Bugs, Guts and SpA

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Disclosures

• Advisory Boards/educational grants/honoraria:
  – Abbvie
  – Takeda
  – Ferring
  – Vifor
  – Janssen
REANIMATE Program

“To give new life to”

NUHS Faecal Microbiota Transplantation Program
November 2013
REANIMATE Team

• Acknowledgements:
• Nick Chew, Reuben Wong, Calvin Koh, Juanda Leo, Roland Jureen, Jonathan Lee, Alex Soh

• Other hospitals participating:
• CGH, TTSH, SGH
Microbiome

• Nobel Laureate Joshua Lederberg
• Collection of microorganisms that cohabit our bodies
• Viruses, fungi, parasites and bacteria
• RNA transcripts outnumber mammalian transcripts 100 to 1

Gut Microbiota and Factors Affecting its Composition

The Microbial Balance

Our Microflora Evolves

Infant

Delivery mode
- Vaginal delivery:
  - Colonization with vaginal microbiota such as *Lactobacillus* and *Prevotella*
- Cesarean delivery:
  - Colonization with skin microbiota such as *Staphylococcus*, *Corynebacterium*, *Propionibacterium*

Feeding method
- Breastfeeding:
  - Increased aerobic organisms, increased *Bifidobacterium*, decreased *Clostridium*, decreased *Bacteroides*
- Bottle feeding:
  - Increased anaerobes and facultative anaerobes, increased *Clostridium*, increased *Bacteroides*

Environmental exposures

Toddler

Relatively sterile gut → Low diversity, unstable, chaotic → Diverse, stable
An example of how bugs can cause problems in the Gut: C. Difficile Colitis

Epidemiology in Singapore

• Paucity of data from Singapore:

  – Lim et al reported 2001 to 2006
    • incidence of CDI increased from 1.49 to 6.64 per 10,000 patient days in a secondary hospital
  – Hsu et al subsequently 2006 to 2008
    • incidence of CDI had fallen from 5.56 to 2.99 per 10,000 patient days in public hospitals


Case 1: Refractory/ relapsing CDI

80 yo lady
CVA, Bedbound
Recurrent admissions for gram negative septicaemia secondary to recto-urethral fistula
Diarrhoea 10-15 times per day; ATN requiring iv fluid and electrolyte replacement; severe excoriations
CD toxin positive with leukocytosis
European Society of Clinical Microbiology and Infectious Diseases: update of the treatment guidance document for *Clostridium difficile* infection

S. B. Debast¹, M. P. Bauer², E. J. Kuipers³, on behalf of the Committee

1) Department of Medical Microbiology, Radboud University Medical Center, Nijmegen; Departments of 2) Infectious Diseases and 3) Medical Microbiology, Centre for Infectious Diseases, Leiden University Medical Centre, Leiden, the Netherlands

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**Non-severe CDI**

- **Oral antibiotic treatment**
  - Metronidazole 500 mg tid 10 days A-I
  - Vancomycin 125 mg qid 10 days B-I
  - Fidaxomicin 200 mg bid 10 days B-I
- **Non-antibiotic treatment regimens**
  - Stop inducing antibiotic(s) + 48hrs clinical observation C-II
  - Immunotherapy with human monoclonal antibodies C-I or immune whey C-II
  - Probiotics D-I
  - toxin binding D-I

**Risk of first recurrence**

- **Oral antibiotic treatment**
  - Vancomycin 125 mg qid 10 days B-II
  - Fidaxomicin 200 mg bid 10 days B-II
  - Vancomycin 500 mg qid 10 days C-II
  - Metronidazole 500 mg tid 10 days D-II

**Multiple recurrences**

- **Oral antibiotic treatment**
  - Pulse/taper therapy vancomycin B-II
  - Fidaxomicin 200 mg bid 10 days B-II

**Non-antibiotic treatment regimens**

- **Oral antibiotic treatment**
  - Faecal transplant (combined with oral antibiotic treatment) A-I
  - Probiotics D-I
  - Passive immunotherapy with immune whey D-I

**Severe disease or complicated course†**

- **Oral antibiotic treatment**
  - Vancomycin 125 mg qid 10 days A-II
  - Fidaxomicin 200 mg bid 10 days B-II

