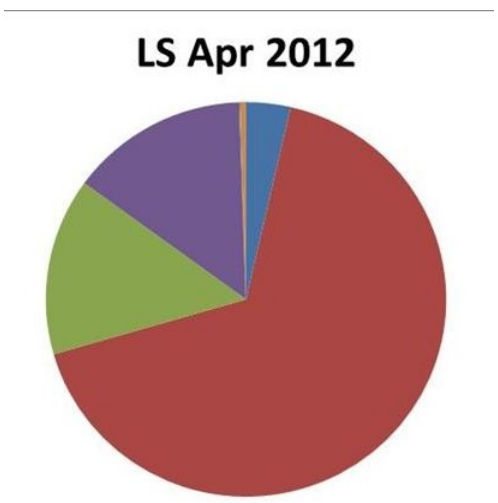
I Found Major Shifts in Microbial Ecology Phyla Between Healthy and Two Forms of IBD

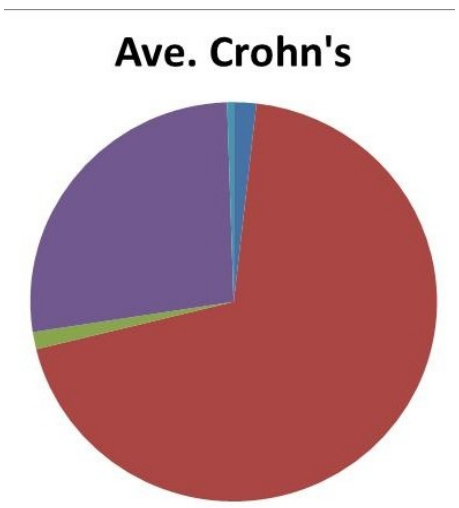




Most Common Microbial Phyla







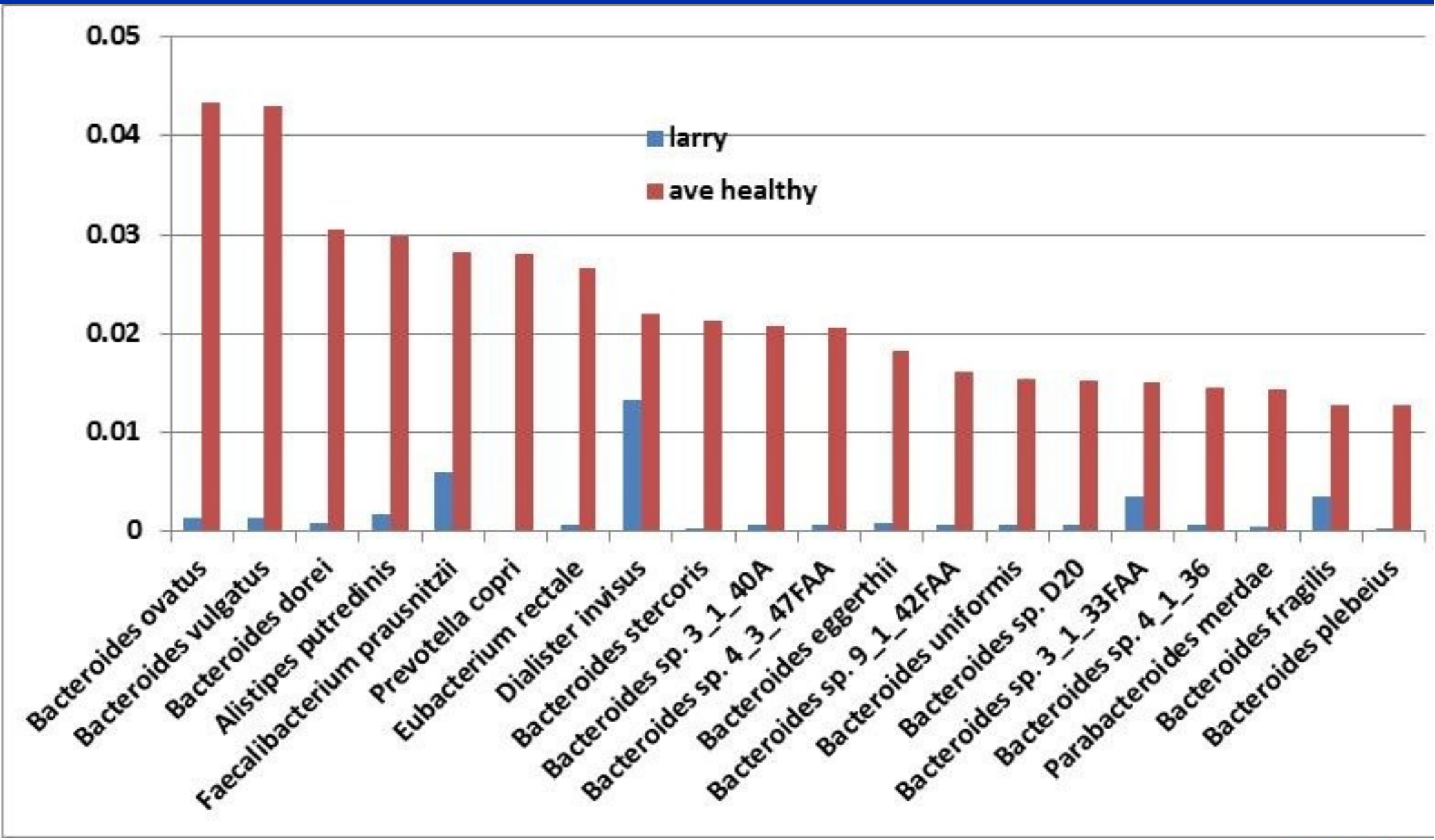
Lessons From Ecological Dynamics II:
Invasive Species Dominate After Major Species Destroyed



