The role of Gut Microbiota in Allergic Disease

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VÄSTERBOTTENS LÄNS LANDSTING

Disclosures

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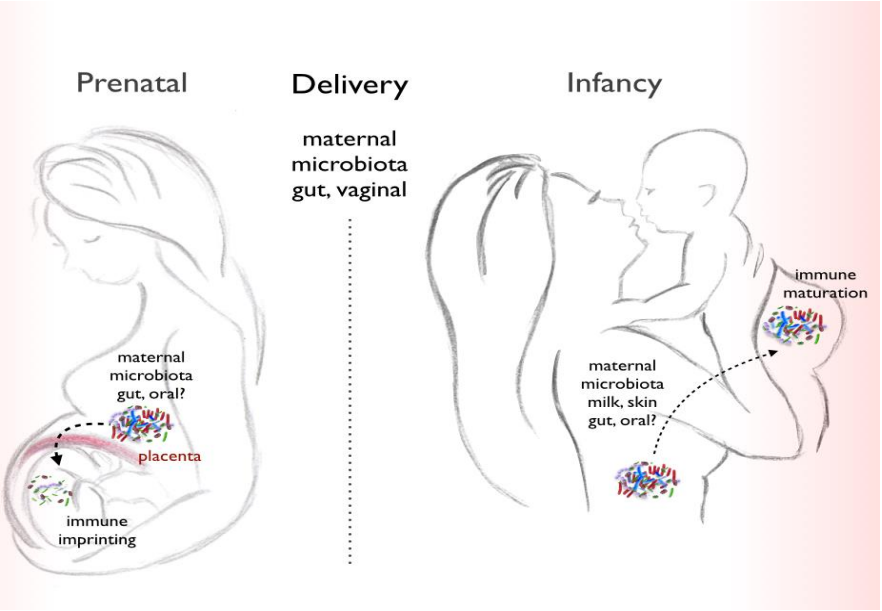
I receive royalties from UptoDate

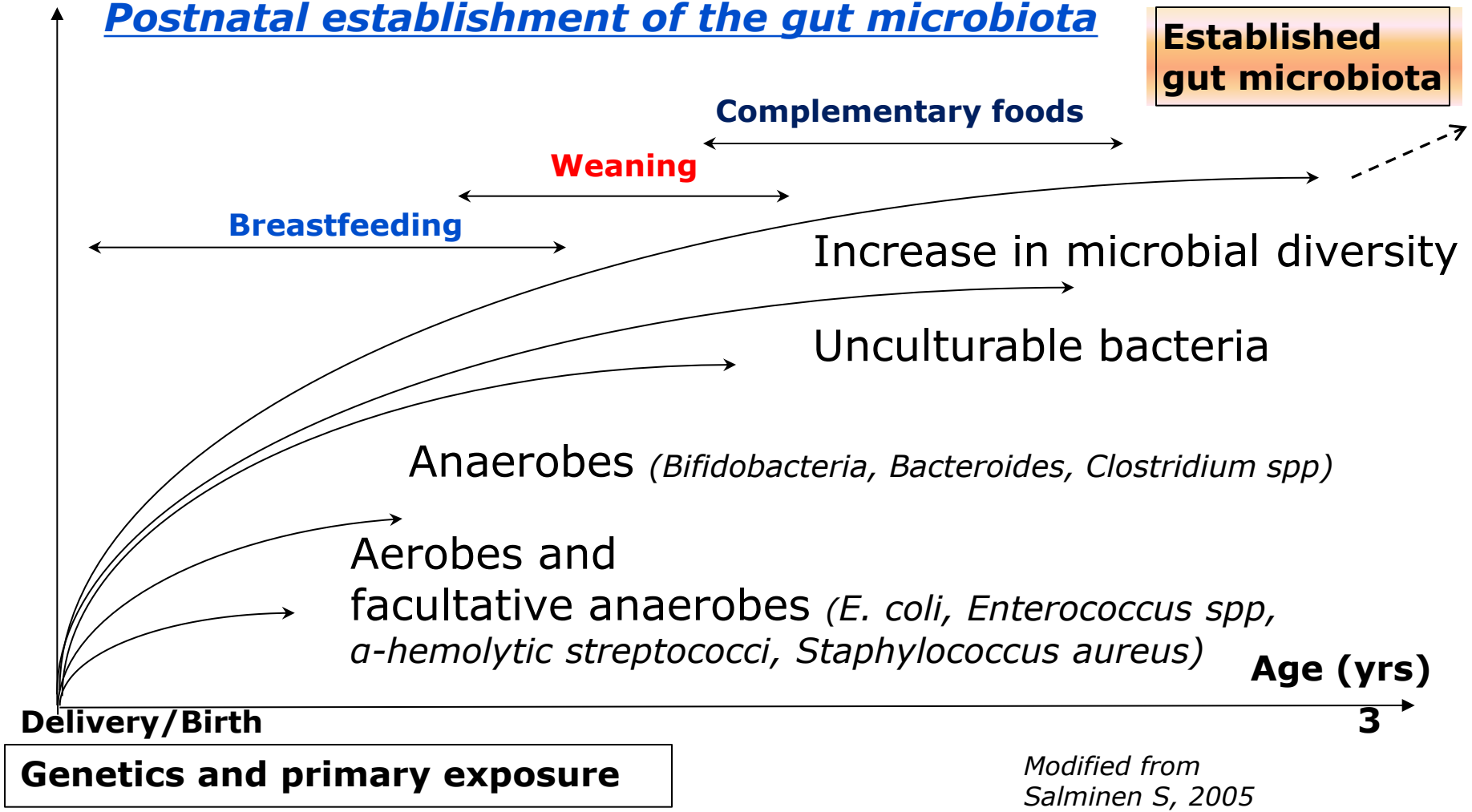
- **Development of early life gut microbiota and immune tolerance**
- **Dysbiosis in early life microbiota and allergic diseases**
- **Current evidence on pre/pro/synbiotics for allergy management**
- **Results from the ASSIGN trial**