**Oral treatment not possible**

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**Non-severe CDI:**
- Metronidazole 500 mg tid iv 10 days A-II

**Severe CDI:**
- Metronidazole 500 mg tid iv 10 days A-II + vancomycin 500 mg qid enteral 10 days B-III
- Tigecycline 50 mg bid iv 14 days C-III
C. Difficile Relapse

Occurs in **20-60%** of successfully treated patients

- Relapse rate similar for vancomycin & metronidazole **~25-30%**
- After 1st relapse, subsequent relapse risk **~40%**
- 2 or more relapses, risk rises to **~60%**

Onset is **1 week - 3 months** after treatment

**Refractory CDI** seen in **10-20%**

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  - Probiotics D-I
  - Toxin binding D-I

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  - Pulse/taper therapy
  - Vancomycin 125 mg qid 10 days A-I
  - Fidaxomycin 200 mg bid 10 days B-II

- **Non-antibiotic treatment regimens**
  - Faecal transplant (combined with oral antibiotic treatment) A-I
  - Probiotics D-I
  - Passive immunotherapy with immune whey D-I

### Severe disease or complicated course

- **Oral antibiotic treatment**
  - Metronidazole 500 mg tid iv 10 days A-II
  - Fidaxomycin 200 mg bid 10 days B-II

### Oral treatment not possible

- **Oral antibiotic treatment**
  - Vancomycin 125 mg qid 10 days A-I
  - Fidaxomycin 200 mg bid 10 days B-II
  - Metronidazole 500 mg tid 10 days D-I

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*CDI: Clostridium difficile infection*
Treatment of Multiple Recurrences

- Frustrating, demoralising to caregivers and patient:
  - Fidaxomicin
  - Infusion of monoclonal antibodies active versus CdA and CdB toxins
  - Vancomycin - tapering, pulse dosing, sequencing with rifaximin*
  - Probiotics - *Saccharomyces boulardii, Lactobacillus* - unproven#
  - Passive treatment with immunoglobulin - anecdotally effective
  - Toxin-binding with cholestyramine or colestipol - unproven^*
  - Fecal reconstitution using donor feces – effective
  - Subtotal colectomy ... reports of C diff ileitis

#Focus on probiotics for CDAD: focus on *Lactobacillus rhamnosus* GG and *Saccharomyces boulardii*. Pharmacotherapy 2007;41(7):1212.
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**CDI**

- **Non-severe CDI**
  - Oral antibiotic treatment
    - Metronidazole 500 mg tid 10 days A-I
    - Vancomycin 125 mg qid 10 days B-I
    - Fidaxomicin 200 mg bid 10 days B-I
  - Stop inducing antibiotic(s) + 48 hrs clinical observation
  - Immunotherapy with human monoclonal antibodies
  - Probiotics D-I
  - Toxin binding D-I

- **Non-antibiotic treatment regimens**
  - Immunomodulator
  - Supportive care

- **Oral antibiotic treatment**
  - Vancomycin 125 mg qid 10 days B-II
  - Fidaxomicin 200 mg bid 10 days B-II
  - Vancomycin 500 mg qid 10 days C-II
  - Metronidazole 500 mg tid 10 days C-I
  - Probiotics D-I
  - Toxin binding D-I

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  - Faecal transplant (combined with oral antibiotic treatment)
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  - Oral treatment not possible

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- Tigecycline 50 mg bid iv 14 days C-III
Faecal Microbiota Transplantation (FMT)  
The new kid on the block?

**Animals**

Coprophagia

**Ancient Medicine**

China  
“Yellow-Dragon Soup”  
Tong-Jin Dynasty 4th Century  
Ming Dynasty 16th Century  
Bedouins of North Africa

**Modern Medicine**

EISEMAN B, SILEN W, BASCOM GS, KAUVAR AJ.  

