COMPREHENSIVE MANAGEMENT OF AGING IN HIV INFECTED SUBJECTS

The gut microbiome in frail and non-frail elders

Comprehensive Management of Aging in HIV infected subjects Workshop

Nov, 25th, 2016

Marc Noguera-Julian, PhD

Outline

- Human microbiome
- Aging & Frailty Syndrome
- Gut Microbiome in frail aging
- Gut Microbiome in extreme longevity
- Gut Microbiome in non-frail aging
- The FRAME Study
- Summary


nature
THE INTERNATIONAL WEEKLY JOURNAL OF SCIENCE
First results from the Human Microbiome Project highlight the healthy variation in our microbial selves
PAGES 194, 207 & 215
FELLOW TRAVELLERS
CLIMATE CHANGE
GET USED TO UNCERTAINTY
Climate modelling faces its limits
PAGE 183
SOLAR SYSTEM
TITAN'S ELUSIVE METHANE
Tropical lakes on Saturn's enigmatic moon?
PAGE 237
NATURE.COM
14 June 2012


SCIENTIFIC AMERICAN
Your Inner Ecosystem
In your body, bacteria outnumber your own cells 10 to 1.
Who's in control?


The Economist
The Catholic church's unholy mess
Paul Ryan: the man with the plan
Generation Xhausted
China, victim of the Olympics?
On the origin of specie
AUGUST 18TH–24TH 2012
Economist.com
Microbes maketh man
How 90% of the cells in your body are bacteria, the benefits of faecal transplants, and other things you would rather not know before breakfast


nature
QUANTUM COMPUTERS
SUPERCONDUCTORS
NUCLEAR PROLIFERATION
OUR OTHER GENOME
A gene catalogue of the human gut microbiome

The human microbiome – Next Generation Sequencing

454 early access — 07/2005

Solexa early access — 01/2007

454 in production — 04/2007

SOLiD early access — 10/2007

454 Titanium — 07/2008

Solexa in production — 07/2008

Illumina GAIIx — 05/2009

454 1K — 12/2009

Illumina HiSeq 2000

PacBio

Ion Torrent

Illumina MiSeq

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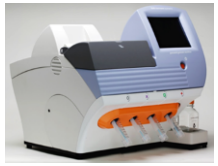
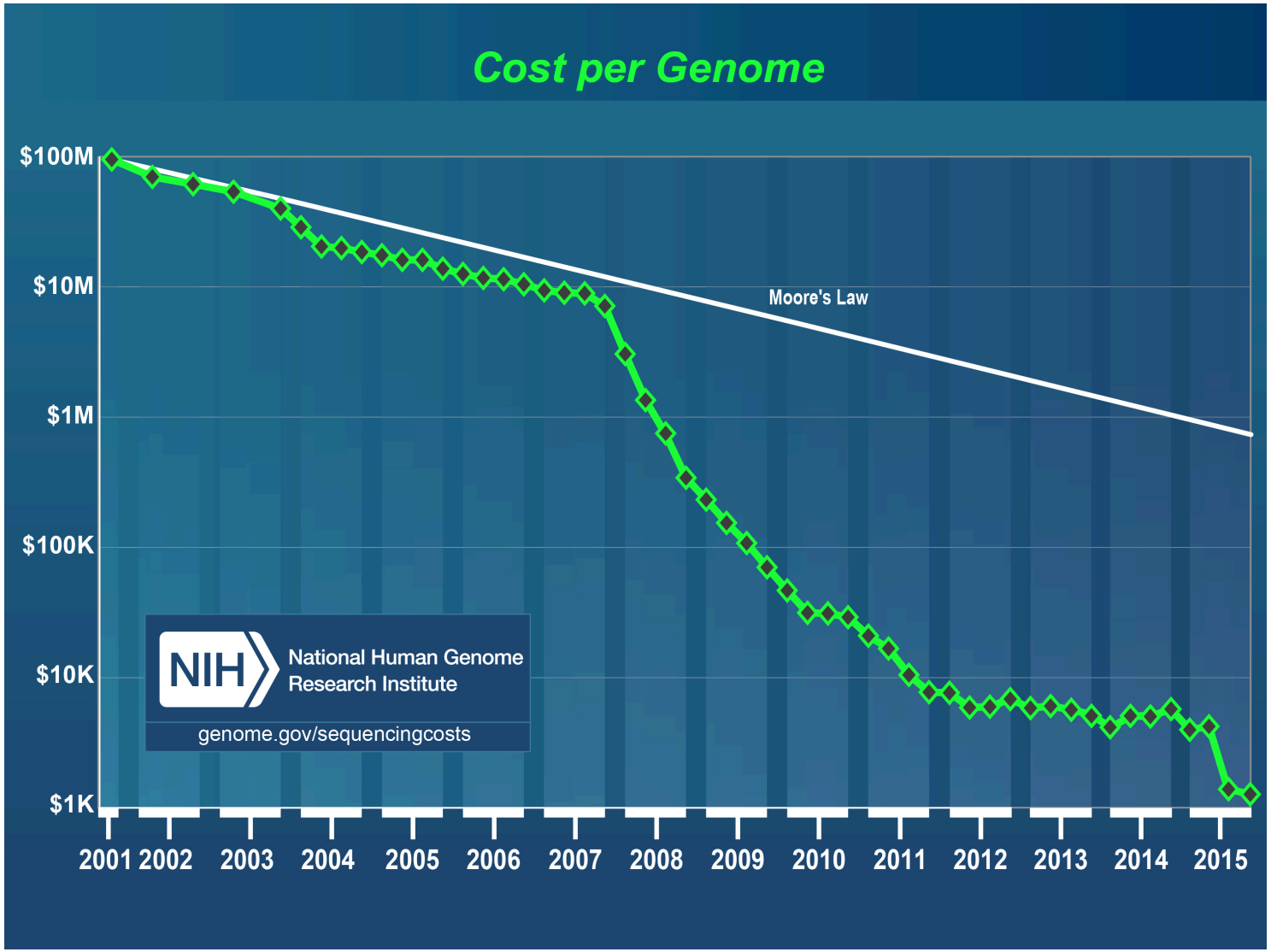
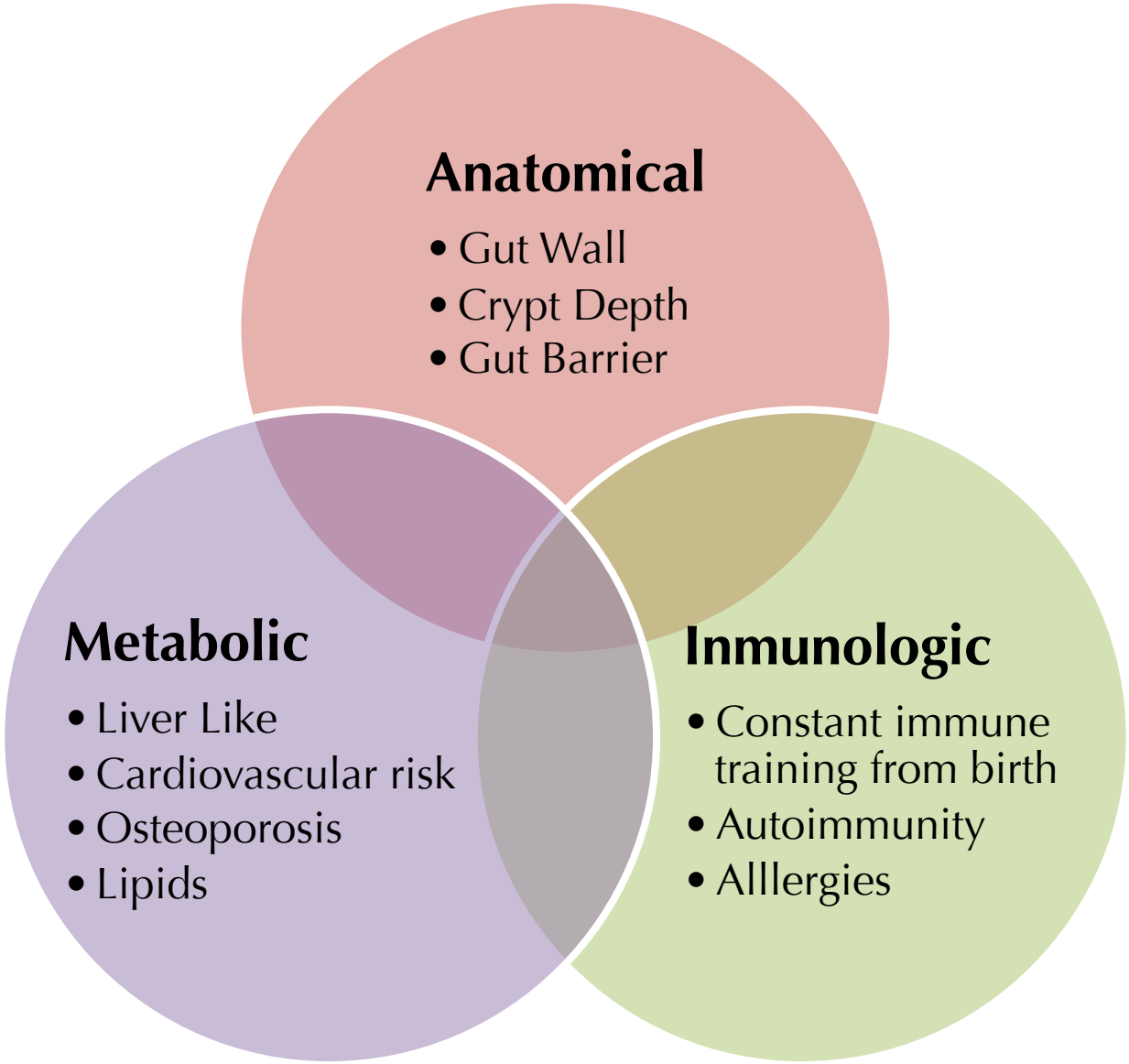


