IBS and FGIDs: the Role of infections and microbiome

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Research Advisory Committee: Gulf War Veterans Illnesses

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Overview

- Interactions between infection and subsequent development of irritable bowel syndrome (IBS); also characterized as post-infection IBS (PI-IBS)
  - Epidemiology & risk-factors
    - Military relevance
  - Pathophysiology
    - Animal and human studies
- Role of microbiota
  - Interactions with peripheral (epithelial, luminal, dietary) factors
  - Bidirectional brain-gut crosstalk

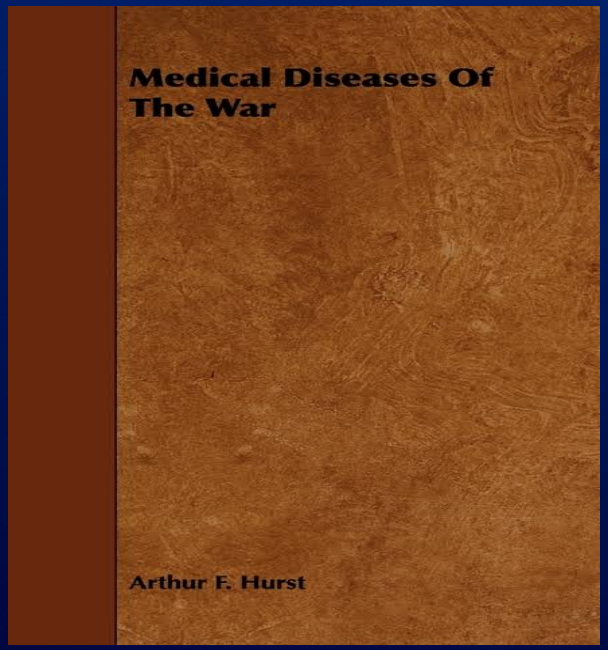
(vi) Colitis and Irritability of the Colon following Dysentery

Patients who have recovered from an acute attack of dysentery frequently remain unfit for a considerable period, which may even extend to years. The symptoms are due to the chronic colitis, which may follow either amœbic or bacillary dysentery after the specific infection has died out, but the possibility that amœbic cysts or even dysentery bacilli may still be present can only be excluded by frequent expert examinations of the stools. In most cases the patient suffers from alternating attacks of constipation and diarrhœa, the latter often being brought on by aperients taken for the relief of the former, or it may follow an indiscretion in diet or exposure to cold. During the periods of constipation, hard scybala coated with mucus are passed. The diarrhœa is accompanied by colic, which is often severe; the stinking fluid fæces contain much undigested food, often with mucus and occasionally a little bright-red blood. The diarrhœa may only last for a few hours, or it may continue for two or three days, the attacks being separated by intervals of several weeks or months. Sometimes chronic diarrhœa is present, especially after amœbic dysentery.

The patient has little appetite and cannot regain his former weight. He complains of constant abdominal discomfort. Slight tenderness is often present, especially over the iliac colon, which can generally be felt as a firmly contracted cord, which contains scybala when constipation is present. The liver is tender and may be slightly enlarged in many of the cases in which the original infection was amœbic. The tongue is dirty and the patient complains of discomfort and fulness immediately after meals. There is no fever, but the pulse is often rapid and symptoms of "soldier's heart" may be present. The patient gets quickly tired and may complain of backache. All the symptoms are aggravated by overwork. In one case the attacks of diarrhœa were immediately preceded by fainting; in another an attack of asthma, from which the patient had suffered for many years, was always a warning that diarrhœa would follow. In both cases the absorption of toxins from the fluid fæces led to symptoms before sufficient fæces had reached the rectum to produce the desire to defæcate.

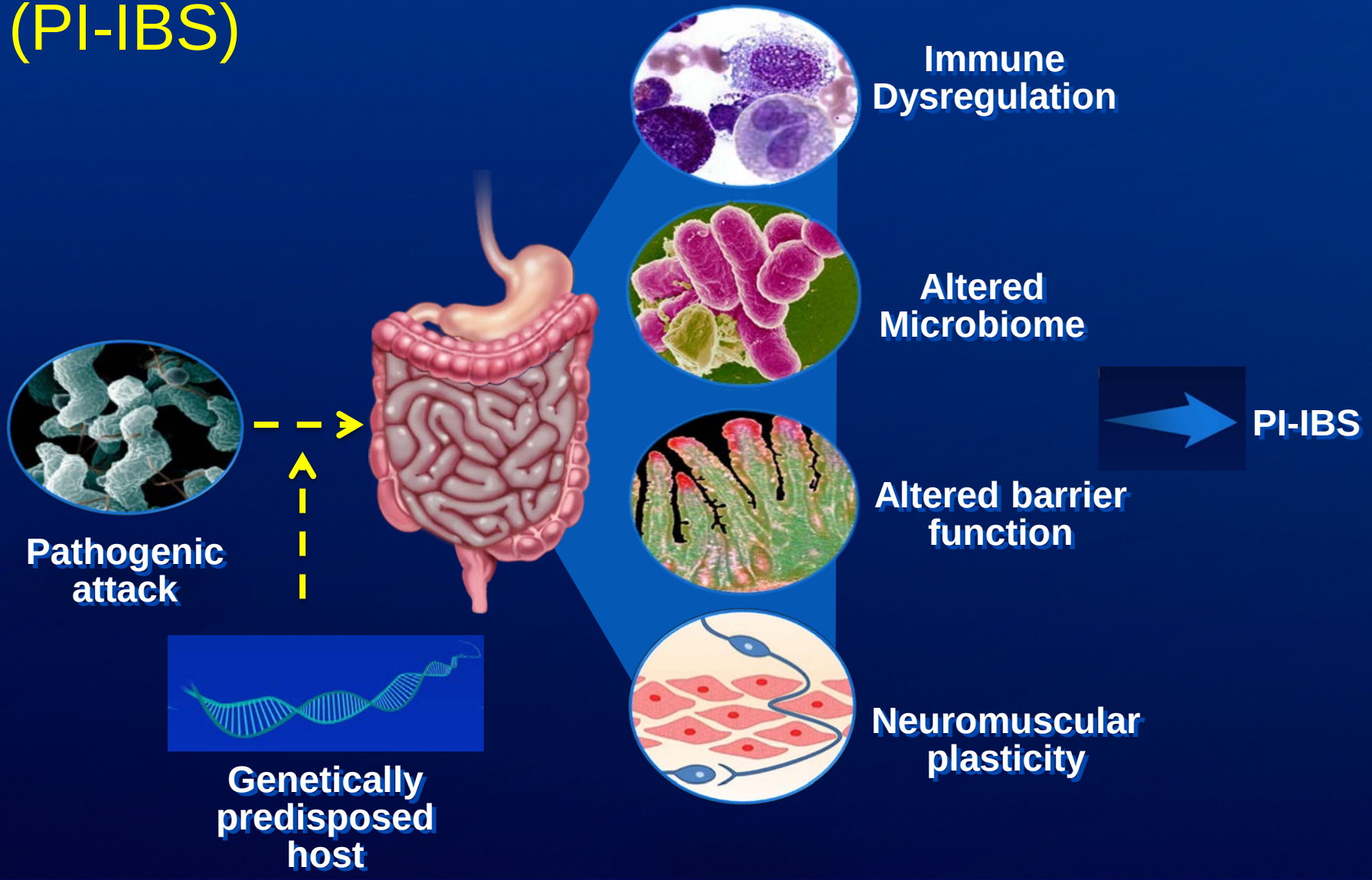
Sometimes the attacks of diarrhœa cease to occur, but intractable constipation remains and the general symptoms persist, though in a lessened degree. In spite of the widespread ulceration in both forms of dysentery, and in spite of its great depth in amœbic cases, I have neither seen nor heard of any case in which the constipation was due to the development of a stricture.

Dr. T. G. Moorhead observed a number of cases in Egypt, most of which came from Gallipoli, in which severe abdominal distension developed from four to eight months after the patient had apparently recovered from an attack of dysentery. They complained of a feeling of fulness in the abdomen, with dyspnœa and general dyspeptic symptoms. The bowels were regular, and nothing



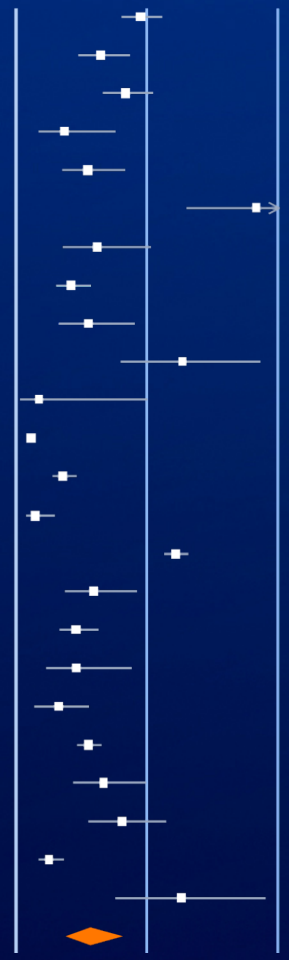
1918

Post-infection irritable bowel syndrome (PI-IBS)



PI-IBS prevalence following bacterial enteritis

Author, Year	n/N	Event Rate (95% CI)
Bacterial		
Bettes, 2014	101/425	0.238 (0.200-0.280)
Cremon, 2014	33/204	0.162 (0.117-0.219)
Nielsen, 2014	56/268	0.209 (0.164-0.262)
Koh, 2012	6/65	0.092 (0.042-0.191)
Youn, 2012	17/124	0.137 (0.087-0.210)
Schwille-Kiuntke, 2011	22/48	0.458 (0.324-0.599)
Lim, 2010	11/71	0.155 (0.088-0.259)
Thabane, 2010	32/305	0.105 (0.075-0.145)
Jung, 2009	12/87	0.138 (0.080-0.227)
Saps, 2008	14/44	0.318 (0.198-0.468)
Piche, 2007	1/23	0.043 (0.006-0.252)
Ruigomez, 2007	167/5894	0.028 (0.024-0.033)
Spence, 2007	49/547	0.090 (0.068-0.117)
Borgaonkar, 2006	7/191	0.037 (0.018-0.075)
Marshall, 2006	417/1368	0.305 (0.281-0.330)
Ji, 2005	15/101	0.149 (0.092-0.232)
Mearin, 2005	31/271	0.114 (0.082-0.158)
Okhuysen, 2004	7/61	0.115 (0.056-0.222)
Wang, 2004	24/295	0.081 (0.055-0.119)
Dunlop, 2003	103/747	0.138 (0.115-0.165)
Parry, 2003	18/108	0.167 (0.108-0.249)
Gwee, 1999	22/109	0.202 (0.137-0.288)
Neal, 1997	23/366	0.063 (0.042-0.093)
McKendrick, 1994	12/38	0.316 (0.189-0.478)
	1200/11760	0.138 (0.094-0.199)