**Veterinary Medicine**: Transfaunicaton
Duodenal Infusion of Donor Feces for Recurrent
Clostridium difficile

Els van Nood, M.D., Anne Vrieze, M.D., Max Nieuwdorp, M.D., Ph.D., Susana Fuentes, Ph.D., Erwin G. Zoetendal, Ph.D., Willem M. de Vos, Ph.D., Caroline E. Visser, M.D., Ph.D., Ed J. Kuijper, M.D., Ph.D., Joep F.W.M. Bartelsman, M.D., Jan G.P. Tijssen, Ph.D., Peter Speelman, M.D., Ph.D., Marcel G.W. Dijkgraaf, Ph.D., and Josbert J. Keller, M.D., Ph.D.
Results – 81% cured after first infusion and 93.8% after second infusion

13/16 DFI recipients cured after 1st infusion and 15/16 after 2nd

4/13 vanco monotherapy cured

3/13 vanco + PEG lavage cured

Overall cure rate ratio
• DFI to vanco alone
  – 3.05 (99.9% confidence interval 1.08-290.05)
• DFI to vanco + PEG
  – 4.05 (99.9% CI 1.21-290.12)

18 who relapse after vanco alone or vanco/PEG received off protocol DFI – 15 cured (11 with one DFI, 4 with two DFI)
Faecal Microbiota – Improved fecal diversity after FMT

Faecal bacterial diversity assayed using 16s r RNA gene PCR amplification before and after fecal infusion

Improved fecal bacterial diversity 14 days following infusion

- Increased Bacteroides (x 2-4), Clostridia (x 2-4) and Proteobacteria (x~100) absolute numbers after DFI (and species diversity within those genera)

**Figure 3. Microbiota Diversity in Patients before and after Infusion of Donor Feces, as Compared with Diversity in Healthy Donors.**

Microbiota diversity is expressed as Simpson’s Reciprocal Index of diversity in fecal samples obtained from nine patients before and 14 days after the first infusion of donor feces, as compared with their donors. The index ranges from 1 to 250, with higher values indicating more diversity. The box-and-whisker plots indicate interquartile ranges (boxes), medians (dark horizontal lines in the boxes), and highest and lowest values (whiskers above and below the boxes).
First FMT in Singapore - May 2014

NUH treats chronic gut infections with stool transplants

SINGAPORE – Fecal matter and eau are not words usually associated with each other. But doctors here have found a murky way to treat those with chronic gut infections by pumping microbes found in faeces from a healthy donor into a patient.

Earlier this year, doctors performed Singapore’s first faecal microbiota transplant (FMT) under the REANIMATE programme and successfully treated two elderly female patients who were chronic sufferers of *Clostridium difficile* (C-diff) infection.

There has been a significant increase in antibiotic-associated C-diff infection, which we spread and sometimes use antibiotics, National University of Singapore (NUS) said in a press conference. About 20 to 40% of those who suffer from C-diff do not respond to usual treatments and are as high as 1% to 2% per cent.

Antibiotics can kill off the majority of bacteria in the gut, leaving resistant ones behind to multiply. One of which is C-diff, a bacterium that releases toxins that damage the intestinal lining. It can cause increased fluid production, inflamed gut lining and even tears in the intestine, which can lead to severe diarrhoea, abdominal pain or even death in severe cases.

“We started looking at other treatments, like possible new drugs. But in terms of antibiotic development, we are not discovering new antibiotics at the rate that we are reinnovating it,” Dr Chew also quoted. “Stool donors are stringently screened and tests are conducted to assess suitability. NUH is not looking to expand its existing pool of donors at the moment, but patients who wish to have their friends or family members become donors can visit the hospital and be screened.”

Don’t pooh-pooh faeces transplant treatment

Method can potentially be used to treat common ailments but has yet to catch on

By LINETTE LAI

A FAECS transplant may smell – and sound like an odd cure, but it can potentially be used to treat common ailments but has yet to catch on.

Patients who typically require this treatment are those who have been put on strong antibiotics, which kill all about two-thirds of patients respond to conventional treatment methods, and even then, the problem may recur.

While the faecal procedure is common overseas, NUH doctors say it has not caught on here due to the “yuck factor”. For, of course, the first step is to collect stool from donors – which is more complex than it sounds.

