Update on the relationship between the microbiome and health/disease

Celina M Dietrich (PhD candidate, UEA and Quadram Institute)

A field is born – The hidden life of microorganisms

1940-50s culture of anaerobic organisms

1960s in vivo exp. using GF mice

1977 Sanger DNA sequencing

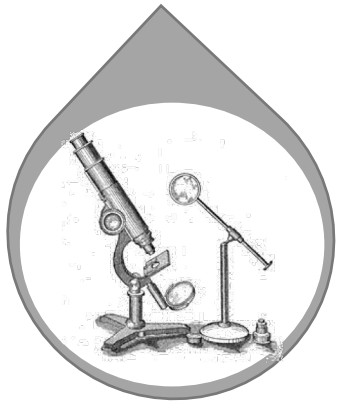
1680 Microscope

1885 Cell culture

"great plate count anomaly"

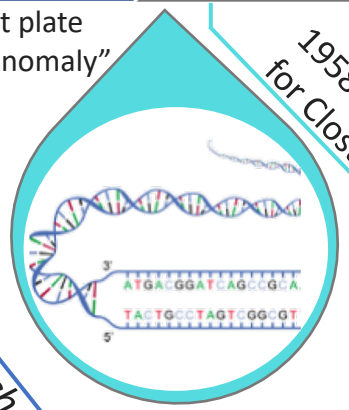
1955 Sequencing

2000 Next-Generation-Seq.



First documentation of human bacteria by Antonie van Leeuwenhoek

1917 isolation of Escherichia coli



1958 faecal microbiota transplantation for Clostridioides difficile infection

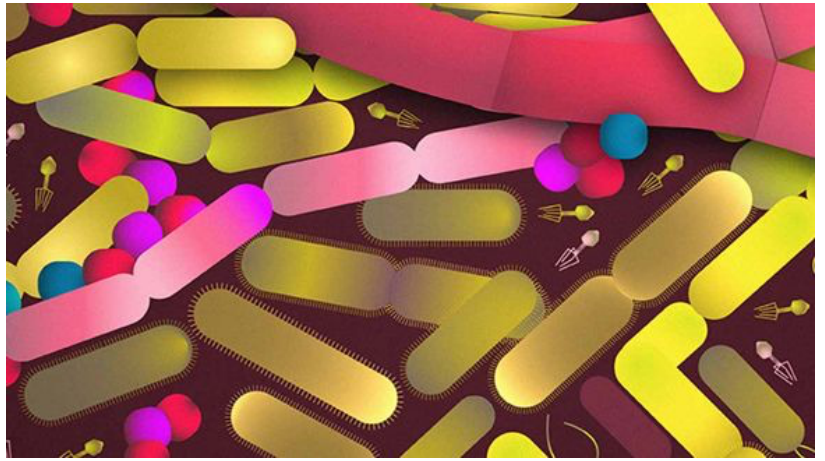
2007-2017 Human Microbiome Project

- Advances in technology have accompanied and enabled this field
- Microbiome research became a hot topic in the last 15 years
- 12 900 publications between 2013-2017

(Pariente, 2019, Jagatia and Relman, 2019)

The human microbiota

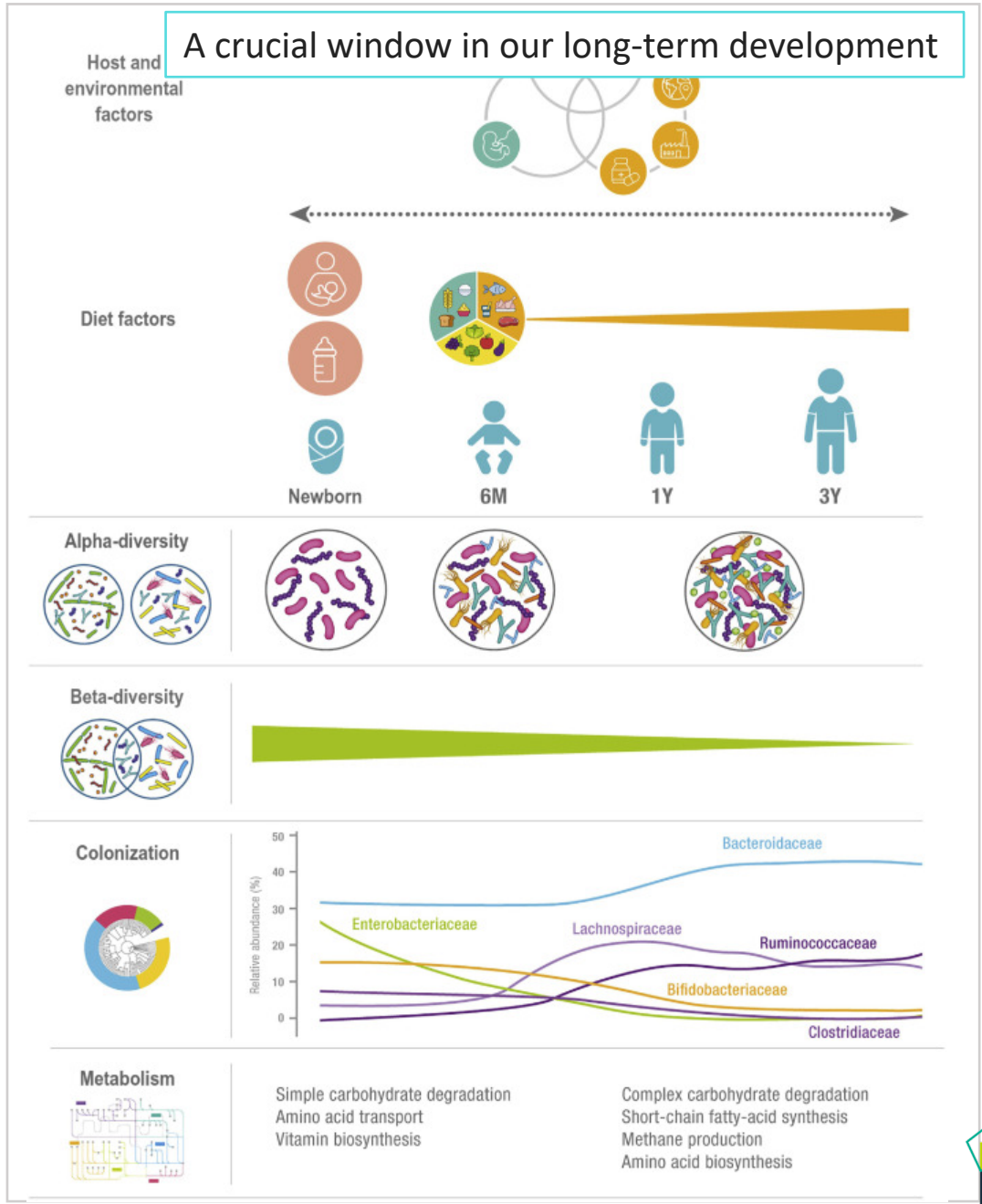
- The human microbiome describes the collective of microbial encoded genes
- 1.3 bacterial cells for every 1 human cell
- Substantial more genetic diversity of bacterial species compared to human genes
- Host-microbiota interactions mainly mutualistic
- Microorganisms perform vital, non-redundant, metabolic and non-metabolic functions
- Variability of the human microbiome over the life-course and between different individuals



Credit: S. Bradbrook / Springer Nature Limited

Early life gut microbiota

- Early life experiences have wide and long-lasting effects that can reach into adulthood
- Development & maturation of the gut microbiota is highly dynamic, influenced by many factors
- Lower diversity & lower functional microbial complexity
- Higher degree of interpersonal variation in gut bacterial diversity compared to adults
- Stabilization and adult-like configuration within first 3 years of life

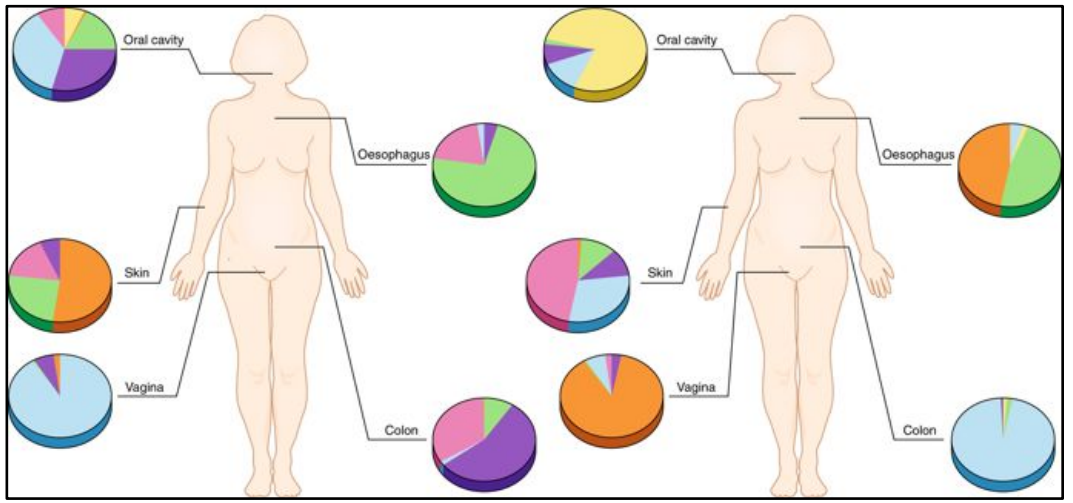


(Tamburini *et al.*, 2016; York, 2019)

Adult life gut microbiota

1) How much variation between adults?
2) Is the microbiota stable over time?
3) Is there a core microbiota?

- Determining our microbiota's role in disease predisposition and pathogenesis will depend critically upon first defining "normal" states
- Microbial communities cluster strongest by body habitat, then by host individual, then by time
- Identifiable core microbiome at the gene level rather than the microbial species level
- ~60% of bacterial strains in the gut remained stable for up to five years



1. Immense variation between body habitats
2. Interpersonal (within-subject) variability: Relatively stable over time.
3. Intrapersonal (between-subjects) variability: Pronounced differences, individual uniqueness.

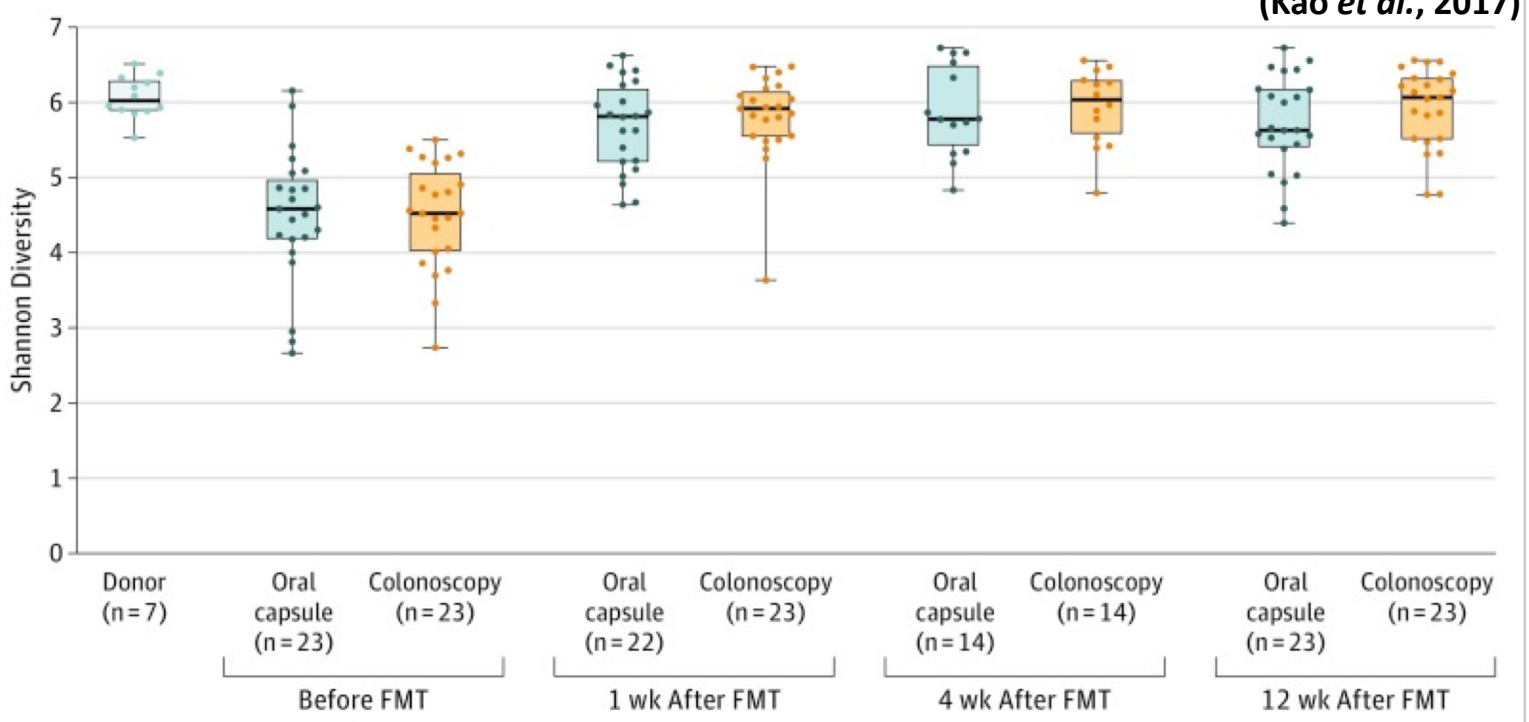
(Costello *et al.*, 2009)