ADAPTED FROM FENG ET AL

NGS Sequencing Costs

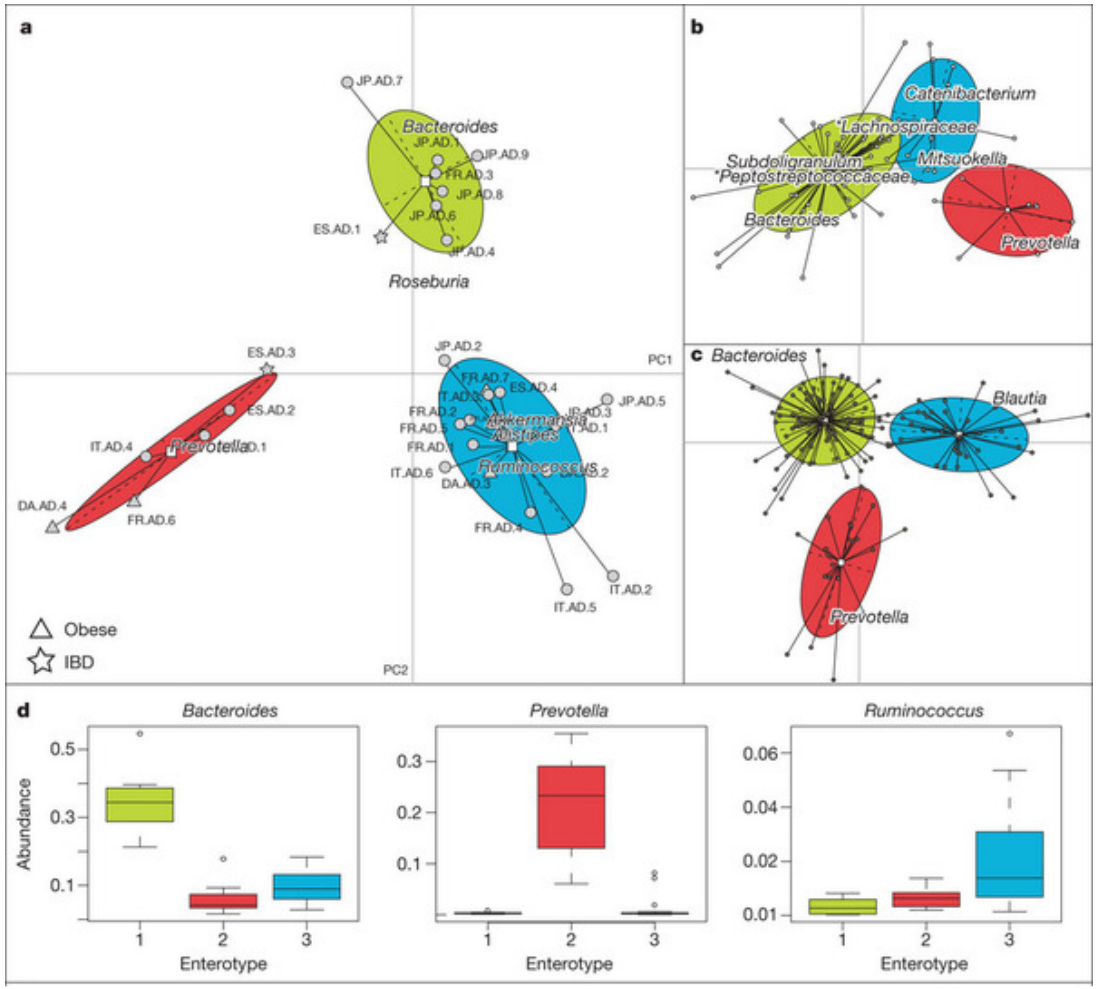


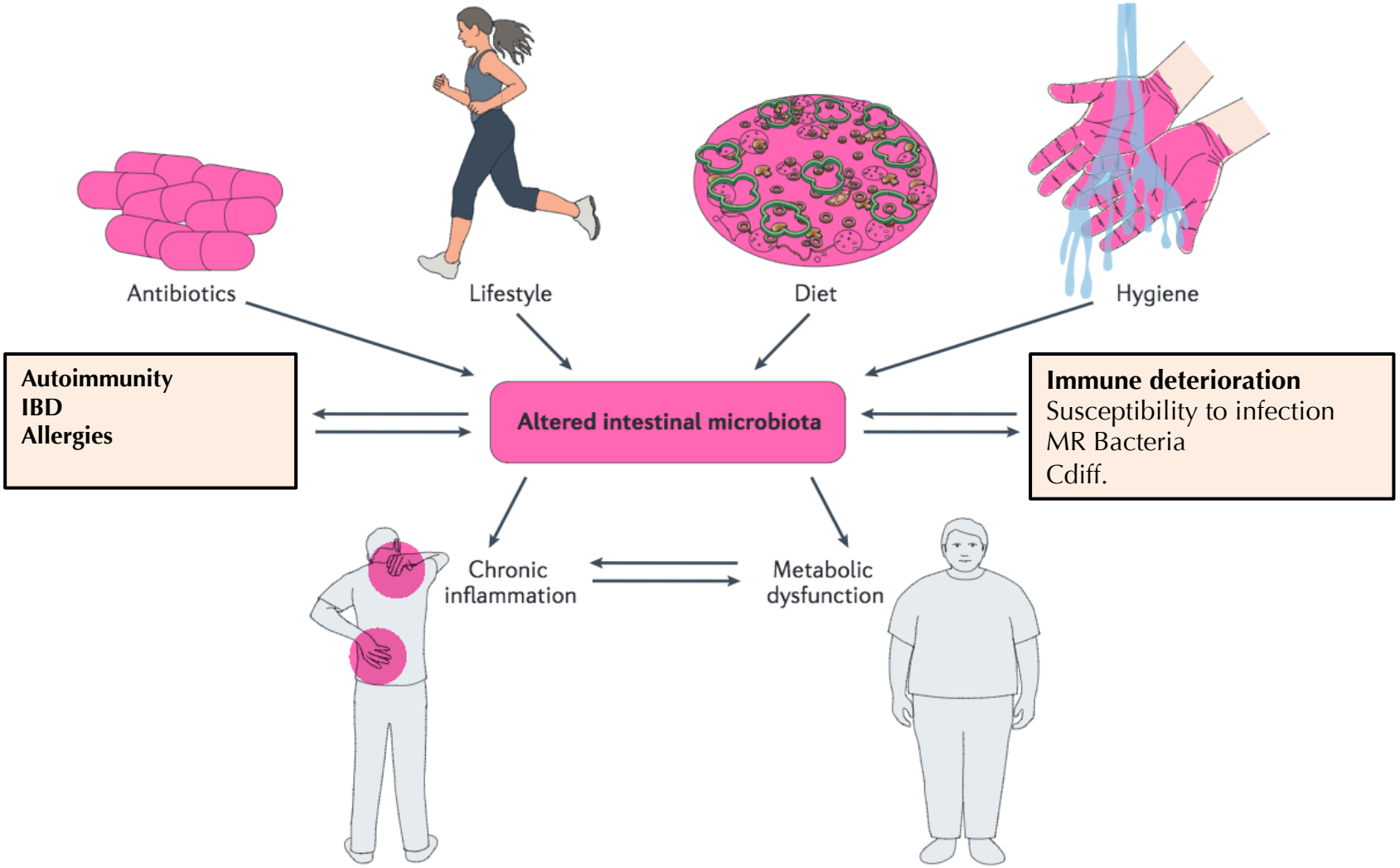

Anatomical
• Gut Wall
• Crypt Depth
• Gut Barrier
Metabolic
• Liver Like
• Cardiovascular risk
• Osteoporosis
• Lipids
Inmunologic
• Constant immune training from birth
• Autoimmunity
• Alllergies

Gut Microbiome's composition is structured

Enterotypes of the human gut microbiome

Manimozhiyan Arumugam[1*], Jeroen Raes[1,2*], Eric Pelletier[3,4,5], Denis Le Paslier[3,4,5], Takuji Yamada[1], Daniel R. Mende[1], Gabriel R. Fernandes[1,6], Julien Tap[1,7], Thomas Bruls[3,4,5], Jean-Michel Batto[7], Marcelo Bertalan[8], Natalia Borruel[9], Francesc Casellas[9], Leyden Fernandez[10], Laurent Gautier[8], Torben Hansen[11,12], Masahira Hattori[13], Tetsuya Hayashi[14], Michiel Kleerebezem[15], Ken Kurokawa[16], Marion Leclerc[7], Florence Levenez[7], Chaysavanh Manichanh[9], H. Bjørn Nielsen[8], Trine Nielsen[11], Nicolas Pons[7], Julie Poulain[3], Junjie Qin[17], Thomas Sicheritz-Ponten[8,18], Sebastian Tims[15], David Torrents[10,19], Edgardo Ugarte[3], Erwin G. Zoetendal[15], Jun Wang[17,20], Francisco Guarner[9], Oluf Pedersen[11,21,22,23], Willem M. de Vos[15,24], Søren Brunak[8], Joel Doré[7], MetaHIT Consortium†, Jean Weissenbach[3,4,5], S. Dusko Ehrlich[7] & Peer Bork[1,25]



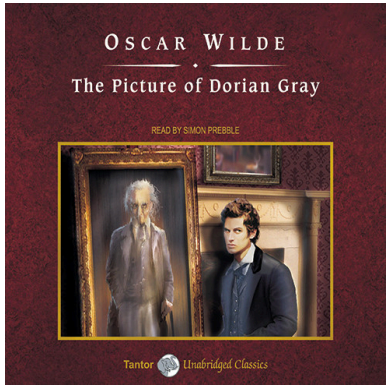


Sommer, Bäckhed. Nat Microb Reviews 2013

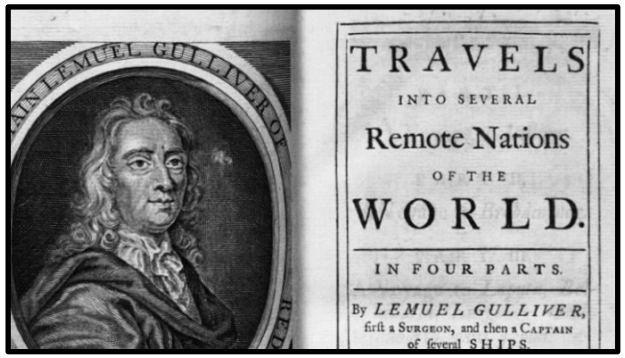
“In the land of frailty, confusion, contradiction, and ambiguity reign supreme.”

Ferrucci et al, J. of Gerontology, 2006, 61, 260-1

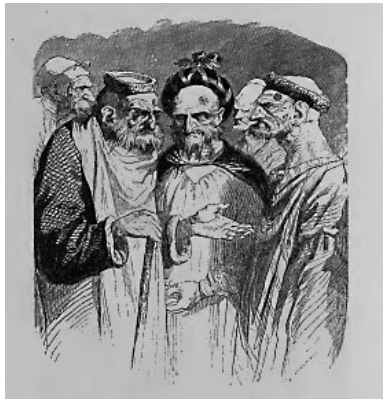
Aging & Frailty



FRAILTY: "liability to be crushed or to decay, either in material or immaterial sense … moral weakness, instability of mind, liability to err or yield to temptation"



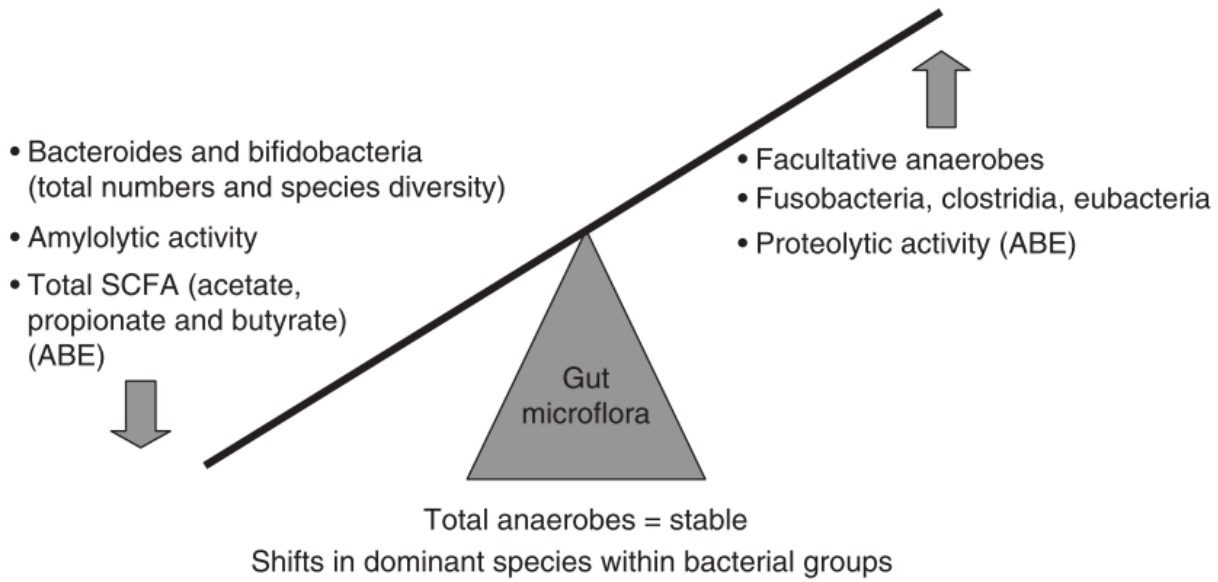
"when they come to fourscore years, which is reckoned the extremity of living in this country, they had not only all the follies and infirmities of other older men, but many more which arose from the dreadful prospect of never dying."



Ferrucci L, Mahallati A, Simonsick EM: Frailty and the foolishness of Eos. J Gerontol A Biol Sci Med Sci 2006, 61:260–1.