Patients must pass a health screening, and only those with no known history of C-diff can be considered. The stool is then processed and given to the patient through an endoscope through gastroscopy or colonoscopy. These microorganisms multiply in the recipient’s colon and help to restore the gut’s ecosystem. It’s a win-win for both parties, with patients feeling better and donors who no longer have to worry about the condition.
Indications for faecal microbiota transplantation

• *C. difficile* infection defined as diarrhea (≥3 loose or watery stools per day for at least 2 consecutive days or ≥8 loose stools in 48 hours) and a positive stool test for *C. difficile* toxin

• Faecal microbiota transplantation can be considered in patients who have had relapsing *C difficile* infection (≥ three episodes or two relapses of *C. Difficile* infection requiring hospitalisation) after adequate courses of antibiotic therapy, including:

  • >14 days of metronidazole or > 14 days of vancomycin

  • FMT may also be considered in patients with refractory CDI, whereby there is inadequate clinical improvement after treatment with first and second-line antibiotics (metronidazole and vancomycin as outlined above)
Contraindications to faecal microbiota transplantation

- Recent chemotherapy
- Human immunodeficiency virus (HIV) infection with a CD4 count of less than 240
- Immunosuppression, such as the prolonged use of prednisolone at a dose of at least 60 mg per day
- Pregnancy
- Use of antibiotics other than for treatment of *C. difficile* infection at baseline
- Need for vasopressor medications for maintenance of normal blood pressure.
- Admission to an Intensive Care Unit.
Faecal Microbiota Transplantation in a Nutshell

Donor Screening
The donor undergoes a stringent screening process to ensure the donor is free from infections and safe for transplant.

Sample Collection
The donor provides samples of his/her faeces and sends it to the hospital.

Sample Processing
The sample is then processed in the lab where the team will remove some solid matters and mix the stool with sterile saline solution to make it liquid.

Transplant into the Recipient-Patient
The liquefied stool is transplanted into the gastrointestinal tract of the recipient-patient with an endoscope, by means of a gastroscope or colonoscopy. Patient remains sedated throughout the procedure.
# Donor Biologic Screening

## Blood Tests

- Cytomegalovirus (IgG and IgM)
- Epstein-Barr Virus (VCA IgM, VCA IgG, VCA, antiEBNA)
- Hepatitis A (total antibodies, and if positive also Hepatitis A IgM)
- Hepatitis B (HBsAg, antiHBs)
- Hepatitis C (anti HCV)
- HIV-1 and HIV-2 (Combined HIV Antigen/Antibody test)
- Human T-lymphotrophic virus types I and II (HTLV) (antibodies)
- Treponema pallidum (EIA total antibody)
- Entamoeba histolytica antibodies
- Strongyloides stercoralis antibodies

## Faecal Tests

- Bacteriological and parasitological evaluation by local standards:
  - Stool culture
  - Ova, cysts and parasites x3
  - Isospora x3 (acid fast)
  - MRSA stool
  - VRE screening
  - CP-CRE screening
  - Giardia antigen
  - Clostridium difficile toxin
  - Rotavirus
  - Cryptosporidium antigen
  - Entamoeba Histolytica antigen
Faecal Processing protocol

Processing
- Approximately 50-60 g faecal material is homogenized in a commercial blender with sterile normal saline solution
- The slurry is passed through a kitchen type stainless steel strainer to remove larger particulate matter
- The resulting material is centrifuged with sterile saline solution
- Sterile pharmaceutical grade glycerol is added to a final concentration of approx. 10%

Storage
- The faecal solution is stored at -80 °C
- Before use the solution is thawed on ice for up to 4 h, and sterile saline is added to a final volume of 250 ml

Quality Control
- One drop of the final thawed suspension is cultured anaerobically, expected to yield heavy mixed growth.
Clear, Colourless and Odourless liquid
Whose Poop to Use?

• Related Donor
  – Less “icky” factor
  – “I’m getting my wife’s stool... part of her now lives in me!”
  – Dysbiosis runs in families and a shared environment

• Unrelated Donor
  – 90-92% vs 70% success rate in CDI eradication; 9% vs 30% recurrence (Hamilton et al)

• Fresh is Better?
  – 92% Fresh vs. 90% Frozen success rate (Hamilton et al)
  – Frozen inoculum via NGT 90% cure rate (Youngster et al)

Top-down or Bottom-up?
The Best Route for Stool Delivery


Lower GIT route found to be more effective than UGIT route in CDI (>85% vs 76%)
Case 2: FMT in an Immunocompromised Host

60 yo male
Myasthenia gravis, ESRD on HD
Myasthenia crisis; complicated by ventilator associated pneumonia and left basilic and subclavian thrombosis
Refractory clostridium difficile infection; WCC 25; diarrhoea 20x/day
Meds: mycophenalate, prednisolone, inotropic support, IVIG
FMT for clostridium difficile infection is safe in immunocompromised patients

