Il Microbioma “tradotto” dal Gastroenterologo

F. Pace

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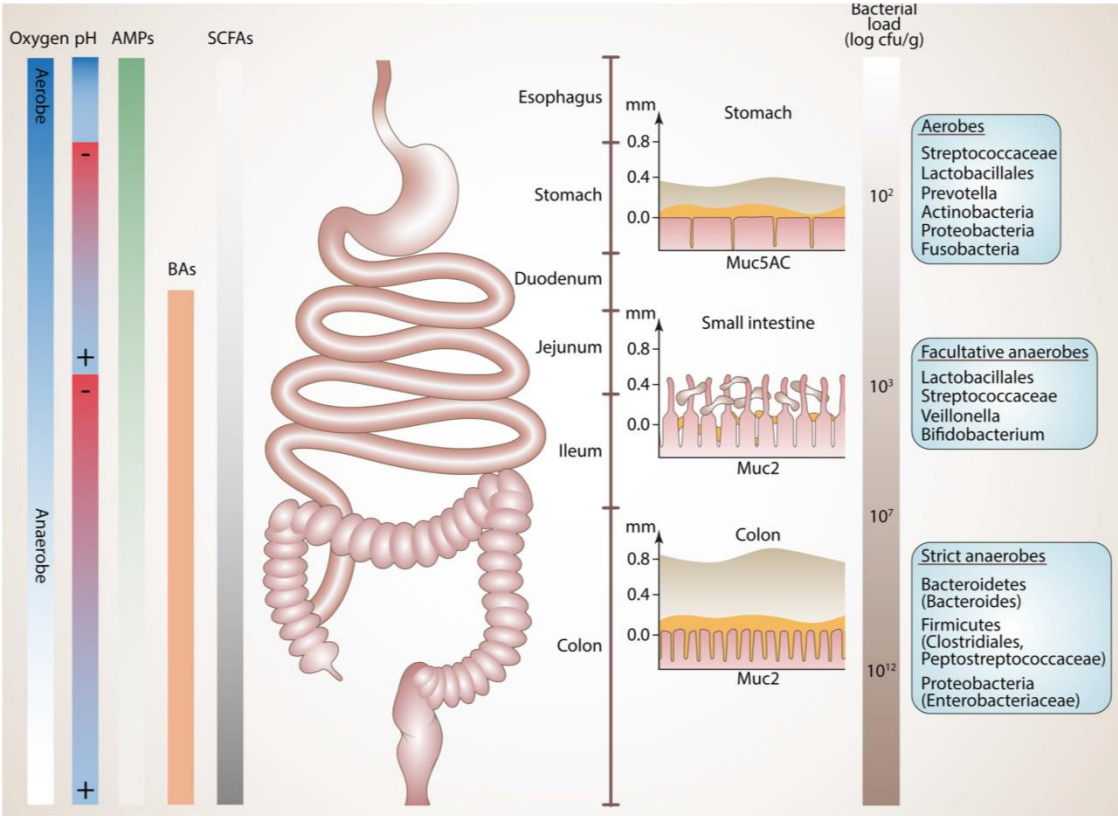
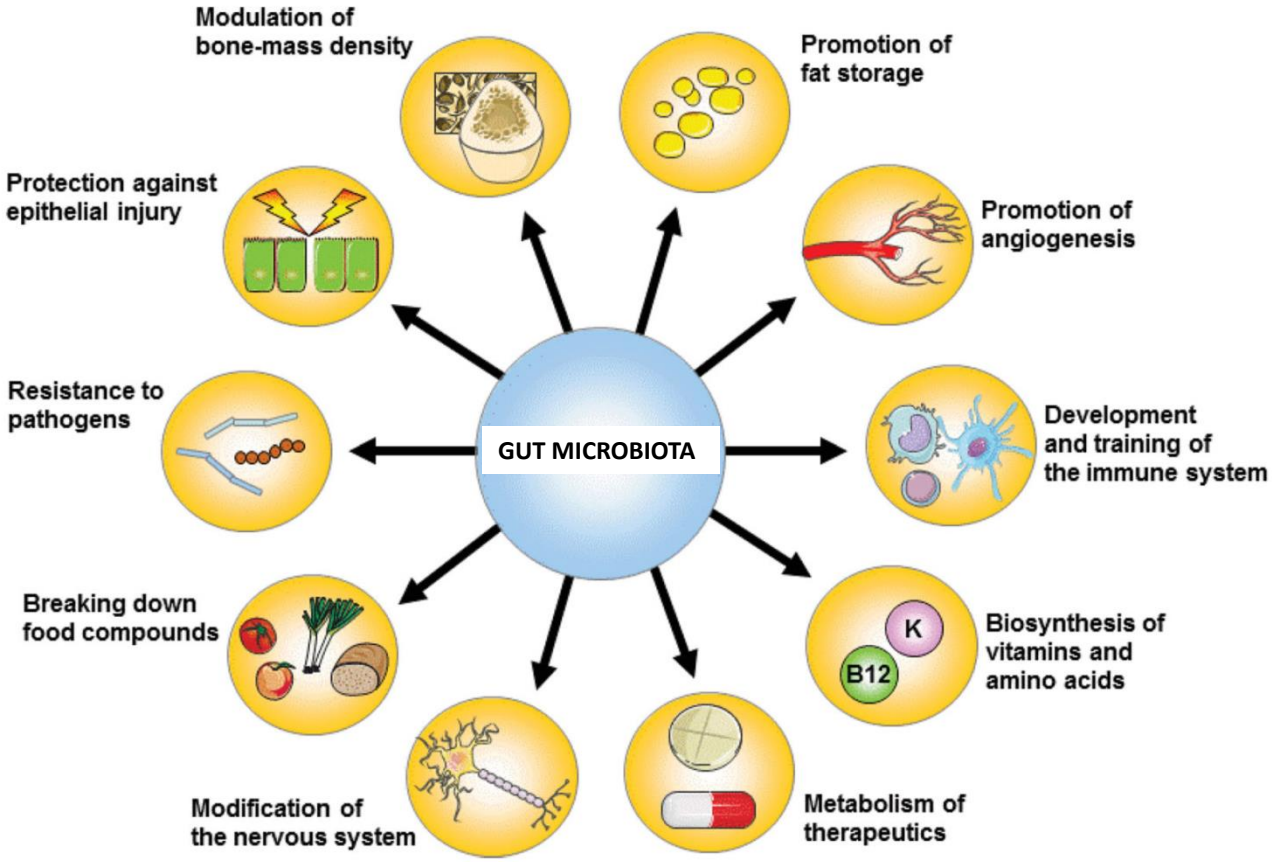
Biogeography and factors shaping the spatial organization of the gut microbiome



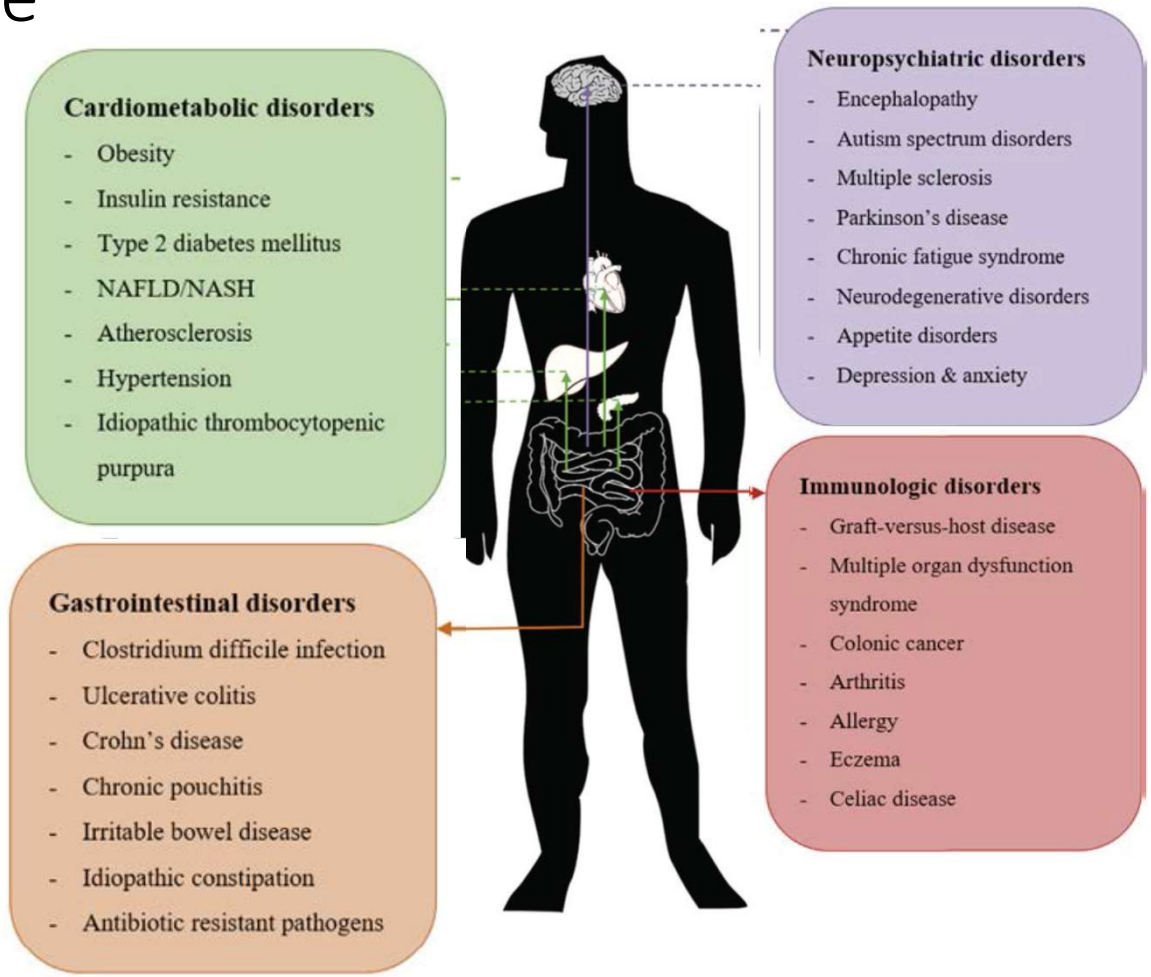
Gorkiewicz et al. *Virchows Archiv* 2018;472:159–172

Main functions of gut microbiota



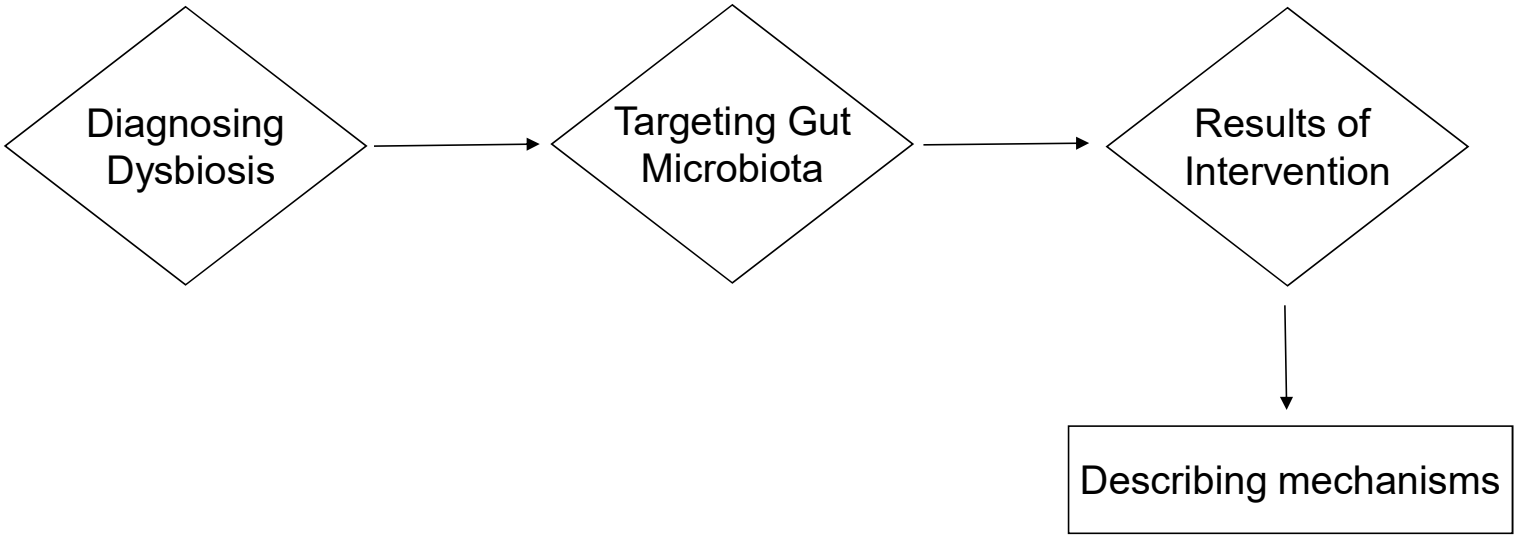
Laukens et al. *FEMS Microbiol Rev* 2016;40:117-32