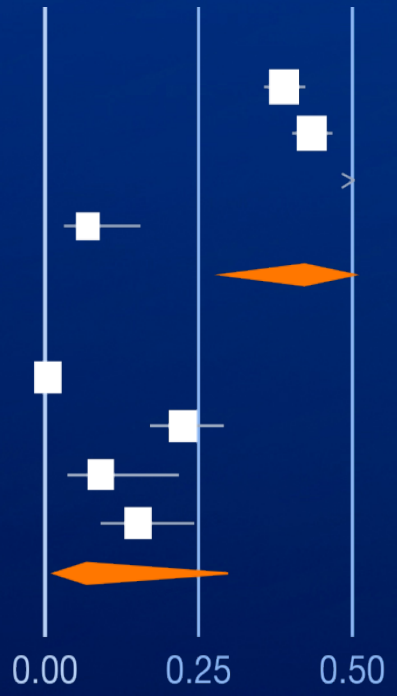


- 45 studies
- 21,421 with enteritis
- Followed for 3 m-10 y

Klem F, Wadhwa A…Grover M, **Gastro 2017**

PI-IBS prevalence following non-bacterial enteritis

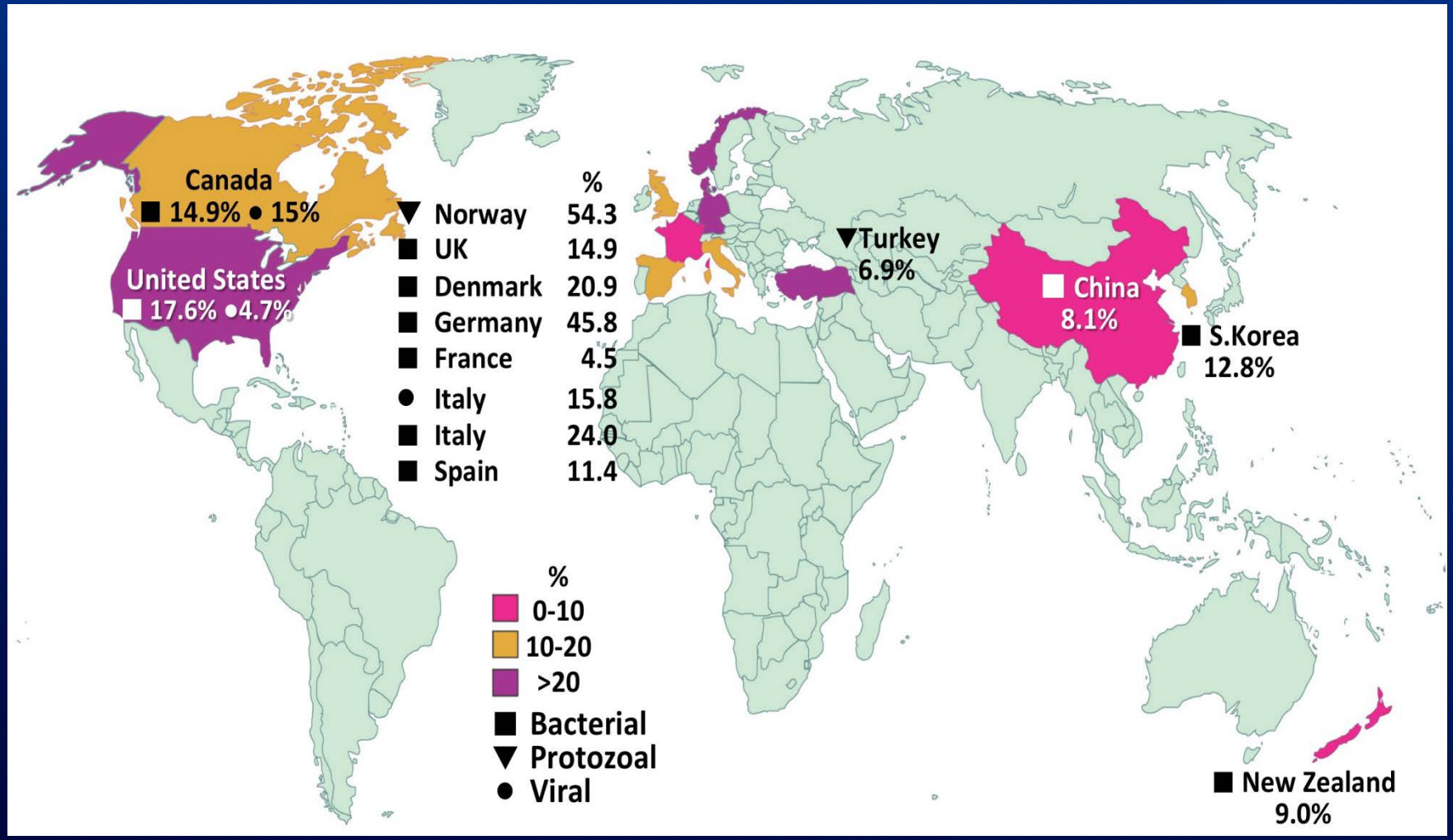
Protozoal/Parasitic		
Hanevik, 2014	291/748	0.389 (0.355-0.424)
Wensaas, 2012	355/817	0.435 (0.401-0.469)
Hanevik, 2009	66/82	0.805 (0.705-0.877)
Soyturk, 2007	5/72	0.069 (0.029-0.156)
	717/1719	0.419 (0.287-0.565)
		I^2=95%
Viral		
Porter, 2012	7/1718	0.004 (0.002-0.009)
Zanini, 2012	40/178	0.225 (0.169-0.292)
Saps, 2009	4/44	0.091 (0.035-0.218)
Marshall, 2007	13/86	0.151 (0.090-0.243)
	64/2026	0.064 (0.011-0.296)
		I^2=97%



PI-IBS relative risk with pathogen type

Subgroups (No. of studies)	Events/Total exposed	Events/Total unexposed	Relative risk	95% CI
Within 12m of exposure				
Overall (23)	500/12831	2397/639635	4.23	3.15-5.69
Organism				
• **Bacterial (10)**	254/7189	261/48340	4.22	2.84-6.25
• **Viral (2)**	53/264	5/147	4.48	1.01-19.95
• **Protozoal (1)**	5/72	0/27	4.22	0.24-73.83
>12m after exposure				
Overall (12)	1363/11439	1060/57240	2.33	1.82-2.99
Organism				
• **Bacterial (7)**	691/8035	758/48291	2.24	1.63-3.10
• **Viral (3)**	26/1839	46/6943	1.19	0.50-2.84
• **Protozoal (2)**	646/1565	256/2006	3.25	2.86-3.69

Worldwide prevalence of PI-IBS



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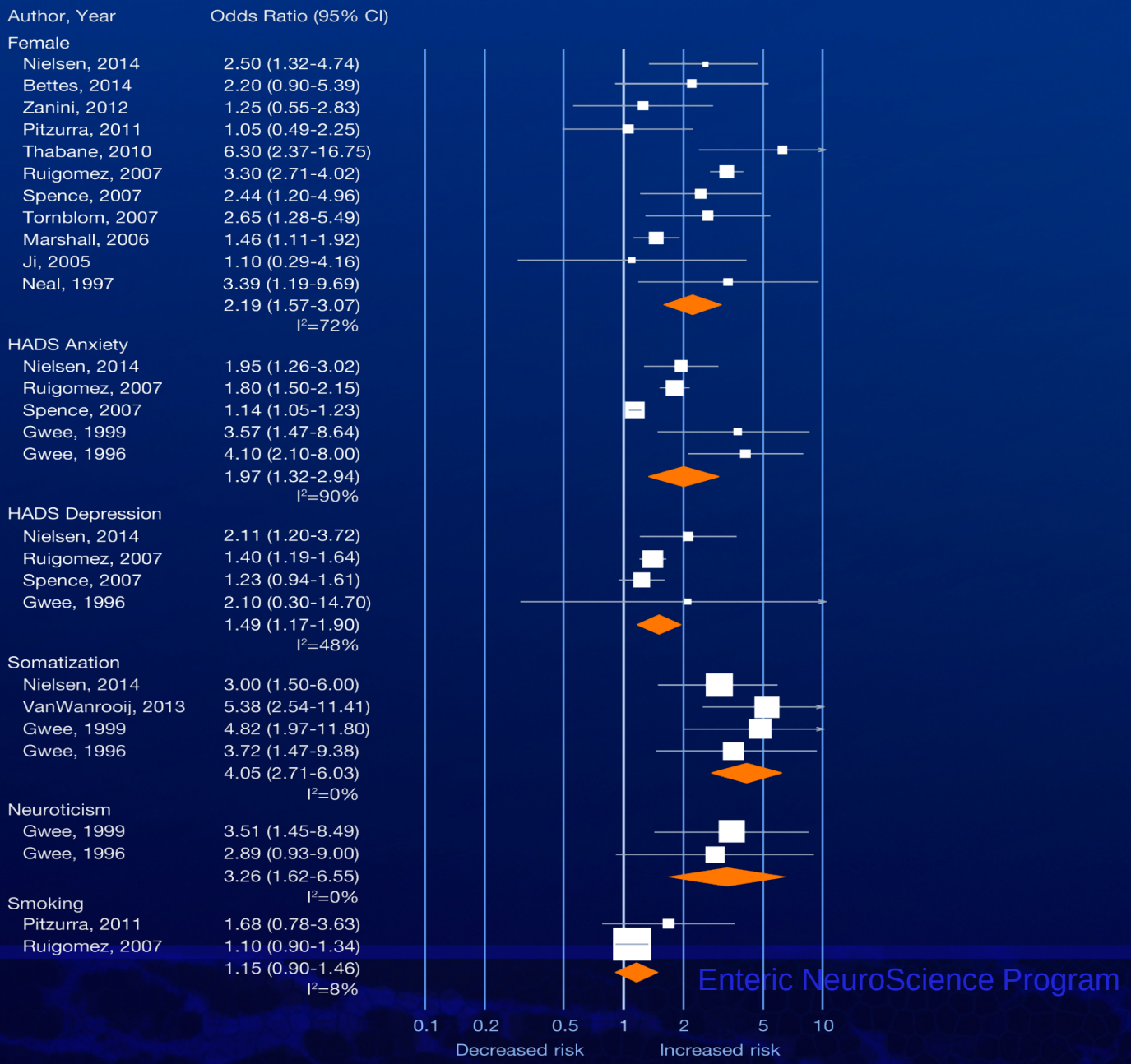
Demographic risk factors for PI-IBS



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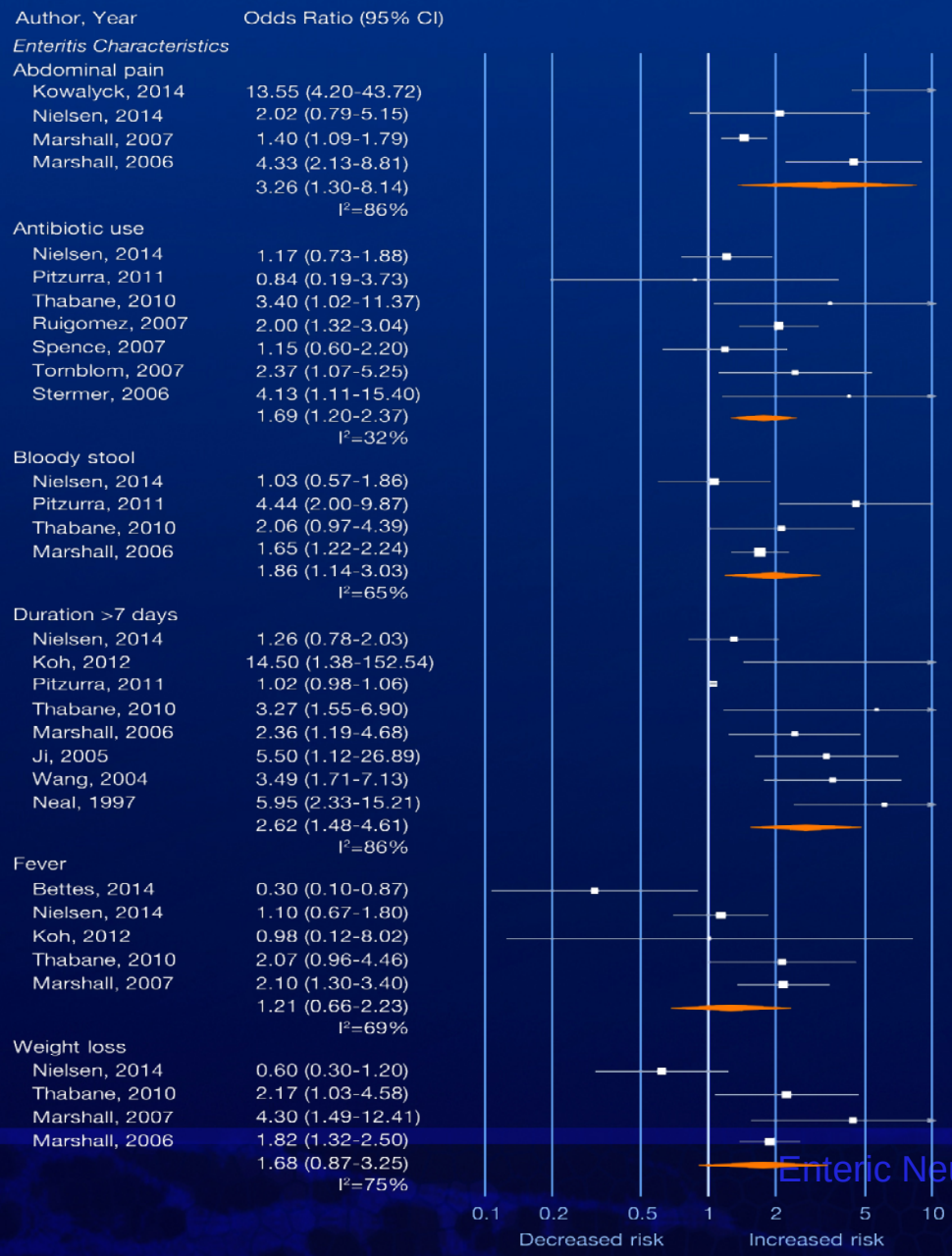
Enteritis episode related risk factors for PI-IBS