Microbiota-targeted treatment options –

Faecal microbiota transplant and phage therapy

Faecal microbiota transplantation (FMT)

- In 1958, Eiseman et al. reported the successful treatment of 'pseudomembranous enterocolitis' using a faecal enema
- FMT accepted rescue treatment for recurrent *Clostridioides difficile* infection (CDI)



Shannon Diversity of Patients With Recurrent *Clostridium difficile* Infection and Donors

- Randomized clinical trial with 116 adults with recurrent CDI
- Primary efficacy endpoint (success rate)
  - 89.5% capsule group
  - 96.6% colonoscopy group
- Non-inferiority of FMT delivery by oral capsules

(Khoruts and Sadowsky, 2016; Kao *et al.*, 2017)

- Safety

- minor adverse events rates: 5.4% capsule group vs 12.5% for colonoscopy group

- Patient Perception & Satisfaction

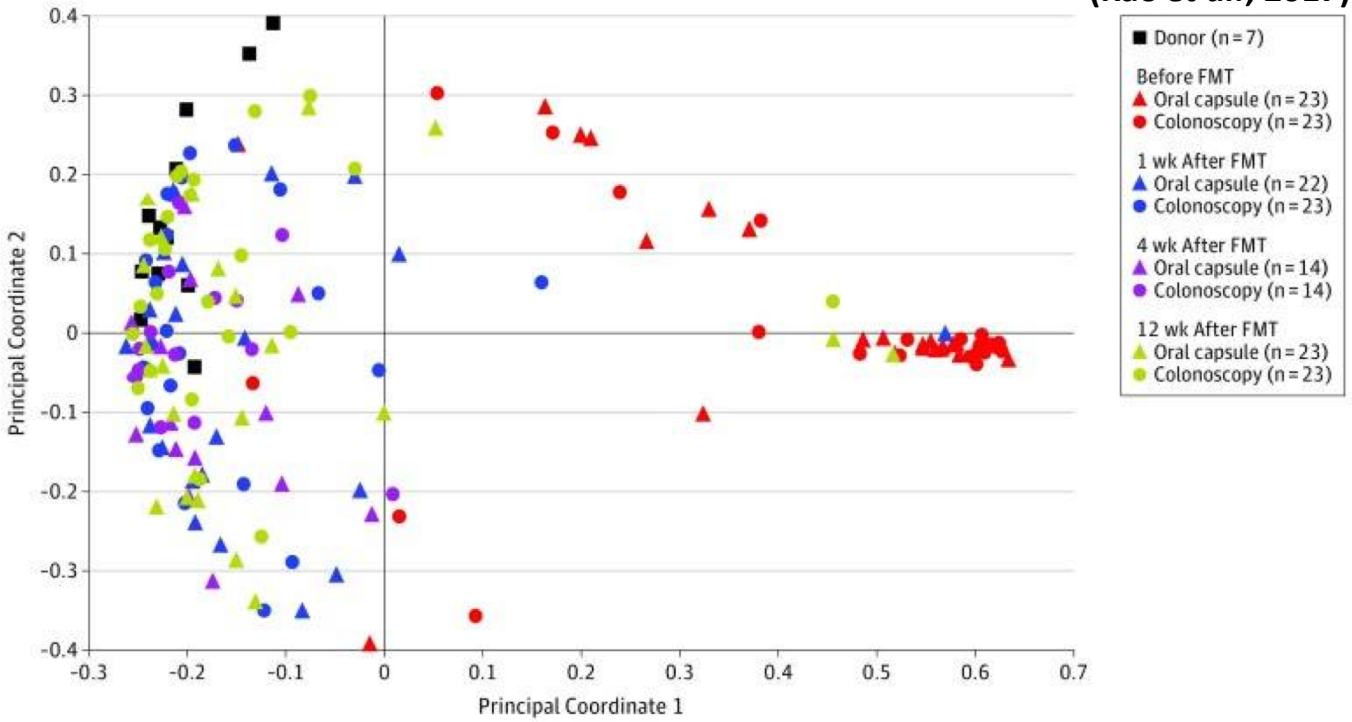
- 97% would undergo the assigned delivery method again if needed

- experience rated “not at all unpleasant” (66% oral capsule vs 44% colonoscopy; difference, 22% [95% CI, 3%-40%]; *P* = .01)

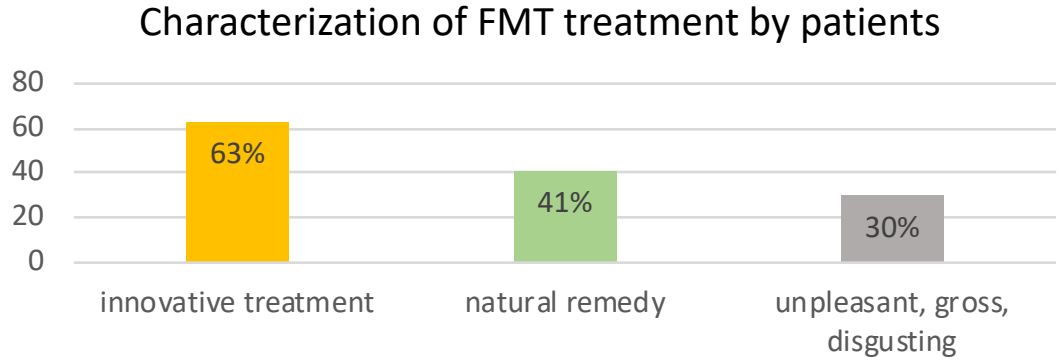
- Cost of intervention

- FMT via colonoscopy = £668
- FMT via oral capsule = £235

(Kao *et al.*, 2017)



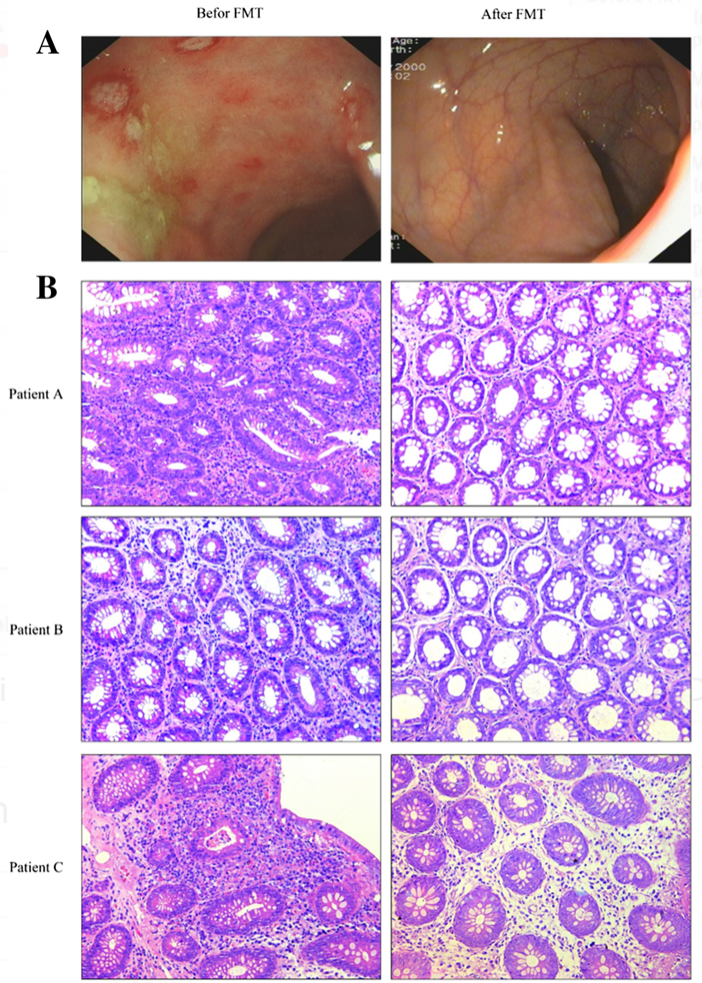
Principal Coordinates Analysis of Patients With Recurrent *C. difficile* Infection and Donors



(Khoruts and Sadowsky, 2016; Kao *et al.*, 2017)

- FMT is also being investigated for other indications.
- First studies in ulcerative and drug-induced colitis has shown some promise.
- FMT from lean donors has been shown to increase insulin sensitivity in men with metabolic syndrome.

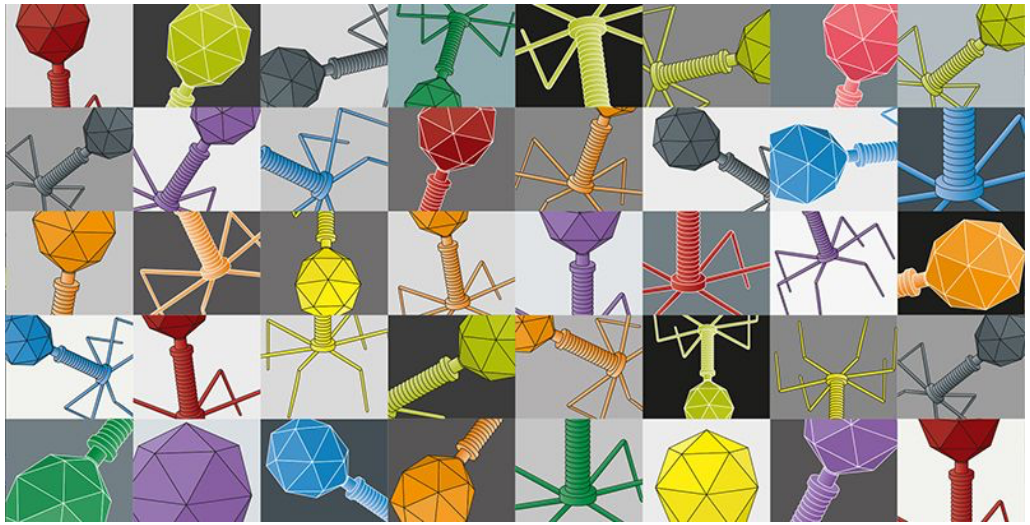
Prospective uncontrolled clinical study of 20 patients with ulcerative colitis



Enteroscopic findings of intestinal mucosa of a patient before and after treatment

(Tian *et al.*, 2019)

Phage therapy – an alternative to antibiotics?