Associations between dysbiosis of gut microbiota and disease

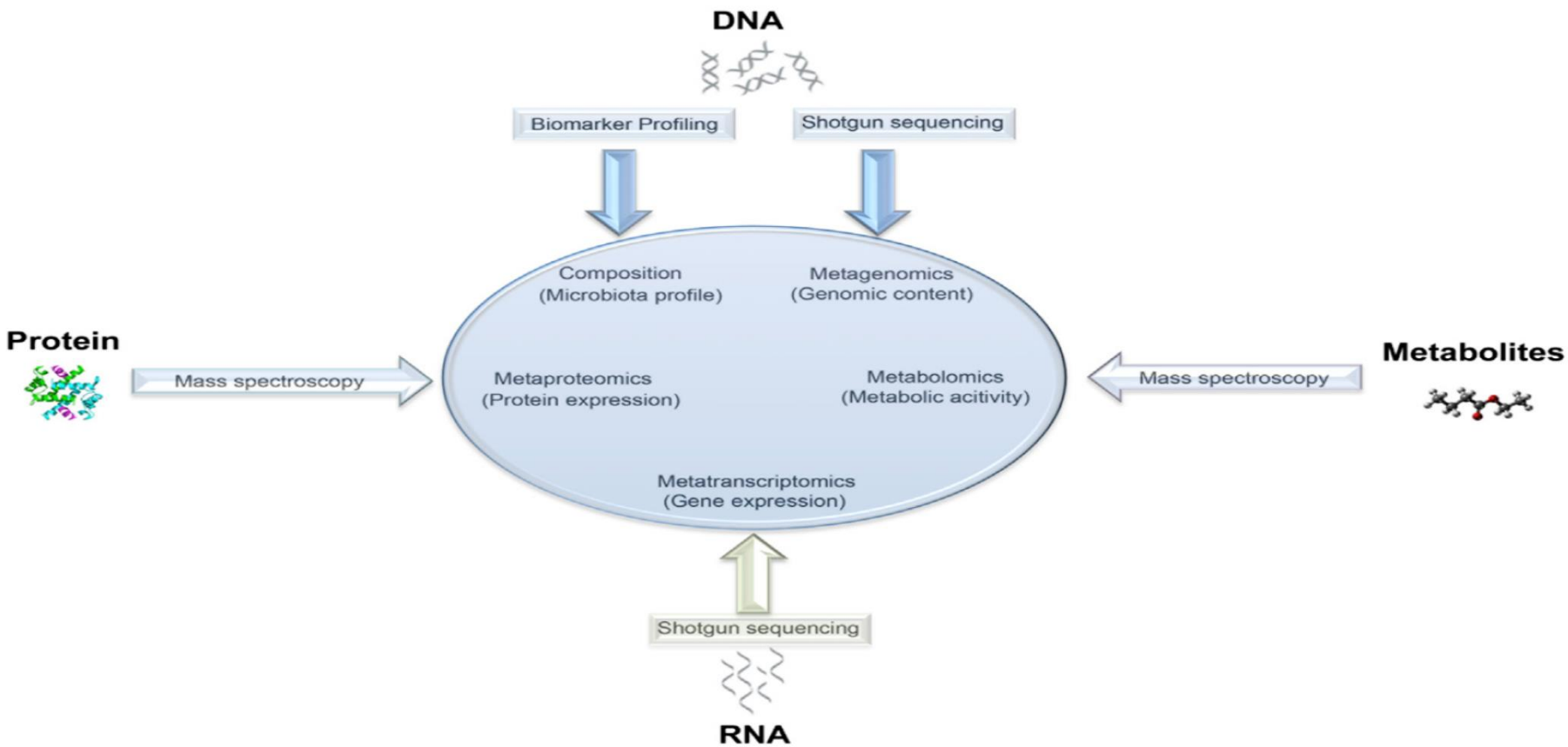


Wortelboer et al, *EBioMedicine* 2019;44: 716–729

Flow chart for gut microbiota intervention by Gastroenterologist

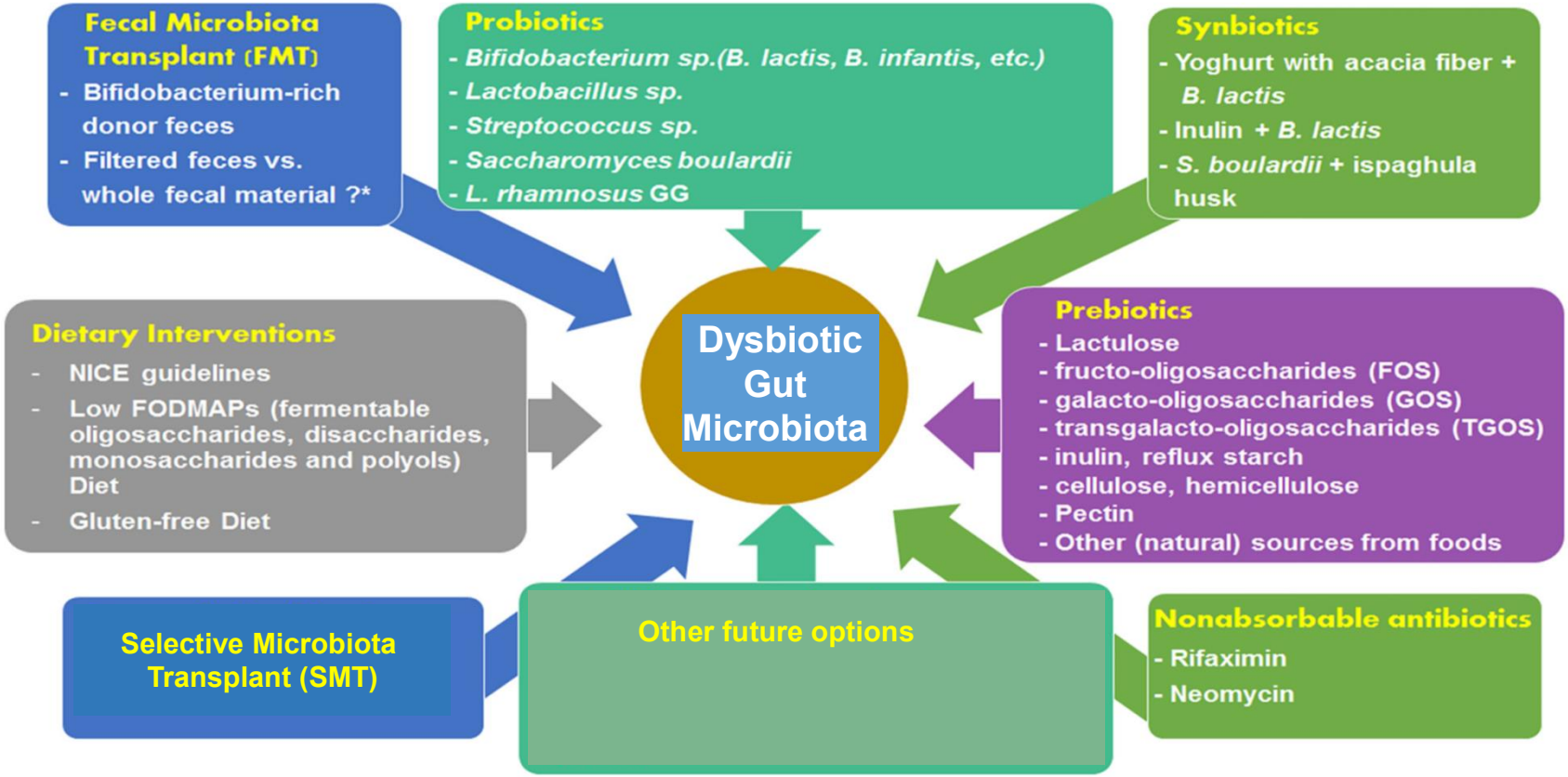


Molecular tools for the study of gut microbiota composition and function. From: *which microbes are there* to *what are they doing ?*



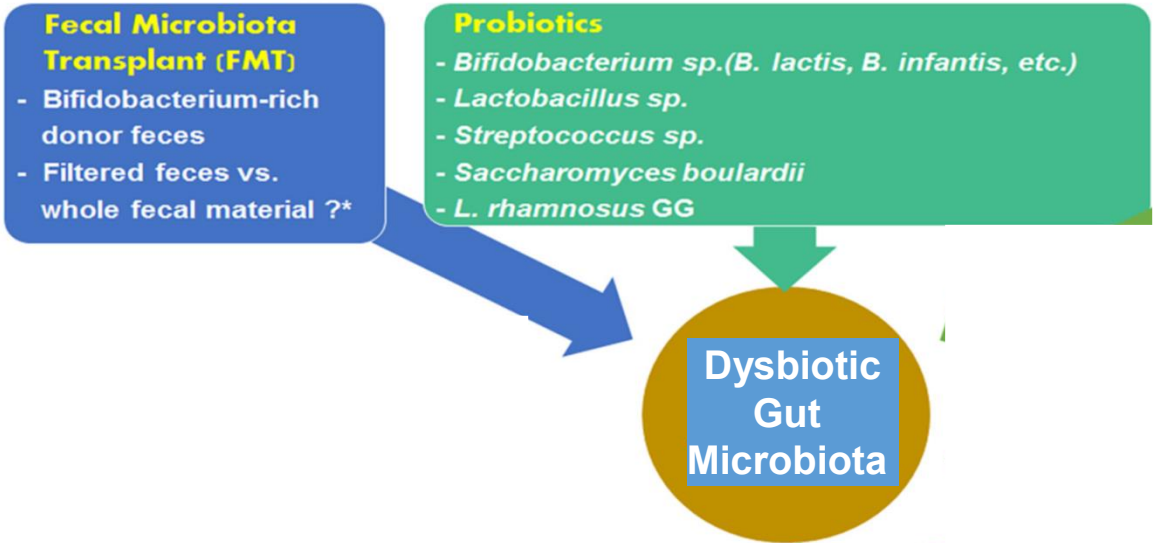
Fujimora et al, *Cell Host Micr* 2015;17:592

Available treatment options for targeting gut microbiota



(modified from) Chong et al, *Front Microbiol* 2019;10:1136

Available treatment options for targeting gut microbiota



(modified from) Chong et al, *Front Microbiol* 2019;10:1136

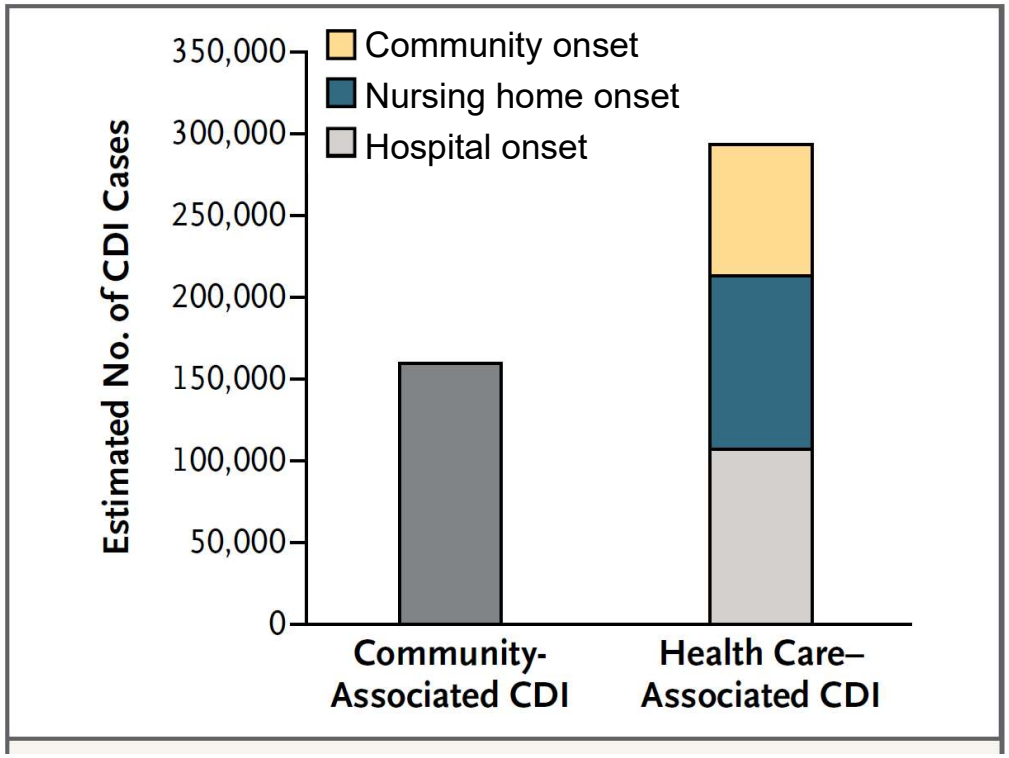
Targeting gut microbiota: 3 examples

- *Cl. difficile* recurrent infections → Mainly FMT
- IBS → Mainly Probiotics
- IBD → Both

1 Incidence and mortality due to CDI in USA (CDC-2011)

In USA (2011):

- **453,000 incident infections**
- **29,000 deaths**
- **9° cause of death** for GI pathology

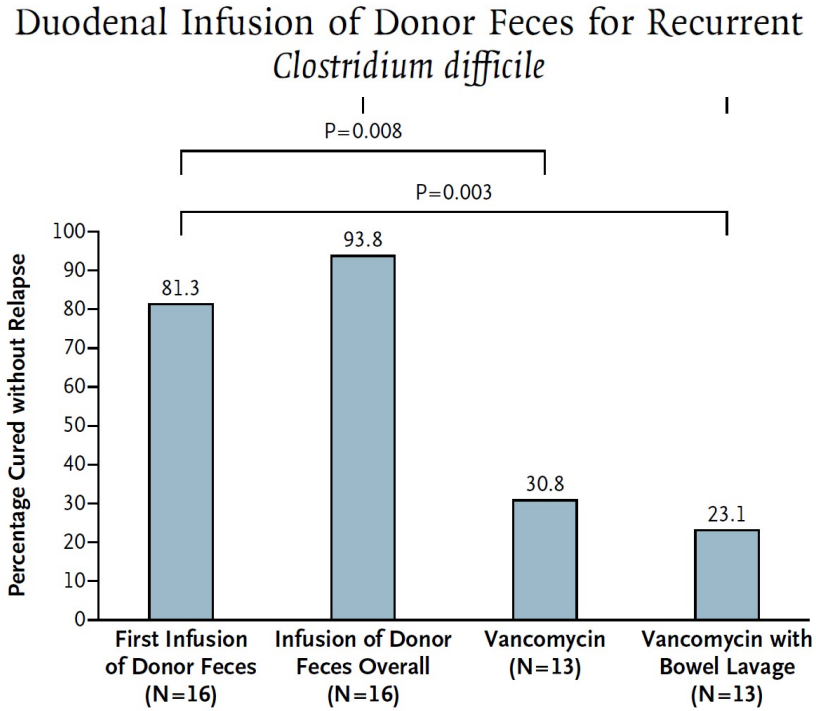


Lessa et al, *N Engl J Med* 2015;372:825-34

FMT in treatment of recurrent *C. difficile* infections (RCDI)

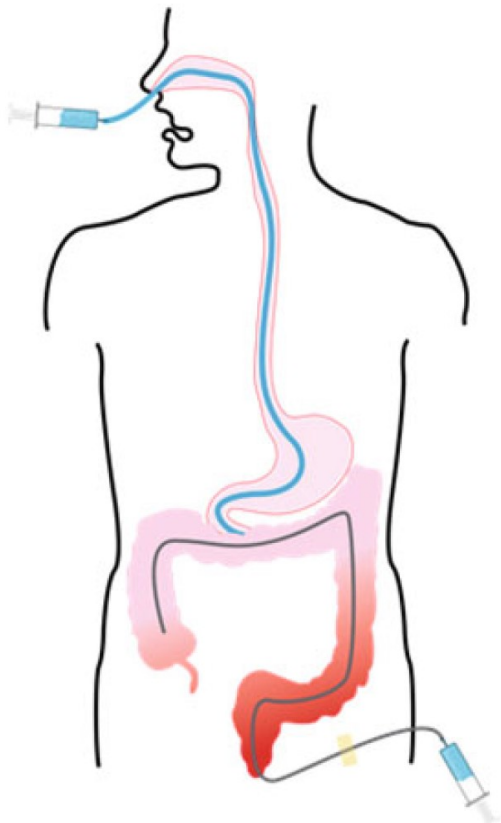
Since 2013, FMT has evolved from an interesting, but little-explored, intervention to a more mainstream therapy of global interest.