Aging & Gut microbiome

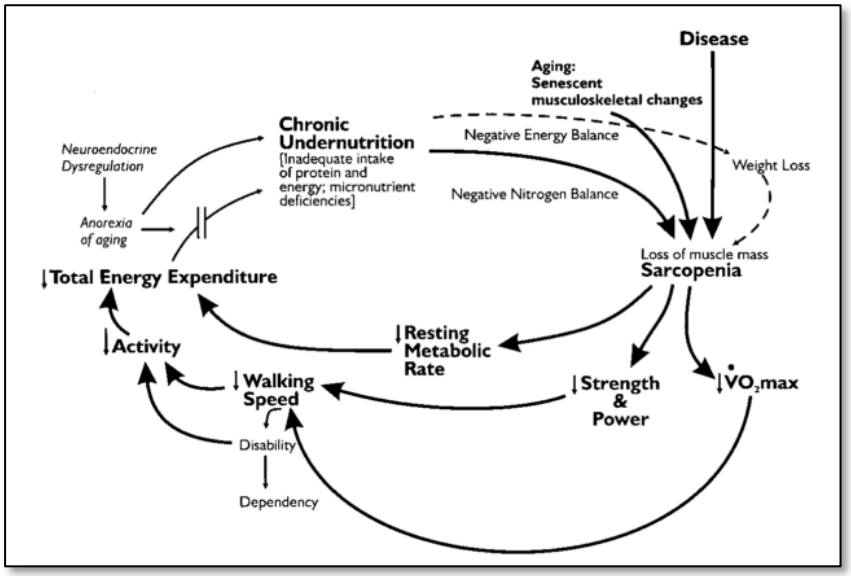
- Changes in the GI tract(mastication function, loss of teeth, swallowing difficulties) can lead to nutritional imbalance
- Decreased intestinal motility and slow intestinal transit results in constipation and reduction of faecal bacterial matter with an associated increase of putrefactive proccesses.
- Age associated inflammation ('inflammaging') is a hallmark of aging and is linked to immune senescence and and increased activation of several pro-inflammatory pathways.

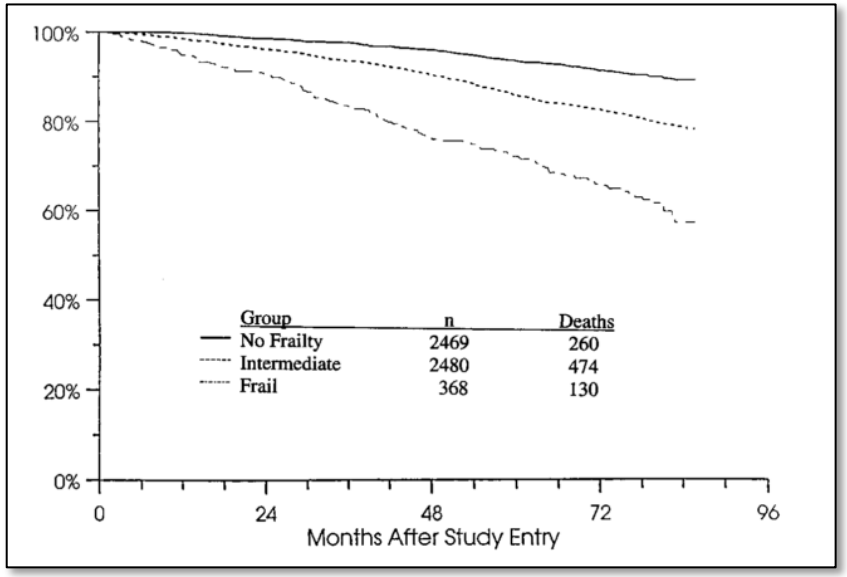


From Woodmansey EJ: Intestinal bacteria and ageing. In Journal of Applied Microbiology. Volume 102; 2007:1178–1186.

FRAILTY

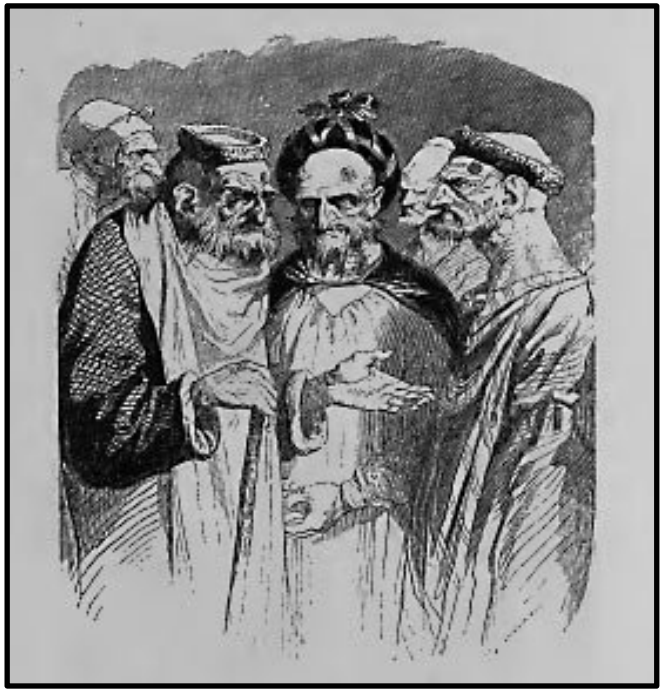
- Frailty is a complex multifunctional syndrome characterized by loss of weight & muscular mass, weakness, functionality loss within and aging background
- Functionally defined by deficit accumulation. Different Scales (Fried[1,2], Edmonton[3], SOF[4]…).
- Prevalence of frailty/Pre-frailty increases with age range from ~7-10% or 20-30% in >65 or >85 year old population.[5]
- Biologically characterized to a decreased function of innate and adaptive immune sytem, dysregulation of several key physiological systems, including the neuroendocrine, musculoskeletal, metabolic and inmune/ inflammatory[6] including increased levels of several inflammation markers(IL-6,CRP).





[1]Fried LP et al. Frailty in older adults: evidence for a phenotype. J Gerontol A Biol Sci Med Sci 2001, 56:M146-56. [2]Bandeen-Roche K, et al: Phenotype of frailty: characterization in the women's health and aging studies. J Gerontol A Biol Sci Med Sci 2006, 61:262–6. [3]Rolfson DB, Majumdar SR, Tsuyuki RT, Tahir A, Rockwood K: Validity and reliability of the Edmonton Frail Scale. Age Ageing 2006, 35:526–9. [4]SOF index: 1. Ensrud KE: Comparison of 2 Frailty Indexes for Prediction of Falls, Disability, Fractures, and Death in Older Women. Arch Intern Med 2008, 168:382. 5Collard RM, Boter H, Schoevers RA, Oude Voshaar RC: Prevalence of frailty in community-dwelling older persons: a systematic review. J Am Geriatr Soc 2012, 60:1487–92. [6]Fulop T, McElhaney J, Pawelec G, Cohen AA, Morais JA, Dupuis G, Baehl S, Camous X, Witkowski JM, Larbi A: Frailty, Inflammation and Immunosenescence. Interdisciplinary topics in gerontology and geriatrics 2015:26–40.

The gut microbiome in frailty



The Struldbrugs
Gulliver's Travels, by Jonathan Swift

Microbiome in Frailty – Bartosch, 2004

Bartosch S, Fite A, Macfarlane GT, McMurdo MET: ***Characterization of bacterial communities in feces from healthy elderly volunteers and hospitalized elderly patients using real-time PCR and effects of antibiotic treatment on the fecal microbiota***. *Appl Environ Microbiol 2004, 70:3575–81.*

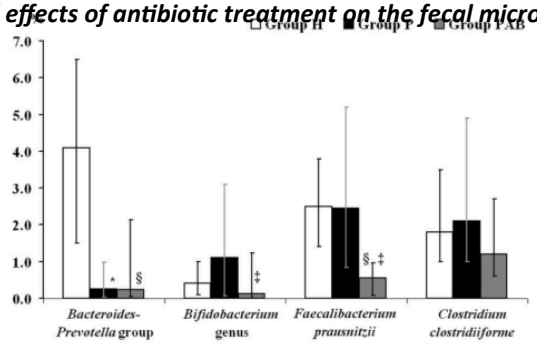


FIG. 1. Percentage of 16S rRNA gene copies of the predominant fecal bacterial groups and species in relation to total bacterial 16S rRNA gene copies (relative abundance). The percentage was calculated for each individual, and the median was determined for each subject group. A nonparametric Mann-Whitney U test was used for pairwise comparison of the subject groups. Error bars represent the interquartile range. *, significant difference ($P < 0.05$) between groups H and P; §, significant difference ($P < 0.05$) between groups H and PAB; ‡, significant difference ($P < 0.05$) between groups P and PAB.

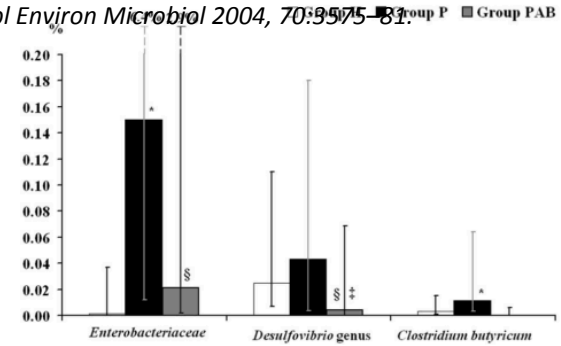


FIG. 2. Percentage of 16S rRNA gene copies of less abundant fecal bacterial groups and species in relation to total bacterial 16S rRNA gene copies (relative abundance). The percentage was calculated for each individual, and the median was determined for each subject group. A nonparametric Mann-Whitney U test was used for pairwise comparison of the subject groups. Error bars represent the interquartile range. *, significant difference ($P < 0.05$) between groups H and P; §, significant difference ($P < 0.05$) between groups H and PAB; ‡, significant difference ($P < 0.05$) between groups P and PAB.

TABLE 4. Summary of findings on fecal bacteria in groups of hospitalized patients compared to the control group

Organism	Shifts observed from control group H to group P			Shifts observed from control group H to group PAB		
	16S rRNA gene copy numbers	Relative abundance	Prevalence	16S rRNA gene copy numbers	Relative abundance	Prevalence
All eubacteria	Significant reduction	Not applicable	No change	Significant reduction	Not applicable	No change
Bacteroides-Prevotella group	Significant reduction	Significant reduction	Decrease	Significant reduction	Significant reduction	Decrease
Bifidobacterium genus	Nonsignificant reduction	Nonsignificant increase	No change	Significant reduction	No change	Decrease
Desulfovibrio genus	Nonsignificant reduction	Nonsignificant increase	No change	Significant reduction	Significant reduction	Decrease
Enterobacteriaceae	Nonsignificant increase	Significant increase	No change	No change	Significant reduction	No change
Faecalibacterium prausnitzii	Significant reduction	No change	No change	Significant reduction	Significant reduction	No change
Clostridium clostridiiforme	Significant reduction	No change	No change	Significant reduction	Nonsignificant reduction	No change
Clostridium butyricum	No change	Significant increase	No change	Significant reduction	Significant reduction	Decrease
Ruminococcus albus	Nonsignificant reduction	No change	Decrease	Significant reduction	Significant reduction	Decrease
Enterococcus faecalis	Nonsignificant increase	Significant increase	Increase	Significant increase	Significant increase	Increase

Microbiome in Frailty – van Tongeren, 2005

*van Tongeren SP, Slaets JPJ, Harmsen HJM, Welling GW: **Fecal microbiota composition and frailty**. Appl Environ Microbiol 2005, 71:6438–42.*

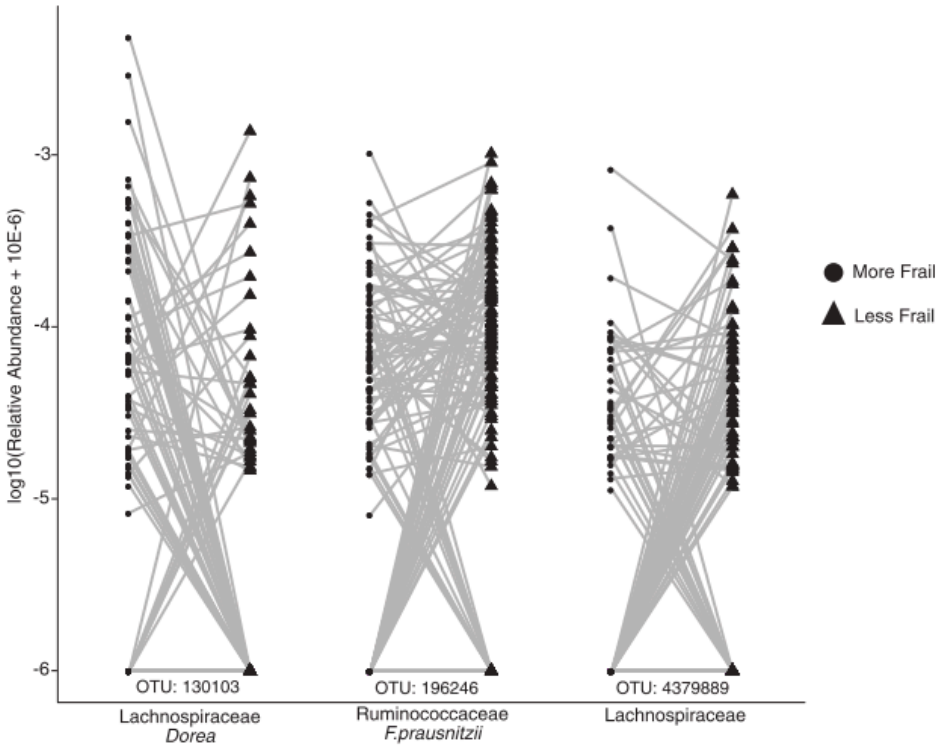
- Cross-sectional study from 23 elderly (median 86 years) in residential care
- Fluorescent in situ hybridization (FISH)
- GFI (Groningen) frailty scale determines

- Fecal samples from volunteers with high frailty scores showed a significant reduction in the number **of lactobacilli** (26-fold).
- At much higher population levels, both **the Bacteroides/Prevotella** (threefold) and the Faecalibacterium prausnitzii (fourfold) groups showed a significant reduction in percentage of total number of hybridizable bacteria in the elderly with high frailty scores.
- In contrast to this, the number of Enterobacteriaceae was significantly higher (sevenfold) in samples from very frail volunteers.