PI-IBS and the military: Millennium Cohort study

- Prospective follow-up of active military service personnel
  - 2-3-fold increased IBS risk found in all models studied
  - Females (hazard ratio=1.8), depression (hazard ratio=2.3), >3 life stressors (hazard ratio=6.8) for PI-IBS development

	OR (95% CI)
No infection and no depression	1.00
Infection and no depression	1.88 (0.65-4.37)
No infection and depression	1.45 (0.43-3.68)
Infection and depression	22.26 (5.30-63.07)

Riddle MS, **Am J Gastro 2016**

PI-IBS and the military: *Campylobacter* as prototypical organism

- *Campylobacter* accounted for nearly one quarter of all diarrheal cases in Southeast Asia
  - Leading cause among US troops deployed to Thailand
  - Leading pathogen during the 2014 Balikatan exercise in the Philippines
- Severe clinical presentation, reduced functional ability, and high incidence of fluoroquinolone resistance
- Relative risk of 3 for PI-IBS development among active duty US military from 1998-2009
  - Persisted after adjusting for branch of military service, ethnicity, or sex

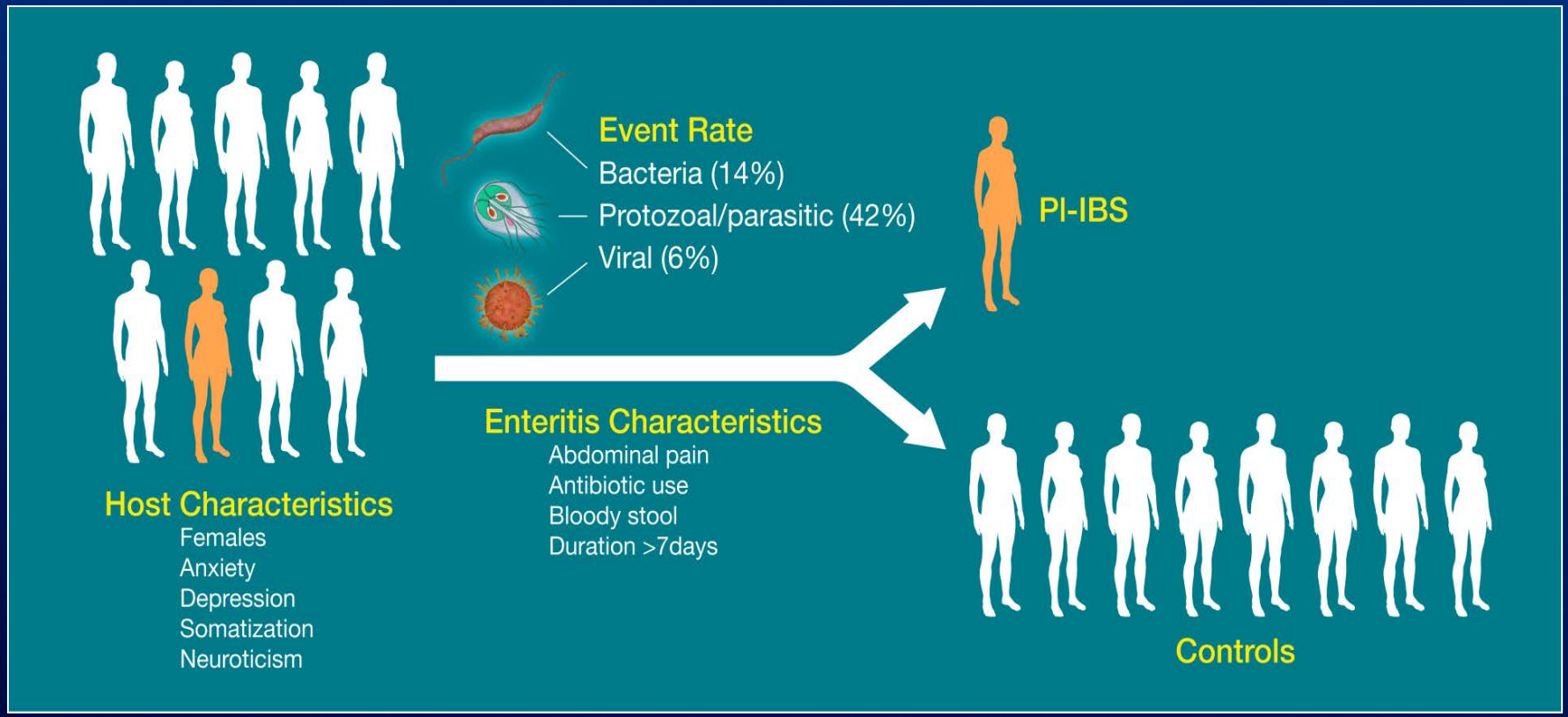
Porter CK, **Am J Gastro 2011**
Lertsethtakarn P, **Mil Med 2016**
Mason CJ, **Trop Dis Travel Med Vaccines 2017**

C. jejuni PI-IBS multivariable risk factor model

Variable	PI-IBS (N=121)	No PI-IBS (N=379)	Univariate PI-IBS OR (95% CI)	P value
Demographic				
Age, mean (SD)	42.0 (15.2)	48.1 (13.1)	0.85 (0.79-0.92)[#]	<0.001
Female gender, n (%)	78 (64.5%)	161 (42.5%)	2.46 (1.61-3.75)	<0.001
***Campylobacter* infection-related**				
Vomiting, n (%)	46 (38.0%)	89 (23.5%)	2.08 (1.34-3.24)	0.001
Fever, n (%)	64 (52.9%)	262 (69.1%)	0.51 (0.33-0.78)	0.002
Duration of diarrhea ≥ 7 days, n (%)	75 (62.0%)	194 (51.2%)	1.82 (1.16-2.84)	0.009
Duration of diarrhea, median (IQR)	8 (5.5, 15)	7 (5, 10)	1.42* (1.15-1.75)	0.001
Hospitalized during enteritis, n (%)	27 (22.3%)	40 (10.6%)	2.43 (1.42-4.16)	0.001
Days to start of antibiotics, median (IQR)	4 (1, 11)	4 (1, 7)	1.17* (1.01-1.35)	0.038

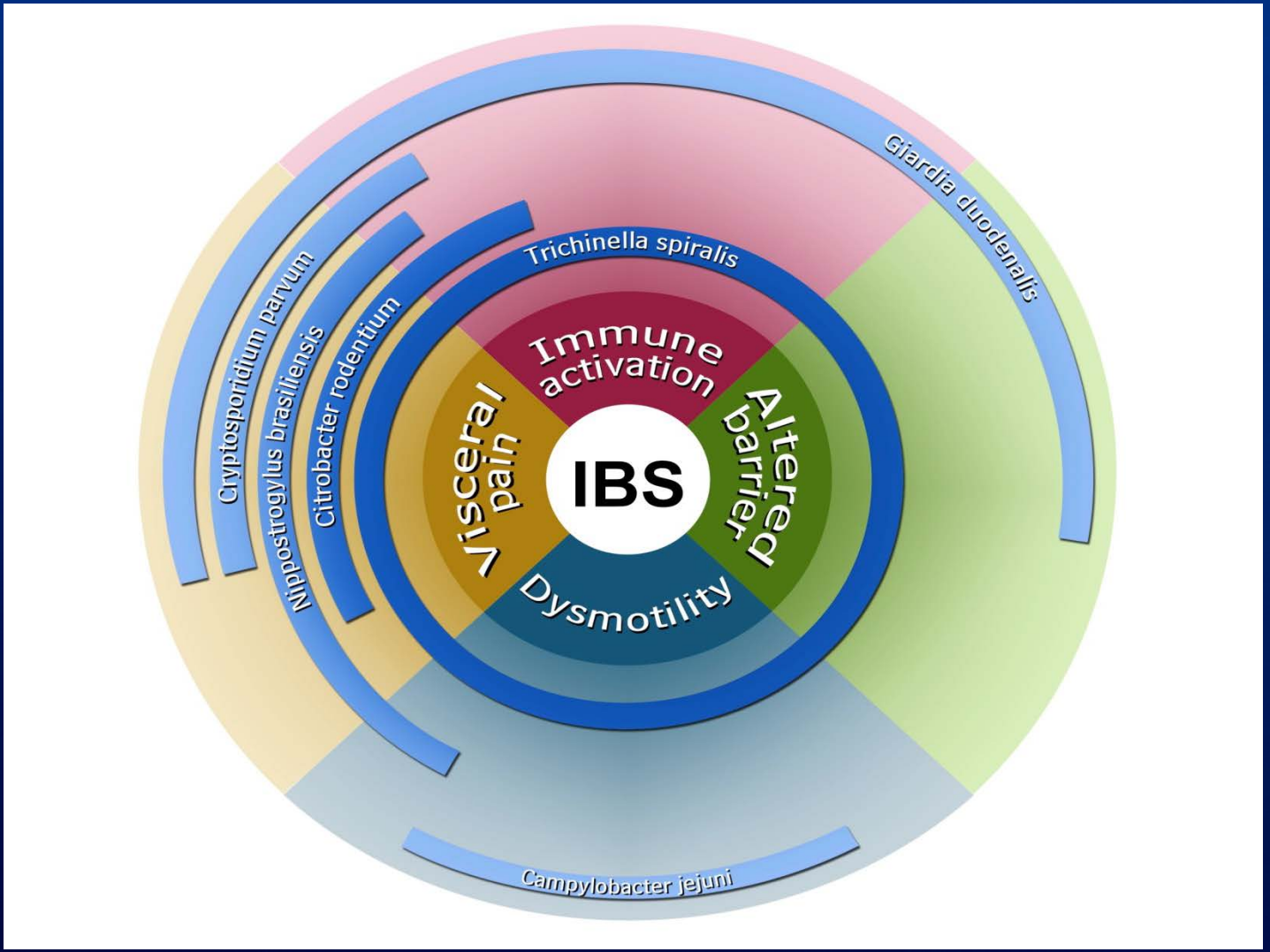
Wiens T…Grover M, **DDW 2017**

Summary: Post-infection IBS



Klem F, Wadhwa A…Grover M, **Gastro 2017**

Animal models of PI-IBS



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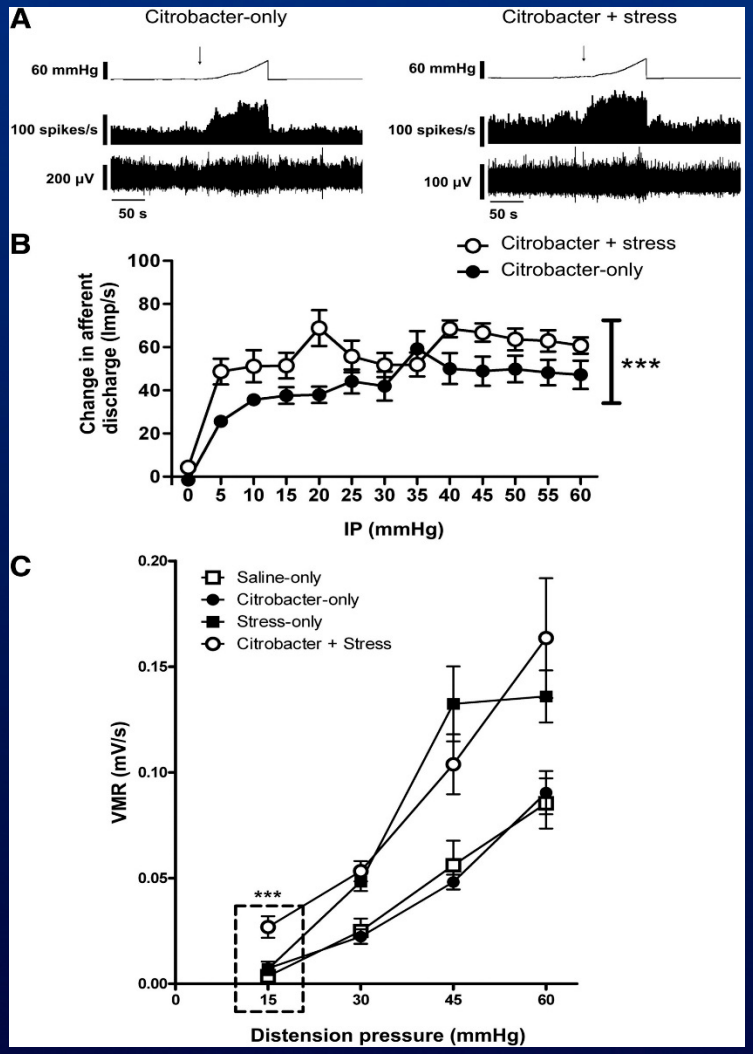
Infection and concomitant stress important for visceral hypersensitivity development



