Biomedical & Life Sciences | Lancaster University

Microbiota

Dysbiosis – a cause or consequence of neurological disease?

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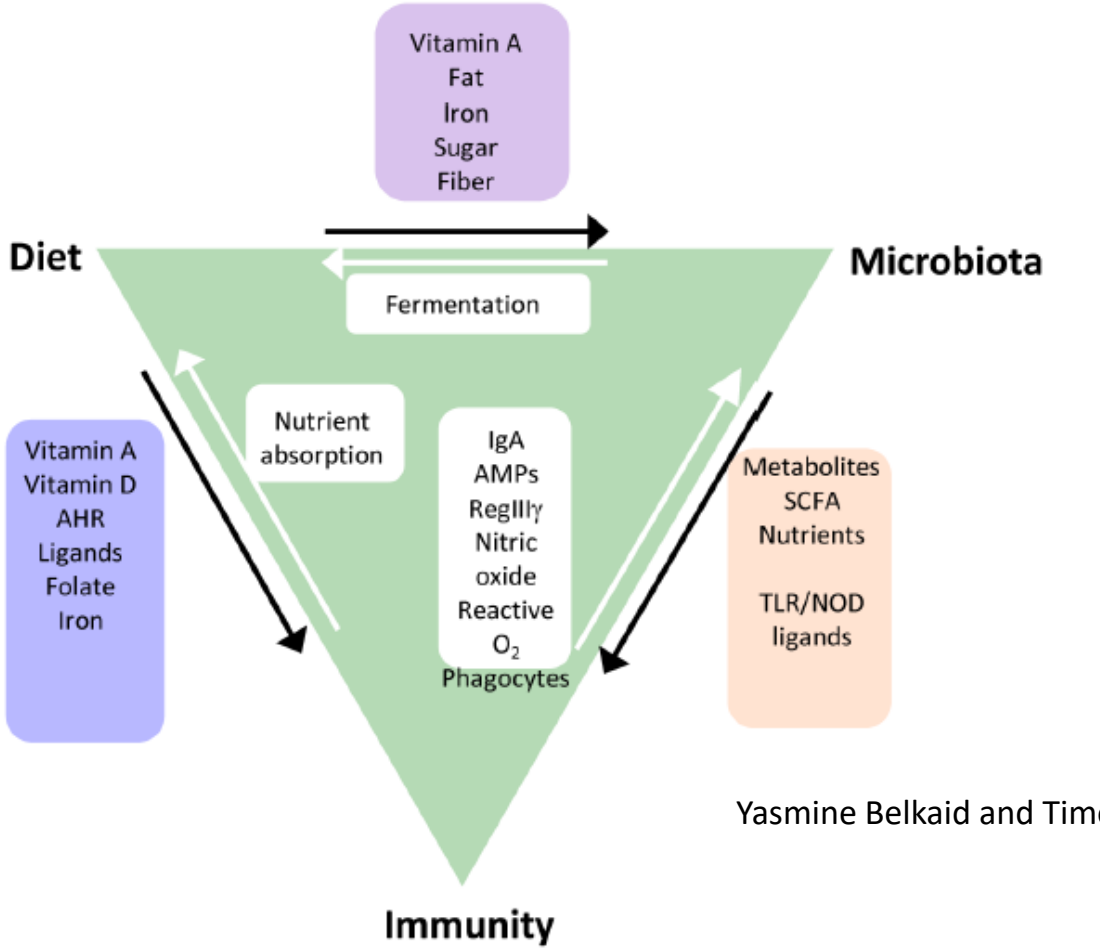
Commensal microflora

Commensalis “sharing the same table”

- $10\text{-}100^{12}$ microorganisms populate the intestine with highest levels in the colon
- Developmental changes in numbers, types and location
  - Bacteroides
  - Clostridium
  - Lactobacillus
  - Streptococci
- Activate Toll-like receptors (TLR) and immune system
- No maintained inflammatory response in healthy individuals