The growth in the use of FMT is due, in large part, to the findings of the Van Nood study



Van Nood et al, *NEJM* 2013;368:40715

Multiple delivering ways of FMT in the GI tract

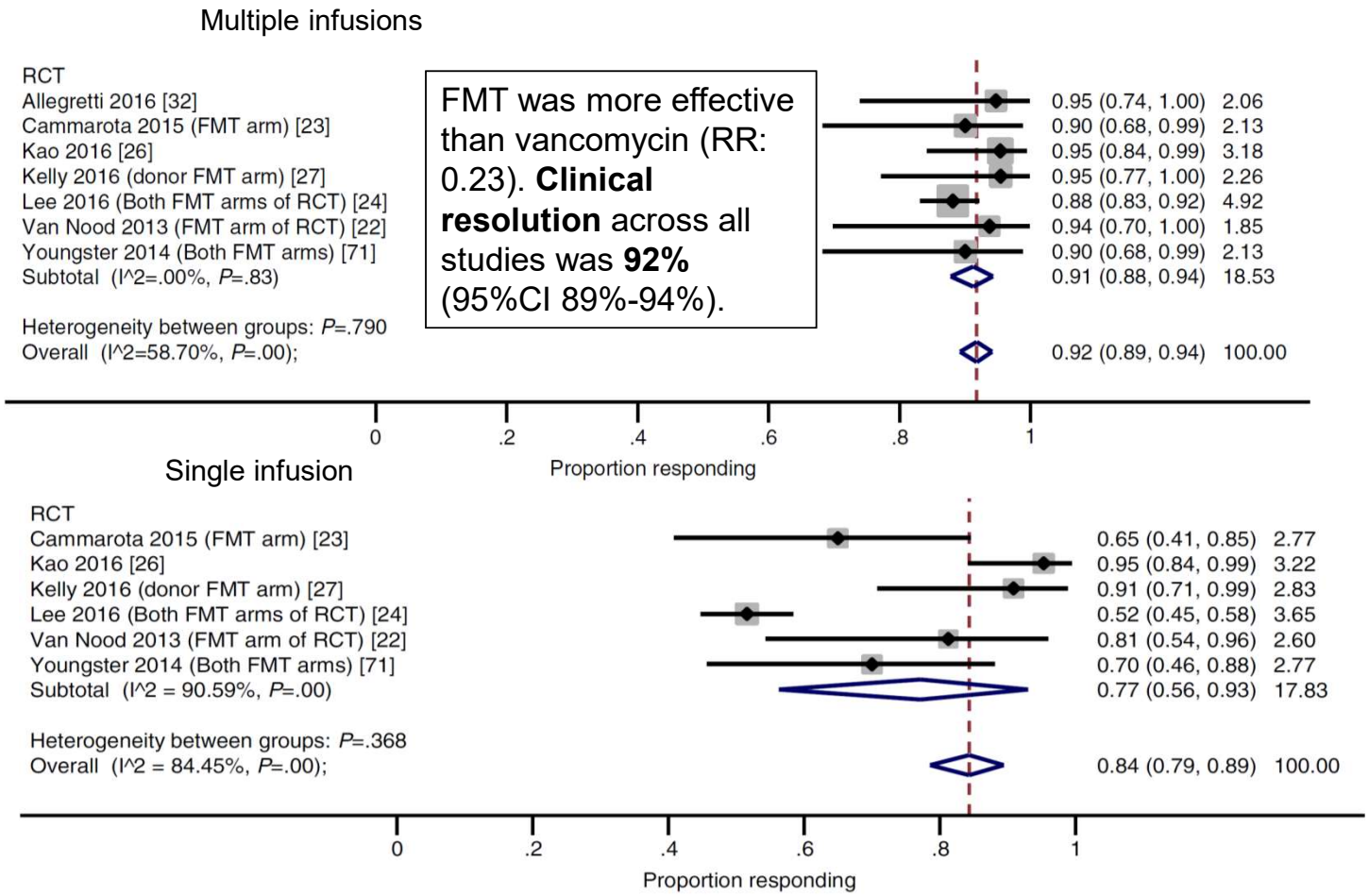


The delivering ways of FMT in g

- Upper-gut
  Capsule
  Drinking
- Mid-gut
  Gastroscopy in duodenum
  Mid-gut tubing
  PEG-J tube
- Lower-gut
  Colonoscopy
  Colonic transendoscopic enter

Zhang et al, *Protein Cell* 2018;9:462–73

Meta-analysis of primary cure rate of FMT for RCDI (RCTs only)



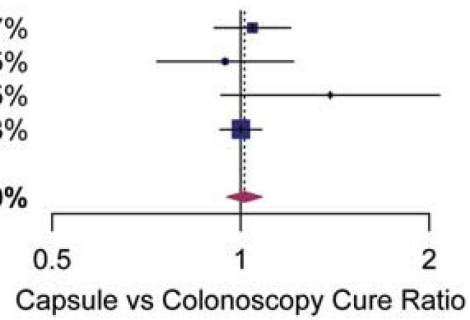
FMT was more effective than vancomycin (RR: 0.23). **Clinical resolution** across all studies was **92%** (95%CI 89%-94%).

Quraishi et al, *Aliment Pharmacol Ther* 2017;46:479–93

FMT: Capsule vs colonoscopy

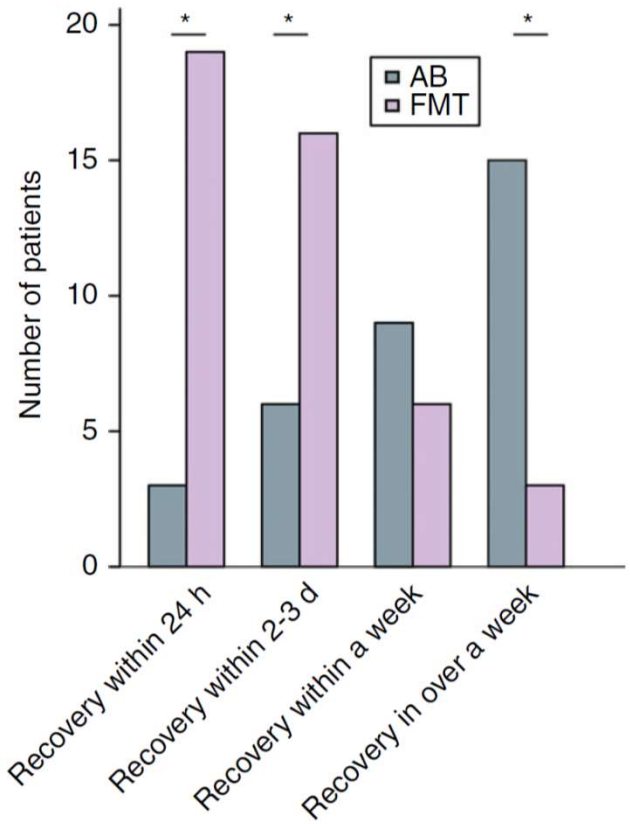
Study	Capsule Cured	Capsule Total	Comparator Cured	Comparator Total	Ratio	95% C.I.	Weight
Greenberg 2018	34	37	44	50	1.04	[0.91; 1.20]	20.7%
Hagel 2016	11	13	43	48	0.94	[0.73; 1.21]	6.5%
Hassoun 2018	11	13	14	23	1.39	[0.93; 2.08]	2.5%
Kao 2017	51	53	50	52	1.00	[0.93; 1.08]	70.3%
Random effects model					**1.01**	**[0.95; 1.08]**	**100.0%**

Heterogeneity: $\chi^2_3 = 2.96$ ($p = 0.40$)



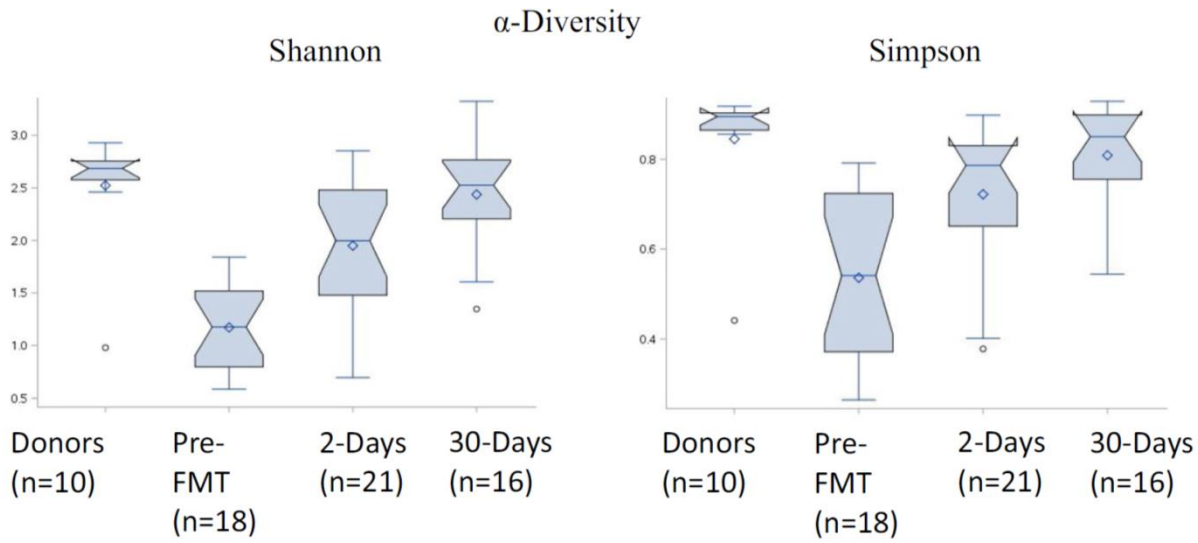
Du et al, J Clin Gastroenterol 2021;55:300–8

Time to recovery in RCDI patients treated with FMT or antibiotics (AB)



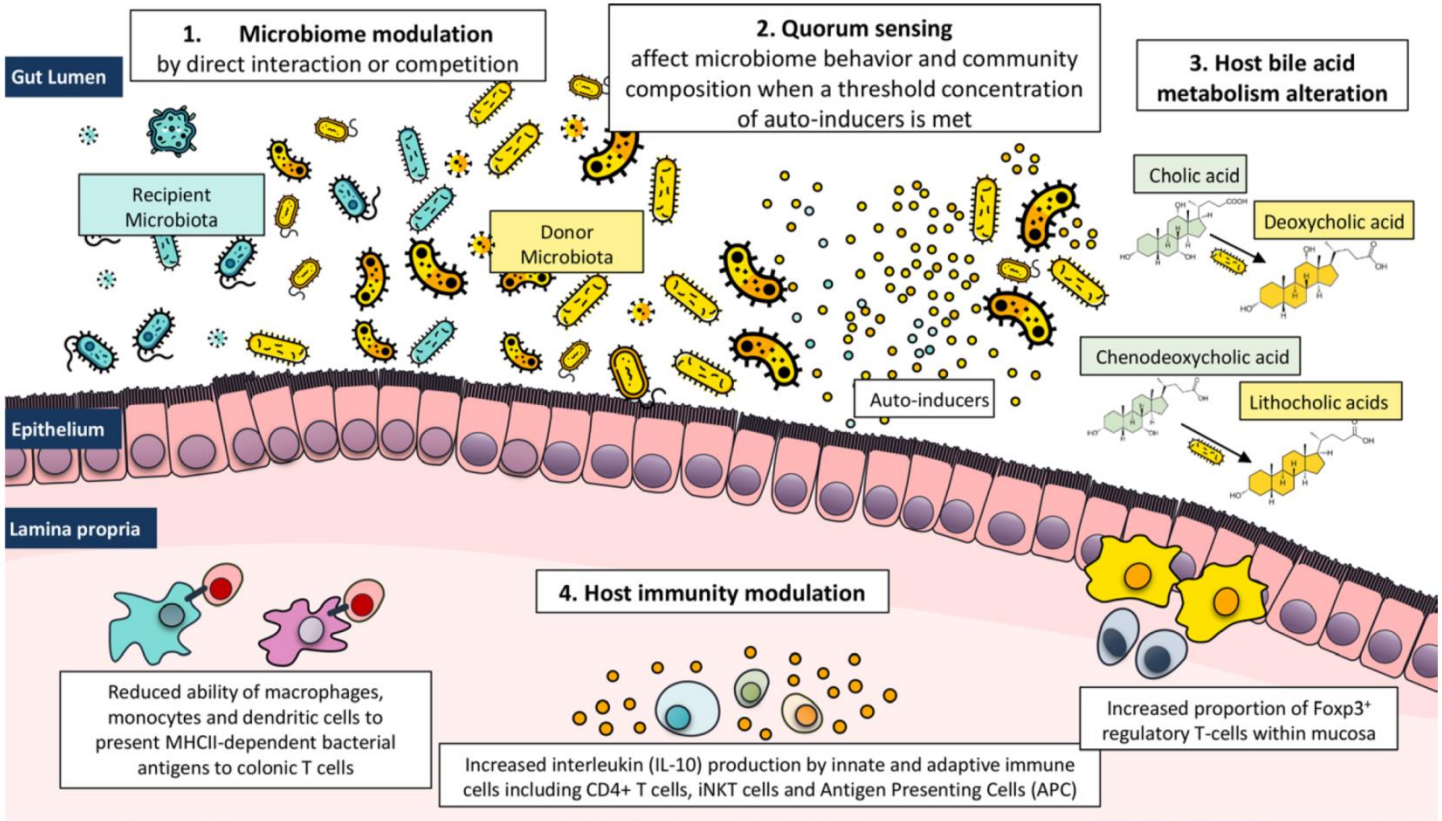
Jalanka et al, *Aliment Pharmacol Ther* 2018;47:371–9