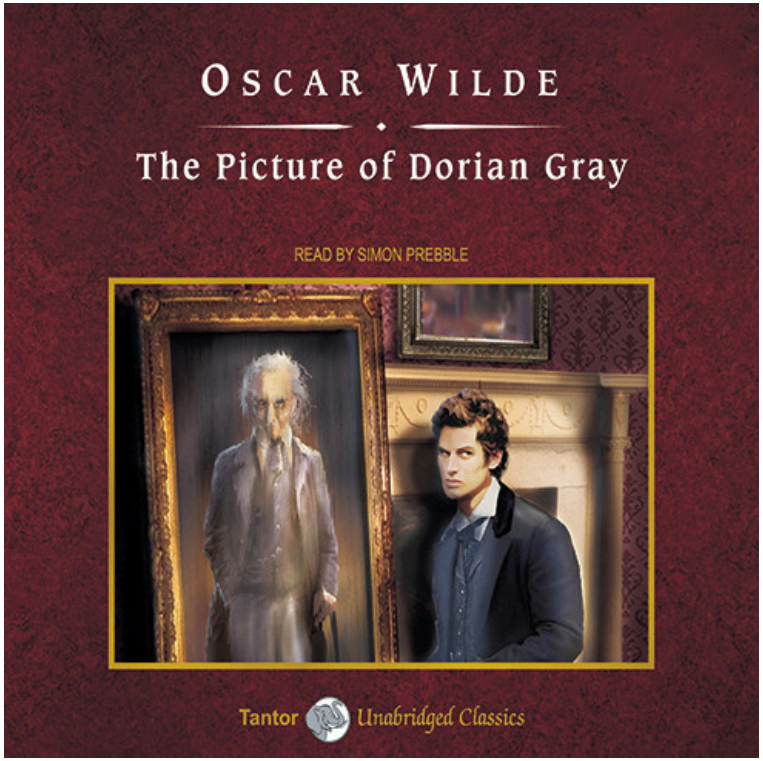
Microbiome signatures in frailty – Jackson, 2016

Jackson M, et al: Signatures of early frailty in the gut microbiota. Genome Med 2016, 8:8.

- Twin Study including 728 female twins with (mean age 63 yo) from twinsUK cohort.
- 16s rRNA sequencing from fecal samples
- Frailty quantified through the Rockwood Frailty Index. Generally low frailty indices.
- Frailty negatively associated with gut microbiota diversity
- *F. Prausnitzii* and *Ruminococcaceae* negatively correlated and *E. dolichum* and *E. lenta.*
- *E. lenta* has been associated to GI disease while *E. dolichum* is enriched in genes involved in simple sugar processing



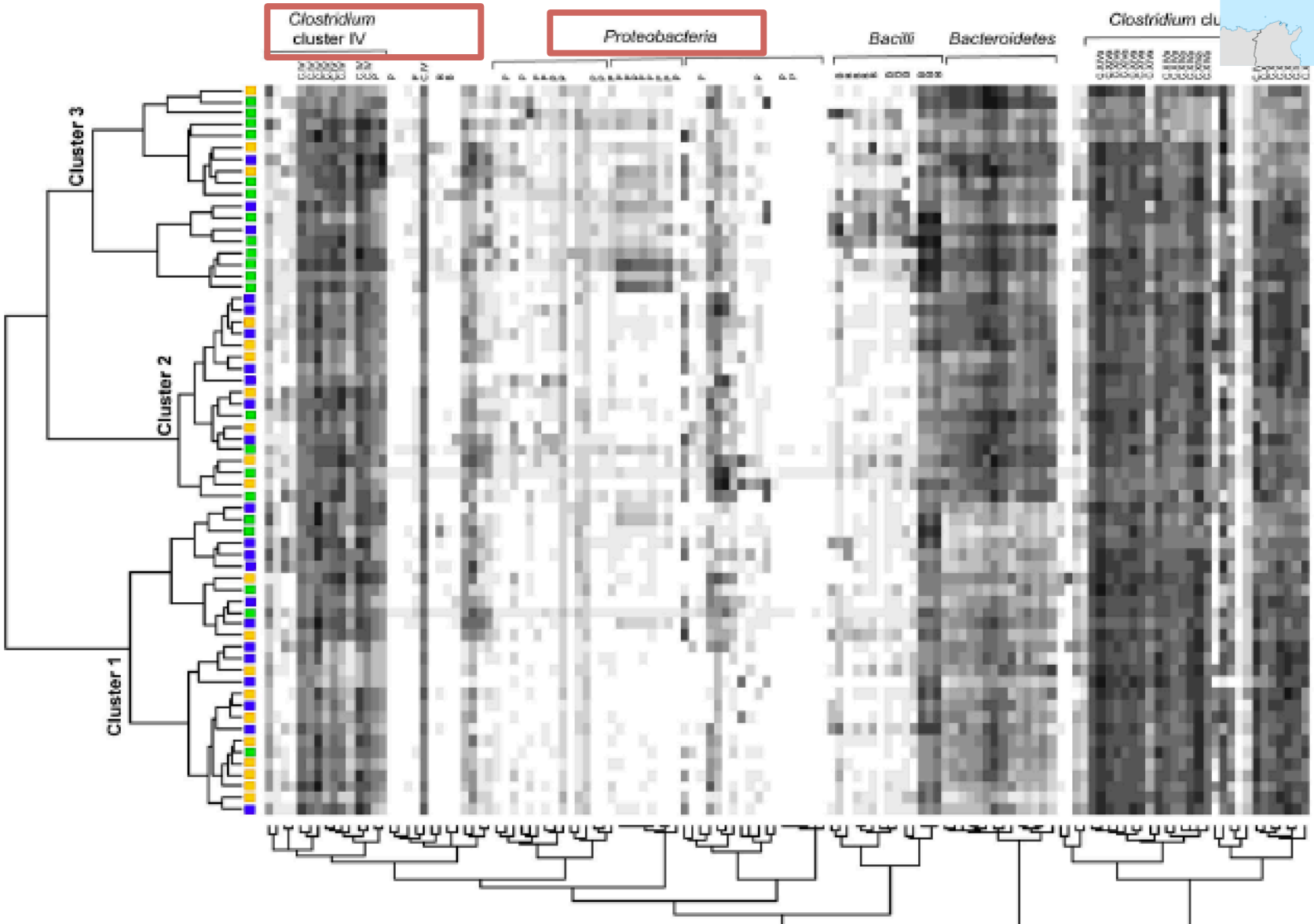
The microbiome in extreme longevity



Ferrucci et al, J. of Gerontology, 2006, 61, 260-1

Microbiome in Centenarian subjects – Biagi, 2010

Biagi E, et al: ***Through ageing, and beyond: gut microbiota and inflammatory status in seniors and centenarians****. PLoS One 2010, 5:e10667.*



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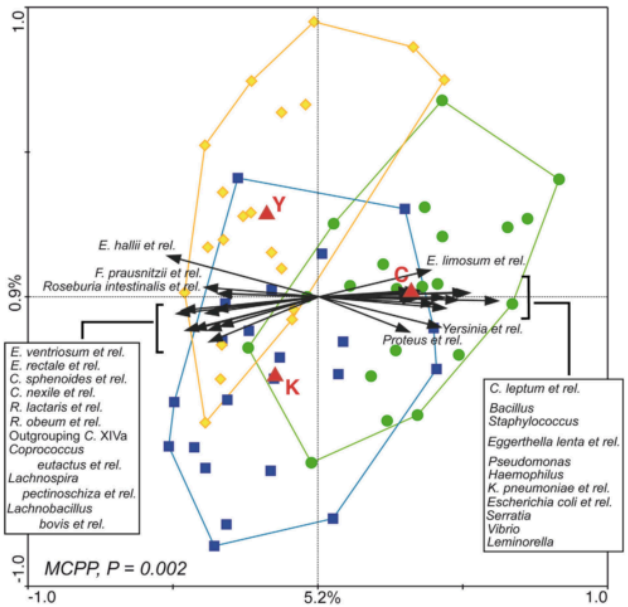


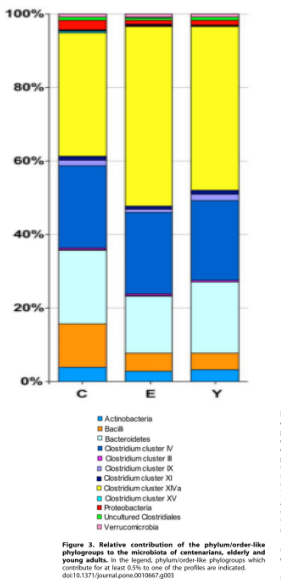
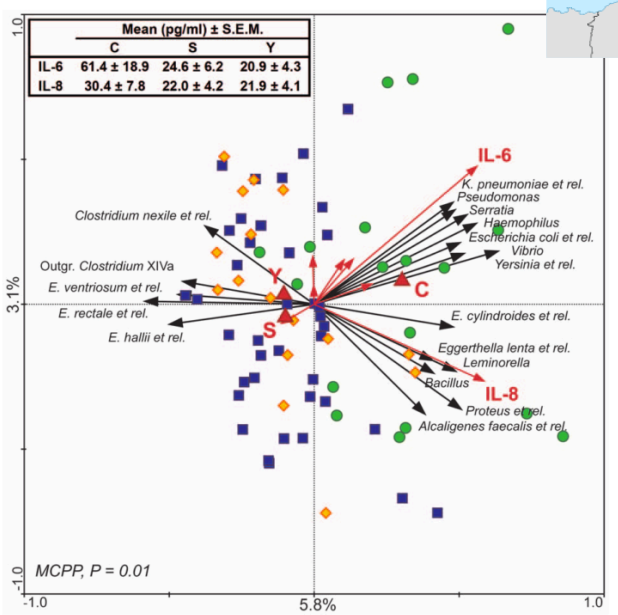