Microbial imprinting may start *in utero*

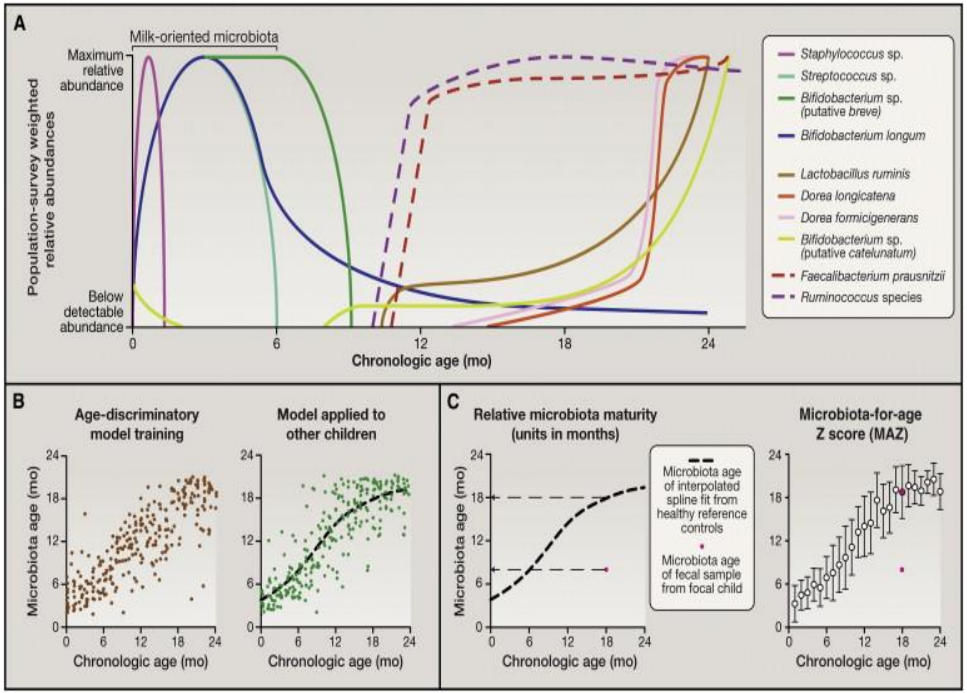
- DNA from bacterial taxa in the feto-placental unit
- Microbes may travel via the blood stream from the oral cavity to the placenta
  (Aagard et al, Sci Transl Med 2014)
- Translocation over the gut epithelium also suggested
- Transmission of administered labeled bacterial strains from the mother to her offspring in mice *(Jimenez et al, Res Microbiol 2008)*