Changes in α-diversity by Shannon index and inverse Simpson diversity index in subjects with recurrent CDI after FMT



DuPont et al, *Dig Dis Sci* 2020;65:741–56

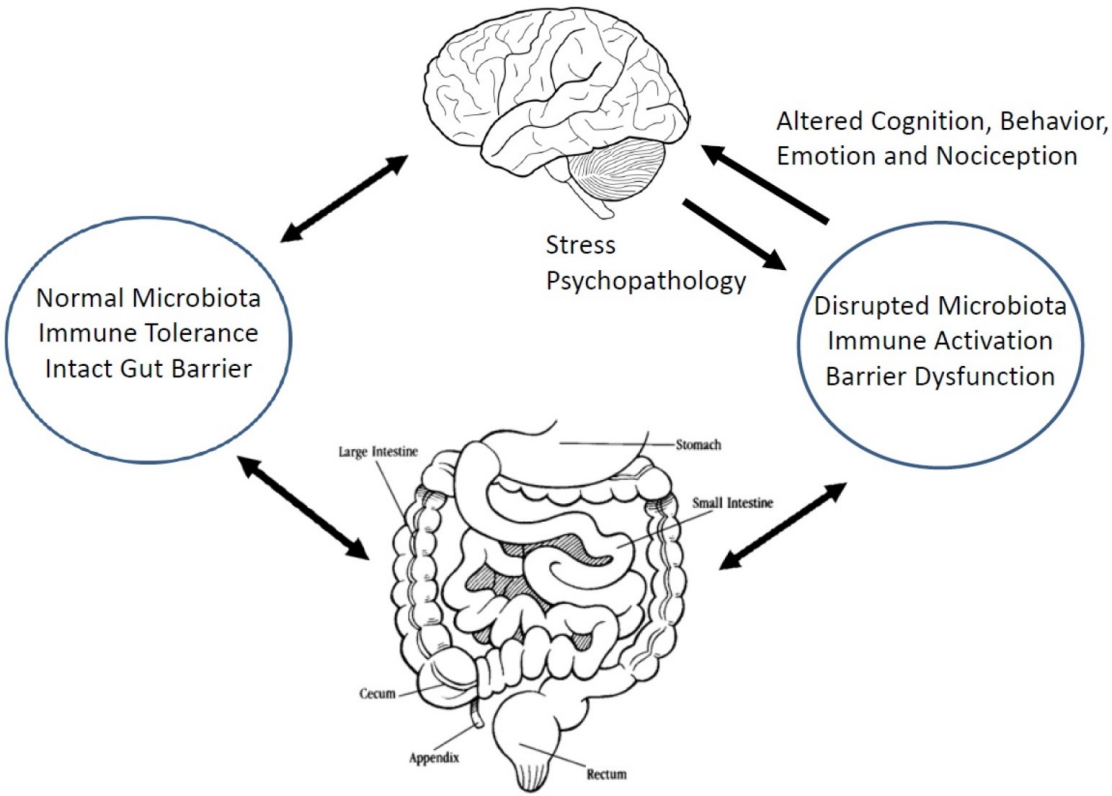
Potential mechanisms of FMT



Ng et al, *Gut* 2020;68:83-91

2

IBS as a disease induced by a disrupted microbiota-brain-gut axis



Quigley E. *J Clin Med* 2018, 7:6; doi:10.3390

Prevalence of dysbiosis in HC, IBS and IBD patients (by GA-map Dysbiosis Test)

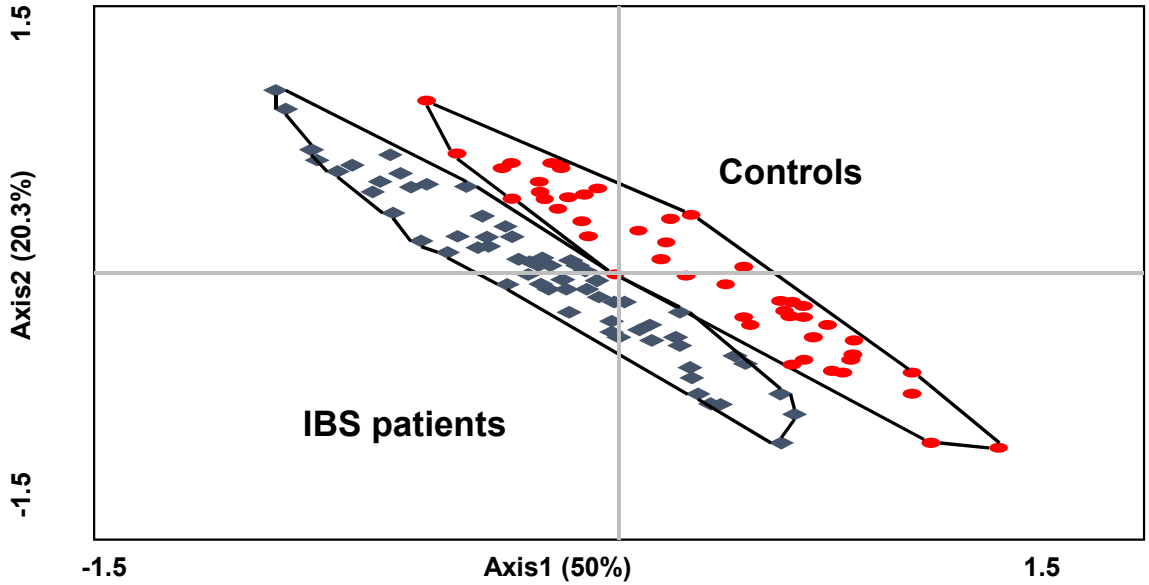
Table 3 | Percentage dysbiosis and mean DI score in validation cohort

Cohort	Total	Dysbiotic, % (95% CI)	DI, mean
Healthy controls	43	16 (±11)	1.72
IBS	109	73 (±8)	2.98
IBS-D	34	76 (±14)	3.03
IBS-C	26	73 (±17)	3.00
IBS-M	3	67	3.33
IBS-U	25	72 (±18)	3.04
IBS-A	20	70 (±20)	2.85
IBD treatment-naïve	80	70 (±10)	3.31
CD	30	80 (±14)	3.60
UC	50	64 (±13)	3.14
IBD remission	55	80 (±11)	3.15
CD	18	89 (±14)	3.65
UC	36	75 (±14)	2.92

Casen et al, *Aliment Pharmacol Ther* 2015; 42: 71–83

Microbiota signature in IBS vs healthy controls

Multivariate analysis (RDA)

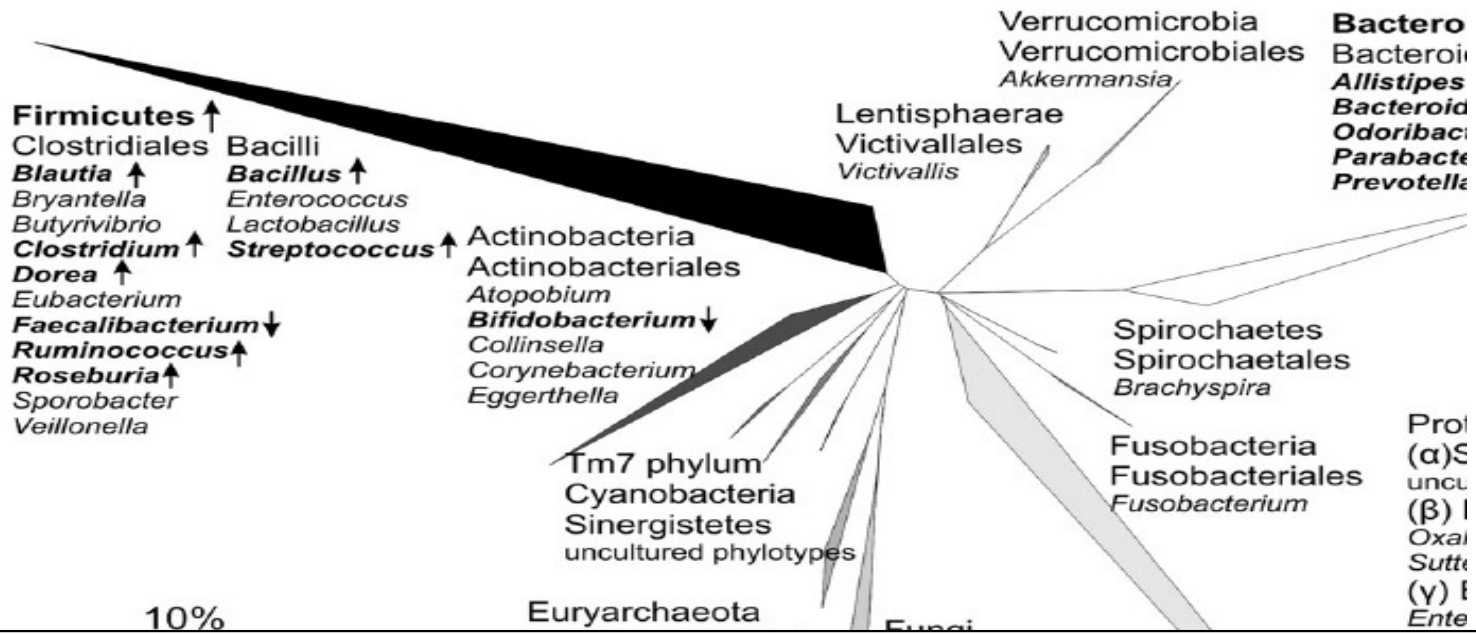


Gut microbiota of IBS patients is significantly different form healthy controls, with a 2 x N increase in *Firmicutes/Bacteroidetes* ratio and a 50% decrease of *Bifidobacteria.*

Rajilic et al, *Gastroenterology* 2011;141:1792

Phylogenetic tree comparing intestinal microbiota of controls vs IBS patients

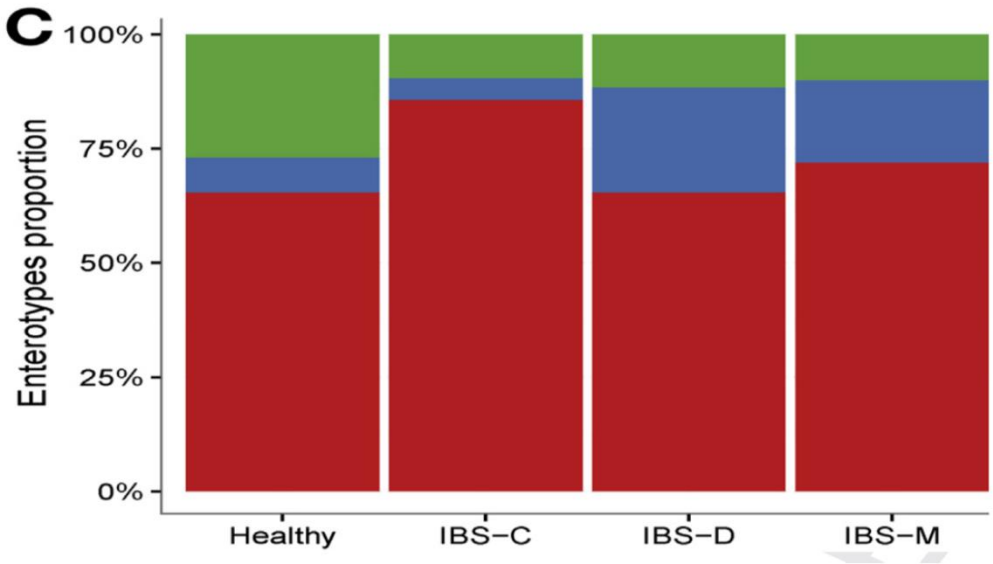
Phylogenetic analysis: HITChip

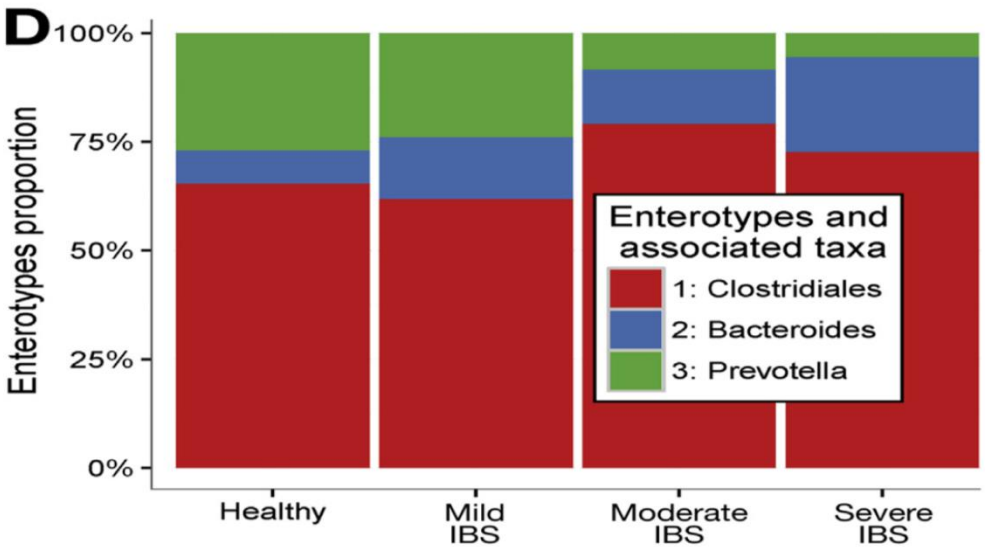


Global and deep molecular analysis of fecal samples indicates that patients with IBS have a different composition of microbiota compared with normals (in general, overall microbial diversity is reduced)