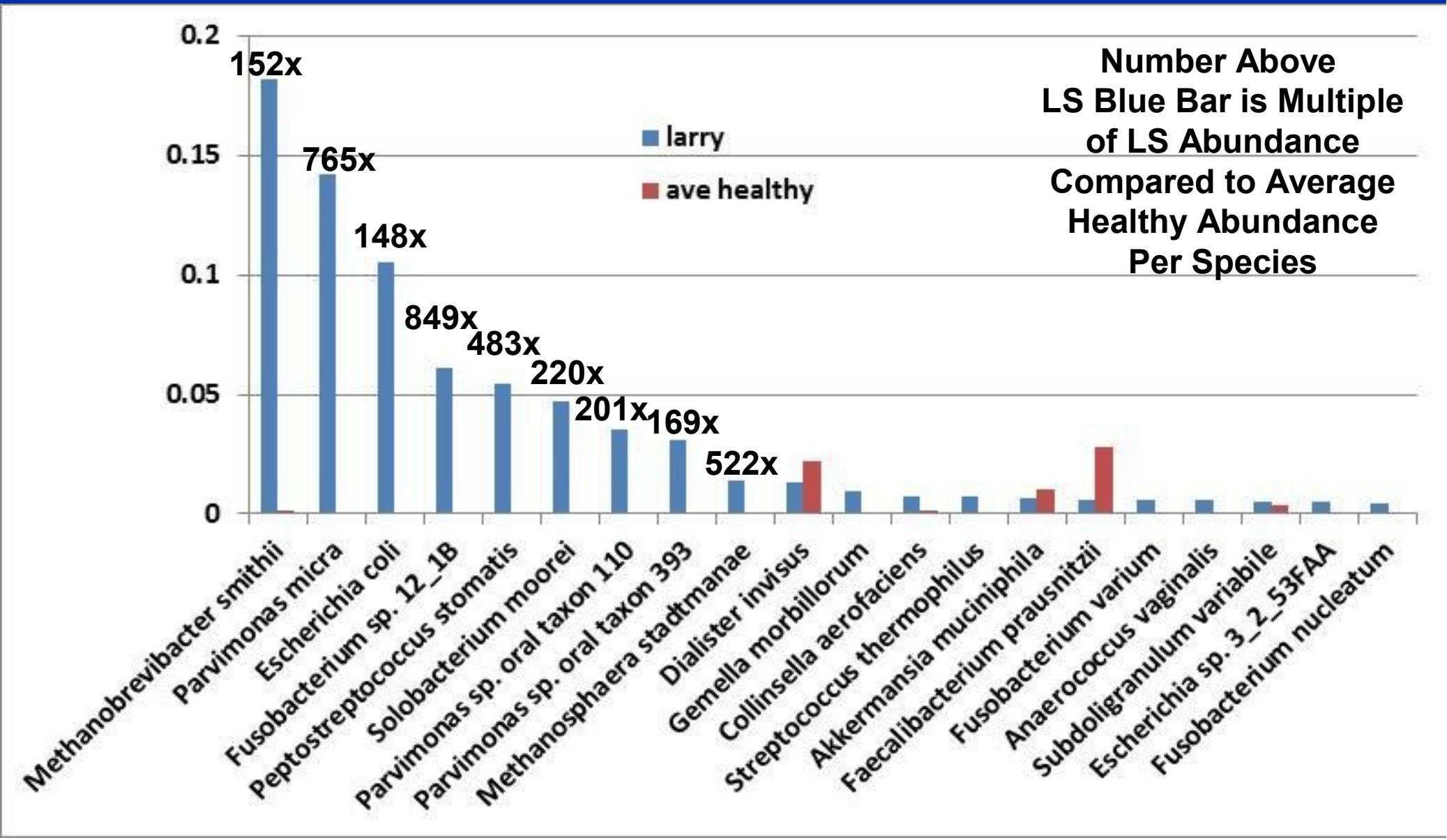
”In many areas following these burns invasive species are able to establish themselves, crowding out native species.”