Multiple roles of microbiota

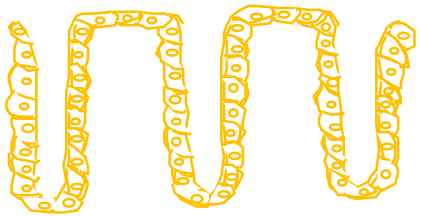


Yasmine Belkaid and Timothy Hand, 2014

Microbiota impact epithelium regeneration and permeability of gut barrier

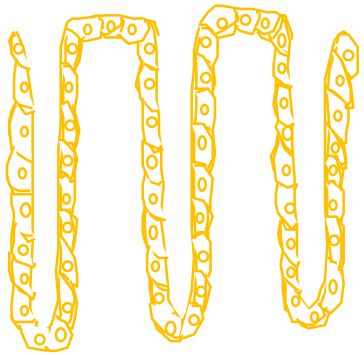
Influence of microbiota

Mucosal Homeostasis



proliferation = apoptosis, anoikis

Mucosal hyperplasia



↑ **Proliferation**

↓ **Apoptosis**

↓ **Anoikis**

Adaptation, Inflammation, Cancer

Mucosal Atrophy

↓ **Proliferation**

Fasting, Germ free, Ulcerative colitis

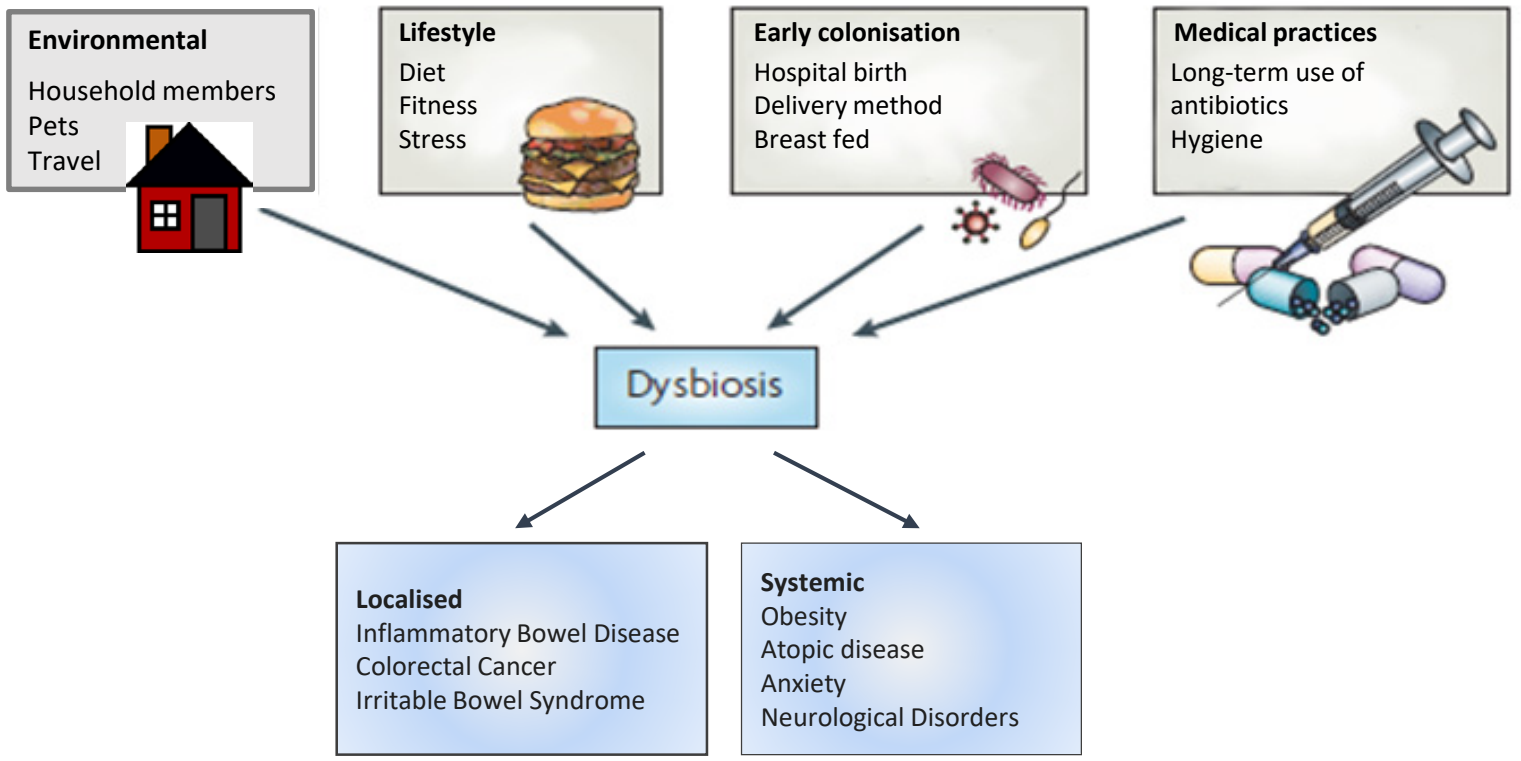
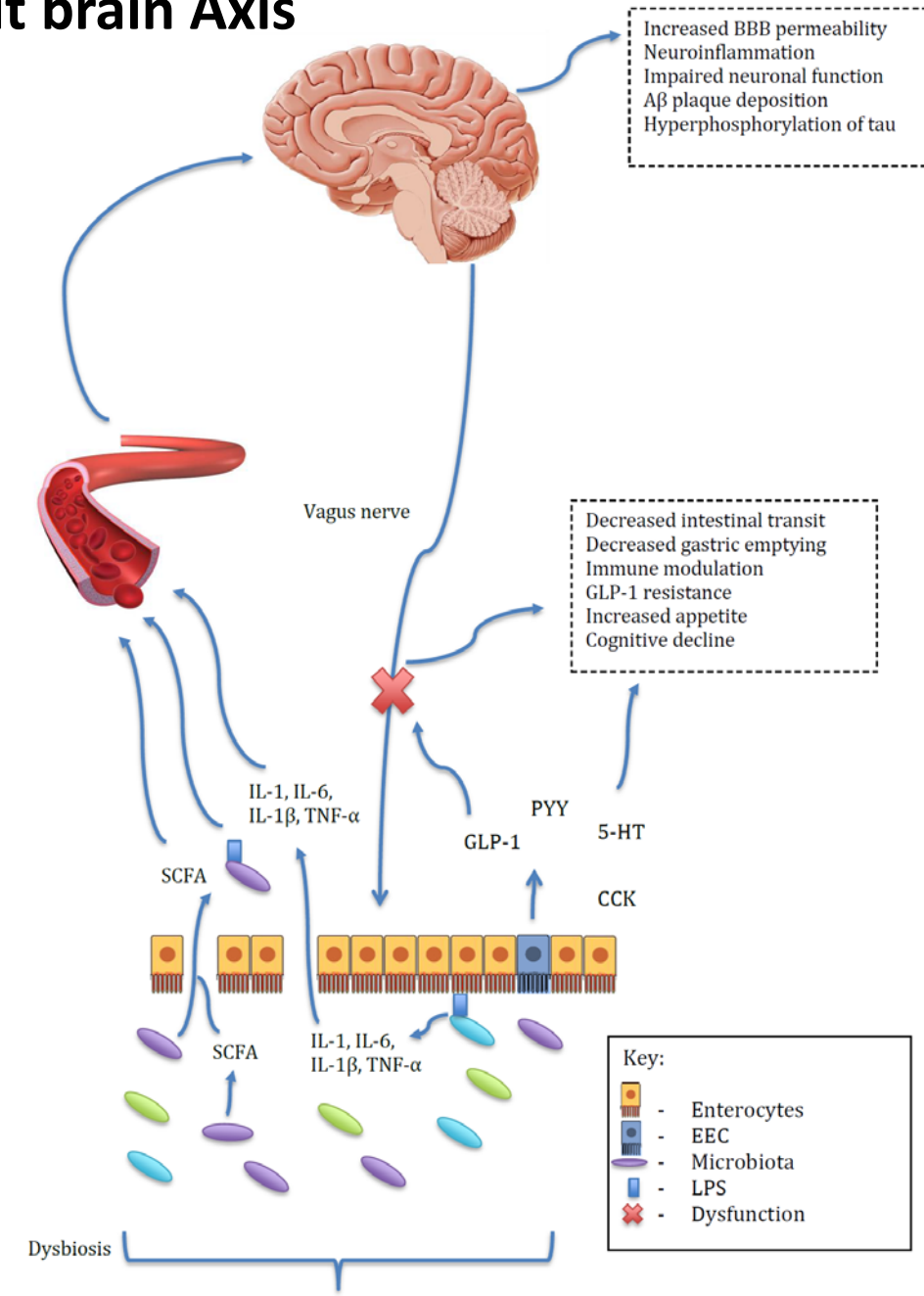