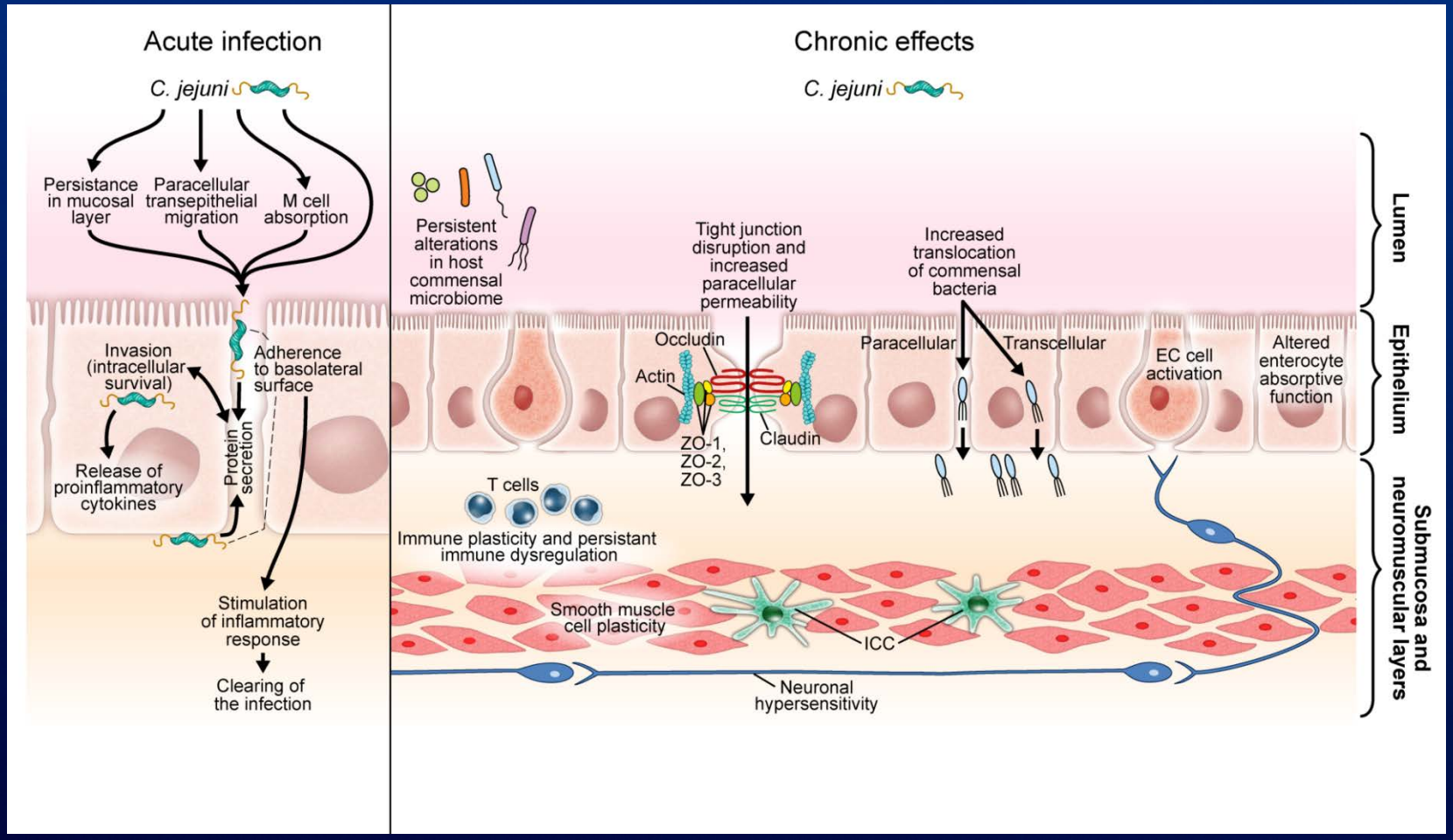
Ibeakanma C…Vanner S, **Gastro 2011**

Pathophysiological findings in human PI-IBS

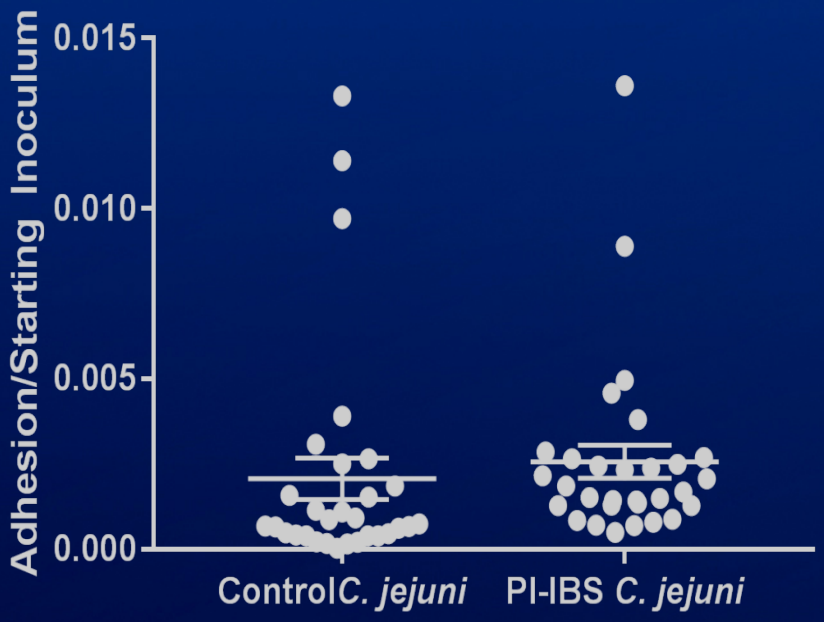
Pathogen	Mucosal cellular changes	Genes	Serum cytokines	Permeability	Mucosal cytokines
Bacterial					
Campylobacter jejuni	1. ↑ rectal EC cells, ↑ LP T lymphocytes[27] 2. ↑ rectal EC cells, ↑ LP CD3, CD8 T lymphocytes, ↑ CD8 IELs, ↑ calprotectin-ir cells[31]	1. ↑ *CCL11*, *CCL13*, *Calpain 8*, *GABRE*; ↓ *NR1D1*, *GPR161*[33] 6 months postenteritis, not PI-IBS	1. ↑ PBMC TNF-α, no difference IL-10, IL-1β; ↑TNF-α rs1800629[33] 2. No difference in IL-18, INFγ polymorphisms[36]	1. ↑ 0–6 h L/M ratio (initially, 12 weeks) not PI-IBS)[31] 2. ↑ 3–6 h Cr^{51} EDTA excretion (initially, 6 months) postenteritis, not PI-IBS[33]	1. No differences in IL-10, TNF-α and IL-1β[33]
Mixed infections	-	1. ↑*TLR9* (rs 5743836), *IL6* (rs206986), *CDH1*(rs16260)[11]	1. ↑ PBMC TNF-α, IL-1β, IL-6, LPS-stimulated IL-6[73]	1. ↑ L/M ratio[71]	1. ↑ rectal mucosal IL-1β[72]
Shigella	1. ↑ Ileal MC, ↑ NSE, substance P, 5-HT-ir nerve fibres[65] 2. ↑ 5-HT-ir EC cells, PYY-ir EC cells, IELs, CD3, CD8 lymphocytes, MC, CD68 cells; ↓ Calprotectin-ir macrophages[66]	-	-	-	↑ Terminal ileal and rectosigmoid IL-1β[65]
Parasitic					
Giardia lamblia	1. ↑ PI-IBS/FD: ↑ CCK-ir cells, ↓EC cells; no difference in duodenal 5-HT or 5-HIAA[58]	-	-	-	-
Unspecified	1. ↑ MC PAR_2 mRNA expression by PI-IBS supernatants[74] 2. ↓ colonic mucosal PAR_4, unchanged PAR_2 expression[75] 3. ↑ EC cells, ↑ LP T lymphocytes. No difference in IELs & MC[76] 4. ↑ mean chronic inflammatory cells in PI-IBS[77]	-	1. TNF-α (G/A, high producer) more prevalent in IBS (both PI and non-PI-IBS) compared to controls. No differences in IL-10 genotype[78]	1. ↑ 0–3 h Cr^{51} EDTA excretion[80]	-

Grover M, **Neurogastroenterol & Motil 2014**

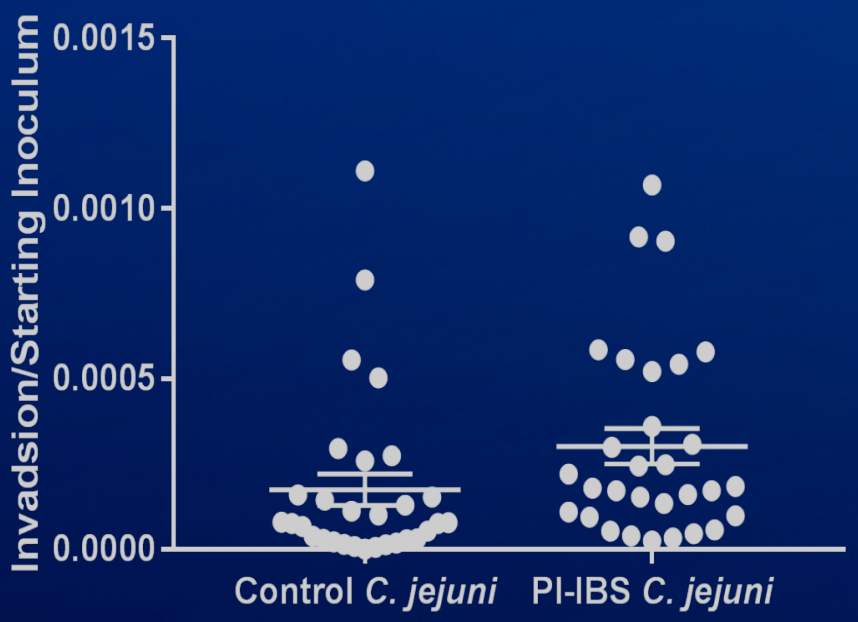
Infections can result in PI-IBS through various mechanisms



PI-IBS causing *C. jejuni* are more adherent and invasive



Control strains: 0.002073 (0.0006121)
PI-IBS strains: 0.002563 (0.0004871)
P = 0.004



Control strains: 0.0001741 (4.655e-005)
PI-IBS strains: 0.0003023 (5.183e-005)
P = 0.004

Peters S…Grover M, DDW 2017

Overview

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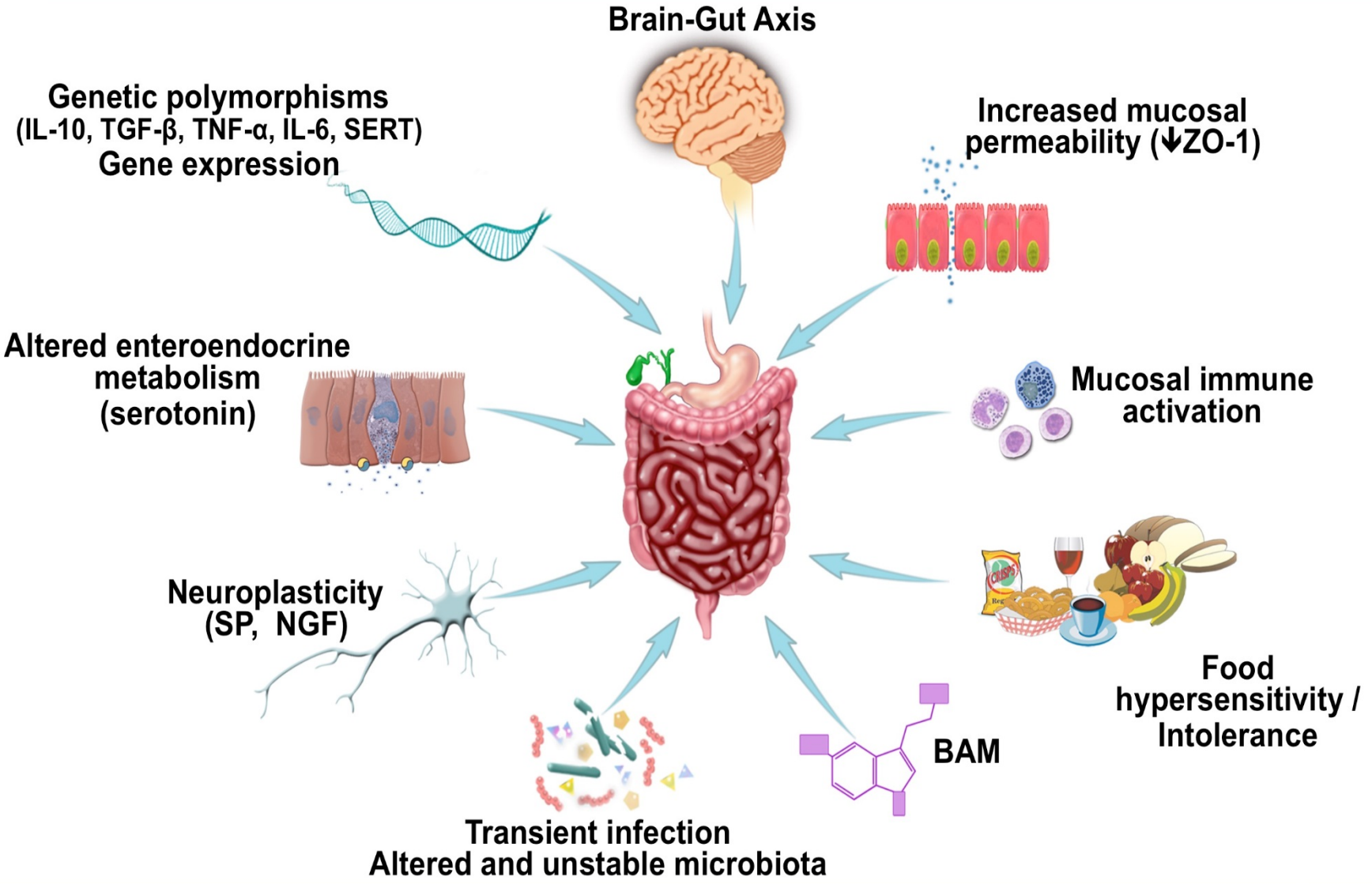
  - Epidemiology & risk-factors
    - Military relevance
  - Pathophysiology
    - Animal and human studies
- Role of microbiota
  - Interactions with peripheral (epithelial, luminal, dietary) factors
  - Bidirectional brain-gut crosstalk