Rajilic et al, *Gastroenterology* 2011;141:1792

Microbial enterotypes according to IBS subgroups (left) and severity (right)

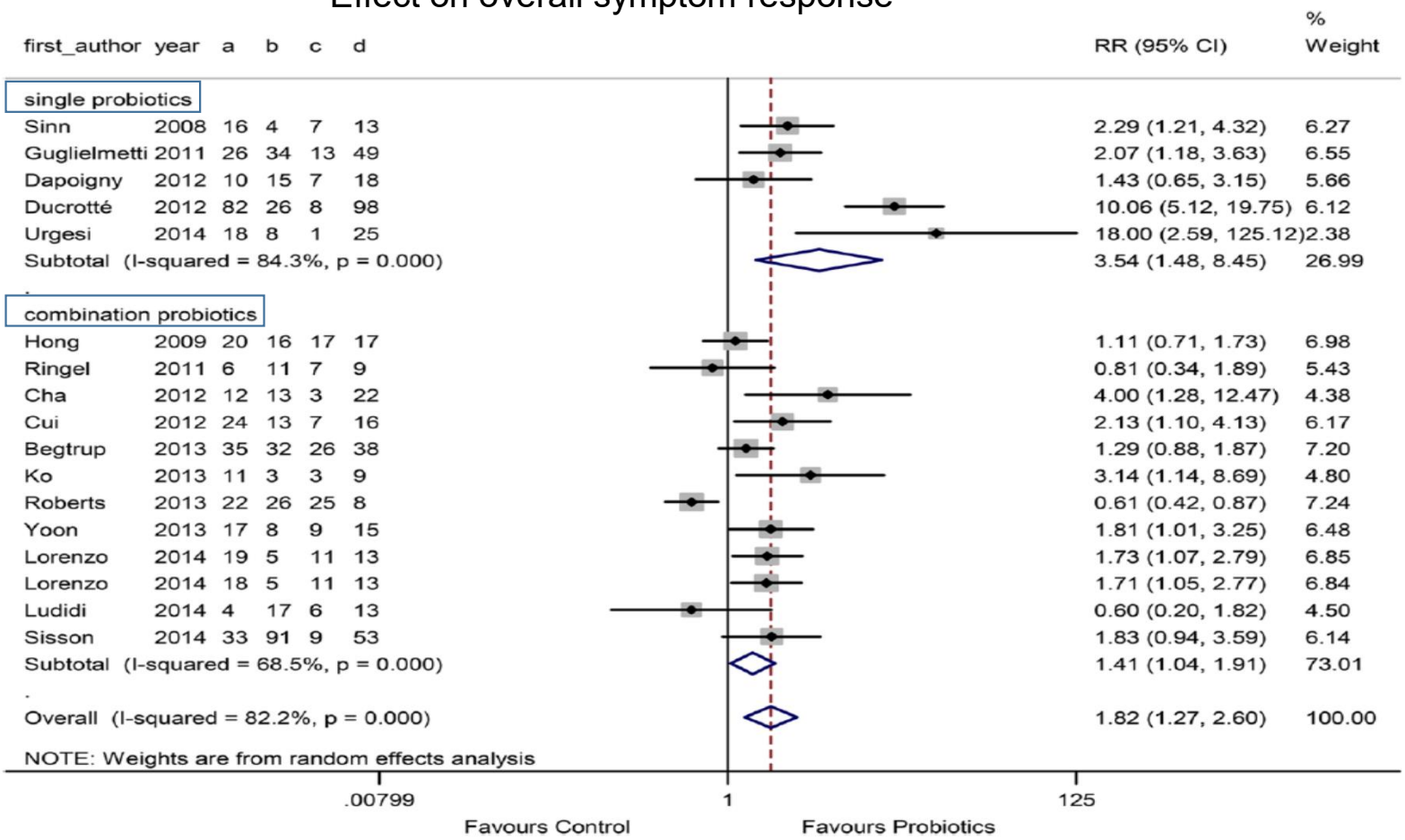




Tap et al, *Gastroenterology* 2017;152:111–123

Single or combination probiotics?

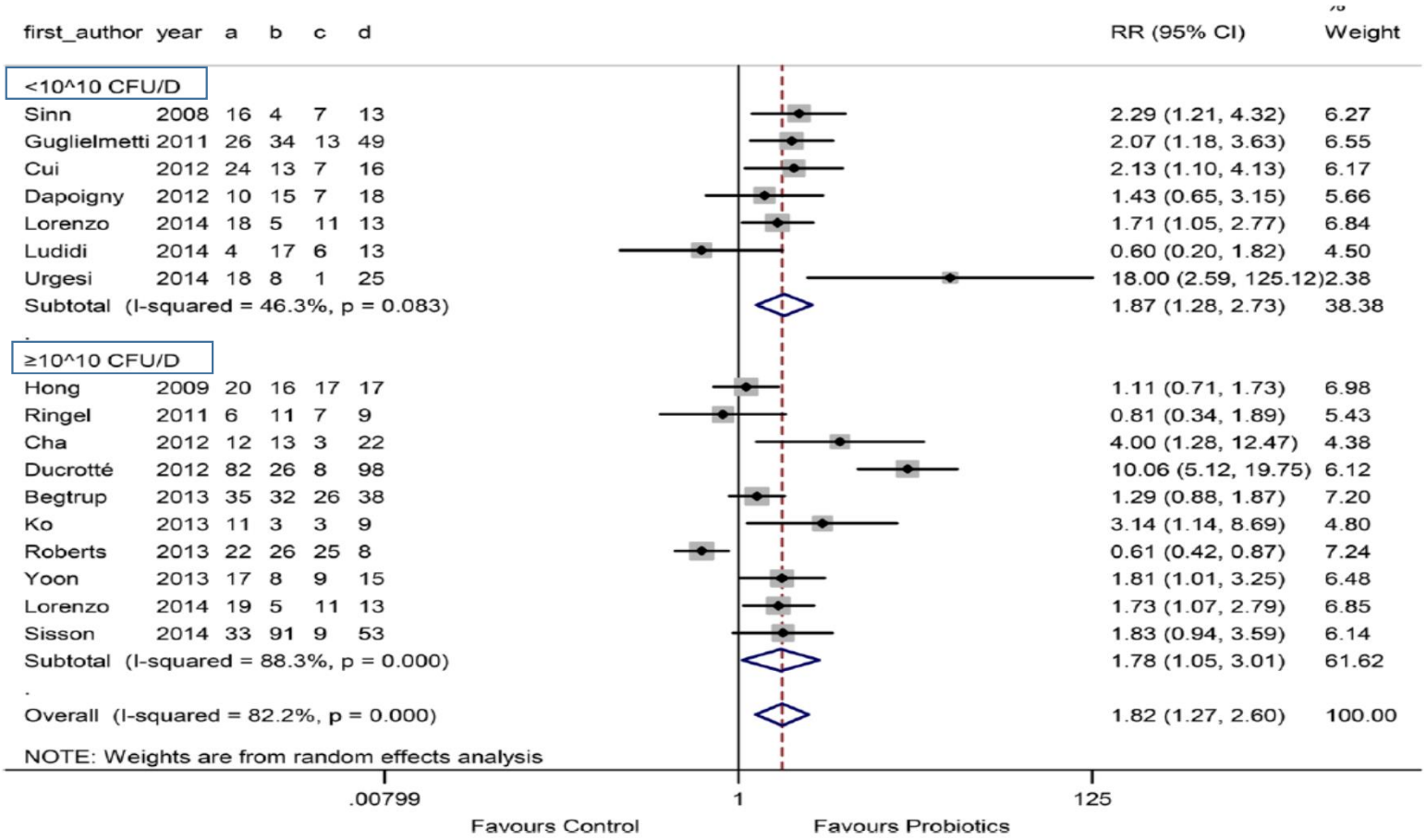
Effect on overall symptom response



Zhang et al. *BMC Gastroenterology* 2016;16:62

Standard or high dose?

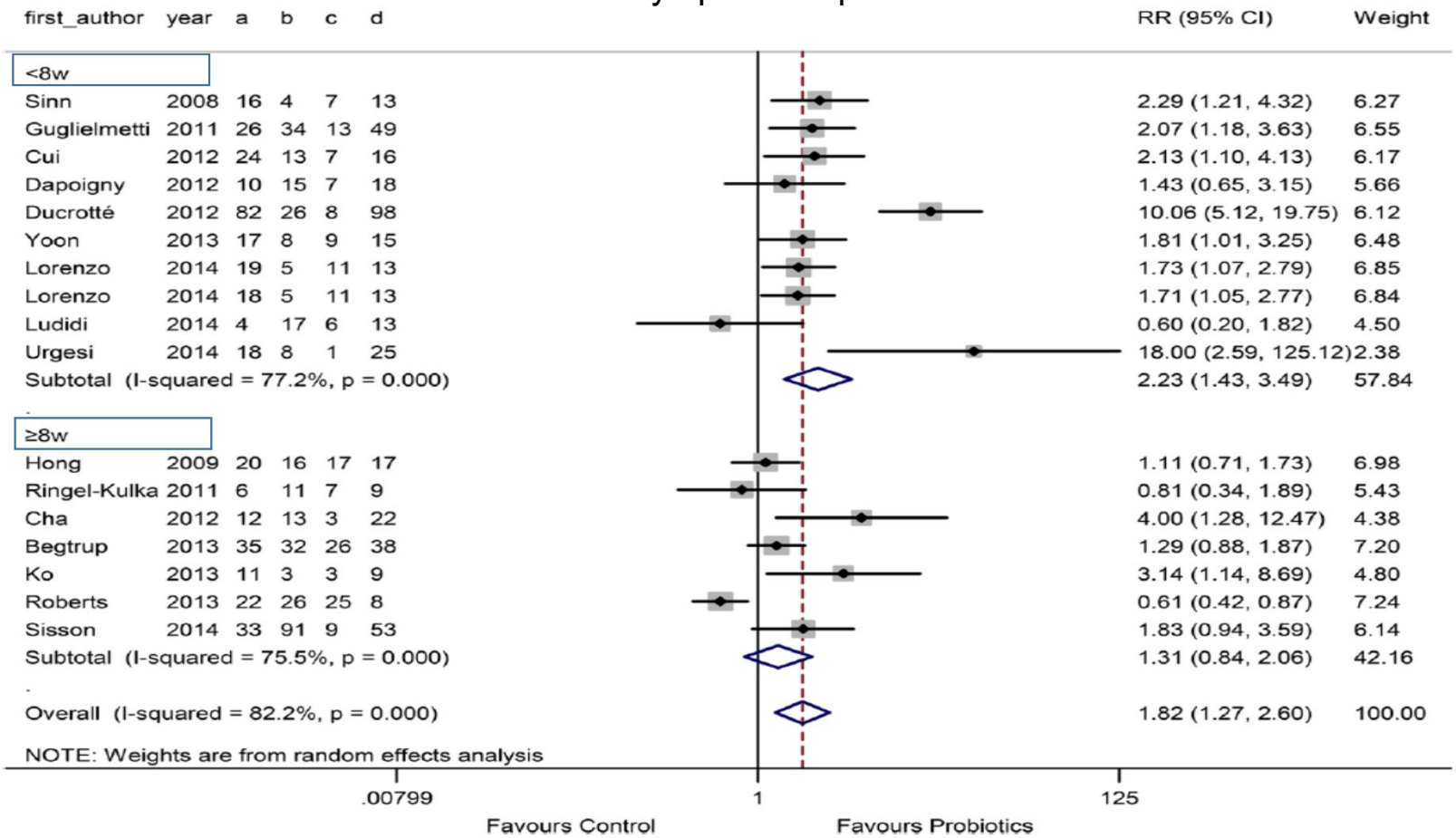
Effect on overall symptom response



Zhang et al. *BMC Gastroenterology* 2016;16:62

Short or long duration?