Figure adapted from Round and Mazmanian, 2009

Gut brain Axis



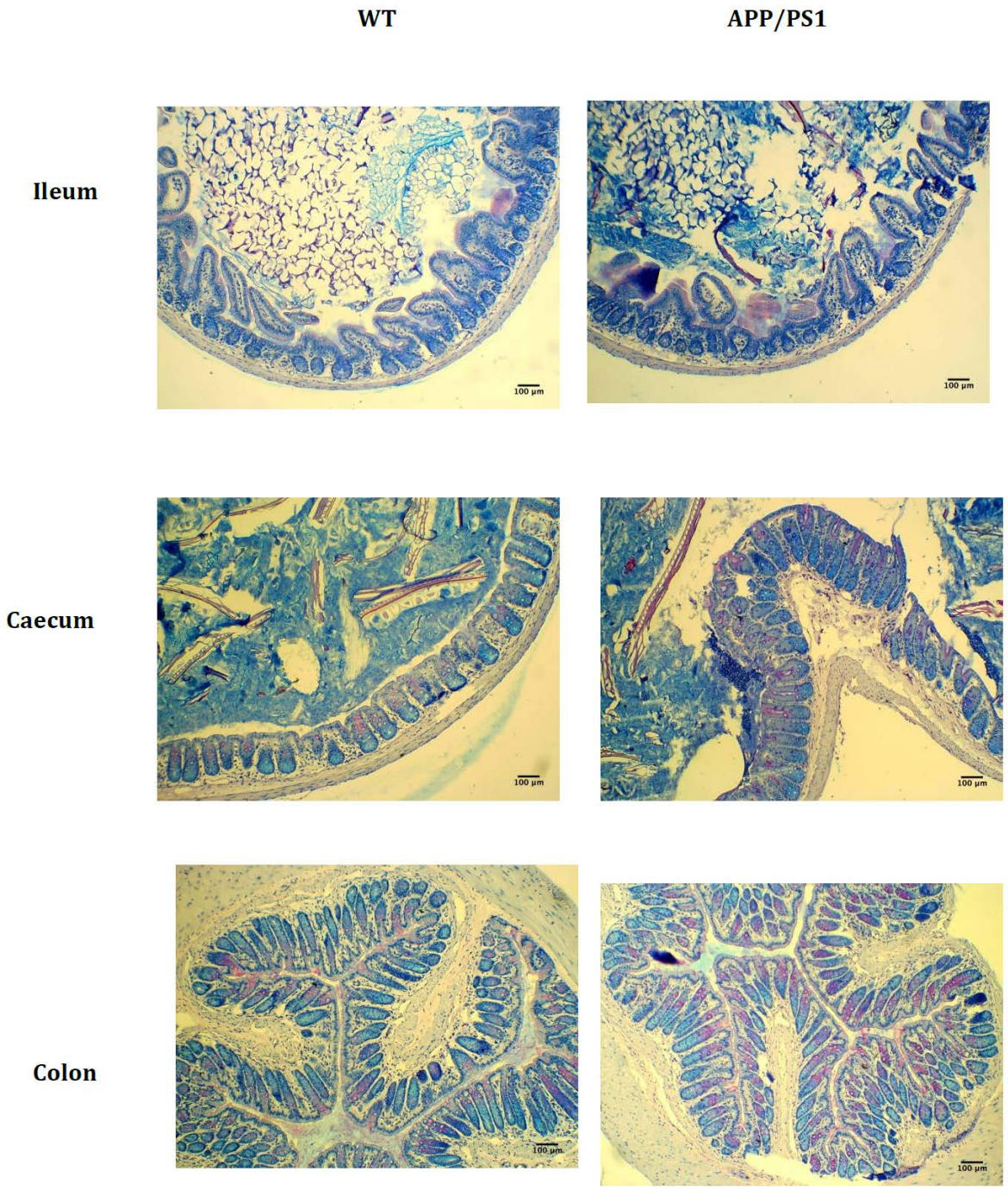
Organism and model	Age	Phylum level	Author
AD patients	Elderly	↓ Firmicutes, Actinobacteria ↑ Bacteroidetes	Vogt et al., 2017
AD patients	Elderly	↓ Bacteroidetes ↑ Firmicutes	Provasi et al., 2017
Mice (APP/PS1)	1 month 3 month 8 month	No significant differences No significant differences ↓ Firmicutes, Verrucomicrobiota, Actinobacteria, Proteobacteria ↑ Bacteroidetes, Tenericutes	Harach et al., 2017
Mice (5xFAD)	6 week 9 week 18 week	No significant differences. ↓ Bacteroidetes ↑ Firmicutes No significant differences	Brandscheid et al., 2017

Table 1. Overview of studies investigating dysbiosis at the phylum level in patients and animal models of AD.

APP/PS1 mouse model of Alzheimer's disease

- Express disease-associated mutant forms of human amyloid precursor protein (APP) and presenilin 1 (PS1)
- Transgenic mice have been able to recapitulate many, although not all, of the key features of AD at a behavioural and cellular level
- Develop amyloid plaques around 7 months of age

PAS stained sections of intestine



Morphometry

	Ileum (villus)	Ileum(crypt)	Caecum	Colon
3 Month	↑ p = 0.31	↑ p = 0.17		↓ p = 0.003
7 Month	↑ p = 0.17	↓ p = 0.71	↑ p = 0.38	↑ p =0.82
15 Month	↑ p = 0.17	↑ p = 0.19	↓ p = 0.38	↓ p =0.82

Table 8: Summary of statistical analysis of villus height and/or crypt depth
Average villus height and/or crypt depth in the ileum, caecum and distal colon in three, seven and fifteen month WT and APP/PS1 mice. ↑ = increase, ↓ = decrease in APP/PS1 compared to WT.

Epithelial cell lineage/function

	Ileum (villus)	Ileum (crypt)	Caecum	Colon
3 Month	↓ p = 0.63	↓ p =0.61		↑ p = 0.52
7 Month	↓ p =0.21	↓ p = 0.21	↓ p = 0.13	↓ p = 0.44
15 Month	↓ p = 0.001	↓ p =0.01	↑ p = 0.34	↑ p = 0.40

Table 9: Summary of statistical analysis of goblet cell numbers
Average number of goblet cells per 100 µm of villus height and/or crypt depth of the ileum, caecum and distal colon in three, seven and fifteen month WT and APP/PS1 mice. ↑ = increase, ↓ = decrease in APP/PS1 compared to WT.