Microenvironment and FGIDs

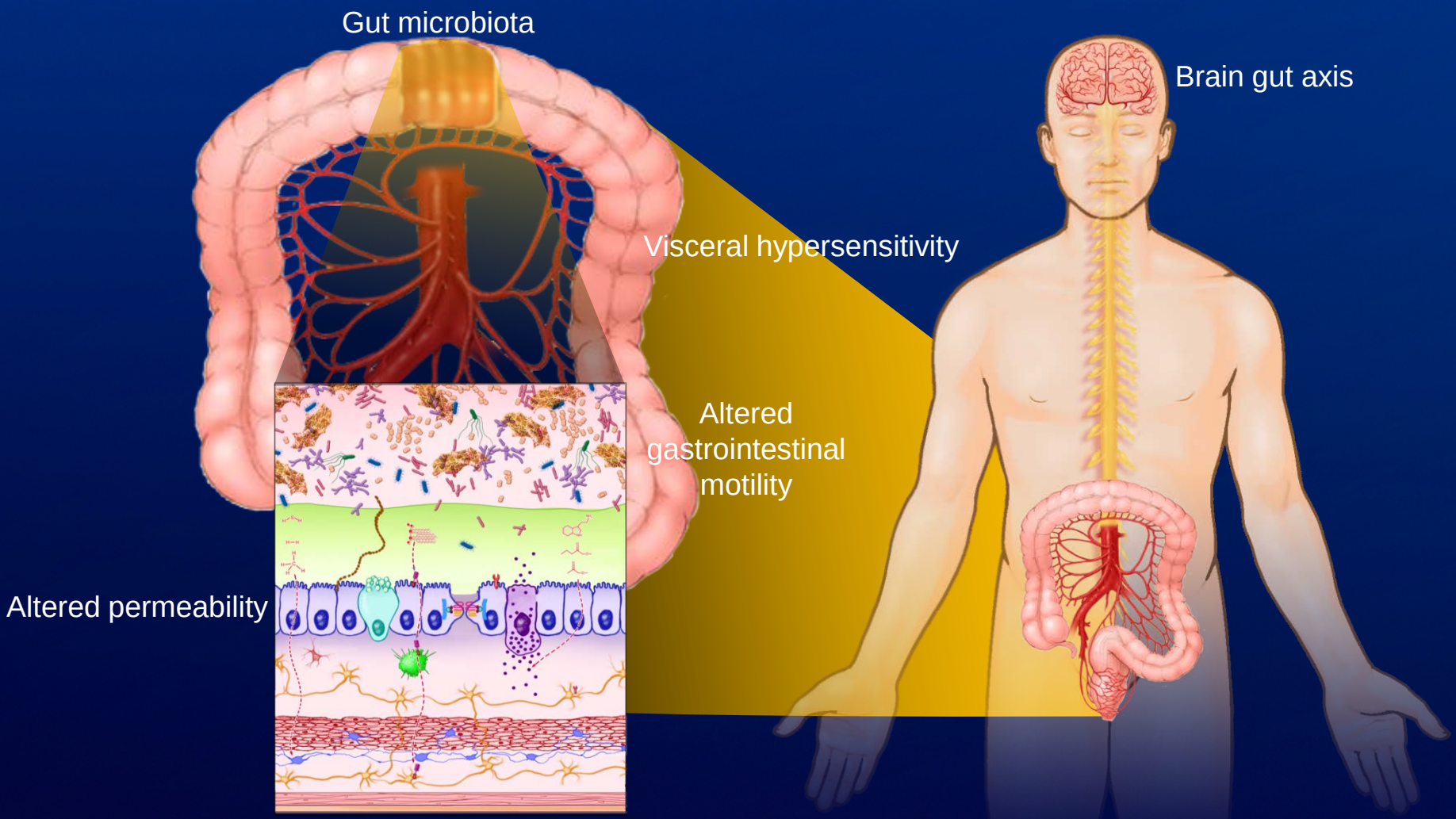


STUDY	POPULATION	KEY RESULTS
Balsari et al	IBS (n=20) Ctris (n=20)	↓ Coliform bacteria ↓ Lactobacillus spp. ↓ Bifidobacterium spp.
Si et al	IBS (n=25) Ctris (n=25)	↓ Bifidobacterium ↑ Enterobacteriaceae ↓ C perfringens
Malinen et al	IBS (n=27) Ctris (n=22)	↓ B catenulatum ↓ Cl coccoides group ↓ Lactobacillus spp. ↑ Veillonella spp. ↑ Lactobacillus spp.
Mättö et al	IBS (n=26) Ctris (n=25)	↑ Coliform bacteria ↑ Aerob to anaerob ratio ↓ Temporal stability
Maukonen et al	IBS (n=24) Ctris (n=16)	↓ Temporal stability ↓ Cl coccoides group
Kassinen et al	IBS (n=24) Ctris (n=23)	↓ Collinsella aerofaciens ↓ Cl cocleatum ↓ Coprococcus eutactus Subgroup-diff (D, C, M)
Rajilić-Stojanović	IBS (n=20) Ctris (n=20)	↑ Proteobacteria and specific Firmicutes ↓ Other Firmicutes, Bacteroidetes and bifidobacteria
Kerkhoffs et al	IBS (n=41) Ctris (n=26)	↓ Bifidobacterium spp. ↓ B catenulatum ↑ Proteobacteria ↑ Firmicutes ↓ Actinobacteria ↓ Bacteroidetes
Lyra et al	IBS (n=20) Ctris (n=15)	↑ R sorques 94% ↓ Cl thermosuccino genes 85% ↑ R bromii-like ↓ R sorques 93% ↑ Cl thermosuccino genes 85%
Tana et al	IBS (n=26) Ctris (n=26)	↑ Veillonella spp. ↑ Lactobacillus spp.
Coding et al	IBS (n=41) Ctris (n=33)	↑ Temporal stability No significant difference Fecal/mucosal
Carroll et al	IBS-D (n=10) Ctris (n=10)	↓ Aerobic bacteria Lactobacillus spp.
Noor et al	IBS (n=11) Ctris (n=22) UC (n=13)	↓ Bacterial species ↓ Biodiversity ↑ Biological variability of predominant bacteria
Malinen et al	IBS (n=44)	R torques 94% symptom severity Other phylotypes neg assoc.
Ponnusamy et al	IBS (n=11) Ctris (n=8)	↑ Diversity in Bacteroidetes & Lactobacilli ↑ Alanine & pyroglutamic acid & phenolic compounds
Rinttila et al	IBS (n=96) Ctris (n=23)	S aures (17%)
Saulnier et al	IBS (n=22) Ctris (n=22) (Children)	↑ γ Proteobacteria Classified IBS subtypes using sets of discriminant bacterial species
Rajilić-Stojanović et al	IBS (n=62) Ctris (n=42)	↑ Proteobacteria and specific Firmicutes ↓ Other Firmicutes, Bacteroidetes and bifidobacteria

"One important limitation of available studies is their descriptive rather than mechanistic nature. Accordingly, studies should be directed at clarifying cause effect relationships between microbiota changes and bowel dysfunction…."

Simŕen M, **Gut 2013**

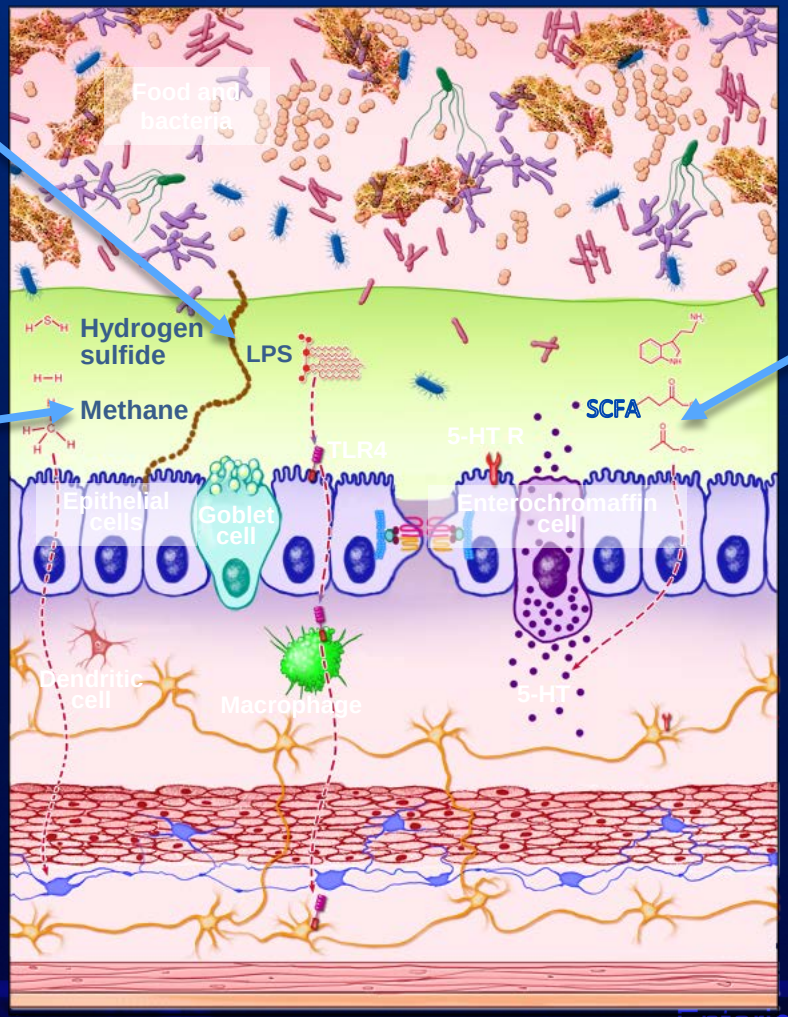
Gut microbiota can influence peripheral mechanisms implicated in IBS