- 75 adult and 5 paediatric patients
- Refractory, recurrent and mixed
- HIV, SOT, IBD on immune-modulators
- Overall cure rate 89%
- SAE in 12 patients (15%)
  - 2 deaths; one related to sedation during FMT
  - One mucosal tear
  - 6 unrelated infections or short lived diarrheal illness
  - 3 flare of IBD


Faecal microbiota transplant for recurrent Clostridium difficile infection

Issued: March 2014

NICE intervention procedure guidance 485
guidance.nice.org.uk/ipg485
• An IND application is not currently needed for the treatment of refractory *C. difficile*, provided that the treating physician obtains adequate informed consent from the patient or his or her legally authorized representative for the use of FMT products.

• Informed consent should include at a minimum, a statement that the use of FMT products to treat *C. difficile* is investigational and a discussion of its potential risks.

September 2014
The Future of FMT
The Future of FMT
The Future of FMT
Preliminary Communication

Oral, Capsulized, Frozen Fecal Microbiota Transplantation for Relapsing *Clostridium difficile* Infection

Ilan Youngster, MD, MMSc; George H. Russell, MD, MSc; Christina Pindar, BA; Tomer Ziv-Baran, PhD; Jenny Sauk, MD; Elizabeth L. Hohmann, MD

**OBJECTIVE** To evaluate the safety and rate of resolution of diarrhea following administration of frozen FMT capsules from prescreened unrelated donors to patients with recurrent *C difficile* infection.

**DESIGN, SETTING, AND PARTICIPANTS** Open-label, single-group, preliminary feasibility study conducted from August 2013 through June 2014 at Massachusetts General Hospital, Boston. Twenty patients (median age, 64.5 years; range, 11-89 years) with at least 3 episodes of mild to moderate *C difficile* infection and failure of a 6- to 8-week taper with vancomycin or at least 2 episodes of severe *C difficile* infection requiring hospitalization were enrolled.

**INTERVENTIONS** Healthy volunteers were screened as potential donors and FMT capsules were generated and stored at −80°C (−112°F). Patients received 15 capsules on 2 consecutive days and were followed up for symptom resolution and adverse events for up to 6 months.

**MAIN OUTCOMES AND MEASURES** The primary end points were safety, assessed by adverse events of grade 2 or above, and clinical resolution of diarrhea with no relapse at 8 weeks. Secondary end points included improvement in subjective well-being per standardized questionnaires and daily number of bowel movements.

**RESULTS** No serious adverse events attributed to FMT were observed. Resolution of diarrhea was achieved in 14 patients (70%; 95% CI, 47%-85%) after a single capsule-based FMT. All 6 nonresponders were re-treated; 4 had resolution of diarrhea, resulting in an overall 90% (95% CI, 68%-98%) rate of clinical resolution of diarrhea (18/20). Daily number of bowel movements decreased from a median of 5 (interquartile range [IQR], 3-6) the day prior to administration to 2 (IQR, 1-3) at day 3 (*P* = .001) and 1 (IQR, 1-2) at 8 weeks (*P* < .001). Self-ranked health scores improved significantly on a scale of 1 to 10 from a median of 5 (IQR, 5-7) for overall health and 4.5 (IQR, 3-7) for gastrointestinal-specific health on the day prior to FMT to 8 (IQR, 7-9) after FMT administration for both overall and gastrointestinal health (*P* = .001). Patients needing a second treatment to obtain resolution of diarrhea had lower pretreatment health scores (median, 6.5 [IQR, 5-7.3] vs 5 [IQR, 2.8-5]; *P* = .02).

**CONCLUSIONS AND RELEVANCE** This preliminary study among patients with relapsing *C difficile* infection provides data on adverse events and rates of resolution of diarrhea following administration of FMT using frozen encapsulated inoculum from unrelated donors. Larger studies are needed to confirm these results and to evaluate long-term safety and effectiveness.