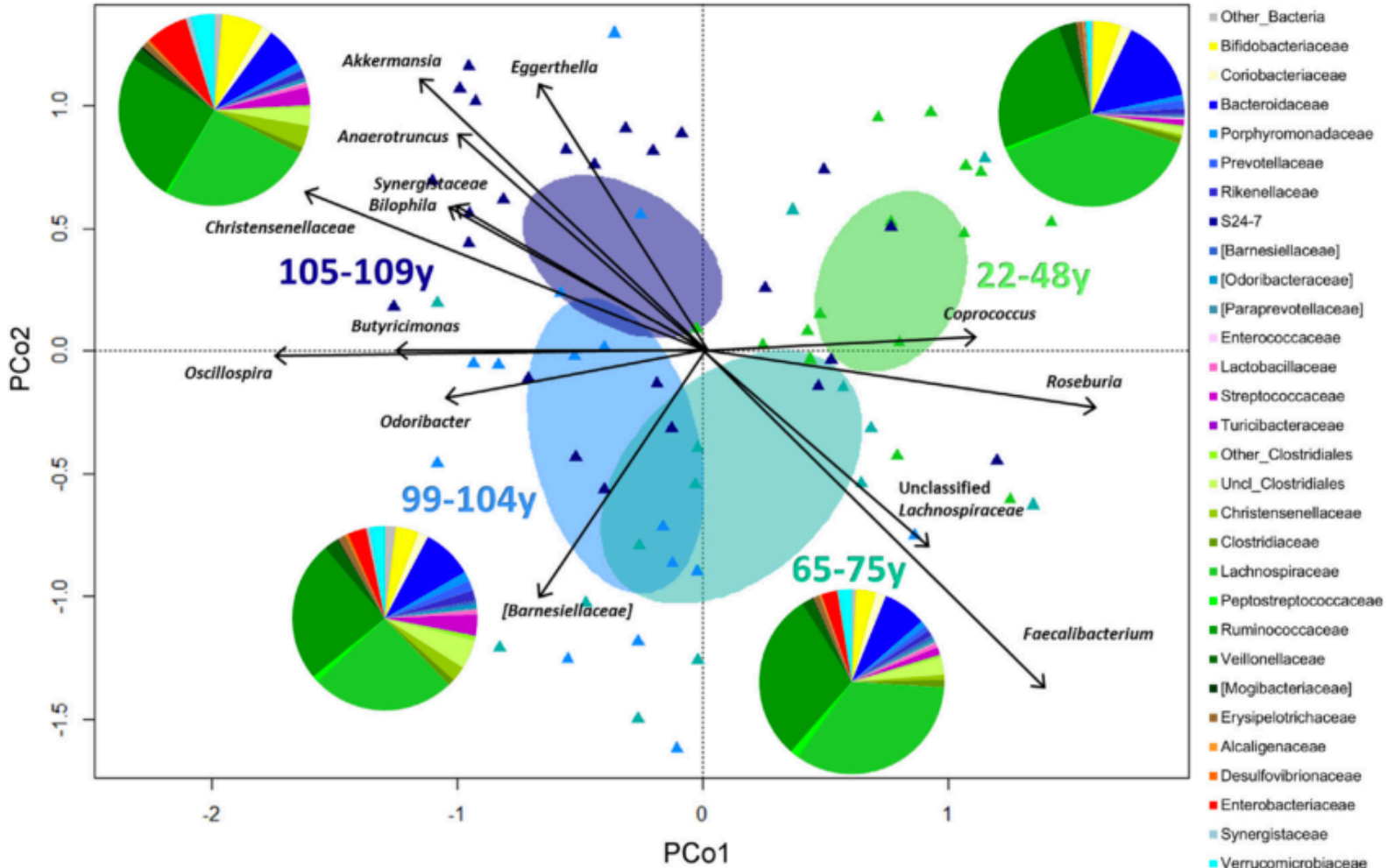
Figure 3. Relative contribution of the phylum/order-like phylogroups to the microbiota of centenarians, elderly and young adults. In the legend, phylum/order-like phylogroups which contribute for at least 0.5% to one of the profiles are indicated.
doi:10.1371/journal.pone.0010667.g003



- Microbial composition and diversity of the gut ecosystem of young adults and seventy-years old people was similar and both differed significantly from that of the centenarians, which showed **lower bacterial diversity**.
- ***Firmicutes*** **phylum rearrangement in centenarians** with an enrichment in anaerobes, notably **pathobionts,** which associates with increased inflammatory status, with a parallel decrease in *F.prausnitzii* and other bacterial species with anti-inflammatory properties.
- Notably, *E. limosum, E. lenta* and *A. Muciniphila* were increased in centenarians.

Microbiome in Centenarian subjects – Biagi, 2016

Biagi E, et al: ***Gut Microbiota and Extreme Longevity. Curr Biol 2016, 26:1480–1485.***

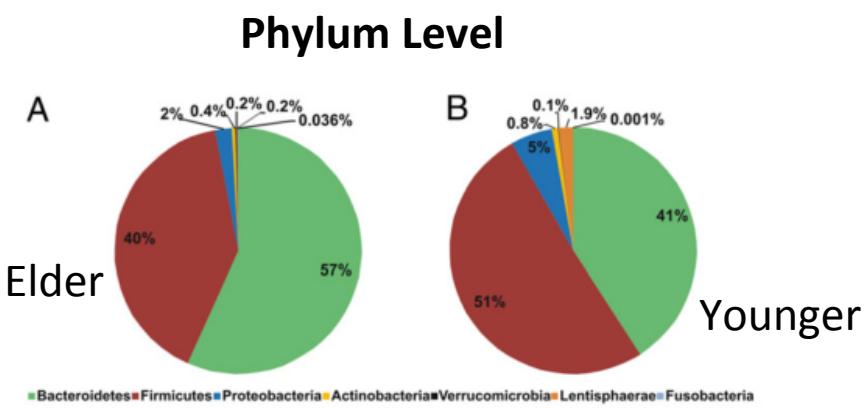


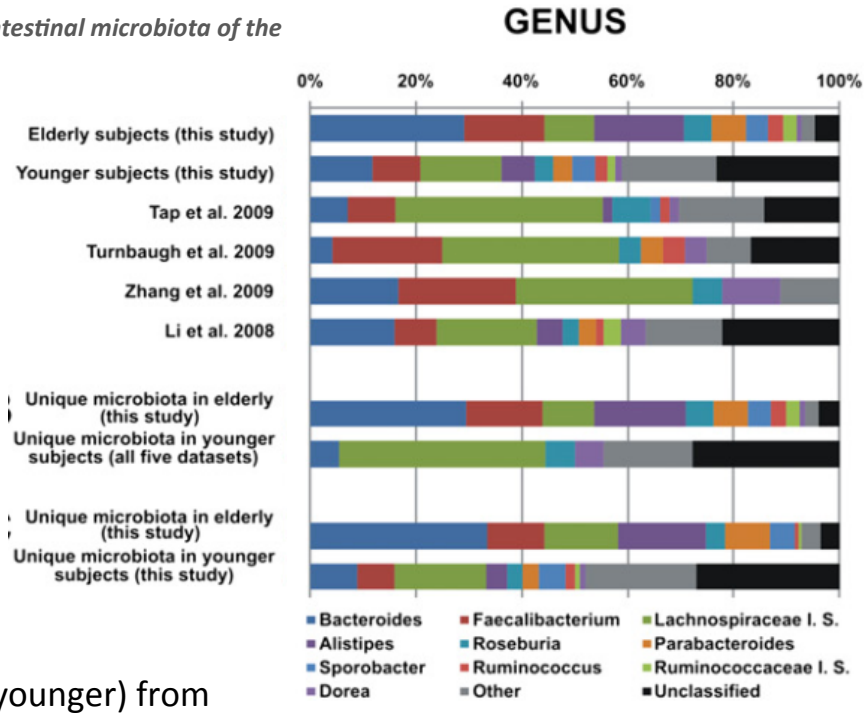
The microbiome through age

Ferrucci et al, J. of Gerontology, 2006, 61, 260-1

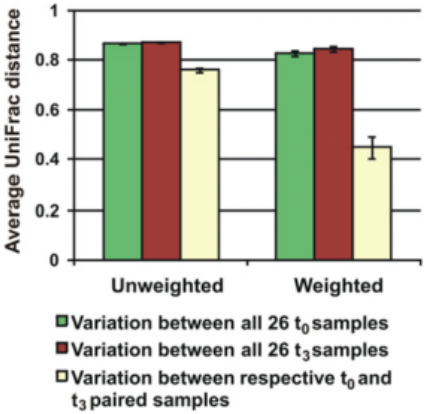
The Frail Microbiome – Claesson, 2011

Claesson MJ, et al: ***Composition, variability, and temporal stability of the intestinal microbiota of the elderly.*** *Proc Natl Acad Sci U S A 2011, 108 Suppl:4586–91.*





- Cross-Sectional study of 170 individuals (161 >65yo & 9 younger) from ELDERMET consortium. 3-month follow-up of 26 individuals. V3-V4 rRNA gene pyrosequencing.
- Different Bacteroidetes/Firmicutes ratio found in young and elder population highly variable microbiome
- A Bacteroidetes dominated and distinct core microbiome was found for elders composed whereas younger subjects had one unique Bacteroides species and were Firmicutes phylum dominated in a highly variable context.
- A given microbiota after 3 mo was more like to localize to its original composition than to any other sample analyzed, thus indicating temporal stability



The Frail Microbiome – Claesson, 2012

Claesson MJ, et al: ***Gut microbiota composition correlates with diet and health in the elderly****. Nature 2012, 488:178–184.*

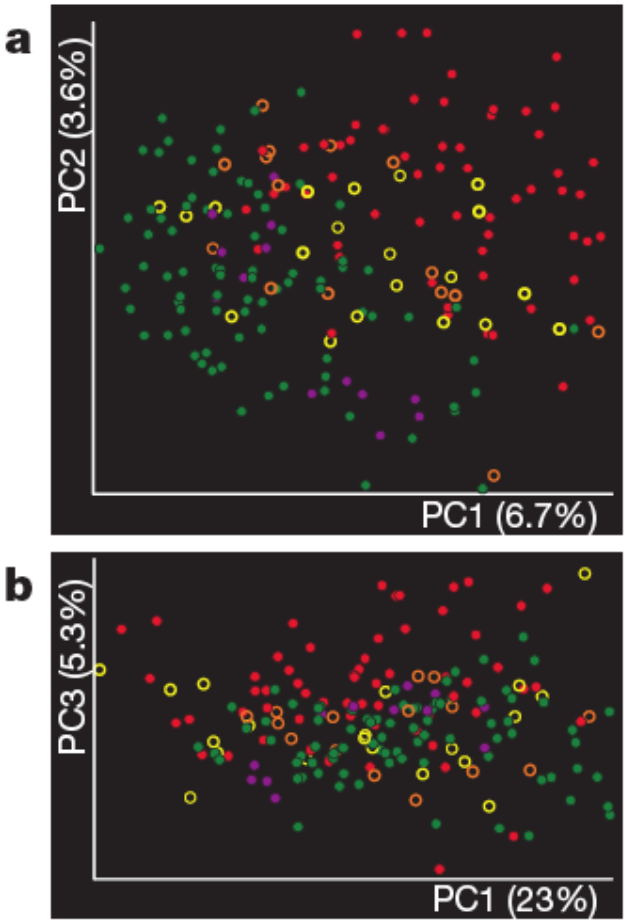
Fecal microbiota characterization of 178 individuals:

- 83 community dwelling subjects (CD)
- 20 attending out-patient day hospital (DH)
- 15 in short-term rehabilitation (RH)
- 60 in long-term residential care, meaning >6 weeks (LC)
- 13 young adults (median age 36 y.o.)(YH)