- As we teeter towards a *post-antibiotic* era, phage therapy could offer novel therapies against multi-drug resistant bacterial infections.
- The current rate of resistance development far exceeds level of antibiotic discovery.
- To date, human phage therapy trials have been largely empirical.
- One of the current challenges of progressing phage therapy into the clinic is the lack of validated and adequately controlled clinical trials.

(Furfaro, Payne and Chang, 2018)

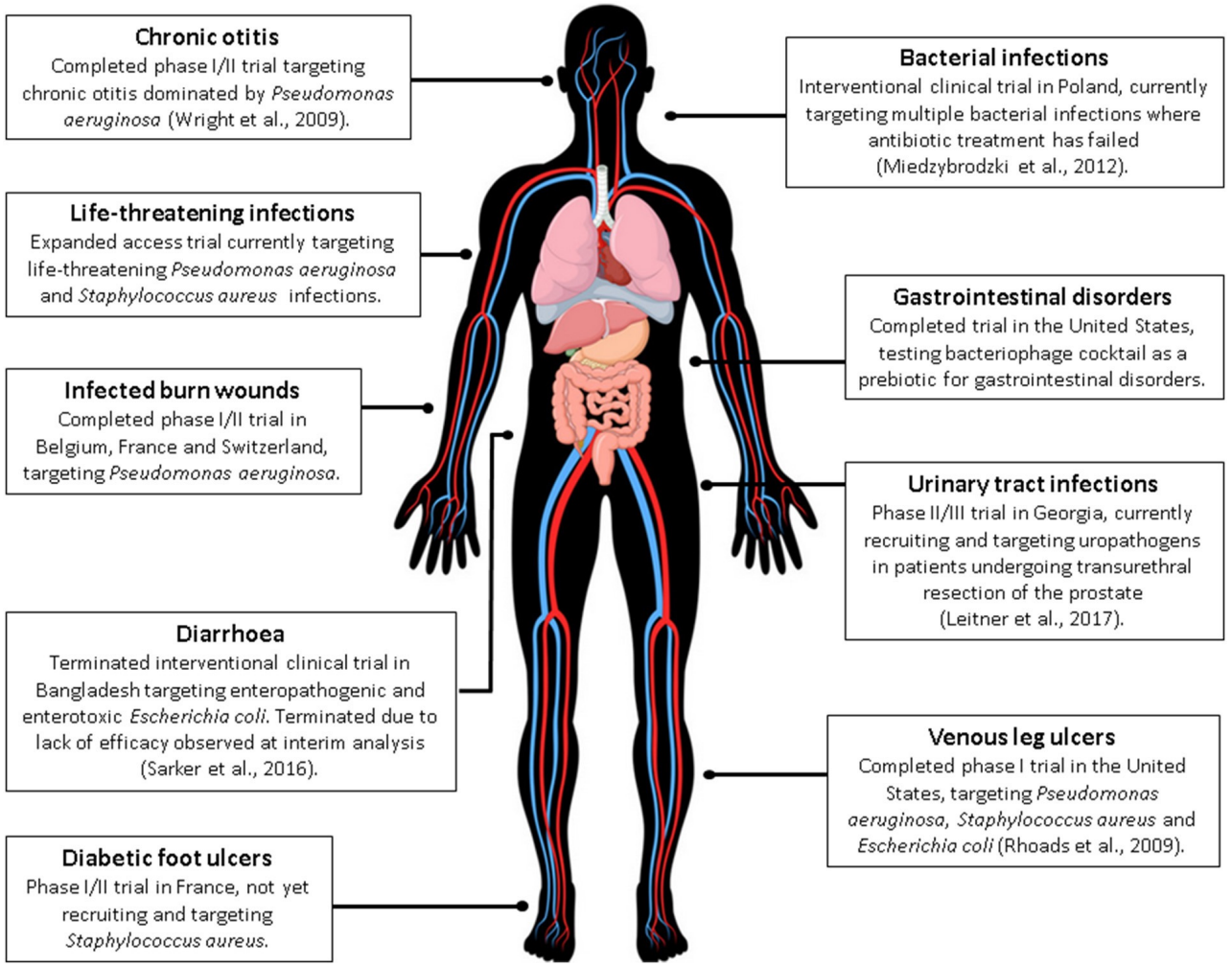
- Key advantages

- High specificity and unlikely to cause secondary infections
- Potential to treat multi-drug-resistant isolates
- Cost and time to select and isolate phages are better

- Key disadvantages

- Lack of established and validated protocols for administration routes, dose, frequency, and duration of phage treatment
- Difficult to obtain regulatory approval (are phages living or not?)
- Sparse data for clinical application

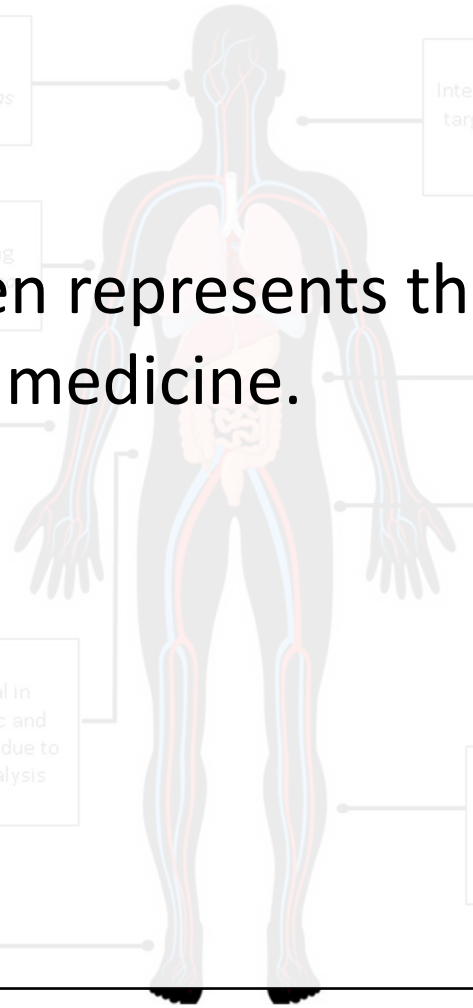
The sparse but diverse landscape of phase I/II clinical phage trials.



(Furfaro, Payne and Chang, 2018)

- Key advantages
- High specificity and unlikely to cause secondary infections
- Potential to treat multi-drug-resistant isolates
- Cost and time to select and isolate phages are better
- Key disadvantages
- Lack of established and validated protocols for administration routes, dose, frequency, and duration of phage treatment
- Difficult to obtain regulatory approval (are phages living or not?)
- Sparse data for clinical application

The sparse but diverse landscape of phase I/II clinical phage trials.



Chronic otitis
Completed phase I/II trial targeting chronic otitis dominated by *Pseudomonas aeruginosa* (Wright et al., 2009).

Life-threatening infections
Expanded access trial currently targeting

Infected burn wounds
Completed phase I/II trial in Belgium, France and Switzerland, targeting *Pseudomonas aeruginosa*.

Diarrhoea
Terminated interventional clinical trial in Bangladesh targeting enteropathogenic and enterotoxic *Escherichia coli*. Terminated due to lack of efficacy observed at interim analysis (Sarker et al., 2016).

Diabetic foot ulcers
Phase I/II trial in France, not yet recruiting and targeting *Staphylococcus aureus*.

Bacterial infections
Interventional clinical trial in Poland, currently targeting multiple bacterial infections where antibiotic treatment has failed (Miedzybrodzki et al., 2012).

Gastrointestinal disorders
Completed trial in the United States, testing bacteriophage cocktail as a prebiotic for gastrointestinal disorders.

Urinary tract infections
Phase II/III trial in Georgia, currently recruiting and targeting uropathogens in patients undergoing transurethral resection of the prostate (Leitner et al., 2017).

Venous leg ulcers
Completed phase I trial in the United States, targeting *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* (Rhoads et al., 2009).

As it stands, phage therapy often represents the epitome of personalized medicine.

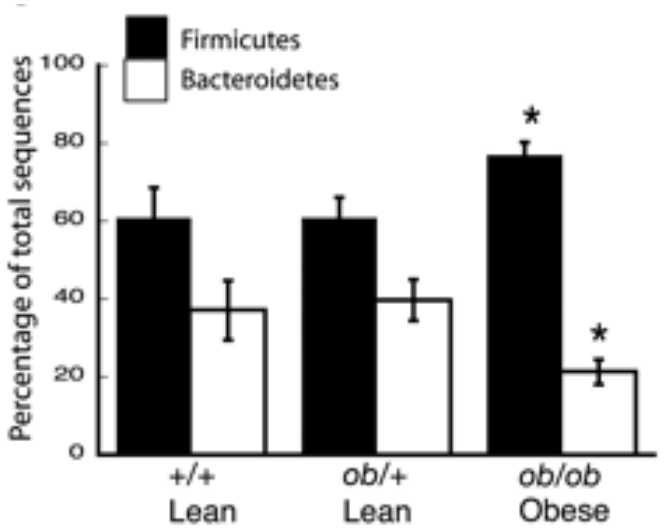
(Furfaro, Payne and Chang, 2018)

Obesity

Evidence from animal research 2004-2005

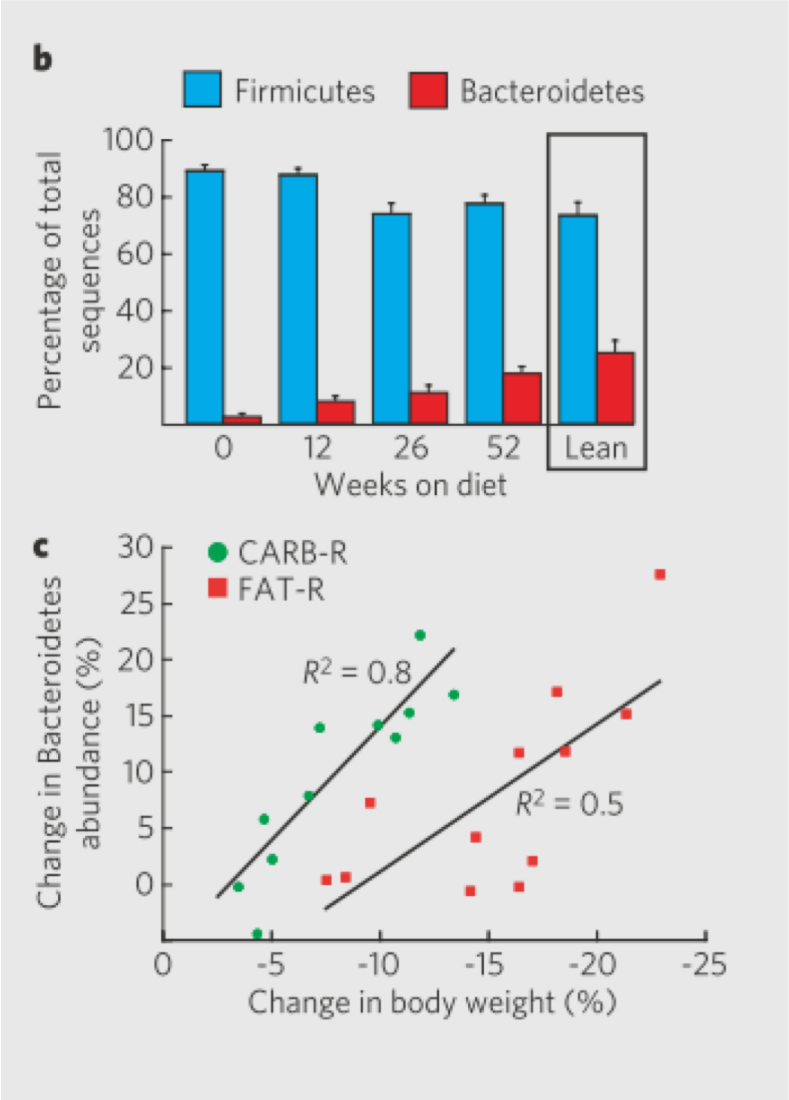
- Reduced body fat in GF mice compared to conventional mice, even though they consumed less food
- Higher Firmicutes/Bacteroidetes ratio in mouse model of obesity
- Obesity-associated microbiota has increased capacity for energy harvest from food
- Phenotype could be transferred through faecal microbiota transplant

- Greater increase in body fat and insulin resistance in germ-free recipients following faecal transplant



The cecal microbiota of obese mice had a statistically significant 50% reduction in Bacteroidetes, relative to lean mice, and a significantly greater proportion of Firmicutes ($P < 0.05$)

(Bäckhed *et al.*, 2004; Ley *et al.*, 2005)



Correlation between body-weight loss and gut microbial ecology.