Effect on overall symptom response



Zhang et al. *BMC Gastroenterology* 2016;16:62

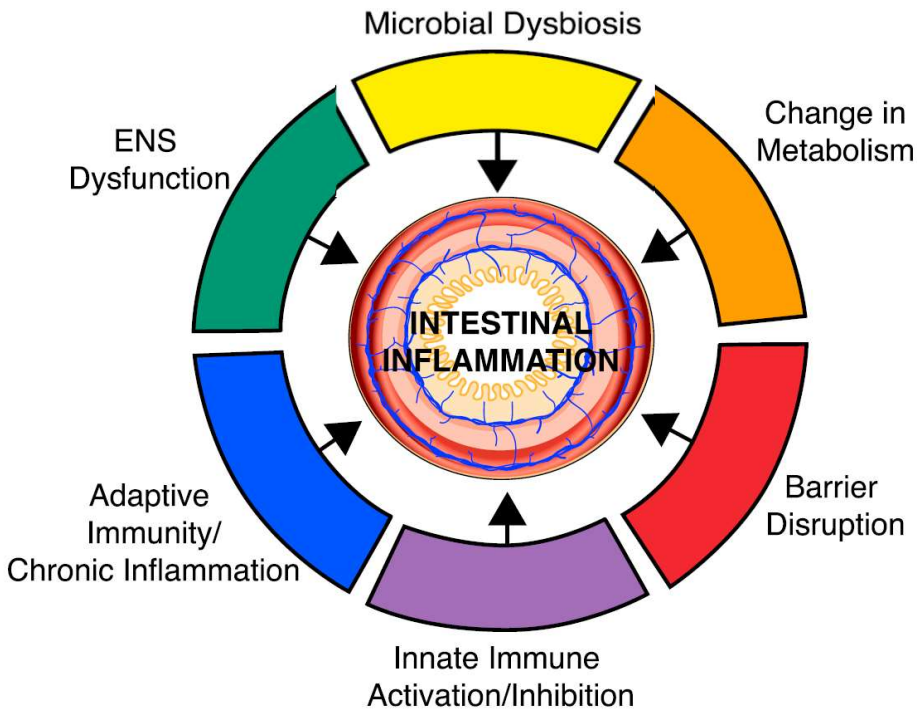
Human studies investigating the effect of probiotics on gut transit time

Reference	N	Population	Intervention	Comparator	Duration	Method of gut transit time assessment	Outcome
Marteau et al., 2002 (8)	36	Healthy women	*B. animalis* DN-173010 (maximum 3.75 × 10^{10} CFU/d)	Fermented milk without probiotics	10 d	ROM technique (20 ROM/d for 3 d, X-ray on day 4)	Gut transit time was significantly lower in the probiotic group than in the placebo group (52 h vs. 61 h, respectively; $P < 0.005$).
Agrawal et al., 2009 (9)	41	Constipation (Rome III for IBS-C)	*B. lactis* DN-173 010 (2.5 × 10^{10} CFU/d)	Nonfermented dairy product	4 wk	ROM technique (24 ROM/d for 3 d, X-ray on day 4)	Gut transit time was significantly reduced in the probiotic group compared with the placebo group (mean difference: −12 h; $P = 0.026$).
Krammer et al., 2011 (10)	24	Constipation (gut transit time >72 h)	*L. casei* Shirota (6.5 × 10^{9} CFU/d)	Milk drink without probiotics	4 wk	ROM technique (20 ROM over 6 d, X-ray on day 7)	Gut transit time was decreased from 96 h at baseline to 77 h after the probiotic consumption ($P = 0.05$). No statistical comparisons were performed between the probiotic and placebo groups at the end of the treatment period.
Waller et al., 2011 (11)	88	Constipation (2–47 stool type at Bristol stool chart and 1–3 stool/wk)	*B. lactis* HN019 (17.2 × 10^{9} CFU/d or 1.8 × 10^{9} CFU/d)	Capsules with rice maltodextrin	14 d	ROM technique (24 ROM/d for 6 d, X-ray on day 7)	Change in WGTT was statistically significant across study groups (high dose: −28 h, low dose: −19 h, placebo: +1 h; $P < 0.001$).
Merenstein et al., 2014 (12)	68	Healthy women	*B. lactis* Bf-6 (5.6 × 10^{10} CFU/d)	Yogurt without probiotics	2 wk	ROM technique (24 ROM/d for 3 d, X-ray on day 4 and 8)	Gut transit time was not different between the probiotic and placebo periods (42 h vs. 43 h, respectively; $P > 0.69$).

[1] *B.*, *Bifidobacterium*; IBS-C, Constipation-predominant irritable bowel syndrome; *L.*, *Lactobacillus*; ROM, radio-opaque marker; WGTT, whole-gut transit time.

Dimidi et al, *Adv Nutr* 2017;8:484–94

3 Multiple intestinal factors involved in IBD pathogenesis



Wlodarska et al, *Cell Host Micr* 2015;577-91

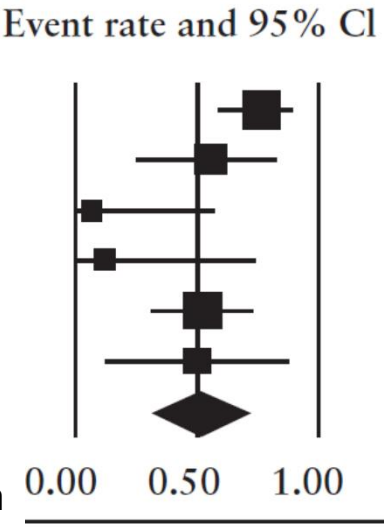
Representative dysbiotic microbiota in IBD

Ulcerative colitis	Crohn's disease
Bacterial diversity ↓	**Bacterial diversity ↓**
Proteobacteria ↑	**Proteobacteria ↑**
Enterobacteriaceae	Enterobacteriaceae ↑
E. coli ↑	Adherent-invasive *E. coli* ↑
	K. pneumoniae ↑
	Pasteurellaceae ↑
	Haemophilus ↑
	Neisseriaceae ↑
Fusobacteria ↑	**Fusobacteria ↑**
F. varium ↑	Fusobacteriaceae ↑
Bacteroidetes ↑↓	**Bacteroidetes**
Bacteroides ↑	Bacteroiales ↓↑
B. vulgatus ↑	
Firmicutes ↓	**Firmicutes ↓**
Clostridiales ↓	Clostridiales ↓
clusters IV, XIVa,XVIII ↓	clusters IV, XIVa ↓
F. prausnitzii ↓	*F. prausnitzii* ↓
E. rectale ↓	*E. rectale* ↓
Ruminococcaceae	Ruminococcaceae ↓
R. gnavus ↑	*R. gnavus* ↑
clusters I, II, IX, XI ↑	
C. perfringens ↑	
Peptostreptococci ↑	Veillonellaceae ↑
Lachnospiraceae	Lachnospiraceae ↓
Roseburia hominis ↓	Erysipelotrichaceae ↓
Bacilli ↑	Bacilli ↑
E. faecalis ↑	Lactobacillus ↓↑
	Actinobacteria
	Bifidobacteriaceae ↓
Viral diversity ↑	**Viral diversity ↑**
	Caudoviales bacteriopahge ↑
Fungal diversity	**Fungal diversity**
Candida ↑	*Candida* ↑

Oka et al, *Dig Dis Sci* 2020;65:757–788

Clinical remission rate in Crohn's disease following FMT

Study name	Event rate	Lower limit	Upper limit	Total
Cui	0.767	0.585	0.884	23 / 30
Suskind	0.556	0.251	0.823	5 / 9
Vermeire	0.071	0.004	0.577	0 / 6
Wei	0.125	0.007	0.734	0 / 3
Vaughn	0.526	0.311	0.732	10 / 19
Goyal	0.500	0.123	0.877	2 / 4
Random	0.518	0.311	0.719	

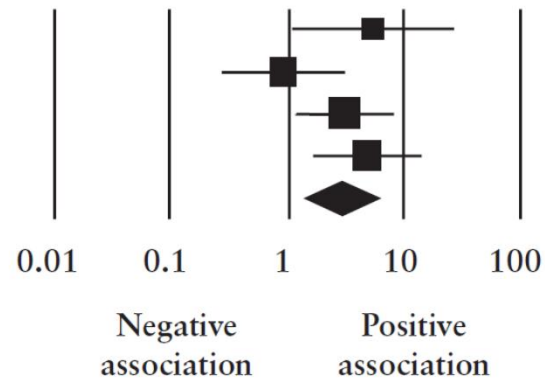


The pooled proportion achieving clinical remission was 52% (95% CI = 31%–72%)

Paramsothy et al, *J Crohn's Col* 2017;1180–1199

Clinical remission rate in UC following FMT

Study name	Statistics for each study				Events / Total	
	Odds ratio	Lower limit	Upper limit	p-Value	Active	Controls
Moayeddi	5.431	1.086	27.151	0.039	9 / 38	2 / 37
Rossen	0.930	0.274	3.158	0.907	7 / 23	8 / 25
Paramsothy	3.130	1.163	8.427	0.024	18 / 41	8 / 40
Castello	4.833	1.633	14.303	0.004	19 / 38	6 / 35
Random	2.885	1.359	6.127	0.006		



The pooled proportion achieving clinical remission was 33% (95% CI = 23%–43%)

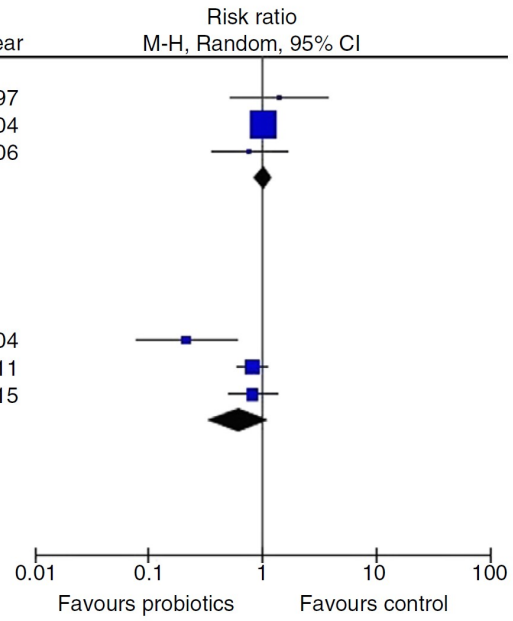
Conclusions: FMT appears effective in UC remission induction, but long-term durability and safety remain unclear. Additional well-designed controlled studies of FMT in IBD are needed, especially in CD and pouchitis.

Paramsothy et al, *J Crohn's Col* 2017;1180–1199

Efficacy of probiotics vs 5-ASA or placebo in inducing remission in active UC

Study or subgroup	Probiotics Events	Probiotics Total	Control Events	Control Total	Weight	Risk ratio M-H, Random, 95% CI	Year
4.2.1 Probiotics vs. 5-ASA							
Kruis (1997)	8	50	6	53	3.5%	1.41 [0.53, 3.79]	1997
Kruis (2004)	92	162	91	165	90.8%	1.03 [0.85, 1.25]	2004
Zocco (2006)	10	65	12	60	5.8%	0.77 [0.36, 1.65]	2006
Subtotal (95% CI)		277		278	100.0%	1.02 [0.85, 1.23]	
Test events	110		109				
Heterogeneity: τ^2=.00, χ^2=.96, df=2 (P=.62), I^2=0%							
Test for overall effect: Z=.25 (P=.80)							
4.2.2 Probiotics vs. control							
Cui (2004)	3	15	14	15	21.0%	0.21 [0.08, 0.59]	2004
Wildt (2011)	15	20	11	12	42.4%	0.82 [0.60, 1.11]	2011
Yoshimatsu (2015)	14	30	17	30	36.5%	0.82 [0.50, 1.35]	2015
Subtotal (95% CI)		65		57	100.0%	0.62 [0.33, 1.16]	
Test events	32		42				
Heterogeneity: τ^2=.22, χ^2=8.19, df=2 (P=.02), I^2=76%							
Test for overall effect: Z=1.50 (P=.13)							

Test for subgroup differences: χ^2=2.27, df=1 (P=.13), I^2=55.9%



Nerwa et al, *Aliment Pharmacol Ther* 2017;46:389–400.

Efficacy of probiotics vs 5-ASA or placebo in preventing relapse in quiescent UC

Study or subgroup	Probiotics Events	Probiotics Total	Control Events	Control Total	Weight	Risk ratio M-H, Random, 95% CI	Year
4.2.1 Probiotics vs. 5-ASA							
Kruis (1997)	8	50	6	53	3.5%	1.41 [0.53, 3.79]	1997
Kruis (2004)	92	162	91	165	90.8%	1.03 [0.85, 1.25]	2004
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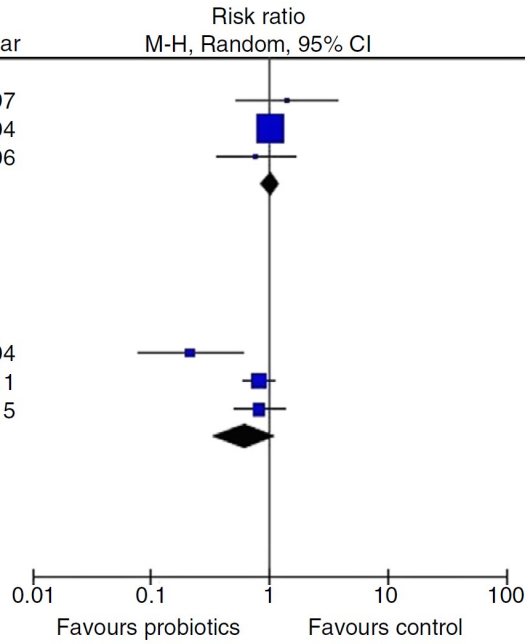


Nerwa et al, *Aliment Pharmacol Ther* 2017;46:389–400.

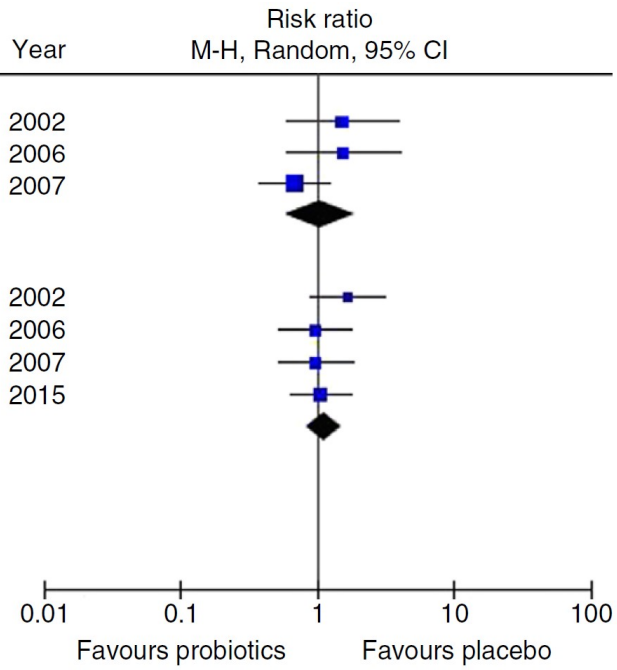
Efficacy of probiotics vs placebo in preventing clinical or endoscopic relapse in CD in remission following a surgical resection

Study or subgroup	Probiotics Events	Probiotics Total	Placebo Events	Placebo Total	Weight	Risk ratio M-H, Random, 95% CI	Year
3.1.1 Clinical relapse of disease activity							
Prantera (2002)	8	23	5	22	26.9%	1.53 [0.59, 3.97]	2002
Marteau (2006)	9	48	6	50	26.8%	1.56 [0.60, 4.06]	2006
Van Gossum (2007)	11	34	17	36	46.4%	0.69 [0.38, 1.24]	2007
Subtotal (95% CI)		105		108	100.0%	1.06 [0.59, 1.92]	
3.1.4 Endoscopic relapse of disease activity (Rutgeerts score 3 or more)							
Prantera (2002)	14	23	8	22	21.5%	1.67 [0.88, 3.18]	2002
Marteau (2006)	14	48	15	50	23.8%	0.97 [0.53, 1.79]	2006
Van Gossum (2007)	12	34	13	36	22.5%	0.98 [0.52, 1.83]	2007
Fedorak (2015)	19	58	19	62	32.2%	1.07 [0.63, 1.81]	2015
Subtotal (95% CI)		163		170	100.0%	1.13 [0.84, 1.52]	
Total events	59		55				