Health state characterization

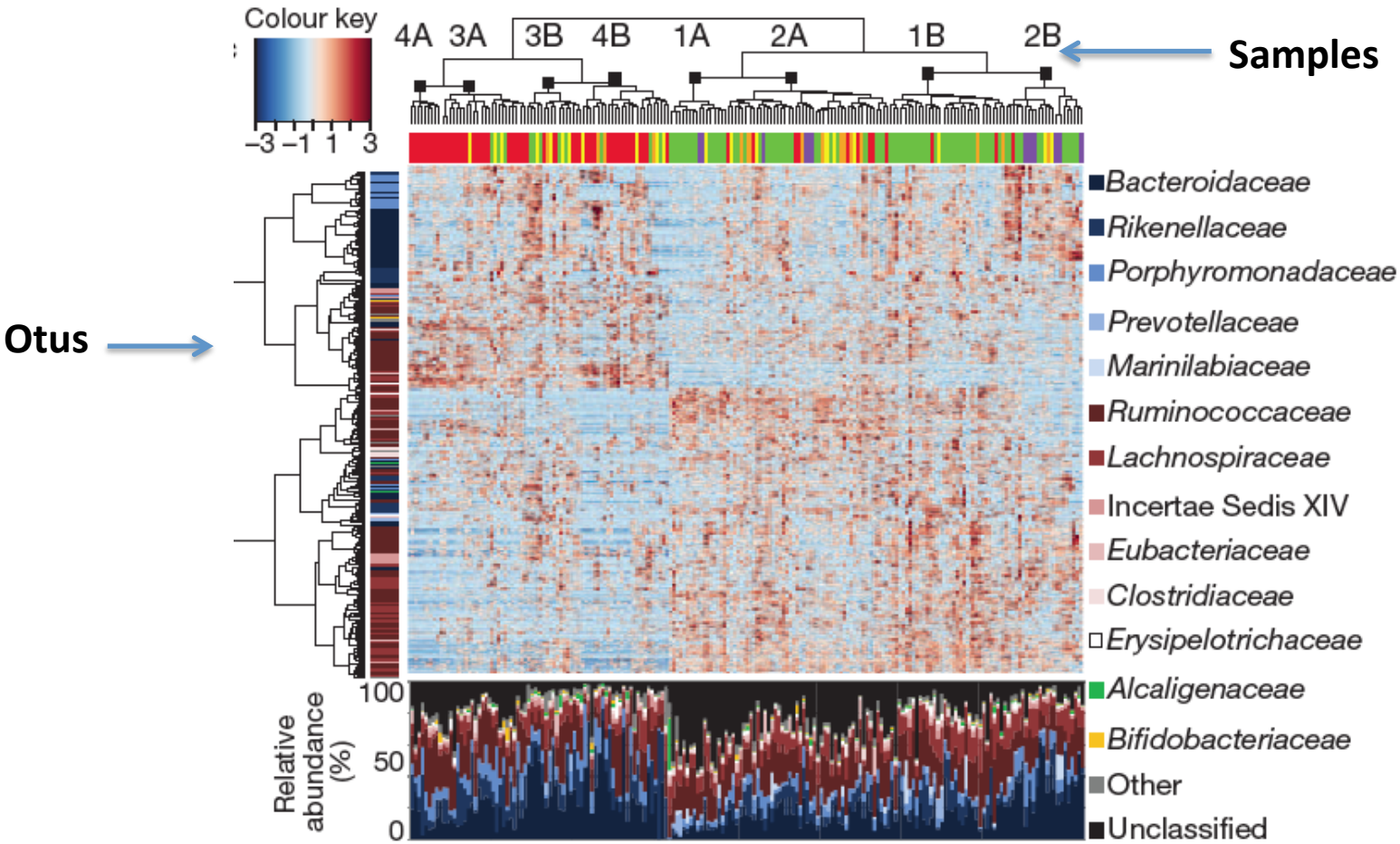
Diet Questionnaires

- The microbiota of older people displays greater inter-individual variation than that of younger adults.
- Clear separation between community-dwelling and long-stay subjects using both weighted and un-weighted analysis
- The faecal microbiota composition from 178 elderly subjects formed groups, correlating with residence location in the community, day-hospital, rehabilitation or in long-term residential care.



Unweighted(a) and Weighted(B) UniFrac PCoA analysis of 47,563 OTUs

Microbiota and Residence Location – Claesson, 2012

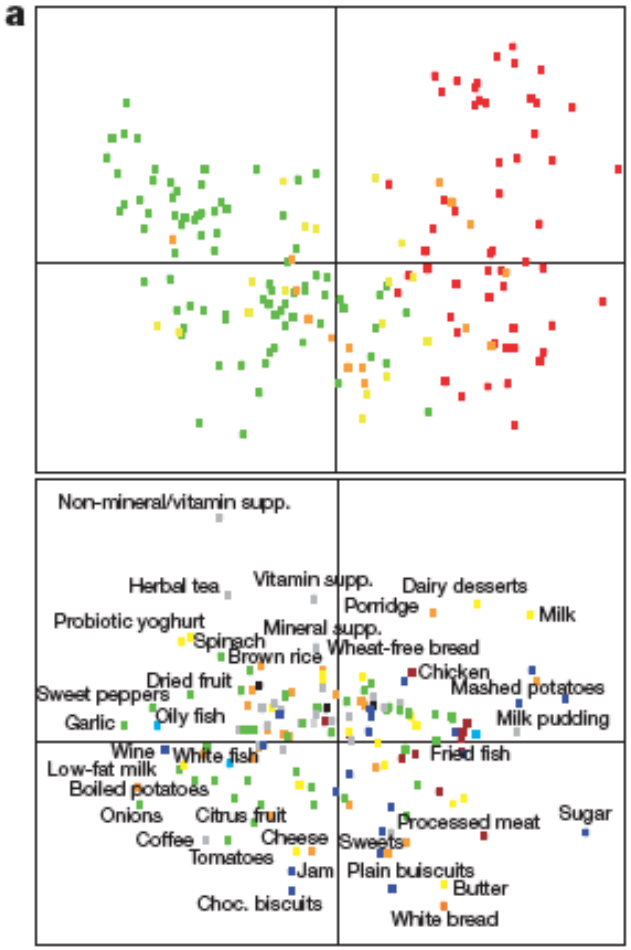


Hierarchical Ward-linkage clustering based on the Spearman correlation coefficients of the proportion of OTUs, filtered for OTU subject prevalence of at least 20%.

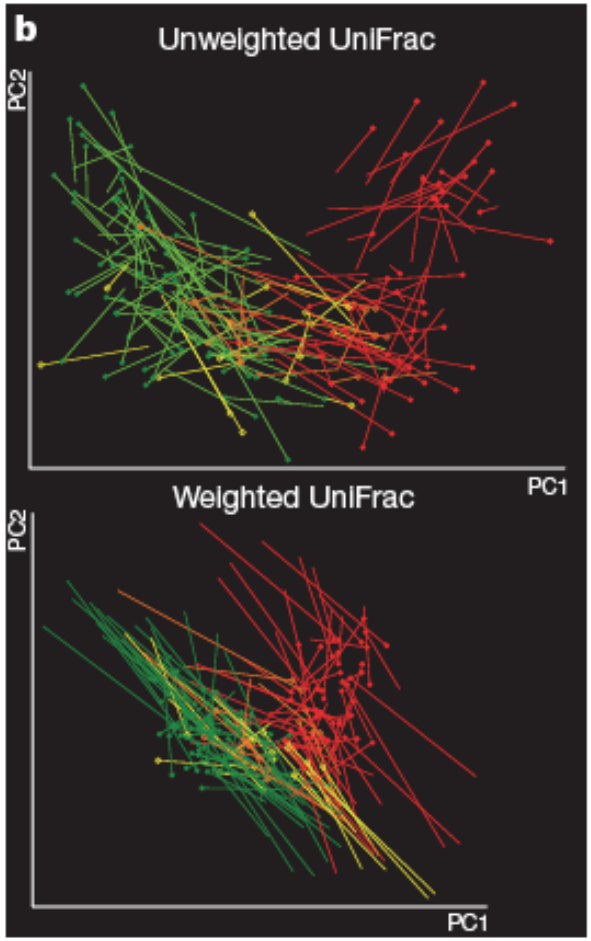
1. Claesson MJ, et al: ***Gut microbiota composition correlates with diet and health in the elderly***. *Nature 2012, 488:178–184.*

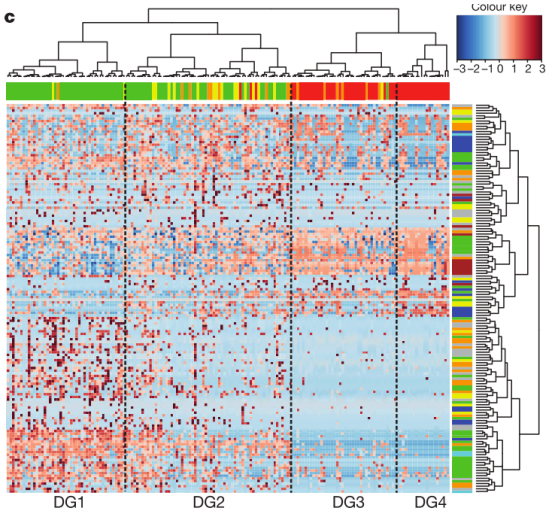
Microbiome & Diet – Claesson, 2012

Claesson MJ, et al: ***Gut microbiota composition correlates with diet and health in the elderly****. Nature 2012, 488:178–184.*



11% Variance Explained





Procrustes analysis: Co-segregation of diet and microbiota along first axis

CD DH RH LC YH

Healthy Diet and Microbiota – Claesson, 2012

Claesson MJ, et al: ***Gut microbiota composition correlates with diet and health in the elderly****. Nature 2012, 488:178–184.*

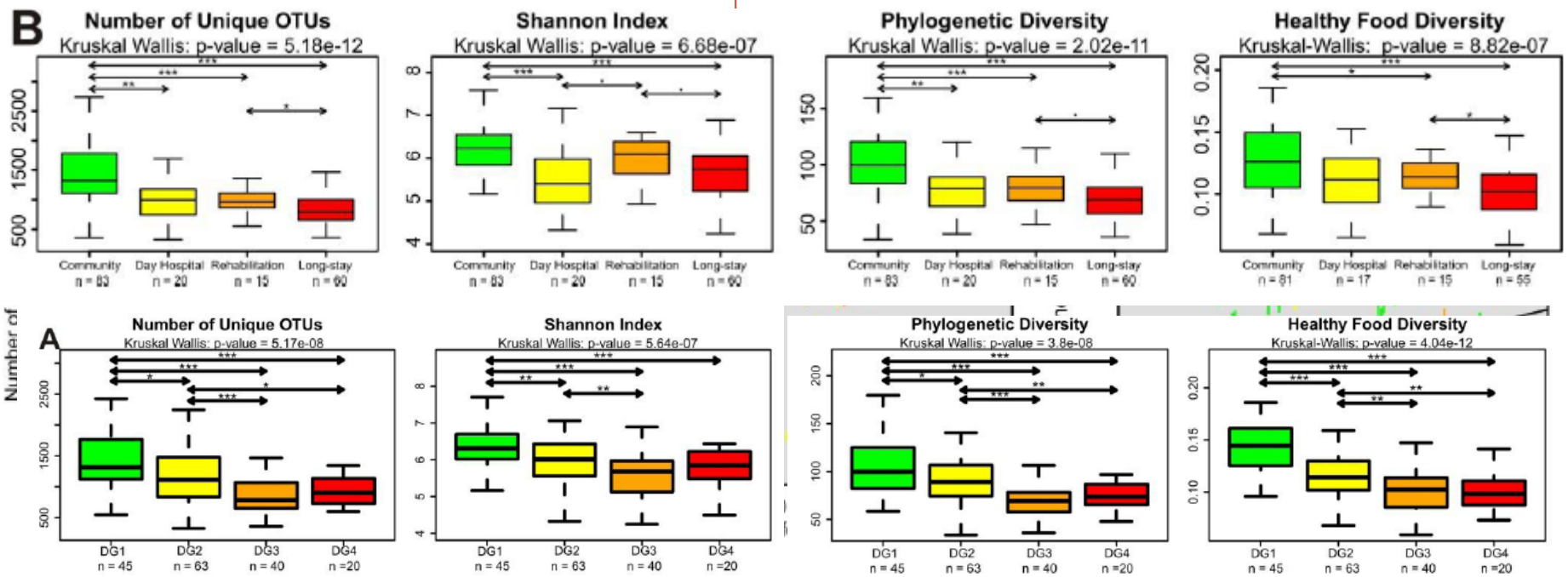
- Healthy food diversity index (HFD) is based on a distribution measure mainly applied in economic and ecological studies. It considers 3 aspects important for healthy food diversity: number, distribution, and health value of consumed foods

The Journal of Nutrition
Nutrient Requirements and Optimal Nutrition

A New Index to Measure Healthy Food Diversity Better Reflects a Healthy Diet Than Traditional Measures[1,2]

Larissa S. Drescher,[3] Silke Thiele,[3*] and Gert B. M. Mensink[4]

[3]Department of Food Economics and Consumption Studies, Christian-Albrechts-University of Kiel, D-24098 Kiel, Germany and [4]Robert Koch-Institute, D-13353 Berlin, Germany



Drescher, L. S et al. A new index to measure healthy food diversity better reflects a healthy diet than traditional measures. 2007. The Journal of Nutrition, 137(3), 647–51.