Evidence from human research

- Decreased relative proportion of Bacteroidetes (to Firmicutes ratio) in obese people compared to lean people
- Ratio normalizes following low-calorie diet and weight loss

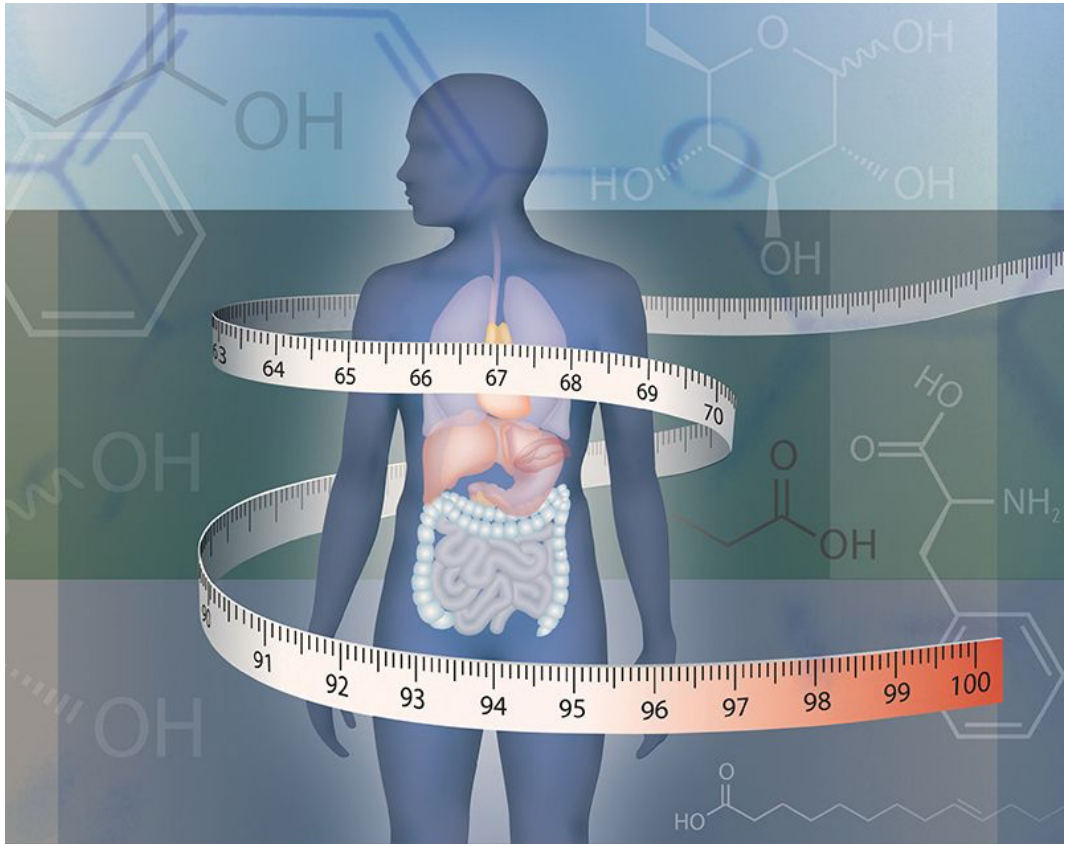
> Obesity has a microbial component, which might have potential therapeutic implications

> Sparked research studies into obesity which produced conflicting results

(Ley et al. 2006)

- Phylum-level signatures were not generalizable
- Significant but weak association between Shannon diversity & evenness and obesity
- Diet was found to consistently alter the gut microbiota
- Change in diet can alter the degradative activity of the colonic microbiota in vivo

> Opened many new research avenues

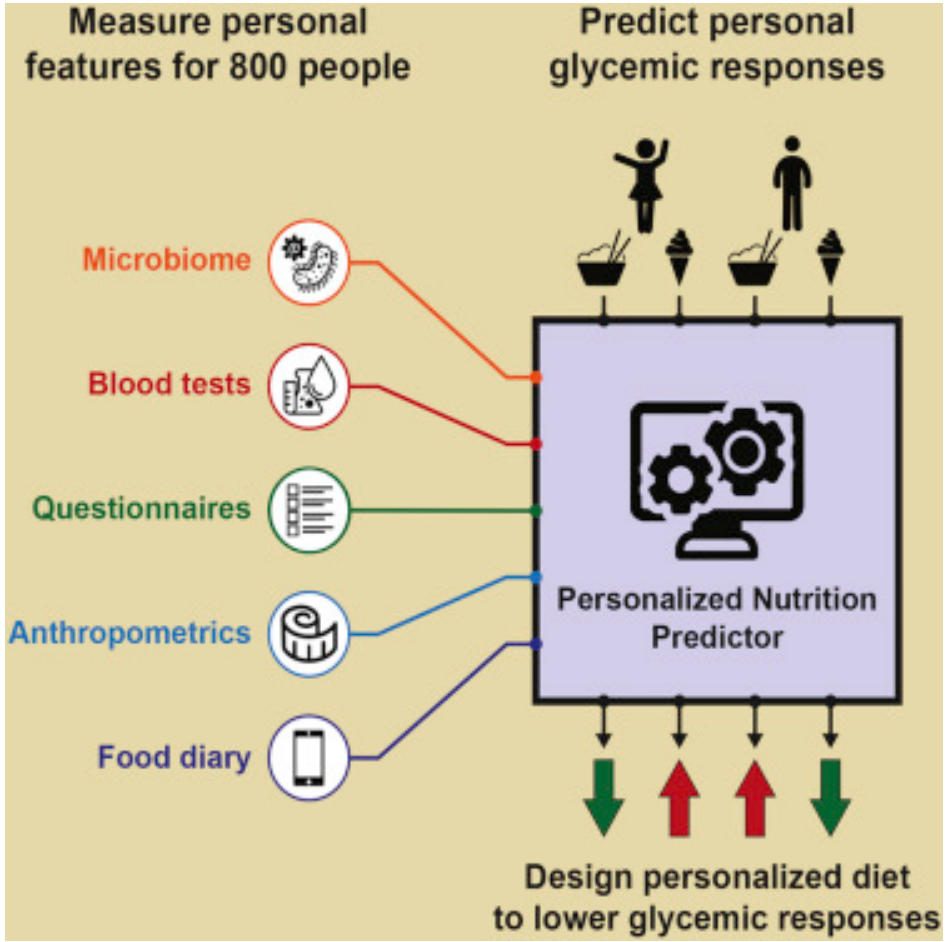


Credit: S. Bradbrook / Springer Nature Limited

"Let food be thy medicine and medicine be thy food"
(Hippocrates, 431BC)

(Ley et al. 2006)

- Research efforts directed to harness this ’power’
- Big data from 800 people: microbiota data, blood parameters, metadata, diet, etc.
- Developed an machine learning algorithm that could predict an individual’s glycaemic response to a particular meal
- Personalized diet for diabetic individuals



(Zeevi *et al.*, 2015; Zhao *et al.*, 2018)

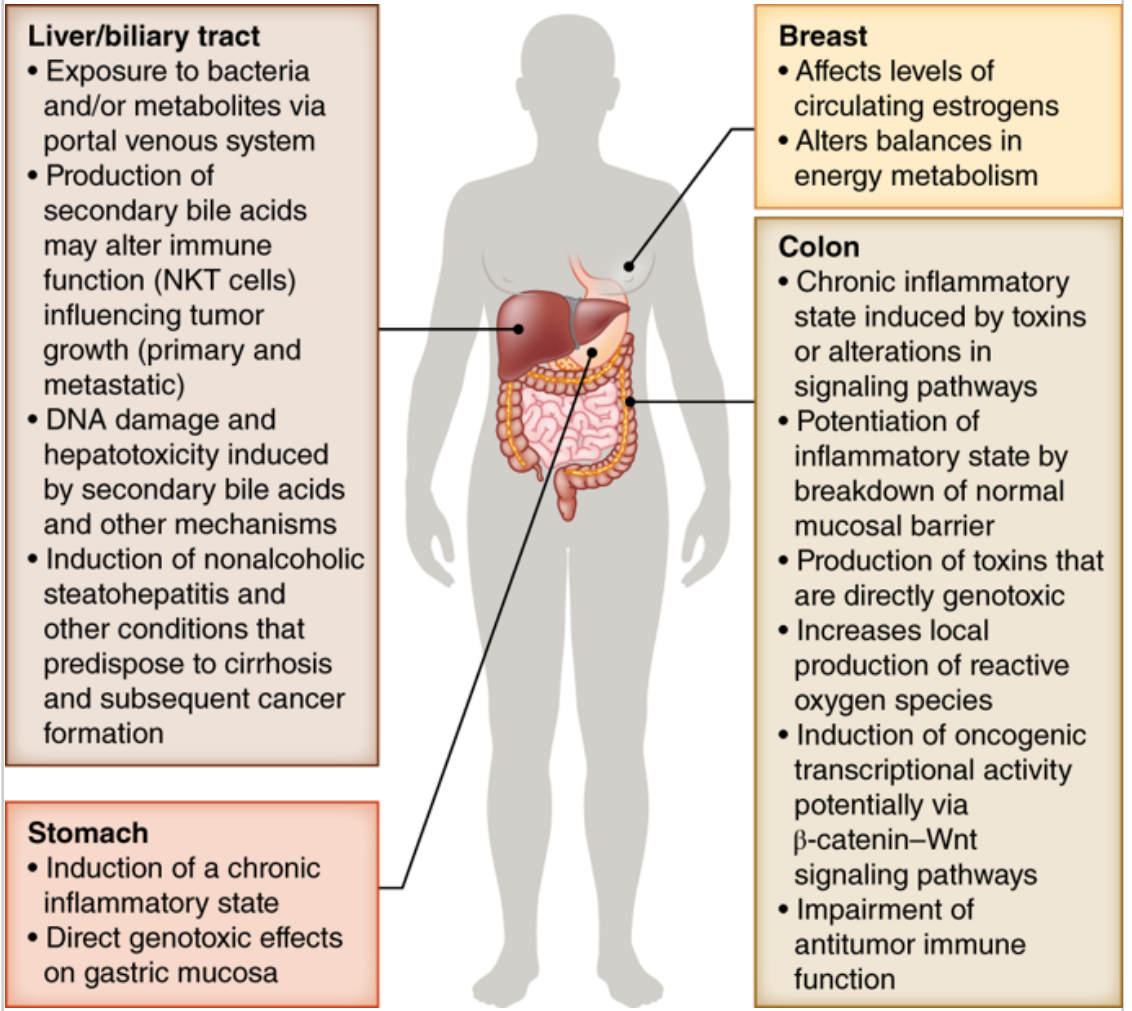
Cancers

- The role of gut microbiota in **cancer development/ growth**

- Oncogenic gut bacteria include *Salmonella typhi and Helicobacter spp.* in biliary cancer and *Helicobacter pylori* (genotoxic effects) in gastric cancer
- Carcinogenesis due to local chronic inflammation
- Gut dysbiosis

- The microbiota is a major factor in determining **immune checkpoint inhibitor therapy** (ICT) response in mice