Denaturing Gradient Gel Electrophoresis (DGGE) analysis

15 month old mice

3 month old mice

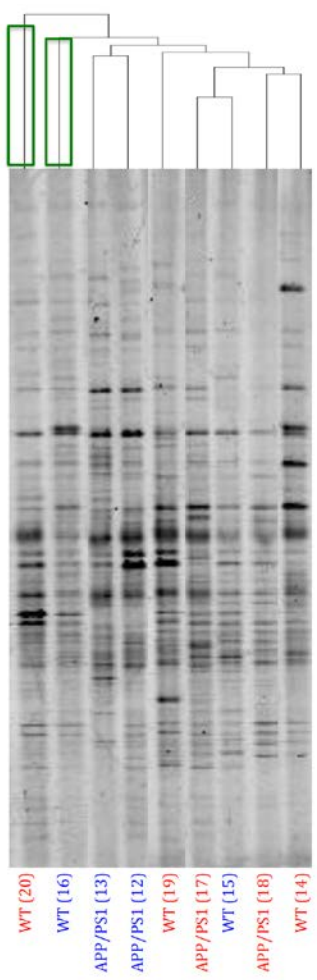
mucosal

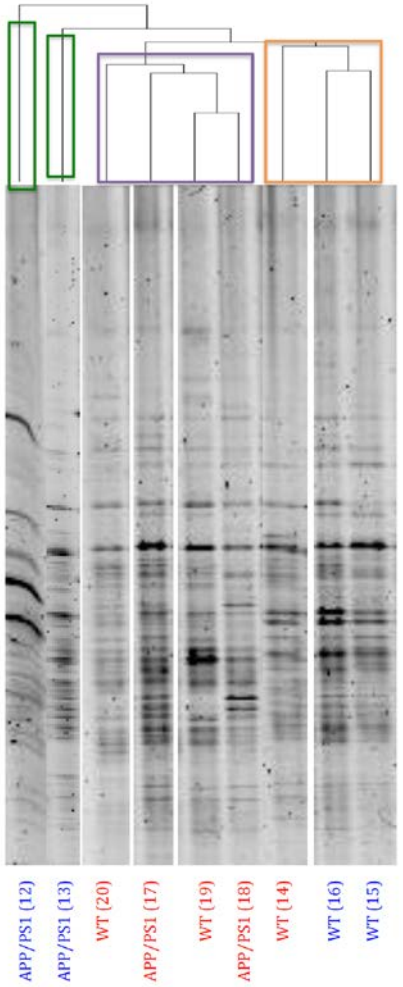
luminal

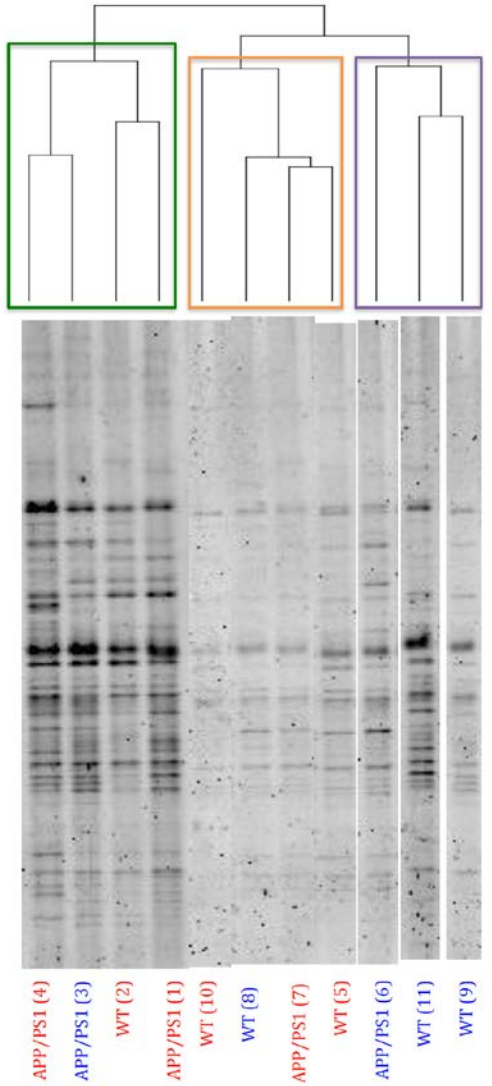
mucosal

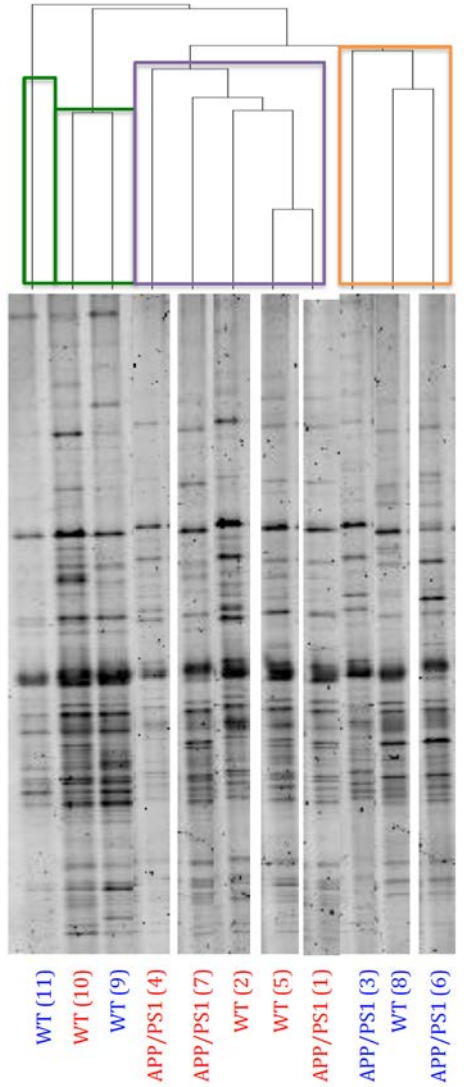
luminal

cecum



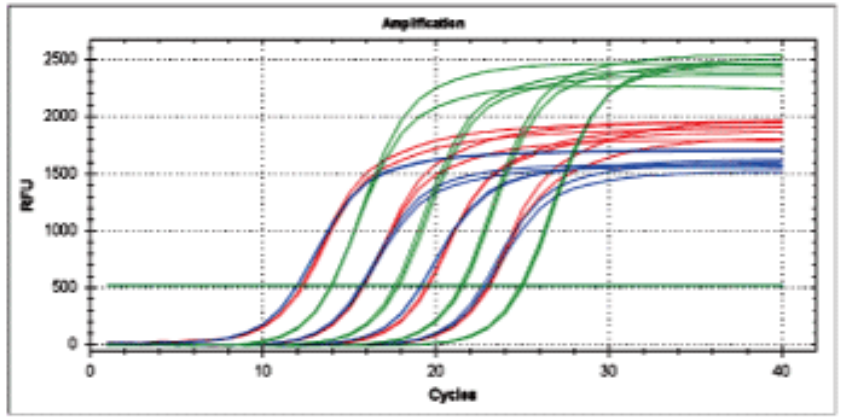


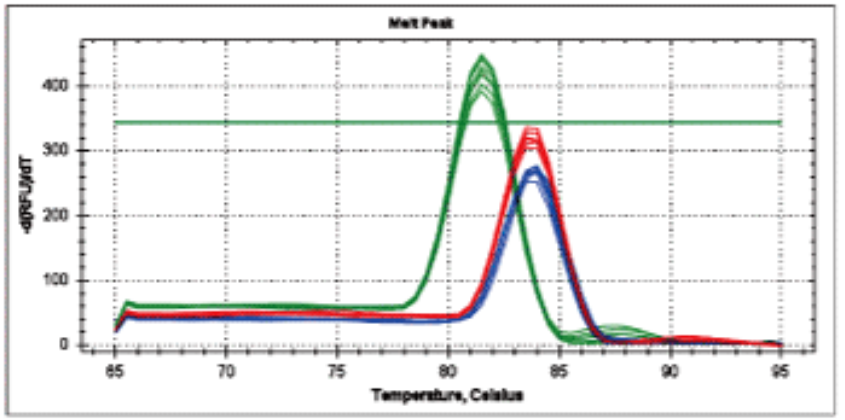




qPCR analysis

Universal	926F	F: 5'-AAACTCAAAKGAATTGACGG-3'
	1062R	R: 5'-CTCACRRCACGAGCTGAC-3'
Firmicutes	928F	F: 5'-TGAAACTYAAAGGAATTGACG-3'
	1040R	R: 5'-ACCATGCACCACCTGTC-3'
Bacteroidetes	798cfbF	F: 5'-CRAACAGGATTAGATACCCT-3'
	cfb967R	R: 5'-GGTAAGGTTCCTCGCGTAT-3'
γ-Proteobacteria	1080γF	F: 5'-TCGTCAGCTCGTGTYGTGA-3'
	γ1202R	R: 5'-CGTAAGGGCCATGATG-3'





Normalised to 16s rDNA (Universal), and the relative abundances of each phylum determined using the delta delta Ct ($2^{-\Delta\Delta Ct}$) algorithm method

Differences in APP/PS1 vs. WT microbiota

DGGE analysis

Mucosal:

	Ileum	Caecum	Colon
3 Month		p = 0.04	
7 Month	p = 0.82	p = 0.82	p = 0.44
15 Month	p = 0.54	p = 0.24	p = 0.83

Luminal:

	Ileum	Caecum	Colon
3 Month		p = 0.66	
7 Month	p = 0.07	p = 0.81	p = 0.49
15 Month	p = 0.78	p = 0.87	p = 0.22