Gut microbial metabolites can influence peripheral mechanisms implicated in IBS

LPS promotes intestinal transit

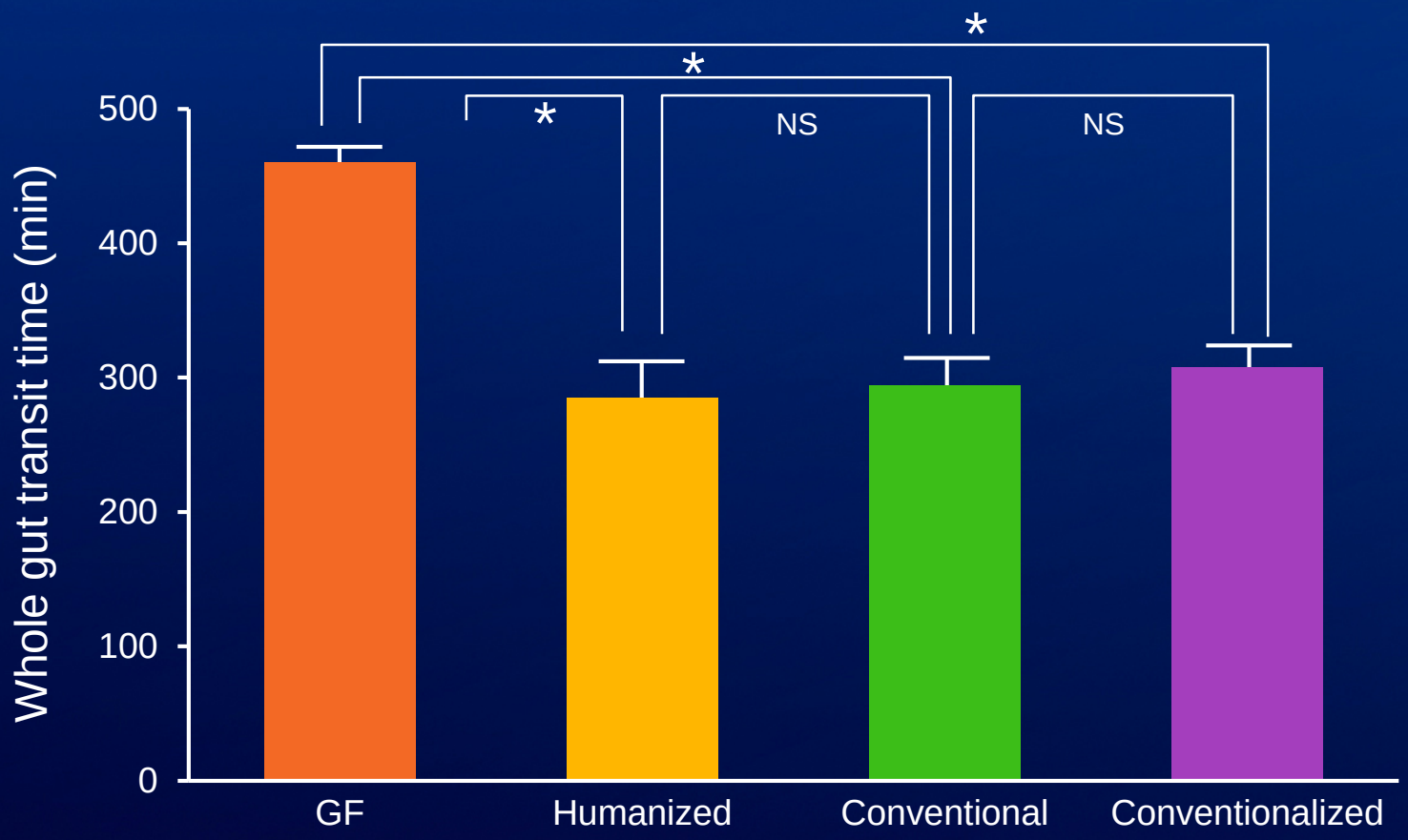
Methane slows intestinal transit



SCFA increase colonic motor function and facilitate water absorption

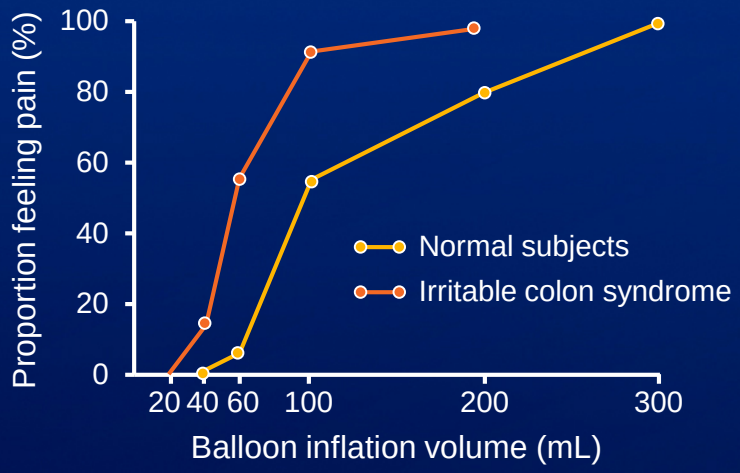
Cherbut C, **Proc Nutr Soc 2003**
Mallappa A, **Gastro 2012**
Pimentel M, **Am J Physiol Gastrointest Liver Physiol. 2006**

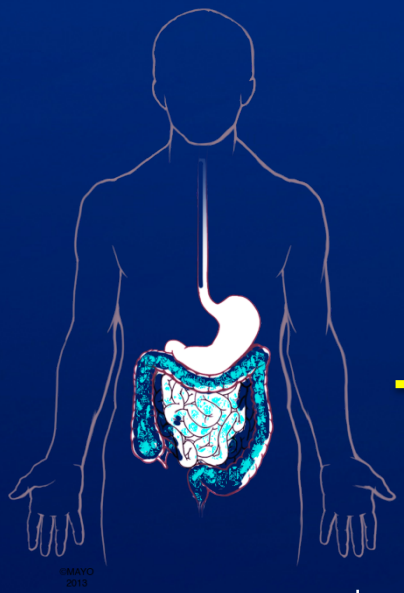
Colonization of germ free mice with complex microbial community shortens GI transit time



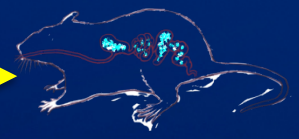
Kashyap PC, **Gastro 2013**

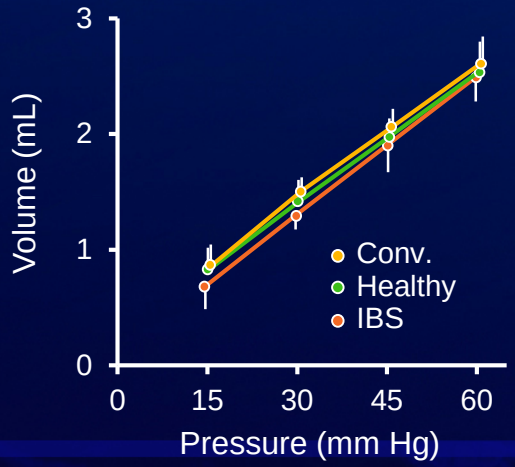
Gut microbiota sufficient to transfer visceral hypersensitivity

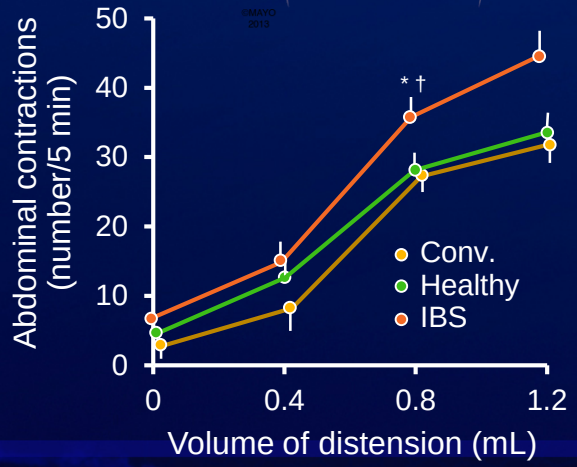




Humanized rodent model

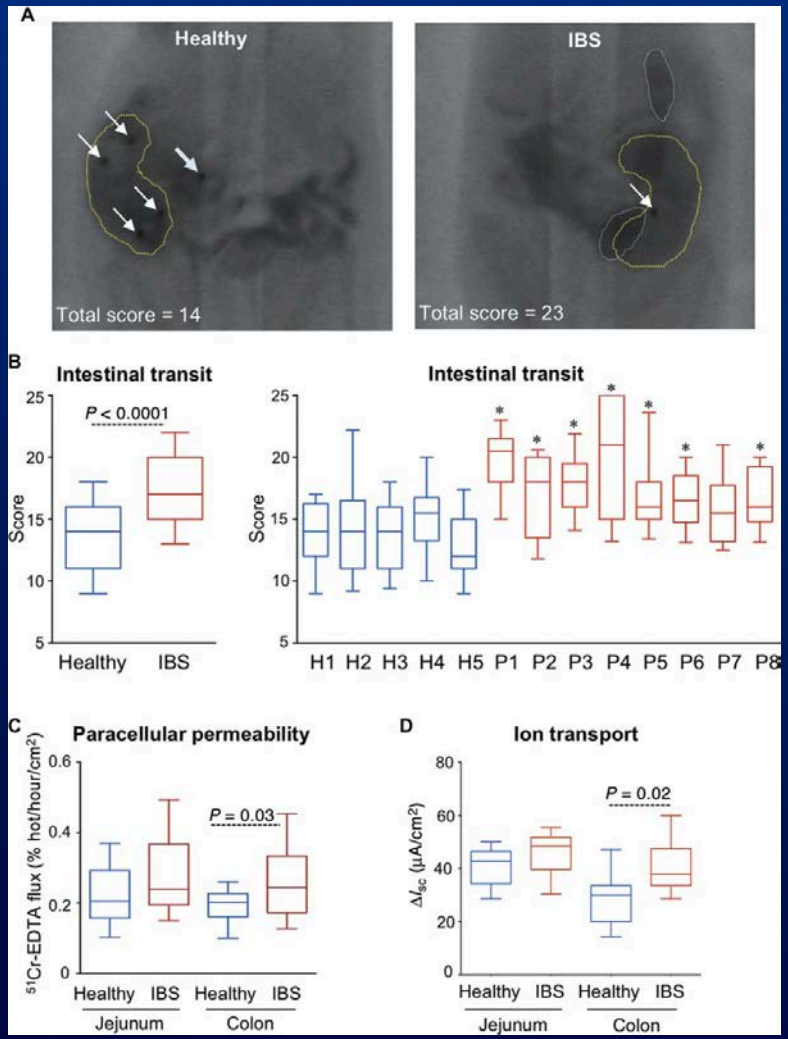






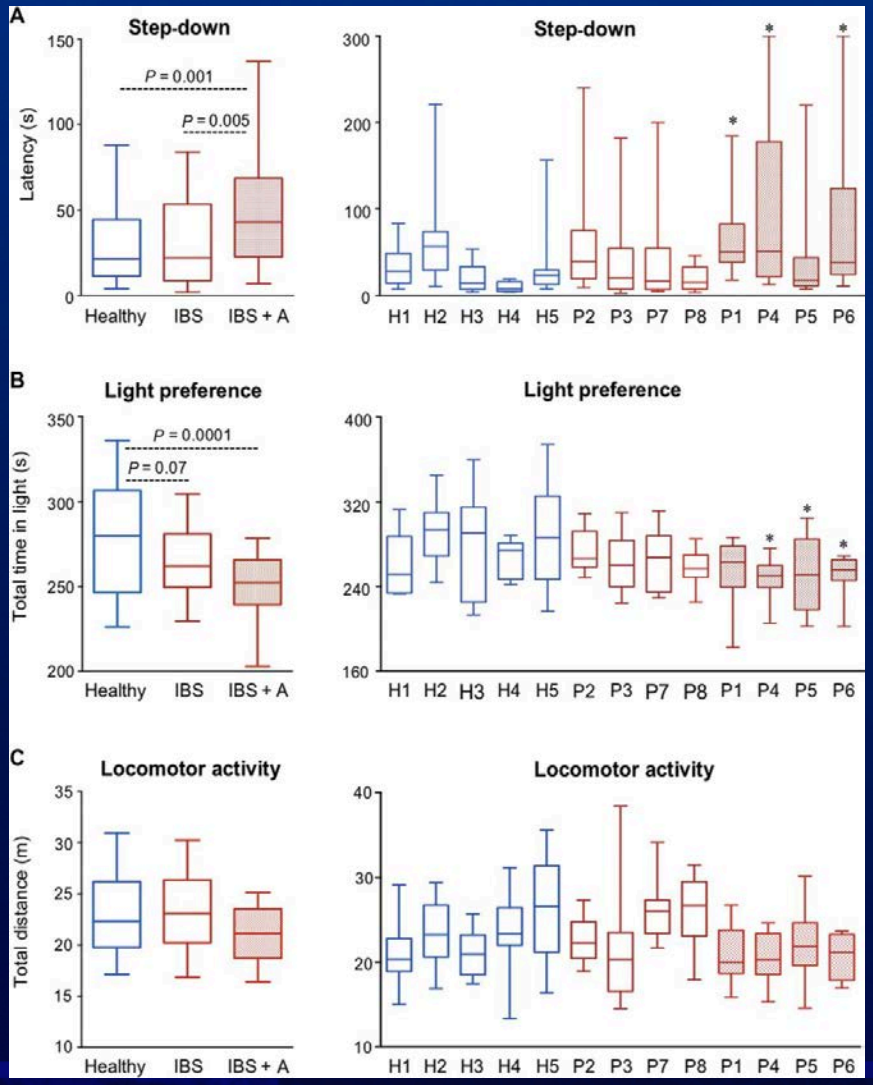
Ritchie J, **Gut 1973**
Crouzet L, **Neurogastroenterol & Motil 2013**