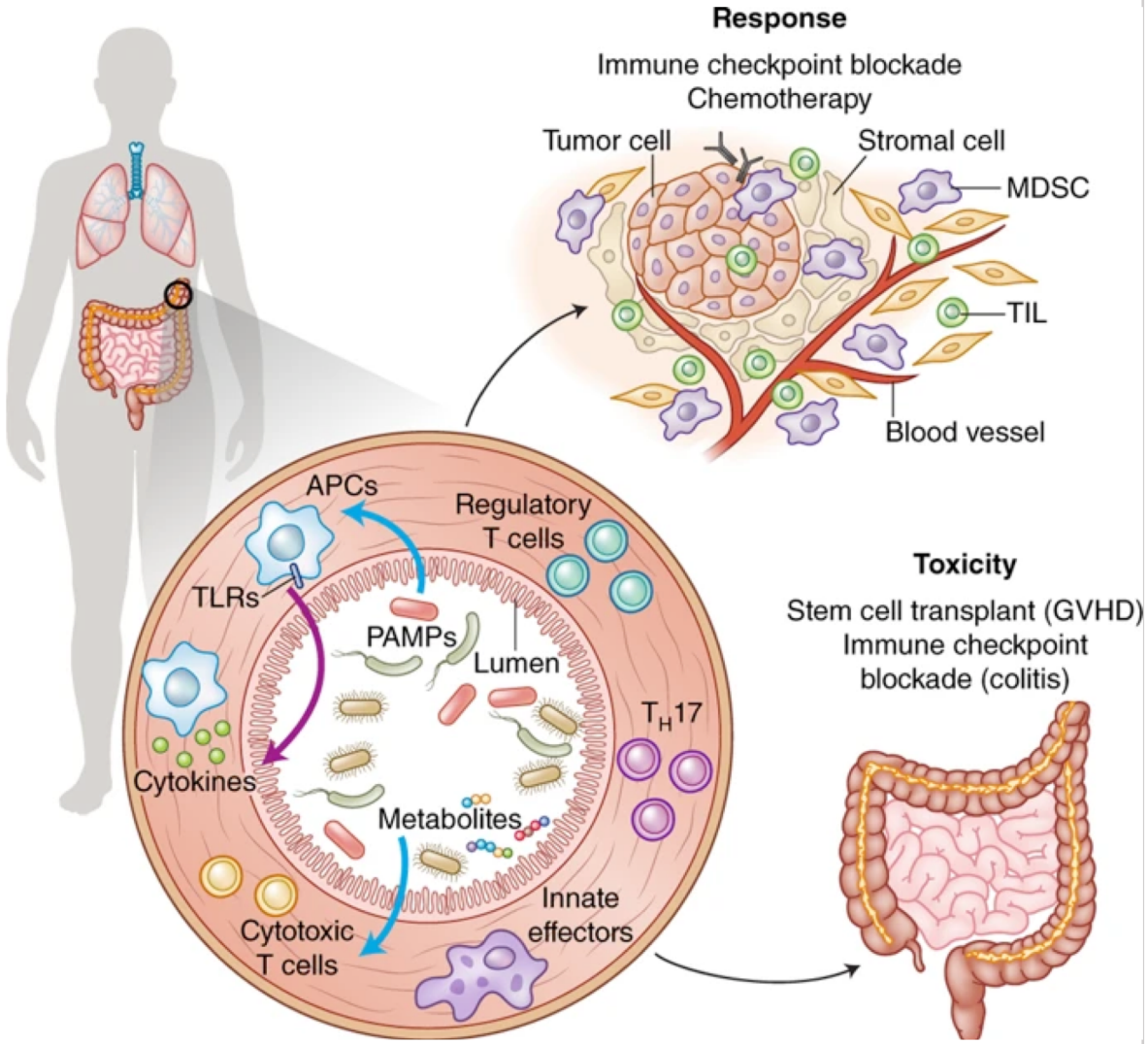
- GF/ antibiotic-treated tumour-bearing mice don't respond to ICT
- Several studies: gut bacteria induced maturation of anti-melanoma dendritic cells and T cells, inhibited tumour growth



Dysbiosis a driver in tumour development and growth – suggested mechanisms

Credit: Debbie Maizels/Springer Nature.

(Helmink *et al.*, 2019)



Credit: Debbie Maizels/Springer Nature.

- Potential mechanisms underlying role in antitumor immunity
  - Production of cytokines
  - Complex interactions of microbial components with the host > Prime adaptive innate immune response
- Modulating immune checkpoint blockade response across several cancer types in humans
  - differential gut microbiota ‘signatures’ exist in patients who respond to treatment
  - favourable signatures: enhanced systemic immunity and intertumoral immune infiltrates
  - ’responder’ phenotypes recapitulated in mice (FMT) could enhance therapeutic response

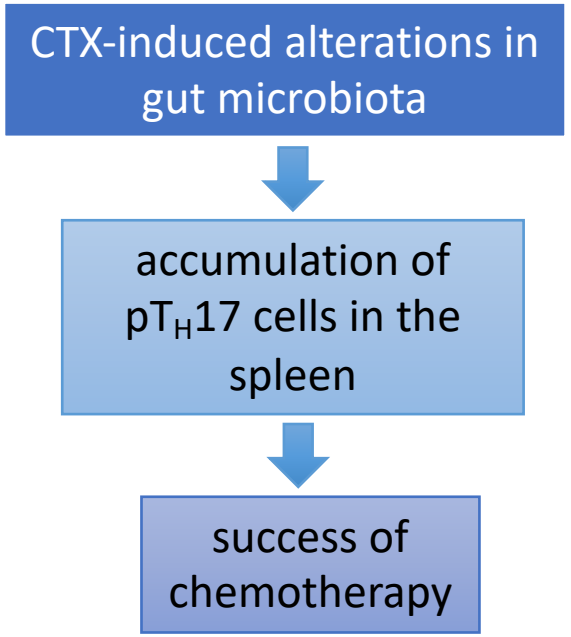
(Helmink *et al.*, 2019)

- **The microbiome and chemotherapy:**

  Microbial modulation of anticancer immune effects of Cyclophosphamide (CTX) in tumor-bearing mice

- Disruption of the intestinal barrier > increased intestinal permeability
- Enabled selective translocation of commensal bacteria into secondary lymphoid organs
- Generation of pTh17 cells and memory Th1 immune response stimulated by translocated bacteria (complex circuitry)
- This correlates with anticancer effects
- Broad-spectrum antibiotics and vancomycin suppressed CTX-induced T_H17 conversion

- Risks associated with antibiotic medication during cancer treatments

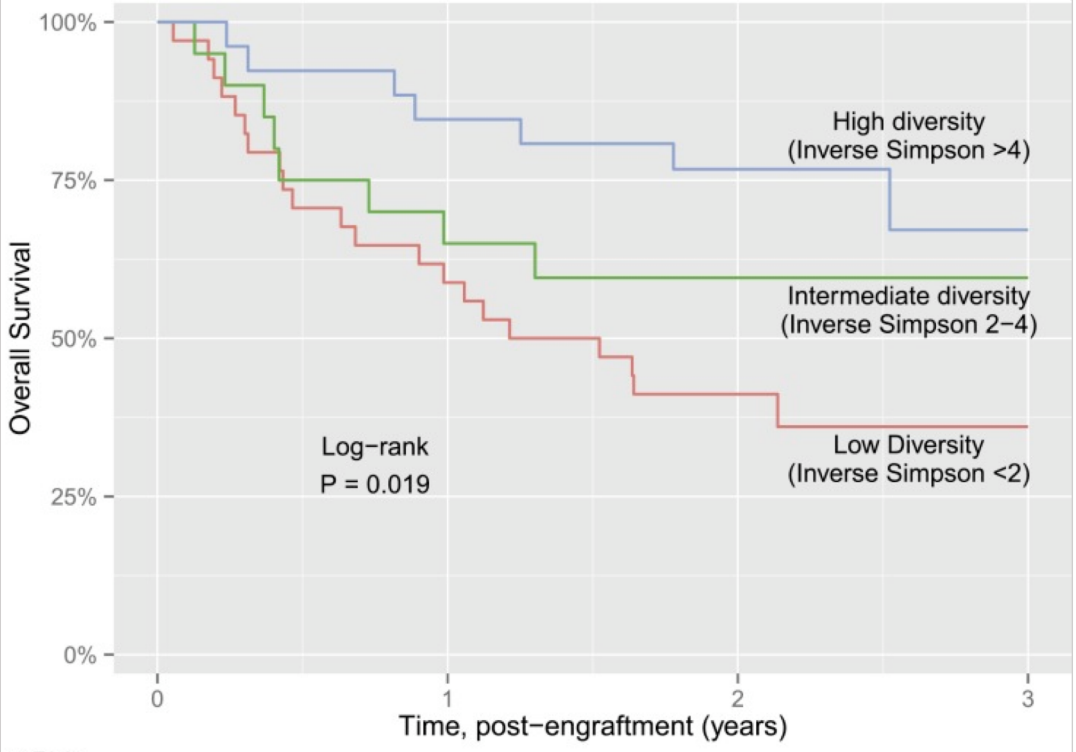


(Viaud *et al.*, 2013)

Allogeneic hematopoietic stem cell transplantation (allo-HSCT)

- Impaired intestinal microbiota with reduced diversity due to allo-HSCT
- Faecal specimens from 80 at the time of stem cell engraftment.
- Overall survival at 3 years = 36% (low), 60% (intermediate), 67% (high diversity groups)
- Microbial diversity is an independent predictor of transplant-related mortality

- Graft-versus-host disease (GVHD): high morbidity and mortality
- Compositional differences in the gut microbiota associated with differing rates of GVHD
- High abundance of gut commensal *Blautia* reduced GVHD-associated mortality



Kaplan-Meier plot of diversity and overall survival and transplant related mortality.

Low diversity group: after multivariate adjustment for other clinical predictors adjusted hazard ratio, **5.25; *P* = .014**

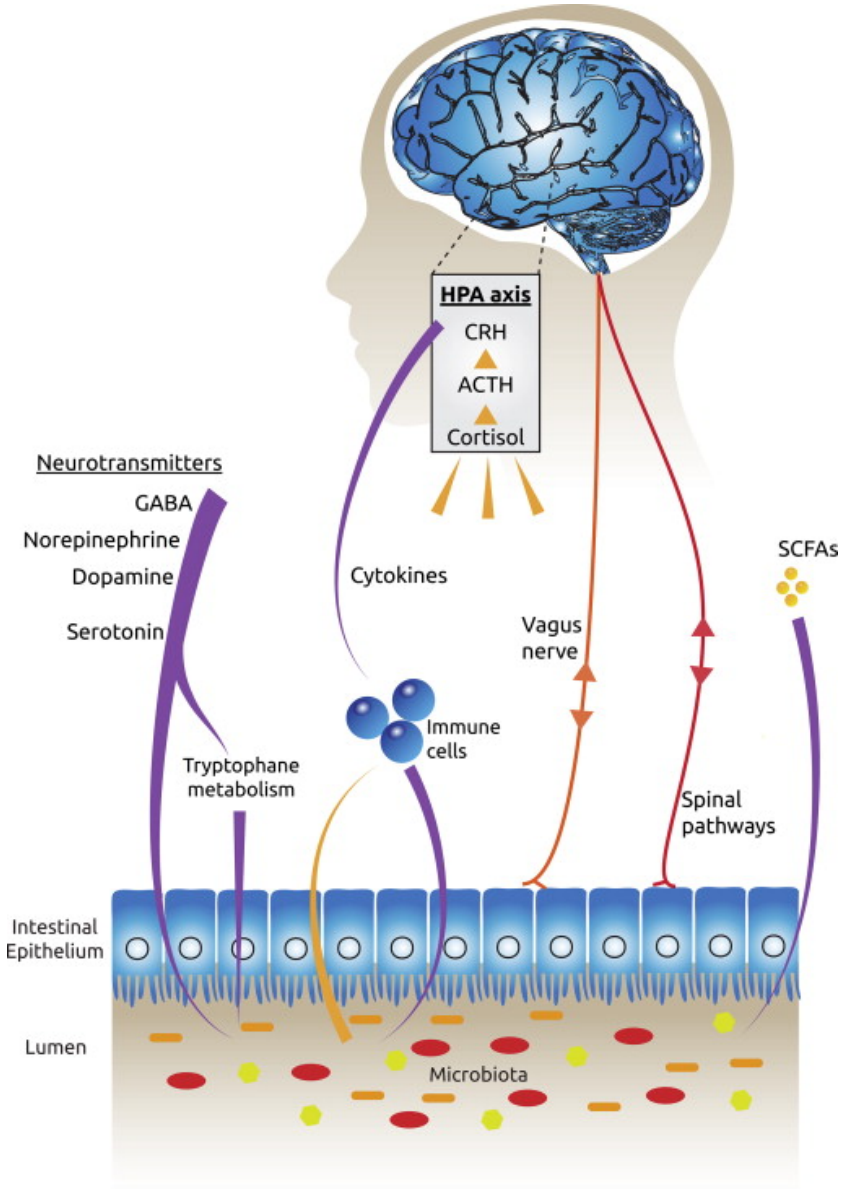
(Taur *et al.*, 2014)