Aging Microbiota & Health Correlations – Claesson, 2012

Claesson MJ, et al: ***Gut microbiota composition correlates with diet and health in the elderly****. Nature 2012, 488:178–184.*

Regression tests of associations between clinical measurements and microbiota composition (Using PCoA PCs)

a Unweighted UniFrac PCoA for all four residence locations

Parameter	PC1			PC2		
	RC range	RC s.d.	*P*	RC range	RC s.d.	*P*
GDT	–0.42	–0.11	0.6	**–2.7**	**–0.54**	**0.037**
Diastolic blood pressure	0.97	0.25	0.81	**–10.1**	**–2.02**	**0.033**
Weight	**–14.6**	**–3.8**	**0.033**	–7.16	–1.43	0.27
CC	**–3.9**	**–1.01**	**0.022**	–2.9	–0.58	0.19
IL-6	**6.71**	**1.7**	**0.006**	**6.1**	**1.22**	**0.007**
IL-8	4.23	1.1	0.43	**13.6**	**2.7**	**0.03**
TNF-α	1.1	0.28	0.31	0.62	0.13	0.72

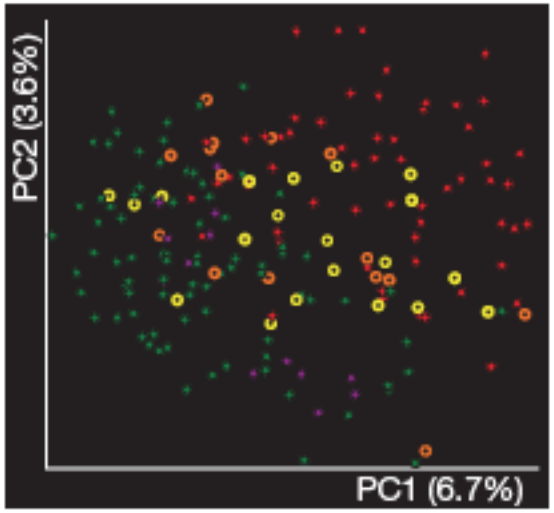
b Unweighted UniFrac PCoA for community-only subjects

Parameter	PC1			PC2		
	RC range	RC s.d.	*P*	RC range	RC s.d.	*P*
MNA	–1.1	–0.26	0.29	**1.9**	**0.5**	**0.006**
Diastolic blood pressure	–8.4	–1.98	0.08	**14.3**	**3.4**	**0.035**
GDT	–0.13	–0.03	0.8	**–1.5**	**–0.35**	**0.02**

c Unweighted UniFrac PCoA for long-stay-only subjects

Parameter	PC1			PC2		
	RC range	RC s.d.	*P*	RC range	RC s.d.	*P*
Barthel	**–6**	**–1.5**	**0.004**	**–4.8**	**–1.3**	**0.036**
FIM	**–30.8**	**–7.8**	**0.046**	**–33.3**	**–4.7**	**0.024**
MMSE	–12.15	–3.08	0.14	**–18.4**	**–4.8**	**0.009**
MNA	–3.87	–0.98	0.23	**–11.2**	**–3**	**0.004**
BMI	–1.2	–0.31	0.69	**–5**	**–1.3**	**0.047**
CC	0.2	0.05	0.93	**–6.8**	**–1.77**	**0.0016**
Diastolic blood pressure	**19.3**	**4.9**	**0.015**	**–12.4**	**–3.24**	**0.034**
Systolic blood pressure	**36.5**	**9.3**	**0.007**	–1.57	–0.41	0.83
Weight	–3.2	–0.81	0.69	**–12.7**	**–3.3**	**0.024**
IL-8	–2.56	–0.65	0.78	**22.31**	**5.84**	**0.006**
CRP	**13.9**	**3.53**	**0.02**	–3.01	–0.8	0.27

a

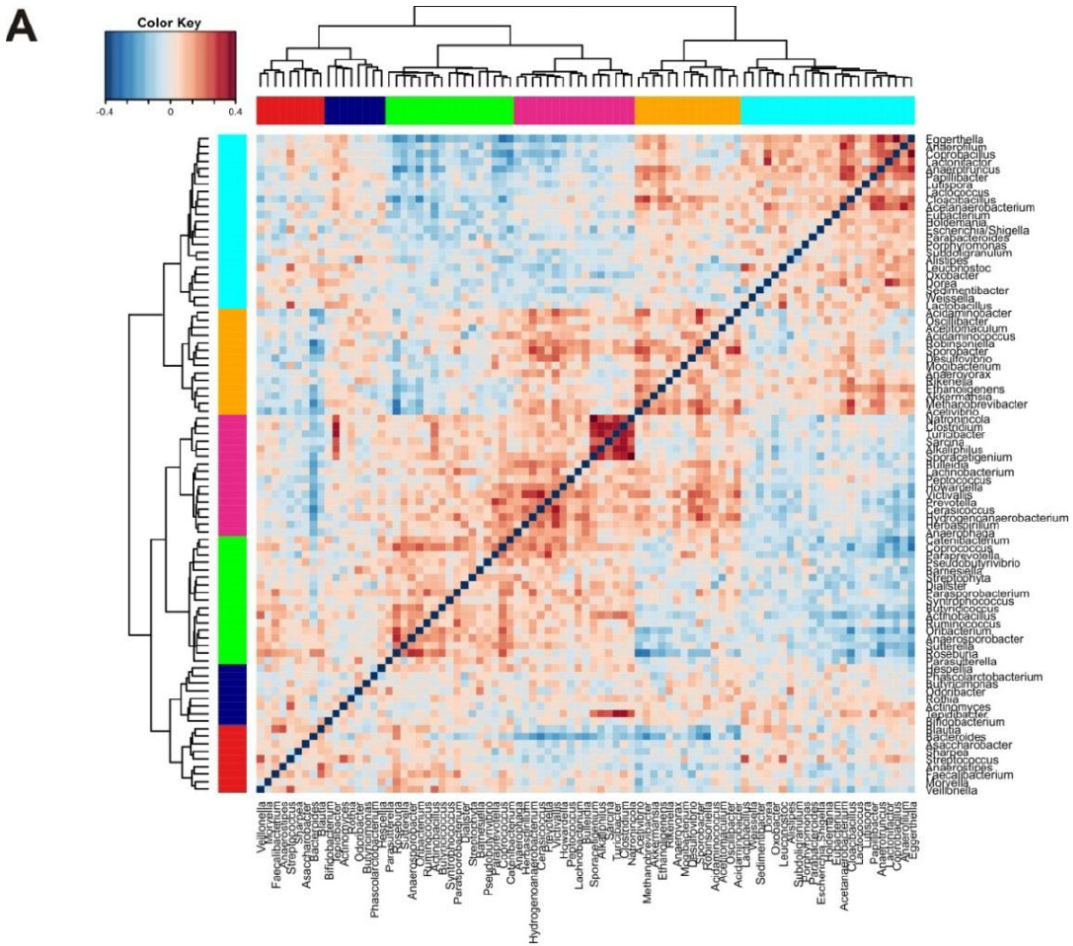


- Charlson Comorbidity index (**CCI**)
- Geriatric depression Test(**GDT**)
- Barthel Index
- Functional Independence Measure (**FIM**)
- Mini-mental state Exam (**MMSE**)
- Mini Nutrititional assessment (**MNA**)

Microbiota structure evolution – Claesson, 2012

Claesson MJ, et al: ***Gut microbiota composition correlates with diet and health in the elderly****. Nature 2012, 488:178–184.*

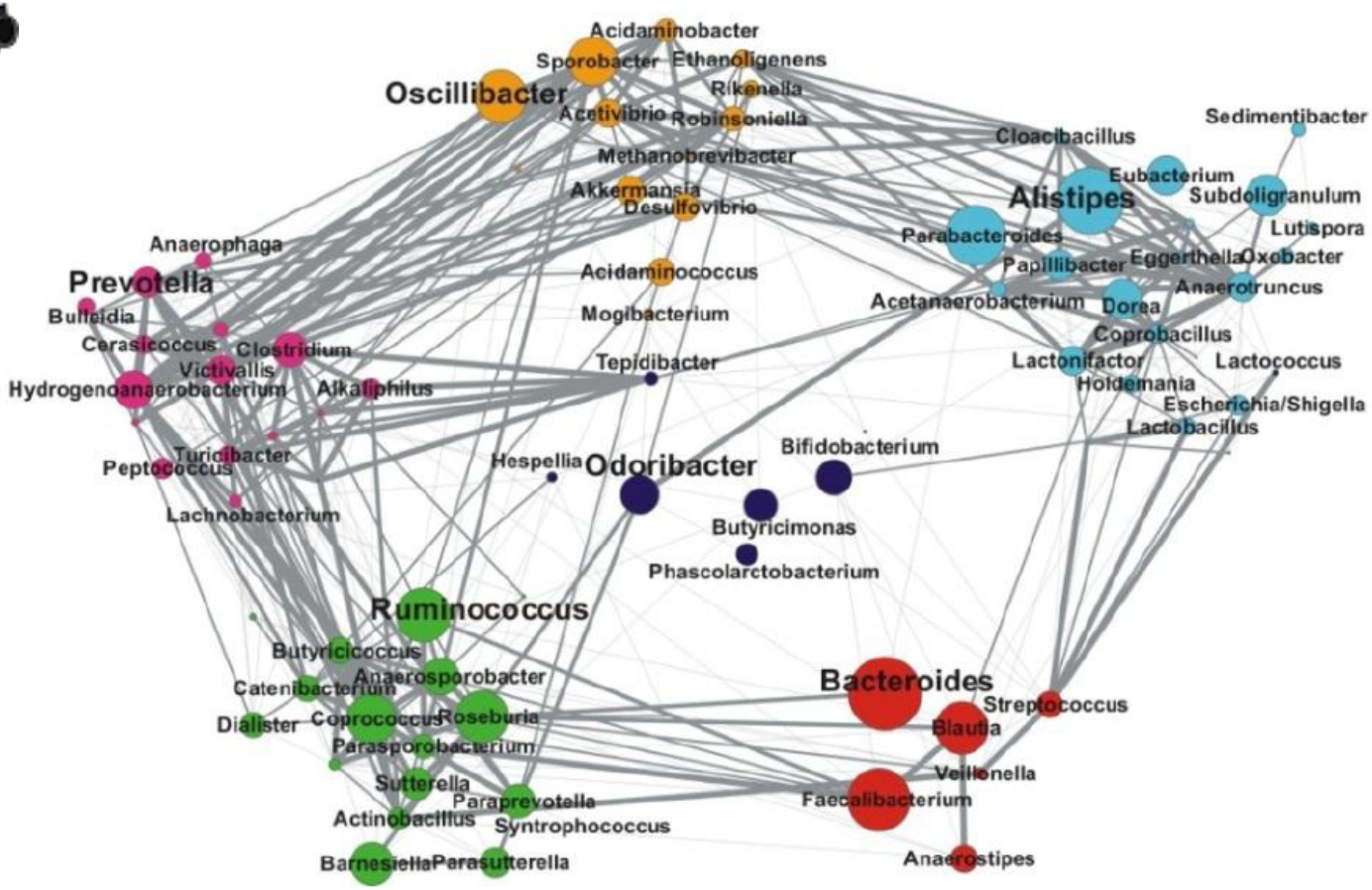
Co-abundance Groups (CAG): Groups of bacteria genera that co-variate along samples



Microbiota structure evolution – Claesson 2012

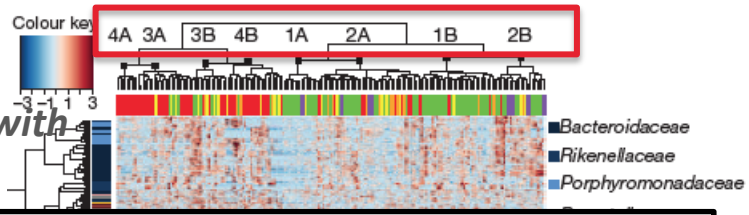
Claesson MJ, et al: ***Gut microbiota composition correlates with diet and health in the elderly****. Nature 2012, 488:178–184.*

Wiggum Plot



Microbiota structure evolves through aging and residency – Claesson, 2012

Claesson MJ, et al: ***Gut microbiota composition correlates with diet and health in the elderly****. Nature 2012, 488:178–184.*