Heterogeneity: τ^2=.00, χ^2=1.92, df=3 (*P*=.59), I^2=0%

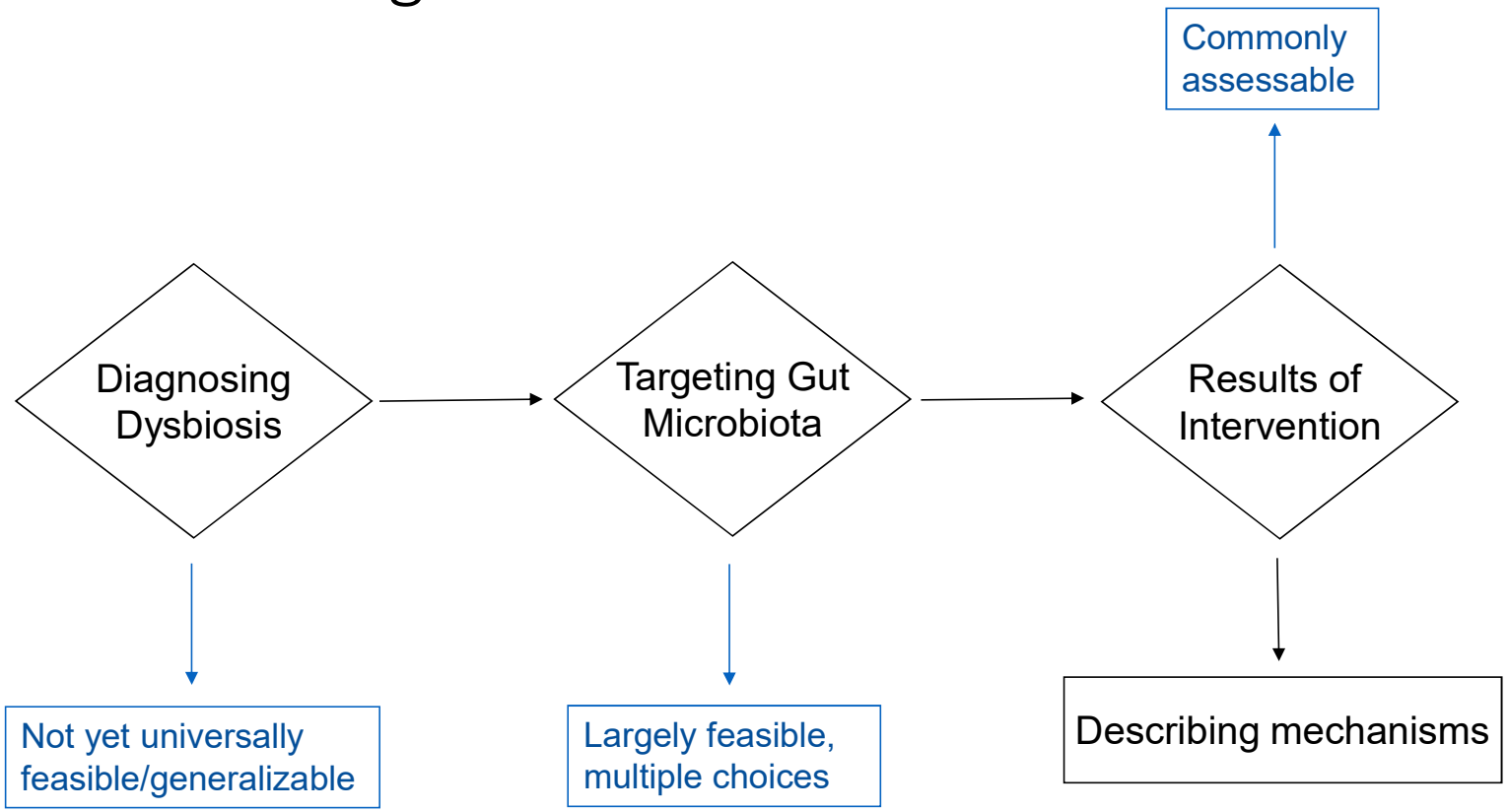
Test for overall effect: *Z*=.79 (*P*=.43)

Test for subgroup differences: χ^2=.22, df=3 (*P*=.97), I^2=0%



Nerwa et al, *Aliment Pharmacol Ther* 2017;46:389–400.

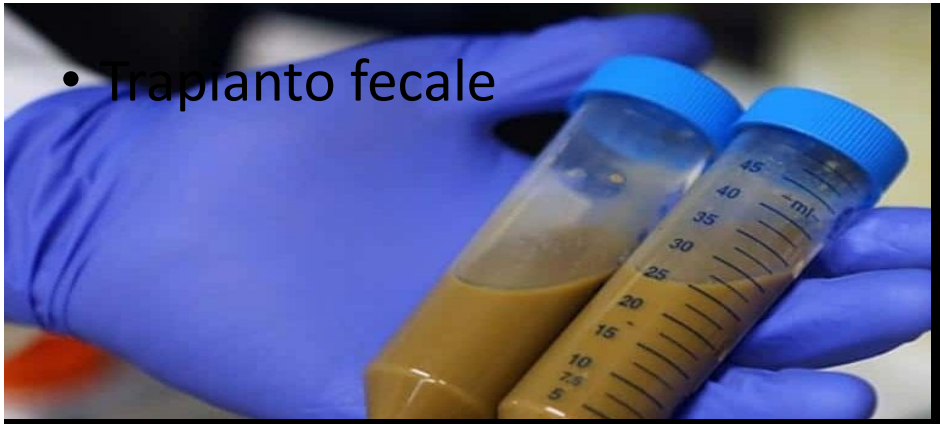
State of art for gut microbiota intervention by Gastroenterologist

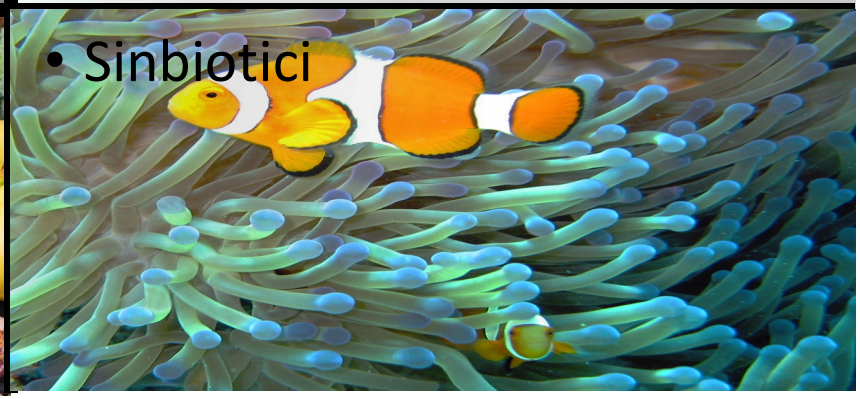


Conclusions

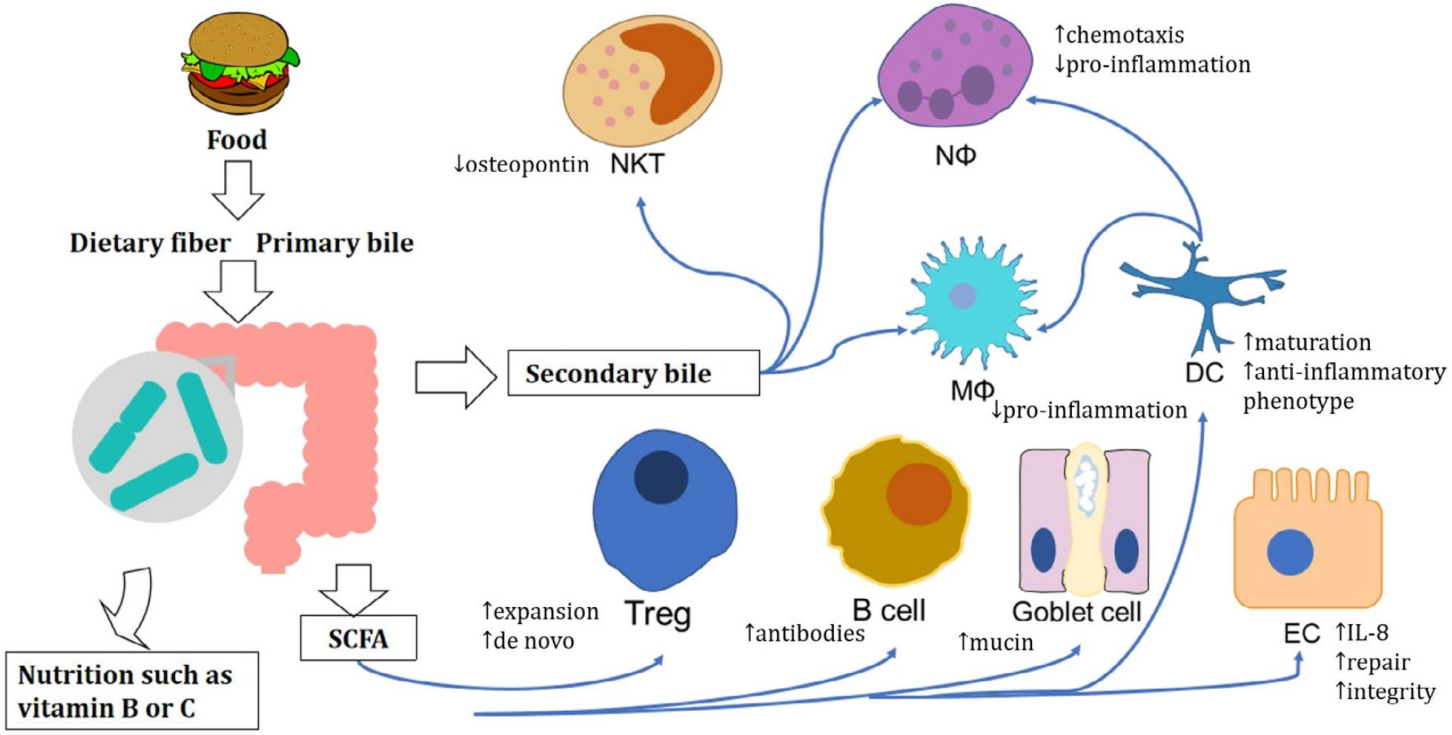
- Gastroenterologists are now able to target gut microbiota in a number of GI diseases (e.g., CDI, IBS, IBD) and in many extra-GI disease, where dysbiosis seems to be pathophysiologically related
- Diagnosing dysbiosis is not an easy task in real-world (Definition of eubiosis unclear- Microbiota composition tells little on function)
- What is needed is to highlight altered mechanisms before gut microbiota-targeted therapy and their normalization after treatment