The Gut-Brain axis

Alzheimer’s disease
Parkinson’s disease

- The Gut-Brain axis: a highly interactive, bi-directional communication system between the gastrointestinal tract and the brain
- Communication through neural, endocrine, immune and metabolic pathways

- Lack of conventional microbiota affects behaviour, gene expression in the brain and the development of the nervous system
- GF and antibiotic-treated mice displayed reduced anxiety-like behaviour
- Animals with altered or absent gut microbiota displayed various molecular differences (BNDF expression, production of neurotransmitters/receptors)



(Carabotti *et al.*, 2015; Bray, 2019)

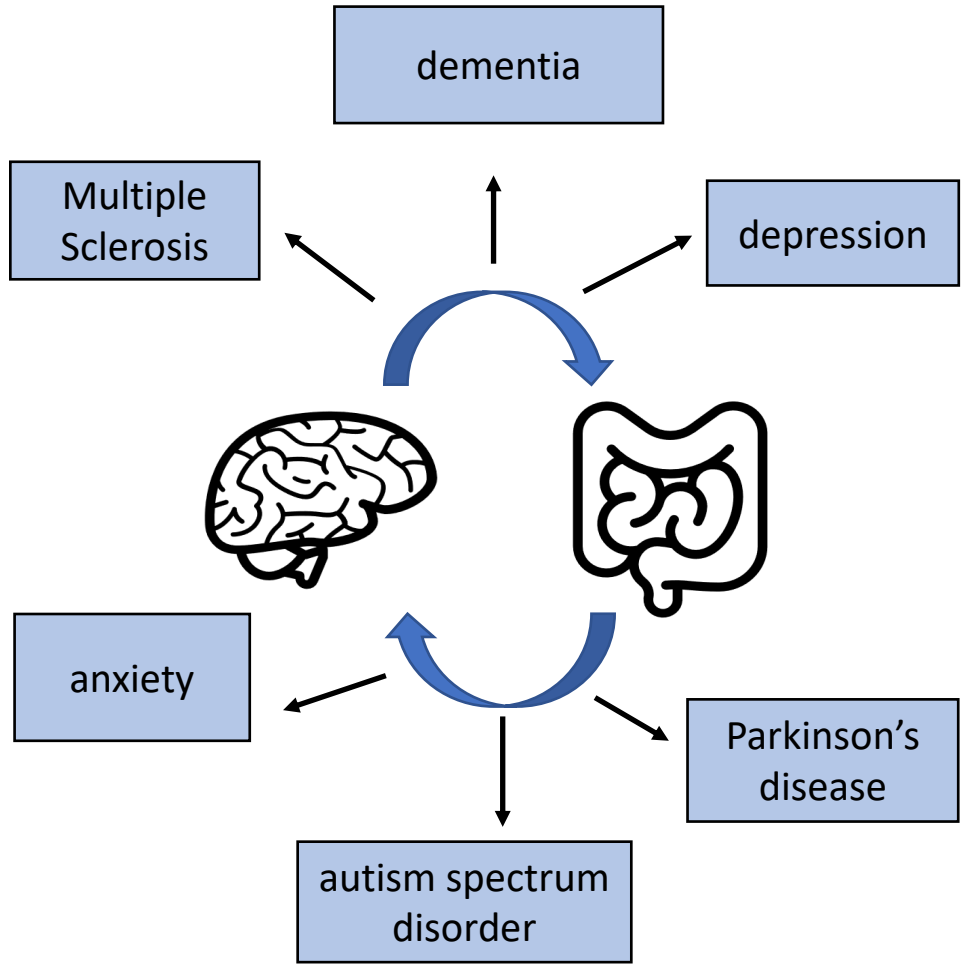
Alzheimer's disease

- Majority of research from animal studies

- Transgenic mouse model (*APP/PS1):* microbial involvement in cerebral amyloid deposition
- *APP/PS1* transgenic mice: ↑*Odoribacter* ↑ *Helicobacter* ↓ *Prevotella* ↓ *Allobaculum* ↓ *Akkermansia*
- Increased permeability in the intestinal and blood-brain barrier

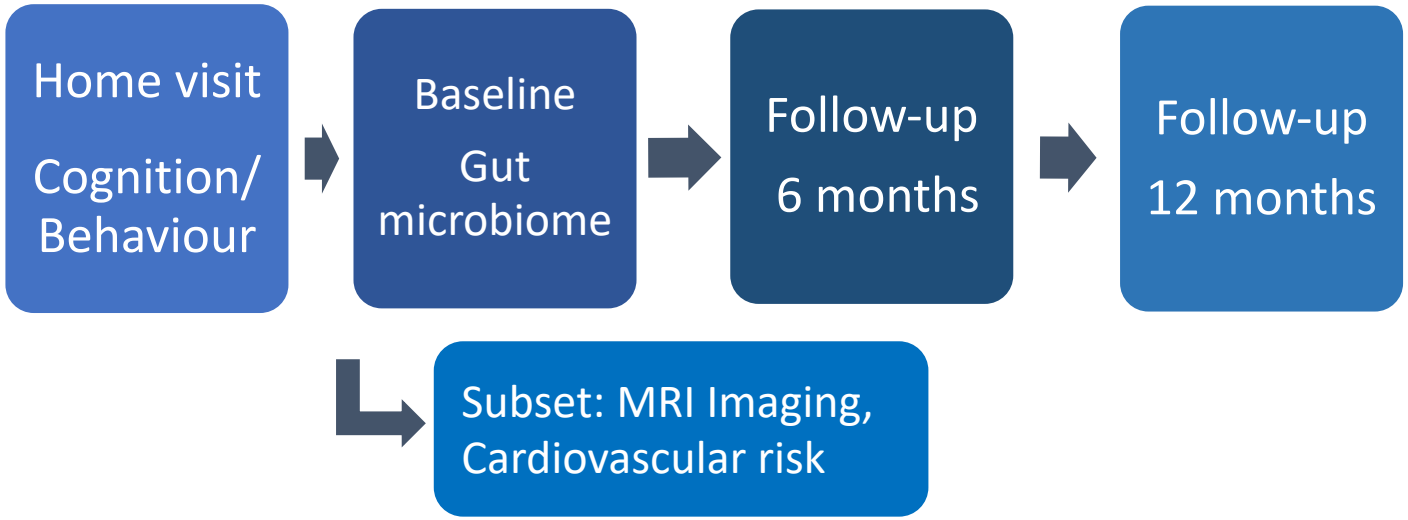
- Few studies in humans with small cohorts

- Decreased richness and diversity
- Changes in relative abundance: ↓Firmicutes,↑ *Bacteroides,*↓ *Bifidobacterium*
- LPS and gram-negative *E. coli* fragments co-localize with amyloid plaques

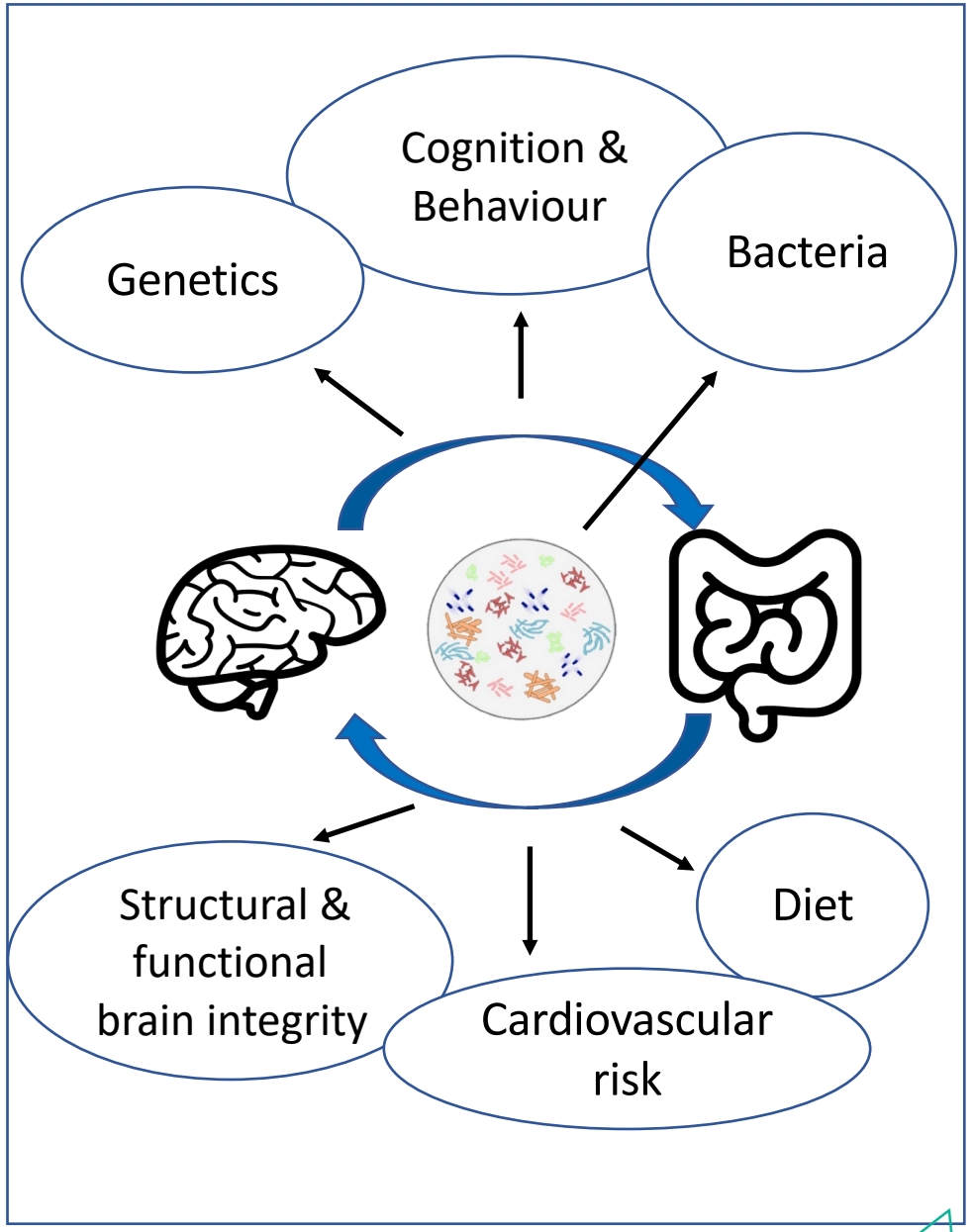


(Harach *et al.*, 2017, Vogt *et al.*, 2017)

- **Design**: Longitudinal tracking (12 months) of participants at different genetic risk of developing dementia.