Transfer of IBS-D microbiota increases transit and alters colonic permeability and secretion



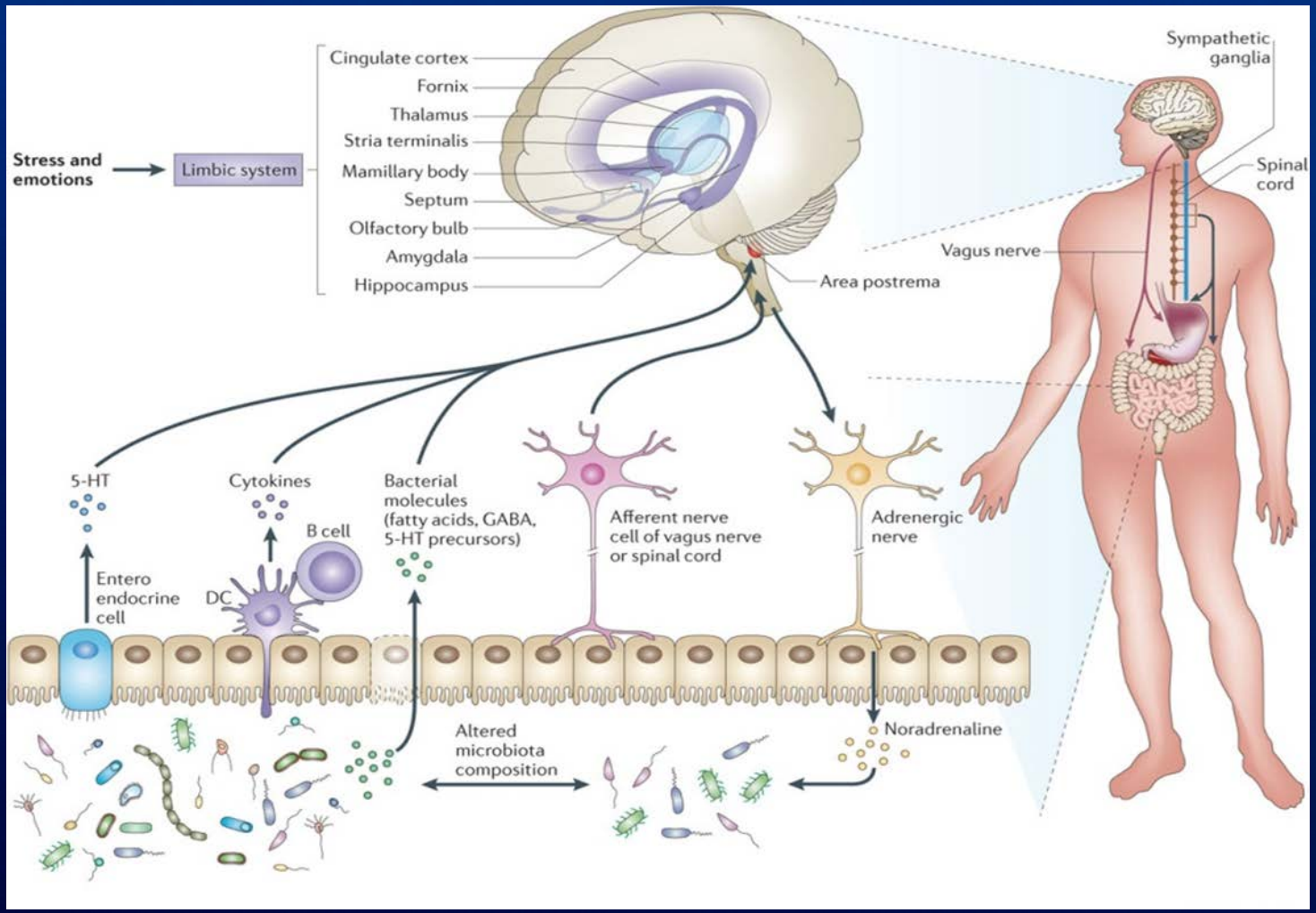
Giada De Palma, **Sci Transl Med 2017**

Transfer of anxiety-like behaviour in mouse recipients of microbiota from IBS-D patients



Giada De Palma, **Sci Transl Med 2017**

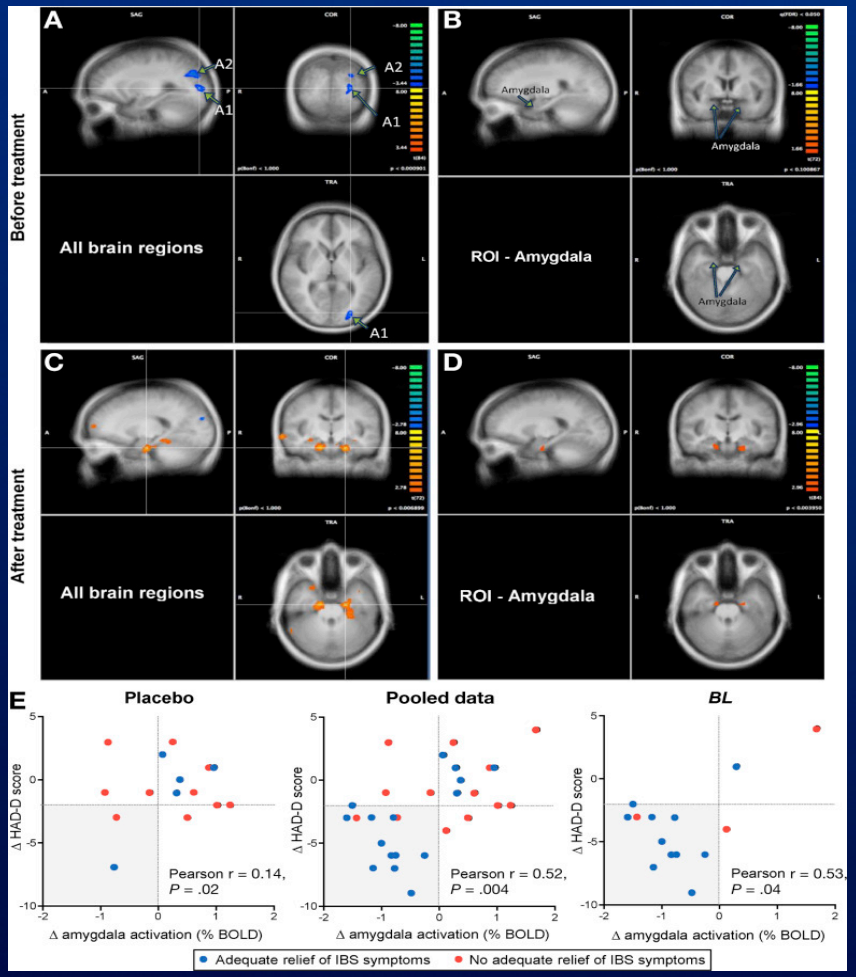
Gut Bacteria/Bacterial Products Can Influence CNS Function



Collins SM, **Nature Reviews Microbiology 2012**
Tillisch K, **Gastro, 2013**

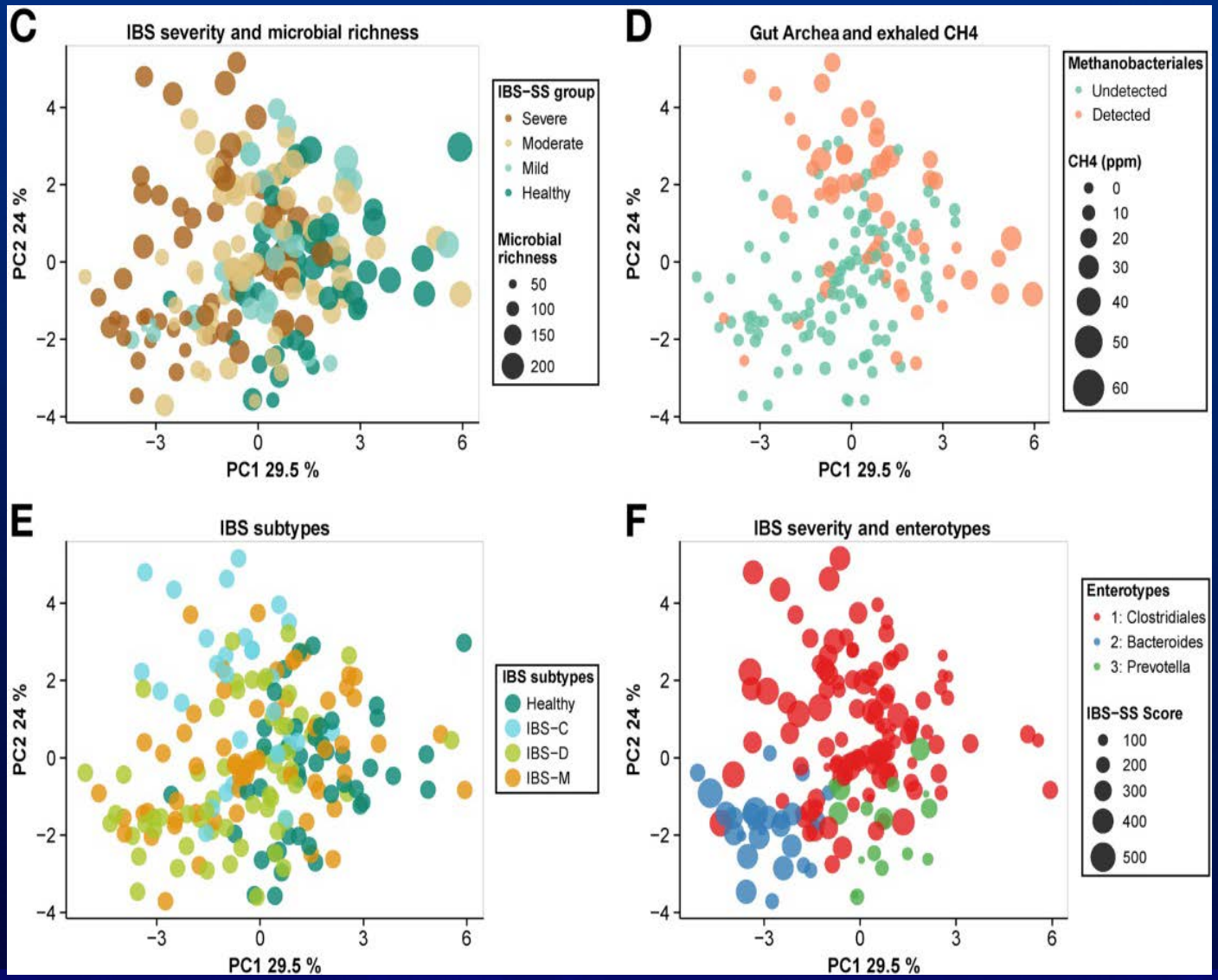
Gut to brain in IBS

- Double blind RCT of IBS-D/M with mild/moderate anxiety or depression using *Bifidobacterium longum* NCC3001 or placebo
- Reduction in depression score and increased quality of life
- BL reduced responses to negative emotional stimuli in multiple brain areas, including amygdala and fronto-limbic regions, compared with placebo