*JAMA*. doi:10.1001/jama.2014.13875
Published online October 11, 2014.
Beyond *C Difficile*...
FMT in IBS and IBD

- Irritable Bowel Syndrome
  1. Case reports/series
  2. Mainly in IBS-D, some IBS-C
  3. Reports improvement in BM and reduced abdominal pain
  4. Awaiting results of ongoing 2 RCTs

- Inflammatory Bowel Disease
  1. Case series
  2. Positive outcomes in Ulcerative Colitis
  3. Disappointing for use in Crohn’s Disease
  4. Recent data at DDW 2014 from ongoing RCTs were conflicting

Is there a role for FMT in SpA?
Therapeutic Potential of FMT

- Multiple sclerosis
- Chronic fatigue syndrome
- Non-alcoholic fatty liver disease
- Obesity
- Atherosclerosis
- Idiopathic thrombocytic purpura
- Insulin resistance/ type 2 diabetes mellitus
- C difficile infection
- Irritable bowel syndrome
- Inflammatory bowel disease

Green: beneficial effect FMT in RCT
Blue: beneficial effect FMT in case series
Black: association between gut microtiota and disease from experimental/observational studies

Smits LP. Therapeutic potential of FMT. Gastro 2013
Microbiota and diet/environment

• High intake of fruit and veg a/w with increased diversity
• Marked differences in fecal microbiota between people brought up in African rural villages from those living in Europe
  – Greater diversity with life in rural environment
  – Predominance of Proteovella vs Bacteroides
• Westernised diet (high in saturated fat and sugar)
  – decreases bacterial diversity esp Firmicutes

Ursell et al, Nutr Rev 2012
De Filipo et al, Proct Natl Acad Sci USA, 2010
Albenberg and Wu,Gastroenterology, 2014
Microbiota and diet/environment

• High protein, low carb/fibre diet
  – Decrease butyrate in fecal short chain FA concentrations within 4 weeks
  – Reduction of Firmicutes
  – Long term adherence may lead to increased risk of colonic disease

• Short term dietary changes tend to produce modest, less permanent changes

• If severe (35% calorie reduction for 6 weeks) increases bacterial diversity

• Increasing bacterial diversity shows reduction in serum sensitive CRP

Cotillard et al Nature 2013
Colorectal Cancer (CRC) and polyposis

- Microbiota plays major role in pathogenesis of CRC
- High cancer development in colon and rare in SB
- Similarity of microbiota in CRC and Crohn’s disease
- Reduced diversity in CRC
  - Higher Proteobacteria and lower Bacteroidetes
- Study of mucosa associated microbiota may be better than just studying fecal microbiota
- Well established association between Strep gallolyticus
  - Found in 20-50% of CRC compared with less than 5% of normal colons

Rhodes et al. Colorectal Cancer, 2014
Colorectal Cancer (CRC) and polyposis

- Fusobacterium nucleatum can interact with E-Cadherin and TLRs, esp when dysplasia has occurred and covering mucus layer is lost
- Inflammatory effects on cellular signalling
- Inhibition of apoptosis

Does the microbiome play a role in SpA?

• Microbiome plays major role in educating immune system

• Strongly implicated in IBD
  – Clinical and genetic overlap between IBD and SpA
  – Bowel and joint disease in HLA B27/human beta2 microglobulin transgenic rats

• Mechanism by which HLA B27 influences microbiome still unknown

James T Rosenbaum et al, Clin Rheumatol 2014
Changes in microbiome linked to IBD

Search of IBD and microbiome yielded more than 900 references

- **Microbial composition**
  - Decrease in alpha diversity
  - Decrease in Bacteroides and Firmicute
  - Increase in Gammaproteobacteria
  - Presence of E Coli
  - Presence of Fusobacterium
  - Decrease in Clostridia, Ruminococcaceae, Bifidobacterium, Lactobacillus
  - Decrease in F prausnitzii

- **Microbial Function**
  - Decrease in SCFAs, Butyrate
  - Decrease in butanoate and propanoatemetabolism
  - Decrease in amino acid biosynthesis
  - Increase in auxotrophy
  - Increase in Amino acid tyransport
  - Increase in oxidative stress
  - Increase in type II secretion system, secretion of toxins

Kostic et al, Gastroenterology, 2014
Absence of NLRP6 results in colitis and alteration of intestinal microbiota

- NLRP6 knockout mouse
- Co-housed
- Germ free wild type mouse

Wild type mouse develops inflammation

Elinav et al, Cell, 2011
Where is the evidence that says SpA is microbiome driven?