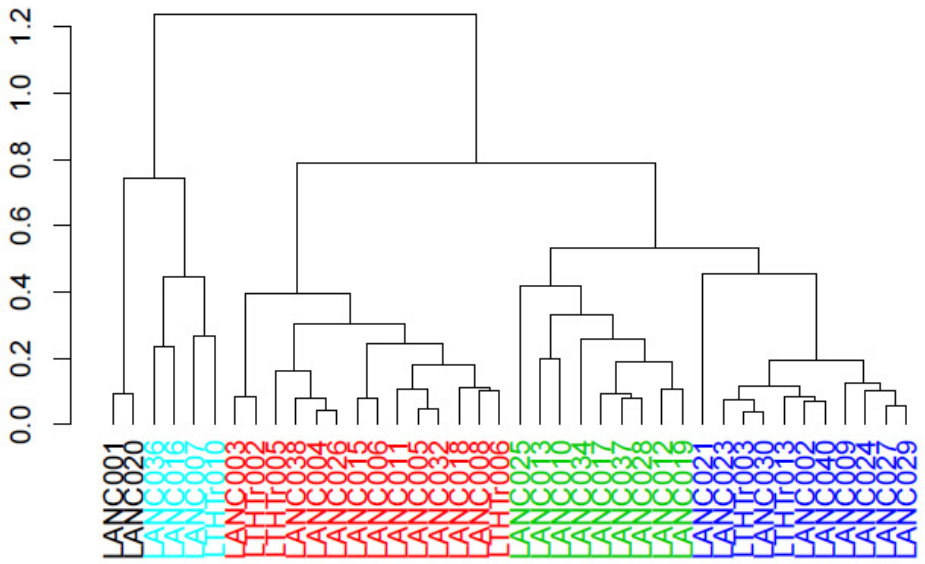
qPCR analysis

		Ileum	Caecum	Colon
3 month	**Firmicutes**		↑ p = 0.86	
	Bacteroidetes		↓ p =0.27	
7 month	**Firmicutes**	↓ p = 0.20	↑ p = 0.92	↓ p = 0.05
	Bacteroidetes	↑ p = 0.33	↑ p = 0.68	↑ p = 0.92
15 month	**Firmicutes**	↓ p = 0.58	↓ p = 0.27	↓ p = 0.80
	Bacteroidetes	↑ p = 0.82	↑ p = 0.31	↑ p = 0.47

Table 11: Summary of statistical analysis of qRT-PCR data
Relative percentage change in the abundance of the two main phyla, Firmicutes and Bacteroidetes from mucosal associated microbiota of the ileum, caecum and distal colon in three, seven and fifteen-month WT and APP/PS1 mice. ↑ = increase, ↓ = decrease in in relative percentage change of phylum in APP/PS1 compared to WT.