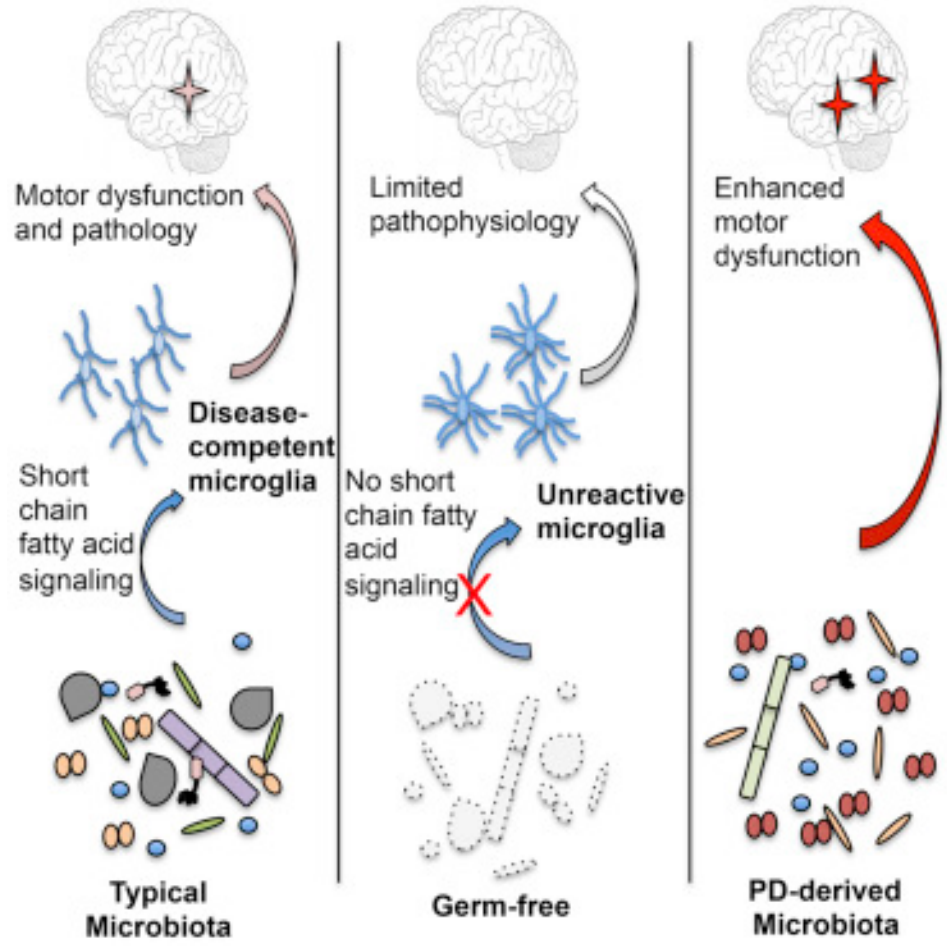
- Participants: N=80 healthy elderly individuals aged 50-75 years.
- Pilot to a large human cohort study



Parkinson's disease

- Second most common neurodegenerative disease in the UK
- Around 10 million people worldwide
- Early gastrointestinal symptomatology
- Presence of alpha-synuclein pathology in the enteric nervous system (prodromal and established cases)
- Synucleinopathy is hypothesized to ascend via the vagal nerve to the central nervous system
- Protective effect of truncal vagotomy for PD (HR 0.85, 95% CI: 0.63–1.14)

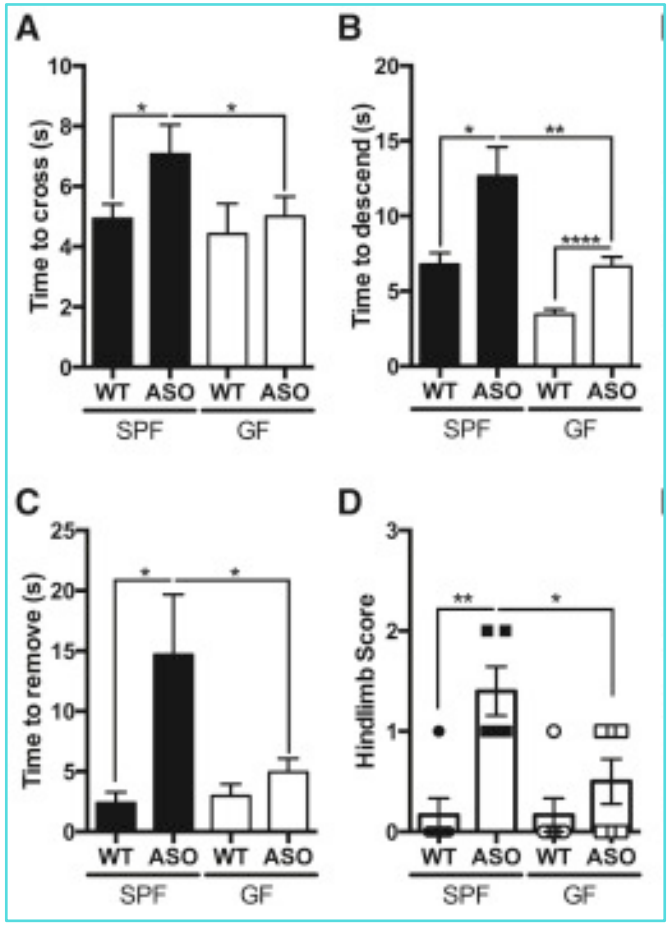
Proposed mechanisms for microbiota role in PD



(Sampson *et al.*, 2016)

- Microbiota is necessary to promote **αSyn pathology**, **neuroinflammation**, and characteristic **motor features** in a validated mouse model (ASO)
- Aggregation of αSyn in the caudoputamen and substantia nigra (fewer in GF-ASO mice)
- Arrest in microglia maturation in GF animals
- Increased production of pro-inflammatory cytokines (ASO mice)
- Motor function deficits (less so in GF-ASO mice)

- Gut bacteria from PD patients promote enhanced motor impairment in ASO mice



SPF-ASO mouse displays deficits in fine and gross motor function, as well as gut motility defects compared to SPF-WT. Germ-free (GF)-ASO motor function tasks resembles SPF-WT.

(Sampson *et al.*, 2016)

- 16 human case-control studies have published gut microbiome composition changes in PD

- Different methodologies

1. Study population
2. Sample transport/storage
3. DNA extraction kits
4. Sequencing method
5. Bioinformatics/ -statistical methods

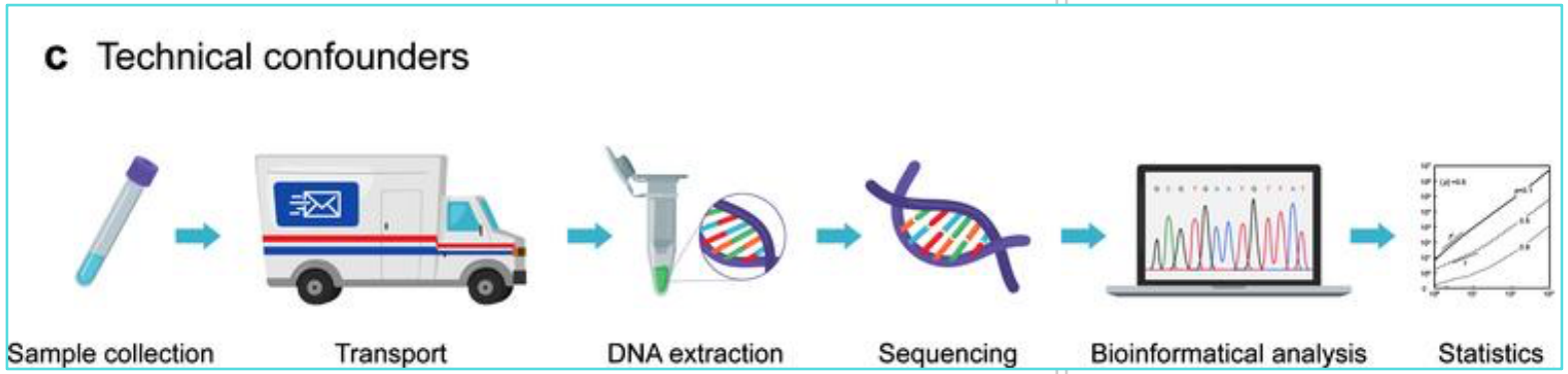
- Differences in microbial community commonly described in terms of alpha and beta diversity, and relative abundance of bacteria

- Disease duration most consistent association with gut microbiome composition

- One follow-up study only

Potential reasons for heterogeneity of results

- Inherent intra- and inter-subject variability of gut microbiome composition
- Methodological inconsistencies between studies



(Boertien *et al.*, 2019)

Research challenges and future directions

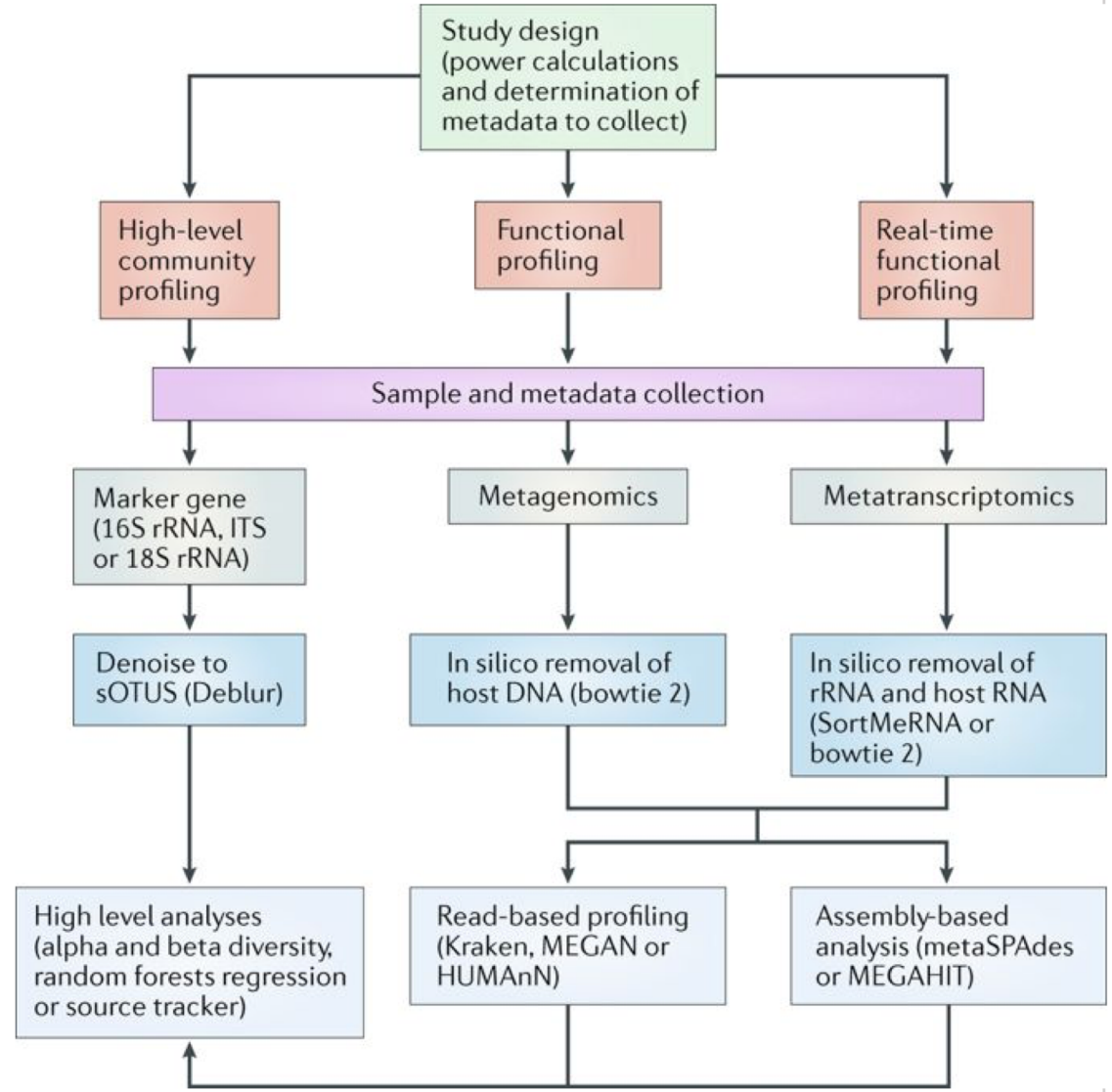
- Massive amounts of data and a bewildering array of computational tools and methods for analysing the data
- Most fundamental issues that concern microbiome studies arise from statistical and experimental design issues
- Rapid advances means best methods are ever changing
- Technical variation > comparability of studies remains an unsolved challenge
- Many confounding factors