Come ripristinare un Microbiota Intestinale alterato



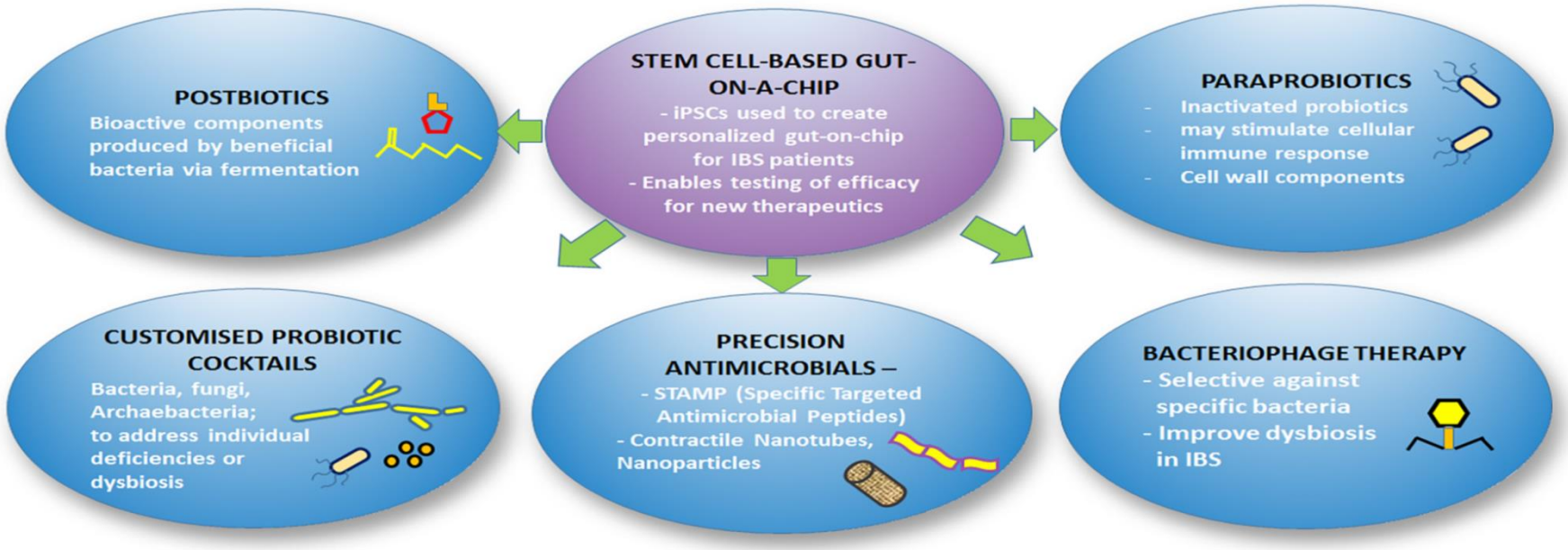


The effect of gut microbiota and metabolites on physiological function



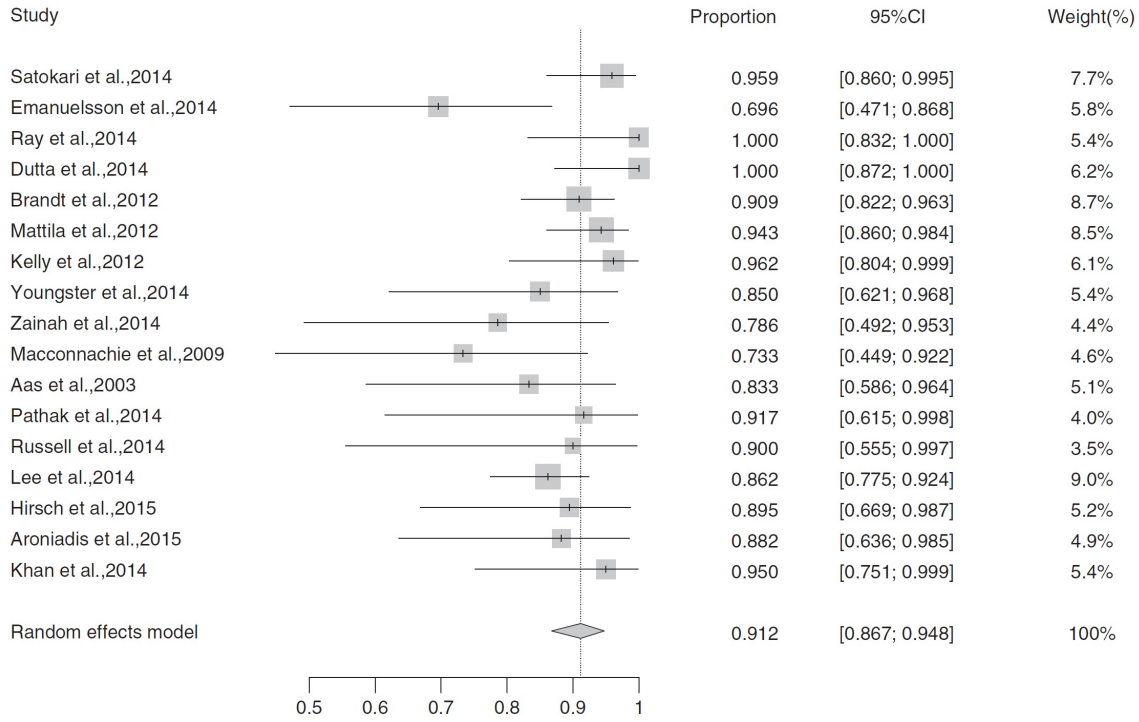
Guo et al, J Dig Dis 2020;21:147–159.

Overview of future microbiome-targeted therapeutics



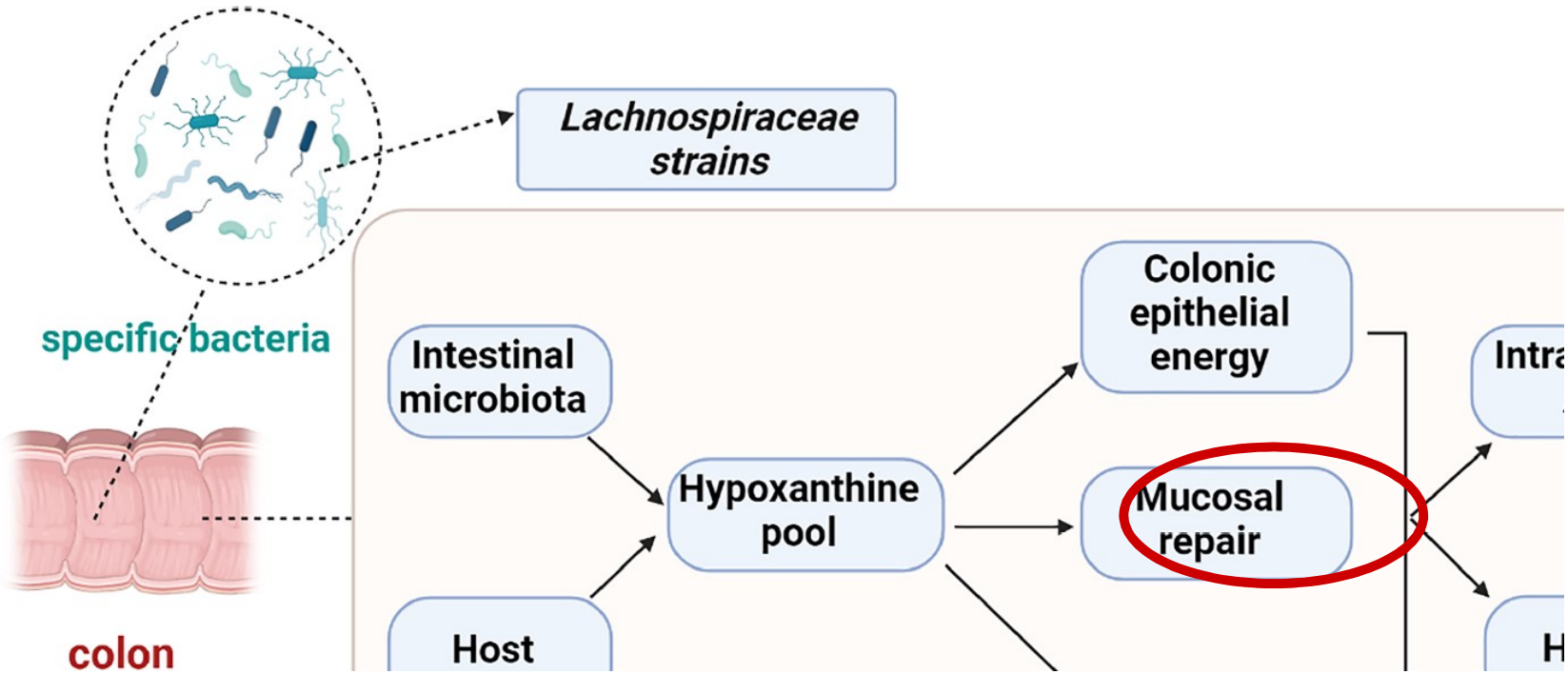
Chong et al, *Front Microbiol* 2019;10:1136

Meta-analysis of primary cure rate of FMT for CDI



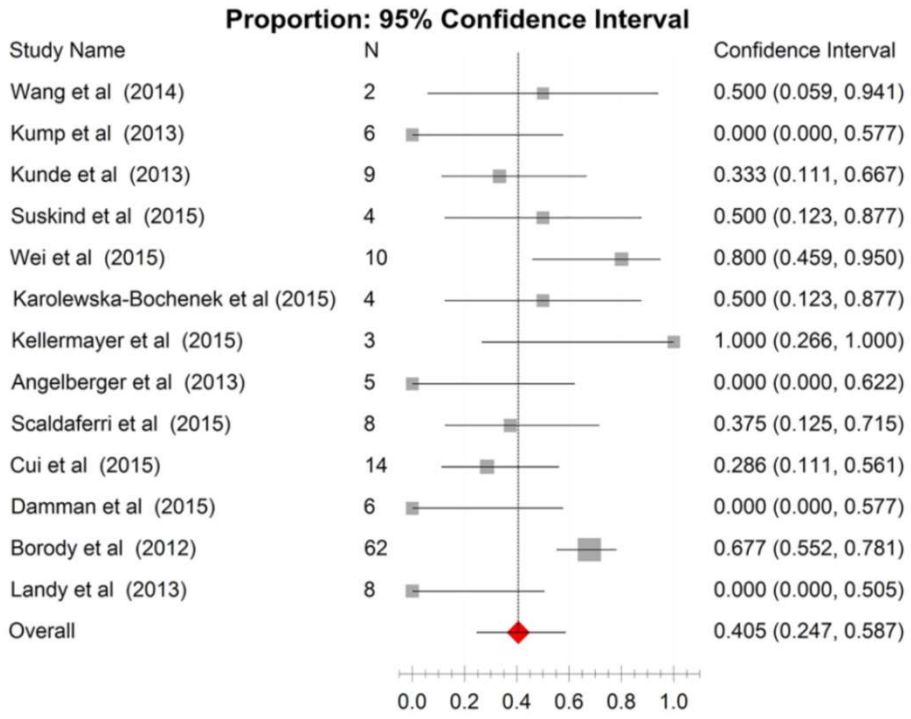
Li et al, Aliment Pharmacol Ther 2016;43:445–457

Potential novel mechanism linking dysbiosis with IBS



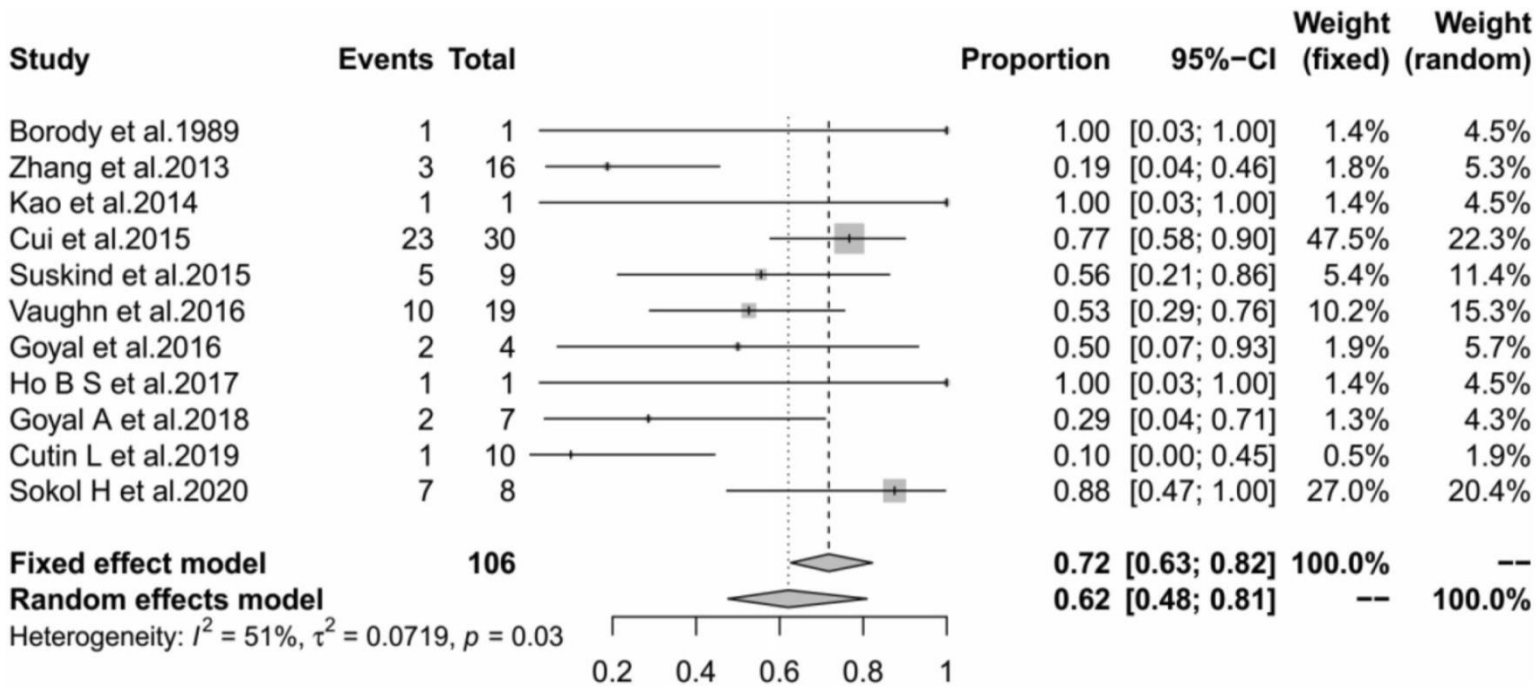
Xiao et al, *Front Cell Infect Microbiol* 2021;11:729346

FMT in UC- a meta-analysis



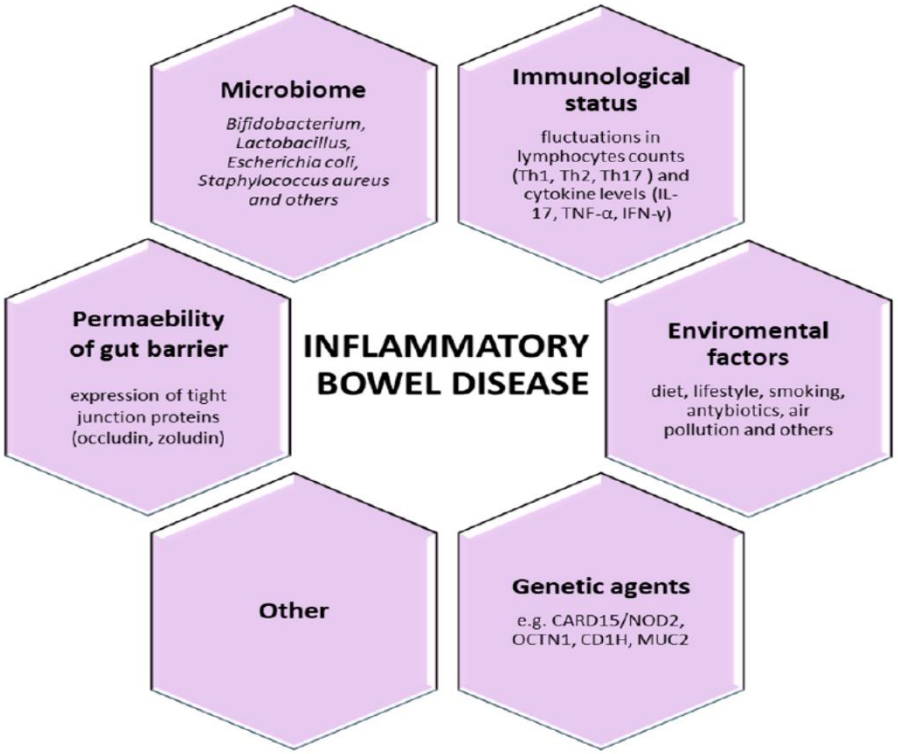
Shi et al, PLoS ONE 2016;11: e0157259

Forest plot of studies evaluating induction of clinical remission post-FMT for CD



Cheng et al, Techn Coloproct 2021;25:495–504

Key players in IBD



Jakubczyk et al, *Nutrients* 2020;12:1973