Source: Ponderosa Pine Fire Ecology
http://cpluhna.nau.edu/Biota/ponderosafire.htm

Almost All Abundant Species (≥1%) in Healthy Subjects Are *Severely Depleted* in Larry's Gut Microbiome

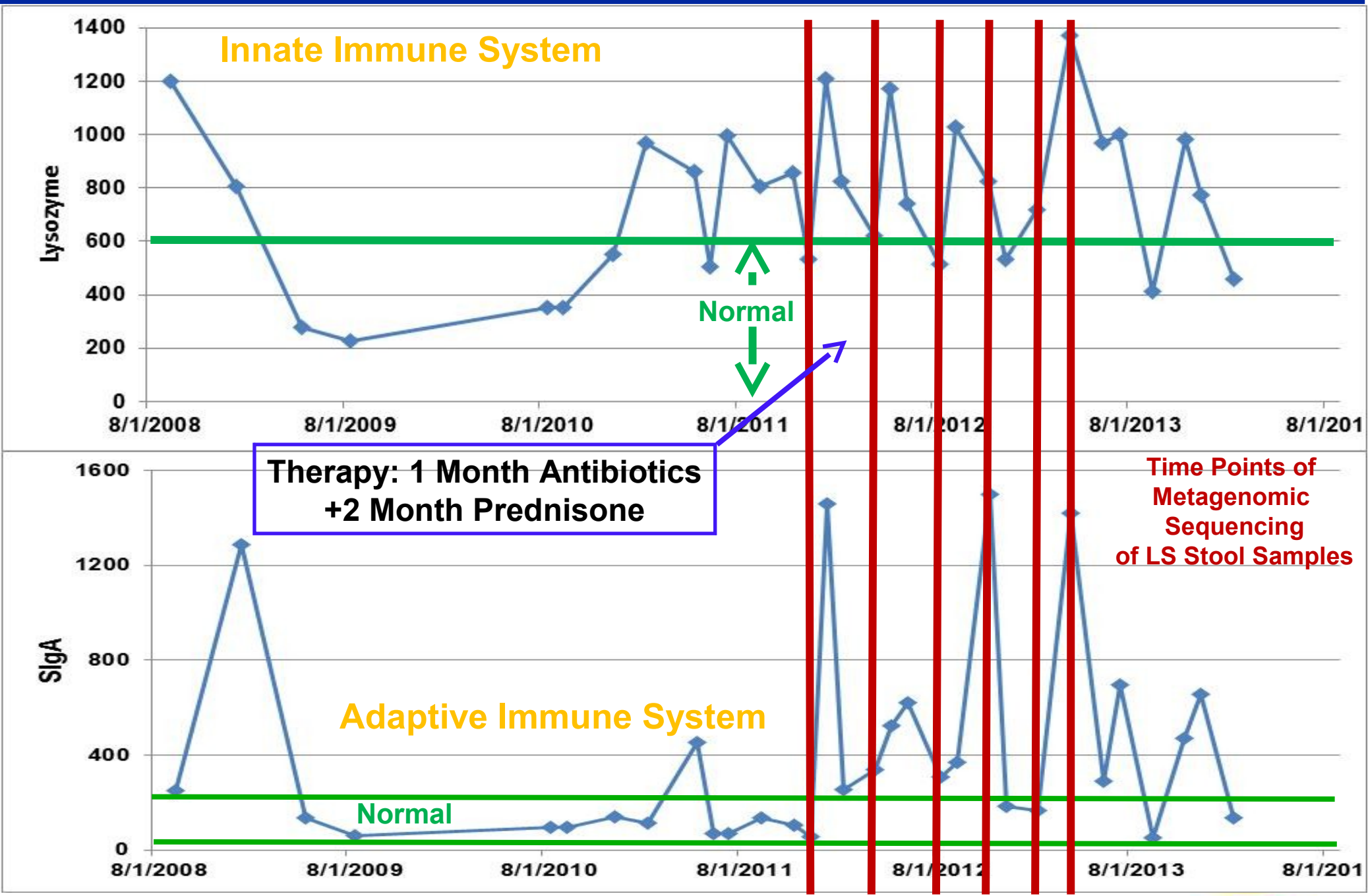