• Associations between IBD and AS
  – 10-20% of patients with IBD develop sacroiliitis identical to AS
  – >50% of patients with AS have microscopic colonic lesions that look like CD
  – Periphearl arthritis and uveitis are characteristic of both AS and IBD

Bluestone et al, Ann Rheum Dis 1975
Mielans et al, J Rheumatol, 1985
When in doubt look at our genes....

- Large number of overlap genes between IBD and AS
- High proportion involved in mucosal immunity
  - RUNX3, EOMES, TBX21
  - Cytokine receptors IL7R and IL23R
- Key regulators of differentiation and activation of innate lymphoid cells
- Critical components of mucosal immune defenses

Cortes A et al, Nat Gen 2013
Strongest Evidence that microbiome is important in SpA comes from Animal data

Transgenic 33-3 rats
Overexpress HLA B27 and Human beta 2 microglobulin
Develop spontaneous diarrhoea and arthritis

Raised in germ free environment

Colitis and arthritis eliminated

Hammer et al, Cell, 1990
Probiotics can maintain the remission whilst colonisation with other bacteria can induce disease.

Colitis and arthritis eliminated by germ free environment

Probiotics Lactobacillus Rhamnosus GG

Maintain remission

Bacteroides thetaiotamicron
Bacteroides vulgatus

Induce diarrhoea and arthritis

Dieleman et al, Gut, 2003
Another example of a mouse model....

SKG mouse model
Features of both SpA and IBD
Shows marked involvement of IL23 dependent immunological pathways

Germ free environment

Unaffected

• Relevance from mouse models will need to be demonstrated specifically in humans
What are the plausible hypotheses out there?

- HLA molecules alone or together with other AS genes determine the microbiome
- HLA B27 transgenic Lewis rats
  - AS features with no colitis
  - Key differences in gut microbiota compared to wild type
  - 16s rDNA sequencing and short fragment sequencing

Unpublished data by Lin, Asquith, Taurog et al

Advances in technology have lowered the cost of next generation sequencing
Does HLA B27 dictate an immune response to an autoantigen?

• No autoantigen identified to explain pathogenesis of AS
• B27 dimerizes on cell surface and can trigger NK cell response
• HLA B27 within cytosol is relatively unstable and can trigger series of events – Unfolded protein response

Could above mechanisms cause alterations in microbiome?

Taurog et al, J Rheumatol, 2010
If HLA molecules shape the microbiome, how could this result in disease?

- Changes in microbiome leads to change in gut permeability
- Leakage of multiple bacterial products which could be arthrogenic

But which comes first? Change in permeability or change in microbiome???

HLA B27 transgenic rat shows increased intestinal permeability
Also demonstrated in patients with AS

Schepens et al, J Nutr 2009
Martinez- Gonzalez et al, Br J Rheumatol 1994
Vaile et al, J Rheumatol 1990
SpA patients have altered microbiota and immune responsiveness to enteric organisms

Methods:
25 children with ERA and 13 controls
Stool and blood collected
16S rDNA sequencing performed
IgA and IgG ELISAs performed on select species of bacteria

Conclusion:
Decreased F. prauznitzii in stools of patients with ERA compared to controls (3.8% vs 10% P=0.008)
Bacteroides and A. muciniphila identified as associative agents in subsets of ERA patients

Differences in the humoral responses to these bacteria may contribute to disease

Stoll et al, Arthritis Research and Therapy, 2014
Discrete Microbial signature of terminal ileum of patients with AS compared to healthy controls

Methods:
Microbial profiles for terminal ileum biopsy specimens compared between AS and controls
Using 16s ribosomal RNA gene sequencing and analysis techniques

Results:
Terminal ileal microbial communities differ significantly (P<0.001) from those in healthy subjects

Driven by higher abundance of 5 families of bacteria and decrease in abundance of 2 families of bacteria

Costello et al, Arthritis and Rheumatology, 2015
Take home messages

• Microbiome is integral to health and disease
• More work needed to confirm how HLA B27 alters the gut microbiome
• Which microbiome population is most important and its function is a mystery
• Understanding microbiome will lead to novel insights into diseases such as IBD and AS
• Is there a role for FMT in SpA?