Cohort ‘Enterotypes’ in Intestinal Microbiota Profiles

A



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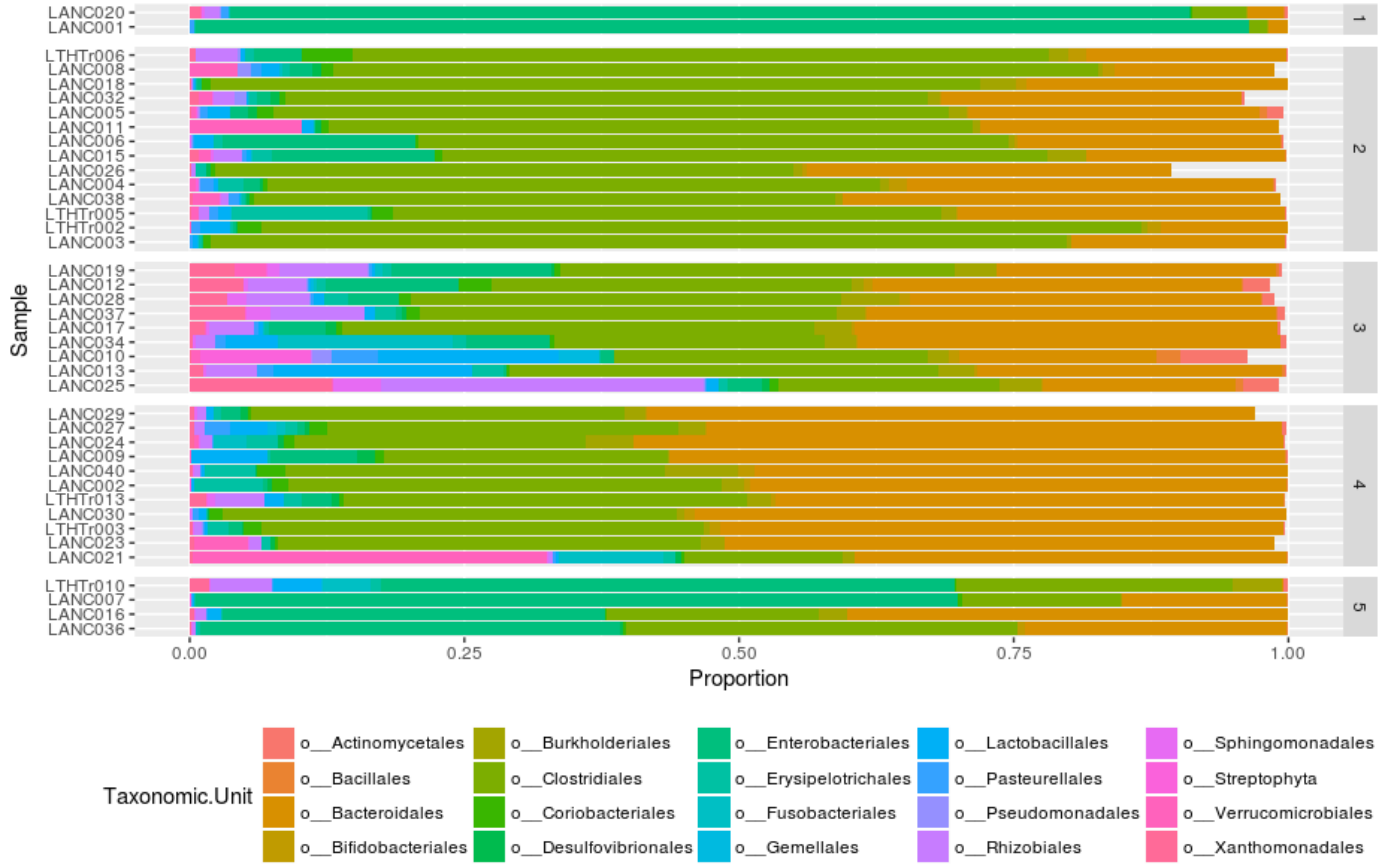
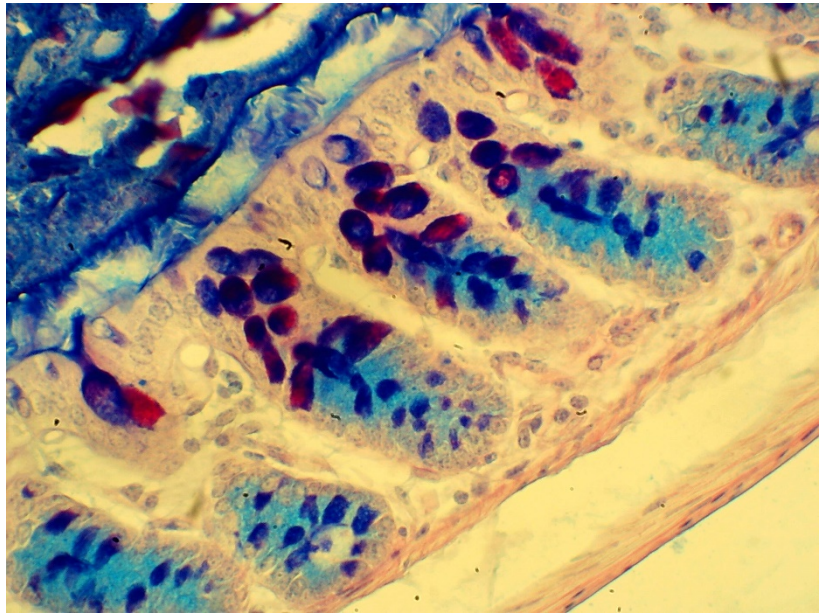
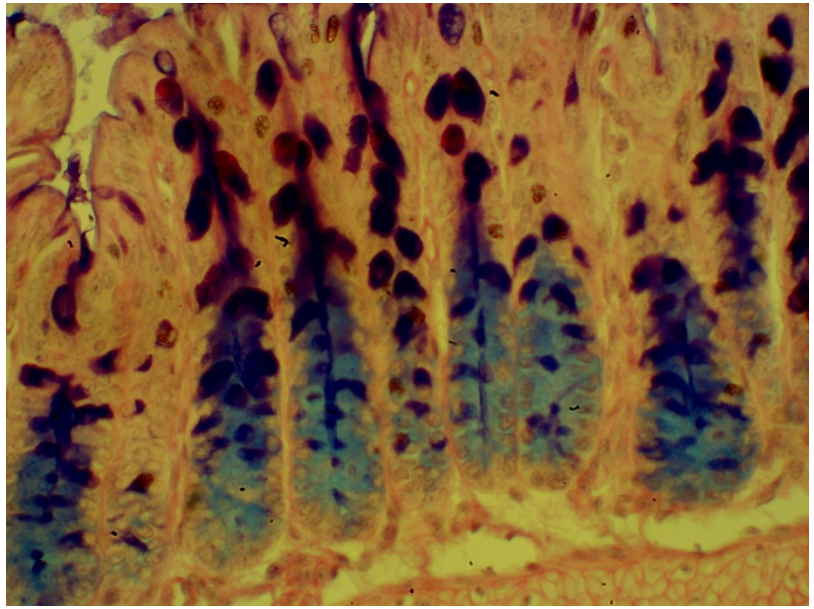


This afternoon - Practical experience
Staining gut tissue





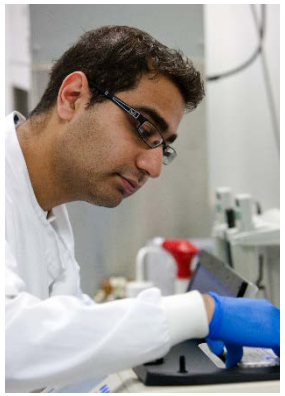
Goblet cells produce antibacterial mucin, impacts interaction with microbiome
Blue = Neutral
Pink = Acidic

Acknowledgements

Lancaster University
Dr Nicole Finn
Dr Jayde Whittingham-Dowd
Dr Emma Beamish
Dr Elisabeth Shaw
Dr Imtiyaz Thagia
Dr Emily Smith

Dr Karen Wright
Dr Neil Dawson
Dr John Worthington
Prof Christian Holscher

NHS Clinical Team
Dr Arnab Bhowmick
Dr Albert Davies
Dr Abhishek Sharma
Judith Johnson



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