Top 20 Most Abundant Microbial Species In LS vs. Average Healthy Subject

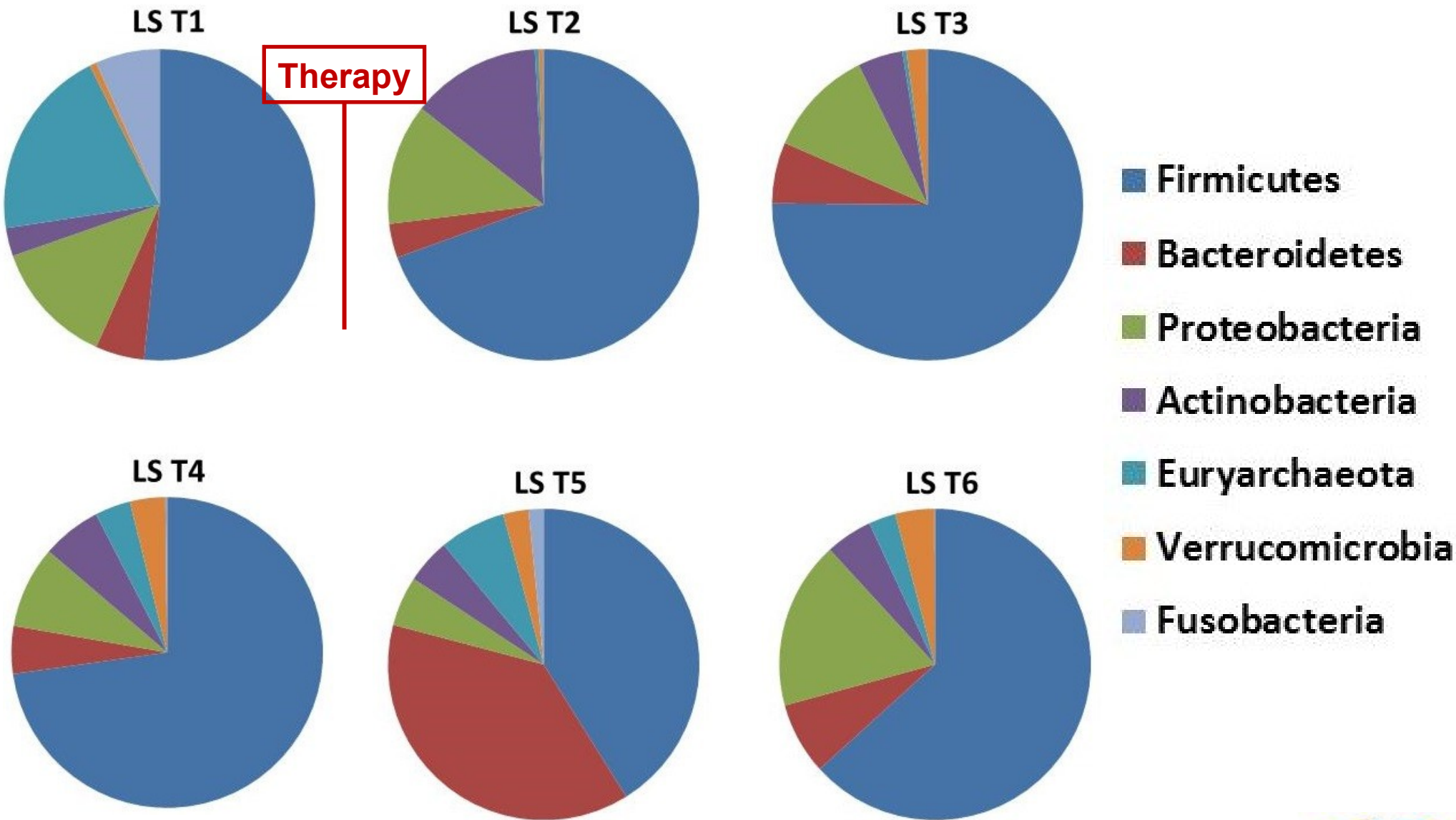


Source: Sequencing JCVI; Analysis Weizhong Li, UCSD
LS December 28, 2011 Stool Sample