Pinto-Sanchez, **Gastro 2017**

Microbial signatures and IBS symptom severity

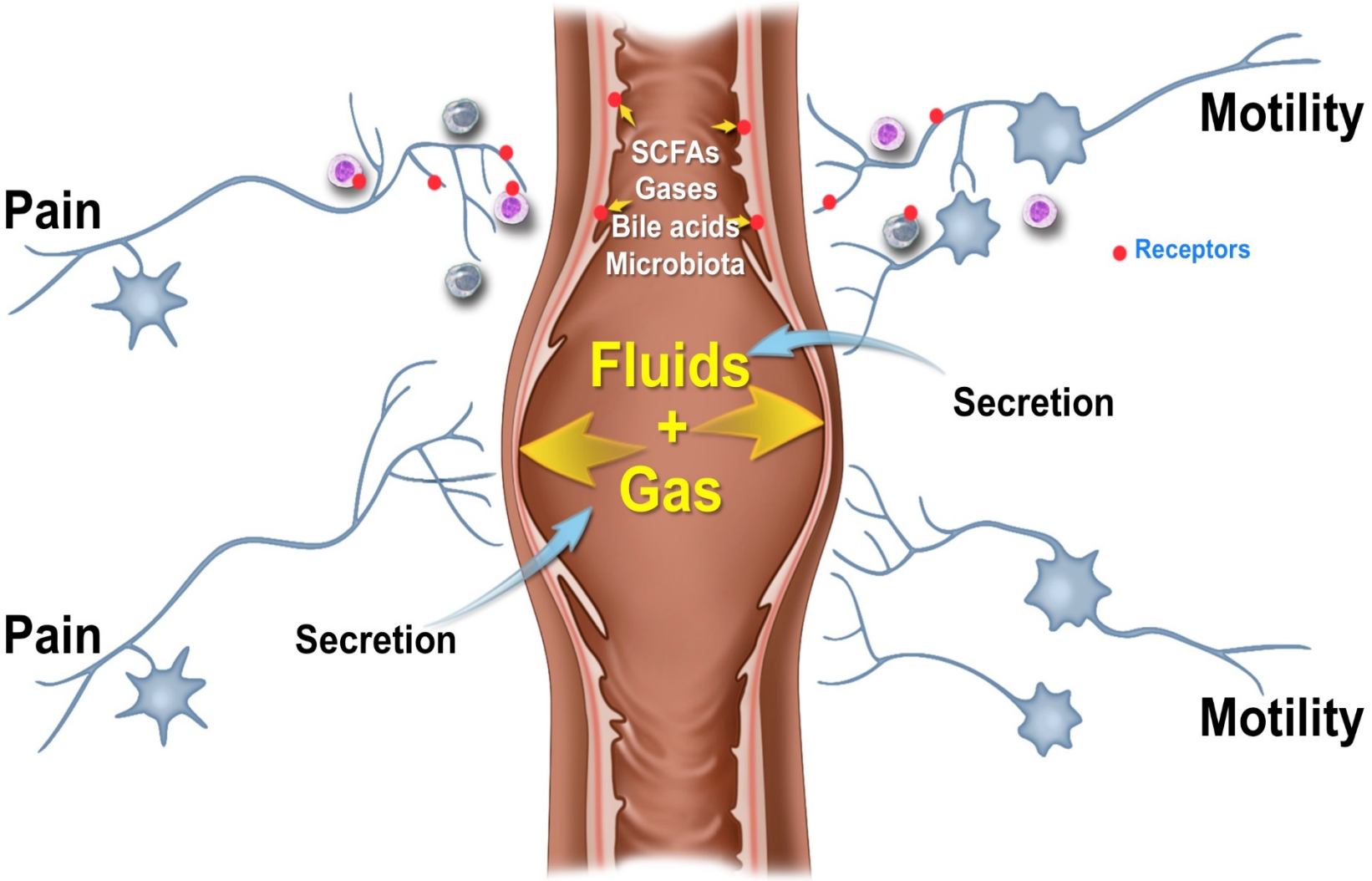


Gut microbial signature for IBS severity linked with

- Lower microbial richness
- Lower levels of exhaled CH_4
- *Bacteroides*-enterotype

Tap J, **Gastro 2017**

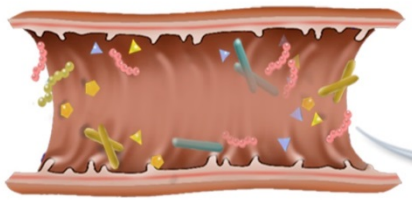
Diet + Microbiome



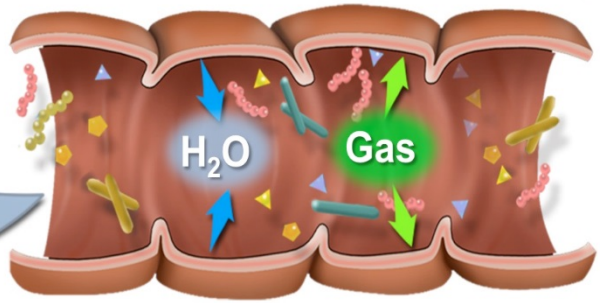
Dietary Factors and Symptom Induction in IBS: Potential Mechanisms

FODMAPs
Gluten
Fibers
Lactose
Food composition

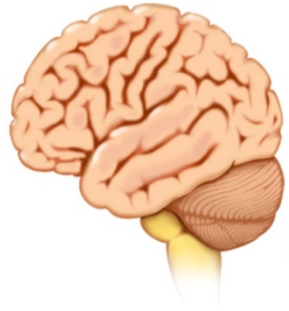
FODMAPs: Fermentable Oligosaccharides, Disaccharides, Monosaccharides and Polyols



Gut microbiota composition



Intestinal distension



Gut inflammation/ immune activity
Barrier defects?
Other factors
Visceral hypersensitivity

Low FODMAP diet in IBS

Controlled trials						
Staudacher *et al*[49]	Placebo-controlled dietary advice RCT (single blind)	Rome III IBS-D, IBS-M, IBS-U	LFD n=51 Sham diet n=53	4 weeks	AR IBS-SSS IBS-QOL	Primary outcome: No difference in AR (LFD 57% vs control 38%; p=0.051) Secondary outcomes: Lower IBS-SSS score (LFD 173 vs control 224; p=0.001) and greater numbers achieving MCID for IBS-QOL (LFD 51% vs control 26%; p<0.023)
Staudacher *et al*[50]	Dietary advice RCT (unblind)	Rome III IBS with bloating or diarrhoea	LFD n=19 Habitual diet n=22	4 weeks	AR GSRS Bristol Stool Form	Primary outcome: Luminal microbiota (see table 3) Secondary outcomes: Greater numbers reporting AR (LFD 68% vs control 23%; p=0.005) Lower bloating, borborygmi, overall symptoms LFD versus control (p<0.05) Greater number of normal stools (LFD 24% vs control 7%; p=0.02)
Harvie *et al*[69]	Dietary advice RCT (unblind)	Rome III IBS	LFD n=23 Waiting list n=27	3 months	IBS-SSS IBS-QOL	Outcomes: Greater reduction in IBS-SSS (LFD 276 to 129 pt vs control 247 to 204 pt; p<0.01), frequency of pain episodes (p<0.01) Greater increase in IBS-QOL score for LFD versus control (p<0.0001)
Pedersen *et al*[88]	Dietary advice RCT (unblind)	Rome III IBS	LFD n=42 Probiotic n=41 Habitual diet n=40	6 weeks	IBS-SSS IBS-QOL	Primary outcome: Greater reduction in IBS-SSS (LFD −75 pt vs control −32 pt; p<0.01) Secondary outcome: No change in IBS-QOL for all groups
Halmos *et al*[73]	Placebo-controlled feeding RCT, crossover (single blind)	Rome III IBS	LFD n=27 Typical diet n=27	21 days	100 mm symptom VAS Stool frequency Stool water content	Primary outcome: Lower overall GI symptoms (LFD 23 mm vs control 45 mm; p<0.001). Secondary outcome: Lower stool frequency in IBS-D in LFD versus control

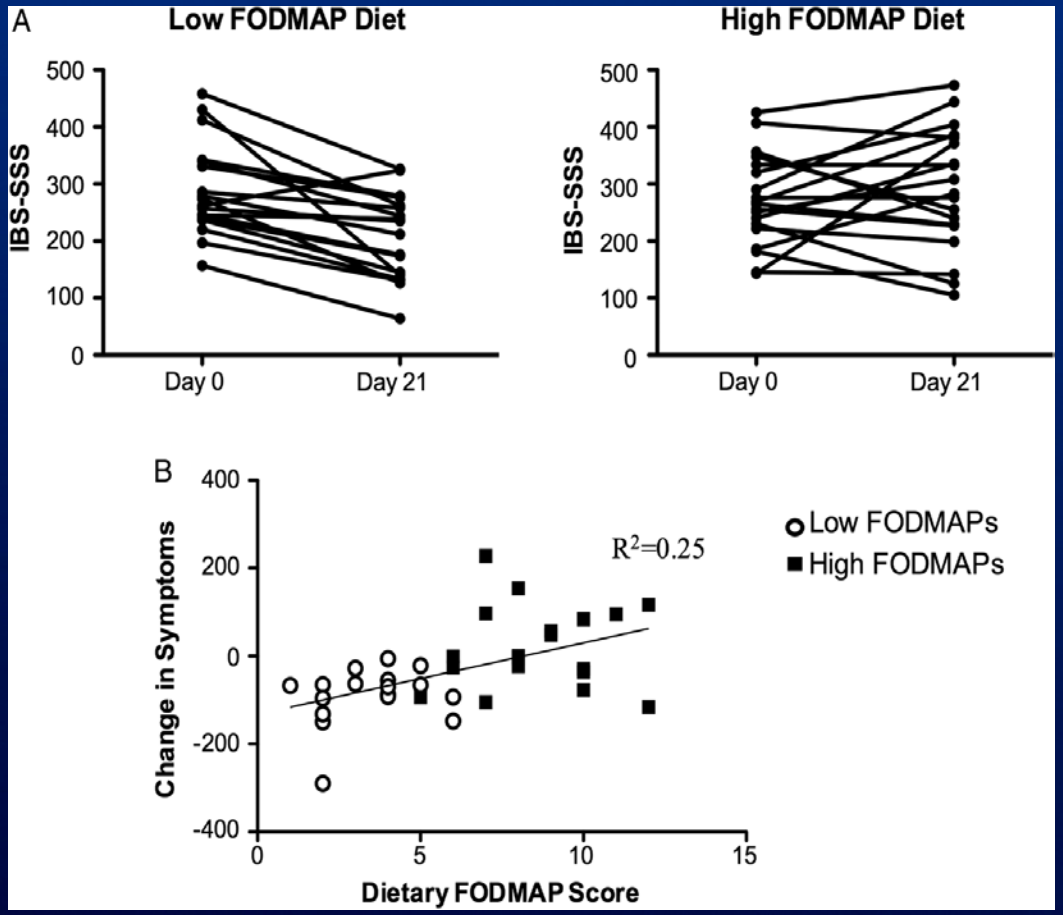
Staudacher H, **Gut 2017**

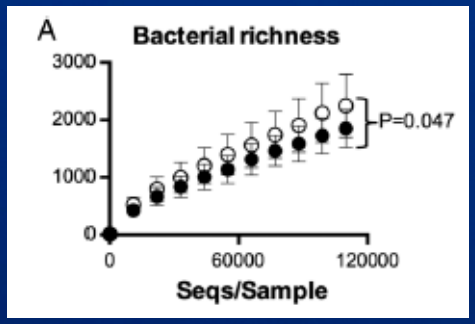
Microbiome signatures predict responders to low FODMAP diet

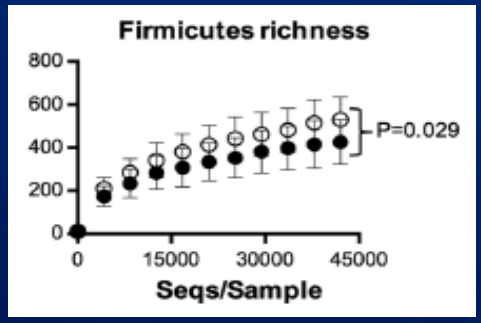
- Responders to the Low FODMAP diet enriched at baseline in OTUs with greater saccharolytic capacity within the family *Bacteroidaceae* (e.g. *Bacteroides*), order *Clostridiales* (e.g. *Ruminococcaceae, Dorea* and *Faecalibacterium prausnitzii*) and family *Erysipilotrichaceae*
- Non-responders enriched at baseline in the genus *Turibacter* from the family *Turicibacteraceae*

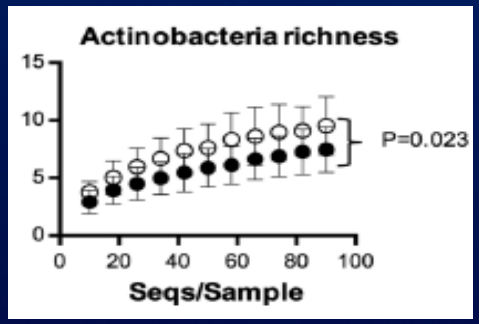
Chumpitazi BP, **Aliment Pharmacol Ther 2015**

High vs Low FODMAP diets and response in IBS symptoms









McIntosh K, **Gut 2017**

Summary

- Intestinal infections are among the most common risk-factors for IBS development
  - Common in active duty military population
  - Psychological stress plays a key role
  - Pathophysiological aspects need further studies
- Microbiome important in pathophysiology of IBS and other functional gut disorders
  - Studies needed to understand interface of microbes and their products with gut physiology
  - Bidirectional brain-gut-microbiome central to understanding the mechanisms and clinical presentation

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