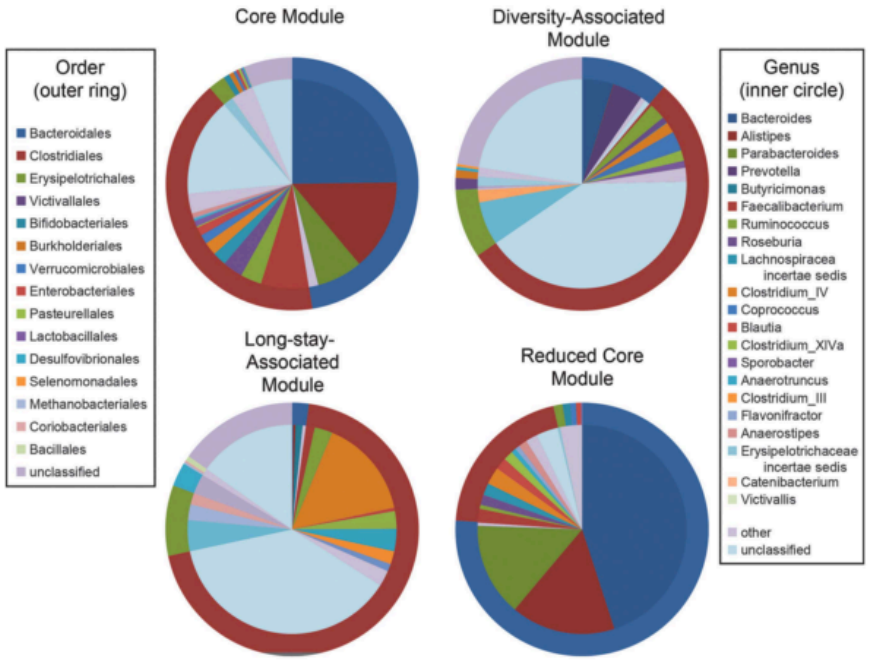
Community

Long Stay

Bacterial modules associated to subject characteristics – Jeffery, 2015

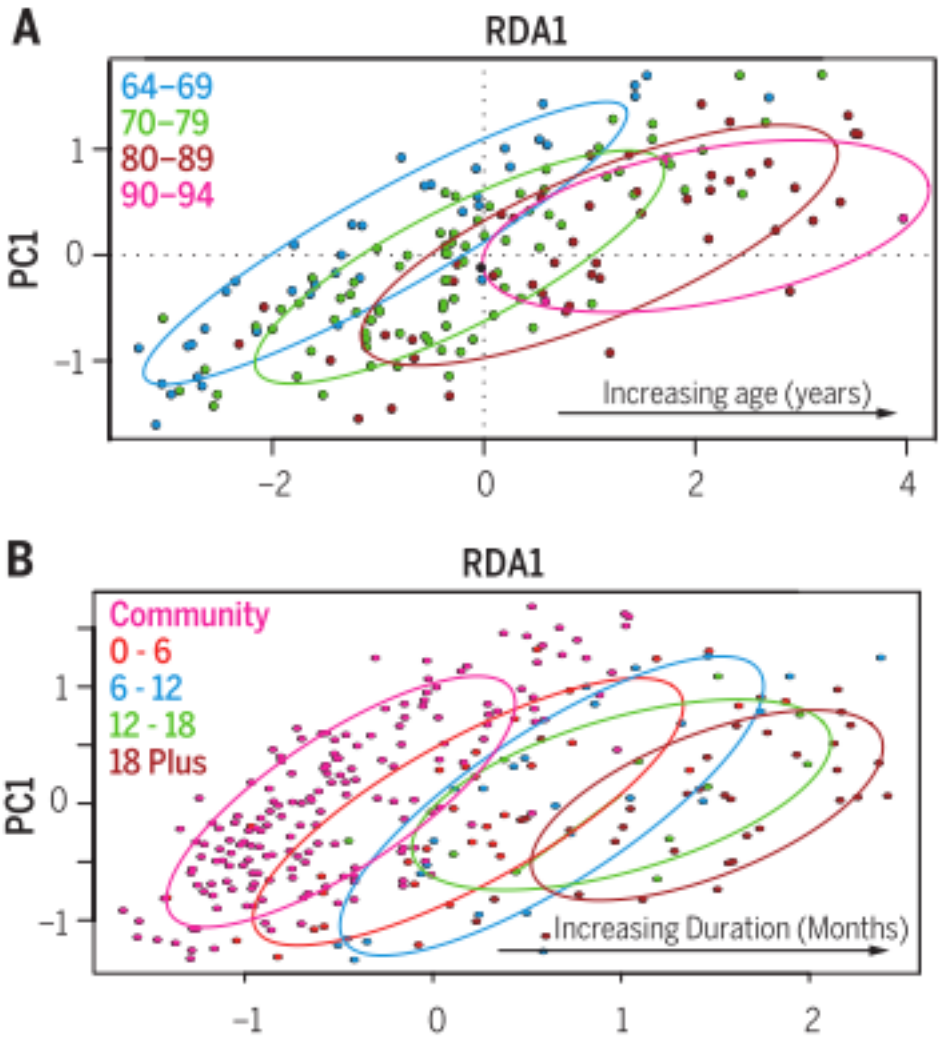
Jeffery IB, Lynch DB, O'Toole PW: Composition and temporal stability of the gut microbiota in older persons. ISME J 2015, 10:170–82.

Four microbiota modules identified by iBBiG (M1, M2, M3 and M9) in 732 samples (371 subjects)
M1: **Core** microbiota(Co), bacterial genera present in most microbiome profiles in high abundance
M2: **Diversity associated** (DA), bacterial genera associated with health or healthy diets and high microbiota diversity present in ~1/3 of the profiles
M3: **Long-stay associated** (LA)
M9: **Reduced Core** (RC). A subset of the Co module



Microbial modules shift through age and residential care – O'Toole, 2015

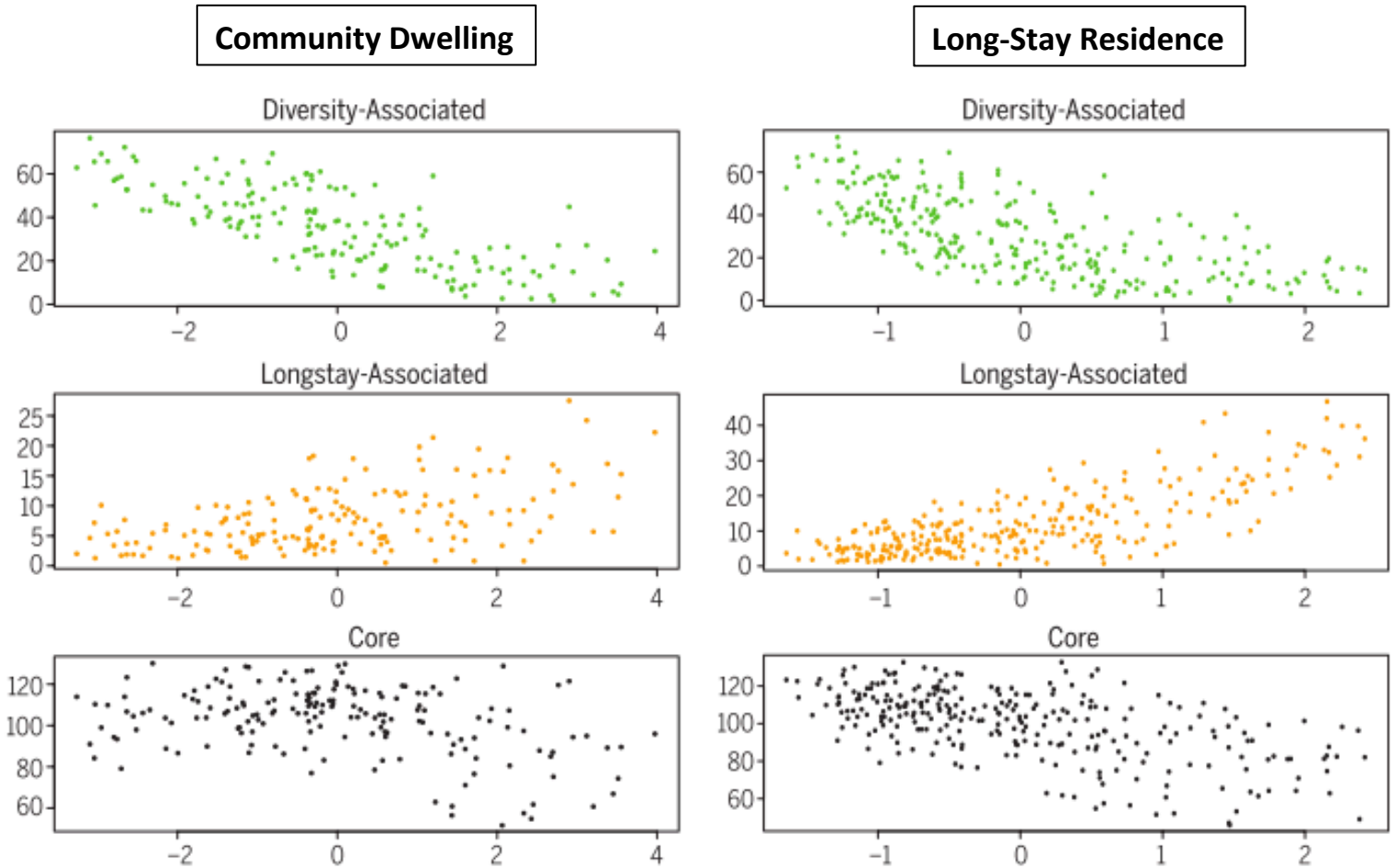
O'Toole PW, Jeffery IB: Gut microbiota and aging. Science (80-) 2015, 350:1214–1216.



Bacterial modules associated to subject characteristics – O'Toole, 2015

O'Toole PW, Jeffery IB: Gut microbiota and aging. Science (80-) 2015, 350:1214–1216.

- Abundance of bacterial modules with aging and length of stay in residential-care
- As elderly people age and/or spend time in a long-stay residential facility, they gain a long-stay–associated defined population of bacteria that are associated with increased frailty



Summary

- Measures of increased inflammation and increased frailty related to both dietary and microbiome composition support a diet– microbiota link to these indicators and underlines and important role of environmental factors related to residence setting
- Elder bacterial microbiome gradually differentiates from that of younger population, is stable over time but shows higher inter-individual variability.
- Bacterial diversity is reduced in non-frail aging and is further reduced in frail aging, but comparatively increased in extreme longevity. Frailty is also characterized by reduction of several aging related bacterial components and an increase in *Proteobacteria* and *Enterobacteria.*
- In long living subject, SCFAs producing bacteria such as Akk. Muciniphila, Clostridium XIVa cluster and Christensenellaceae show to be consistently increased compared to "young" population.
- A loss of F. Prausnitzii is associated with frailty whereas and an enrichment A. muciniphila is correlated with longevity, respectively. These species have been shown to be important players in other diseases with an important inflammatory component.
- Importantly, aging/frailty associated microbiome changes are highly population-specific and may depend on a combination environmental factors such as residence, psychosocial factors, diet and presence of co-morbidities.

The FRAME Study - The gut microbiome in frail aging

Study Design

Study Population:
- Approx 200 individuals
- 65 yo or older
- With no GI diseases

Microbiome Characterization
- Sequencing of 16s rRNA V3-V4 region with NGS(Illumina)
- High-Throughput Whole (Meta)Genome sequencing
- Metabolomics: Restricted to SCFA

Clinical Evaluation:
- Frailty Scales:
  - Fried
  - OARS
  - Barthel
  - Pfeiffer
  - Get up & Go
  - Depression
- Blood & Hormone Test
- Immune and Inflammation markers
  - sCD14 & LBP (Translocation)
  - Zonuline (Gut Perm)
  - IFABP (Enterocyte damage)
  - CRP, D-dimer, IL-6, IP10, G-CSF

Nutritional Information:
- PREDIMED Questionnaire.
- 14 point scoring.

Screening visit: Consent and material
Baseline Visit: Evaluation and sample collection

Data Collection:
- On-line data collection
- Secure/Encrypted DB
- OpenClinica data forms designed for this study

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