Comparing Changes in Gut Microbiome Ecology with Oscillations of the Innate and Adaptive Immune System

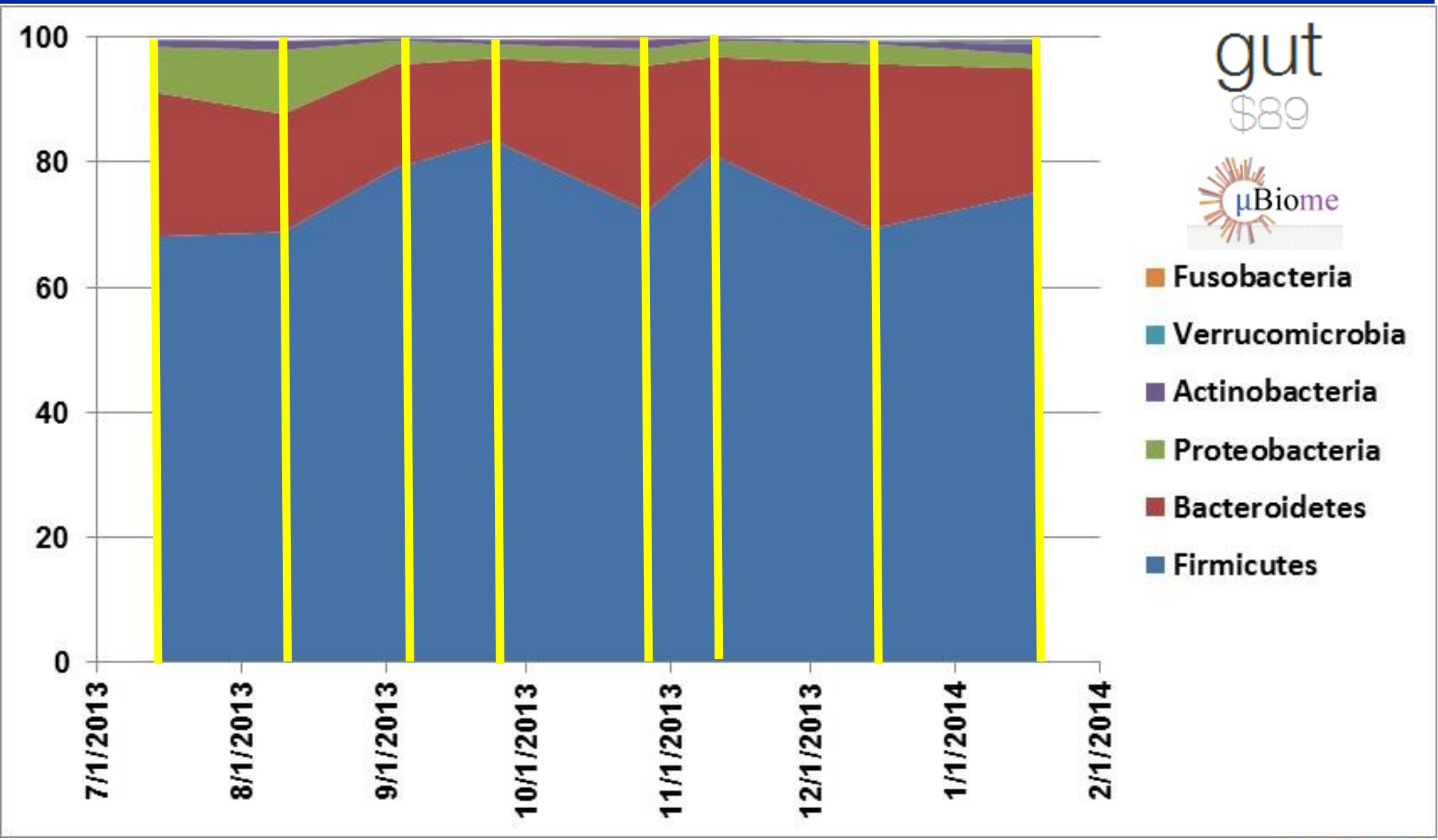


Time Series Reveals Autoimmune Dynamics of Gut Microbiome by Phyla



Six Metagenomic Time Samples Over 16 Months

Inexpensive 16S Time Series of Microbiome Now Possible Through Ubiome



Data source: LS (Yellow Lines Stool Samples); Sequencing and Analysis Ubiome

Thanks to Our Great Team!

UCSD Metagenomics Team

Weizhong Li
Sitao Wu

JCVI Team

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