(Knight *et al.*, 2018)

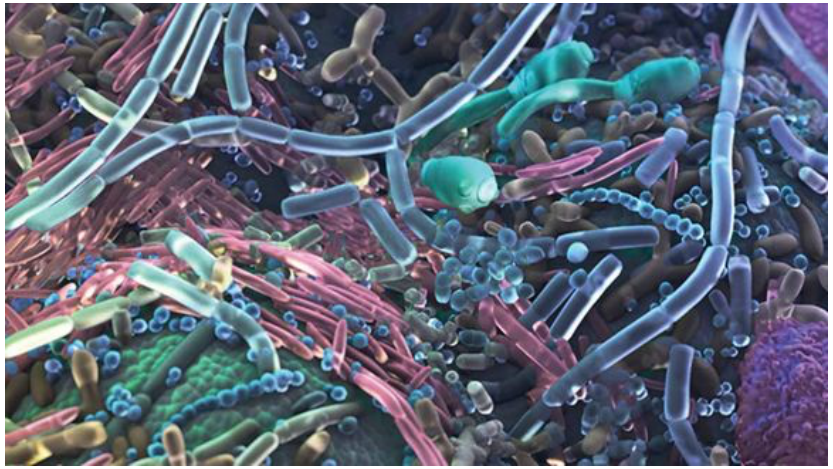
- Example workflow for microbiome data generated from

- 16S ribosomal RNA sequencing: high-level view with low resolution of microbial community
- Whole metagenome sequencing: high taxonomic resolution and assembly of whole microbial genomes (functional capacity at gene level)
- Metatranscriptomic (RNA) sequencing: transcription profile to assess functional activity of microbes

> The choice of method: trade-offs between cost, robustness, possible biases, resolution



(Knight *et al.*, 2018)



Credit: Panther Media GmbH / Alamy Stock Photo

- Large population studies have greatly advanced our knowledge
- Identification of factors that shape the microbiome
- Advanced the establishment of research methods and standards: yet no gold standard

- A ”normal” gut microbiota
- Predominantly single-time point analysis study designs in small cohorts
- Cost considerations
- Strength mainly from animal work
- Correlation or causation?
- New avenues to personalized medicine

(Knight *et al.*, 2018)

Take home message

1. There is tremendous opportunity for research at every level to advance our understanding of this complicated ecosystem.
2. It is becoming increasingly clear that commensal microbiota play a major role in health and disease.
3. This opens exciting new avenues for multifaceted strategies with an unprecedented potential to improve patient outcomes across many conditions and diseases.

Acknowledgements

My PhD supervisors
Prof Michael Hornberger (left)
Prof Simon Carding (right)

Associated lab groups at the Quadram Institute and Medical School.

NRP Science Links Seed Corn funding.


UEA
University of East Anglia


Quadram
Institute
Science Health
Food Innovation

THANK YOU

References

Bäckhed, F. *et al.* (2004) 'The gut microbiota as an environmental factor that regulates fat storage.', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 101(44), pp. 15718–23. doi: 10.1073/pnas.0407076101.

Bhattarai, Y., Muniz Pedrogo, D. A. and Kashyap, P. C. (2016) 'Irritable bowel syndrome: A gut microbiota-related disorder?', *American Journal of Physiology - Gastrointestinal and Liver Physiology*. American Physiological Society, pp. G52–G62. doi: 10.1152/ajpgi.00338.2016.

Boertien, J. M. *et al.* (2019) 'Increasing Comparability and Utility of Gut Microbiome Studies in Parkinson's Disease: A Systematic Review', *Journal of Parkinson's Disease*. IOS Press, pp. S297–S312. doi: 10.3233/JPD-191711.

Bondar, T. (2019) *Regulation of mucosal immunity by the microbiota*. Available at: https://www.nature.com/articles/d42859-019-00014-2 (Accessed: 22 January 2020).

Bray, N. (2019) *The microbiota–gut–brain axis*. Available at: https://www.nature.com/articles/d42859-019-00021-3 (Accessed: 22 January 2020).

Carabotti, M. *et al.* (2015) 'The gut-brain axis: Interactions between enteric microbiota, central and enteric nervous systems', *Annals of Gastroenterology*. Hellenic Society of Gastroenterology, 28(2), pp. 203–209.

Chen, G. *et al.* (2019) 'Mesoporous coxsn(1 - x)o2 as an efficient oxygen evolution catalyst support for spe water electrolyzer', *Royal Society Open Science*, 6(4). doi: 10.6084/m9.figshare.

Chu, H. and Mazmanian, S. K. (2013) 'Innate immune recognition of the microbiota promotes host-microbial symbiosis', *Nature Immunology*, pp. 668–675. doi: 10.1038/ni.2635.

Collins, S. M. (2014) 'A role for the gut microbiota in IBS', *Nature Reviews Gastroenterology and Hepatology*. Nature Publishing Group, pp. 497–505. doi: 10.1038/nrgastro.2014.40.

Costello, E. K. *et al.* (2009) 'Bacterial community variation in human body habitats across space and time', *Science*, 326(5960), pp. 1694–1697. doi: 10.1126/science.1177486.

El-Salhy, M. *et al.* (2019) 'Gut microbiota Efficacy of faecal microbiota transplantation for patients with irritable bowel syndrome in a randomised, double-blind, placebo-controlled study', *Gut*, 0, pp. 1–9. doi: 10.1136/gutjnl-2019-319630.

Furfaro, L. L., Payne, M. S. and Chang, B. J. (2018) 'Bacteriophage Therapy: Clinical Trials and Regulatory Hurdles', *Frontiers in cellular and infection microbiology*. NLM (Medline), p. 376. doi: 10.3389/fcimb.2018.00376.

Harach, T. *et al.* (2017) 'Reduction of Abeta amyloid pathology in APPPS1 transgenic mice in the absence of gut microbiota', *Scientific Reports*. Nature Publishing Group, 7. doi: 10.1038/srep41802.

Helmink, B. A. *et al.* (2019) 'The microbiome, cancer, and cancer therapy', *Nature Medicine*. Nature Publishing Group, pp. 377–388. doi: 10.1038/s41591-019-0377-7.

References

Ivanov, I. I. *et al.* (2008) 'Specific Microbiota Direct the Differentiation of IL-17-Producing T-Helper Cells in the Mucosa of the Small Intestine', *Cell Host and Microbe*, 4(4), pp. 337–349. doi: 10.1016/j.chom.2008.09.009.

Jagatia, A. and Relman, D. A. (2019) *The origins of human microbiota research*. Available at: https://www.nature.com/articles/d42859-019-00073-5 (Accessed: 22 January 2020).

Kao, D. *et al.* (2017) 'Effect of oral capsule– vs colonoscopy-delivered fecal microbiota transplantation on recurrent Clostridium difficile infection: A randomized clinical trial', *JAMA - Journal of the American Medical Association*. American Medical Association, 318(20), pp. 1985–1993. doi: 10.1001/jama.2017.17077.

Khoruts, A. and Sadowsky, M. J. (2016) 'Understanding the mechanisms of faecal microbiota transplantation', *Nature Reviews Gastroenterology and Hepatology*. Nature Publishing Group, pp. 508–516. doi: 10.1038/nrgastro.2016.98.

Knight, R. *et al.* (2018) 'Best practices for analysing microbiomes', *Nature Reviews Microbiology*. Nature Publishing Group, pp. 410–422. doi: 10.1038/s41579-018-0029-9.

Ley, R. E. *et al.* (2005) 'Obesity alters gut microbial ecology', *Proceedings of the National Academy of Sciences*, 102(31), pp. 11070–11075. doi: 10.1073/pnas.0504978102.

Ley, R. E. *et al.* (2006) 'Microbial ecology: Human gut microbes associated with obesity', *Nature*, 444(7122), pp. 1022–1023. doi: 10.1038/4441022a.

Lynch, S. V. and Boushey, H. A. (2016) 'The microbiome and development of allergic disease', *Current Opinion in Allergy and Clinical Immunology*. Lippincott Williams and Wilkins, pp. 165–171. doi: 10.1097/ACI.0000000000000255.

Ochoa-Reparaz, J. *et al.* (2018) 'Microbiome and Allergic Diseases', 9, p. 1584. doi: 10.3389/fimmu.2018.01584.

Pariente, N. (2019) 'A field is born', *Nature Research 2019*. Nature Publishing Group.

Proctor, L. M. *et al.* (2019) 'The Integrative Human Microbiome Project', *Nature*. Nature Publishing Group, 569(7758), pp. 641–648. doi: 10.1038/s41586-019-1238-8.

Sampson, T. R. *et al.* (2016) 'Gut Microbiota Regulate Motor Deficits and Neuroinflammation in a Model of Parkinson's Disease', *Cell*. Cell Press, 167(6), pp. 1469-1480.e12. doi: 10.1016/j.cell.2016.11.018.

Tamburini, S. *et al.* (2016) 'The microbiome in early life: Implications for health outcomes', *Nature Medicine*. Nature Publishing Group, pp. 713–722. doi: 10.1038/nm.4142.

Taur, Y. *et al.* (2014) 'The effects of intestinal tract bacterial diversity on mortality following allogeneic hematopoietic stem cell transplantation', *Blood*. American Society of Hematology, 124(7), pp. 1174–1182. doi: 10.1182/blood-2014-02-554725.

Teo, S. M. *et al.* (2015) 'The infant airway microbiome in health and disease impacts later asthma development HHS Public Access', *Cell Host Microbe*, 17(5), pp. 704–715. doi: 10.1016/j.chom.2015.03.008.

Tian, Y. *et al.* (2019) 'Fecal microbiota transplantation for ulcerative colitis: a prospective clinical study', *BMC Gastroenterology*, 19(1), p. 116. doi: 10.1186/s12876-019-1010-4.

Viaud, S. *et al.* (2013) 'The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide', *Science*, 342(6161), pp. 971–976. doi: 10.1126/science.1240537.

Vogt, N. M. *et al.* (2017) 'Gut microbiome alterations in Alzheimer's disease', *Scientific Reports*. Nature Publishing Group, 7(1). doi: 10.1038/s41598-017-13601-y.

York, A. (2019) *Microbiota succession in early life*. Available at: https://www.nature.com/articles/d42859-019-00010-6 (Accessed: 22 January 2020).

Zeevi, D. *et al.* (2015) 'Personalized Nutrition by Prediction of Glycemic Responses', *Cell*. Cell Press, 163(5), pp. 1079–1094. doi: 10.1016/j.cell.2015.11.001.

Zhao, L. *et al.* (2018) 'Gut bacteria selectively promoted by dietary fibers alleviate type 2 diabetes', *Science*. American Association for the Advancement of Science, 359(6380), pp. 1151–1156. doi: 10.1126/science.aao5774.