West CE, Jenmalm MC, Kozyrskyj AL, Prescott SL, Expert Rev Clin Immunol 2016

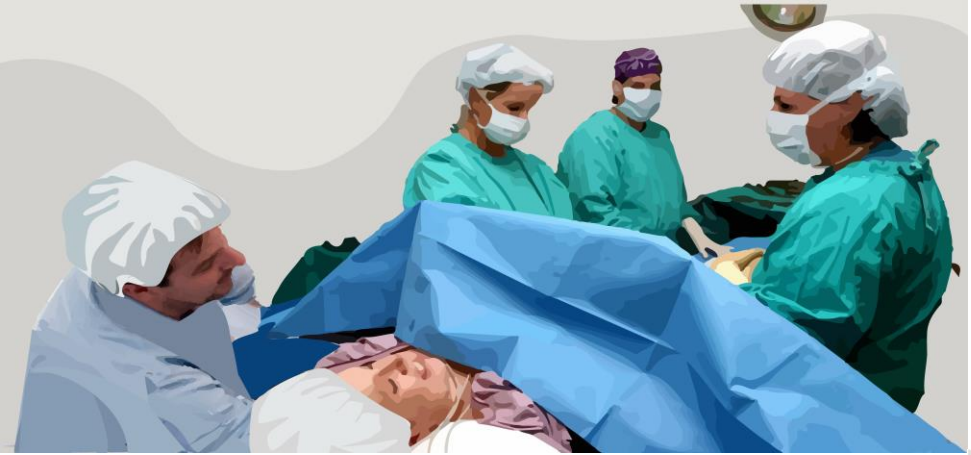

Postnatal establishment of the gut microbiota
Established gut microbiota
Complementary foods
Weaning
Breastfeeding
Increase in microbial diversity
Unculturable bacteria
Anaerobes (Bifidobacteria, Bacteroides, Clostridium spp)
Aerobes and facultative anaerobes (E. coli, Enterococcus spp, α-hemolytic streptococci, Staphylococcus aureus)
Age (yrs)
Delivery/Birth
3
Genetics and primary exposure
Modified from Salminen S, 2005

Predictable microbiome development



- The early gut microbiota abundant in bifidobacteria
- Microbes that characterize early stages of development are more capable of metabolizing nutrients associated with breast feeding
- Later stages have a gut microbiota enriched in genes that can help to digest solid foods

Subramanian S et al. Cell 2015; 161:36-48.



Caesarean section delivery

↑ *Staphylococcus*
↑ *Clostridium difficile*
↓ *Bacteroides*
↓ Diversity

Dominguez-Bello et al, Proc Natl Acad Sci USA 2010;107:11971–11975.
Jakobsson H, Andersson AF et al, Gut 2014;63:559–566.
Bäckhed F, Wang et al, Cell, Host and Microbe 2015;17:690–703.

Vaginal delivery

↑ *Lactobacillus*
↑ *Prevotella*
↑ *Sneathia*

•Caesarean section delivery associated with increased risk of allergic and autoimmune disease *(Kristensen K et al, JACI 2016;137:587-90)*

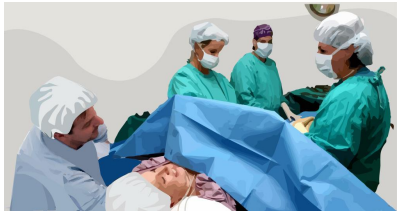
Gut microbiota changes faster than our immune system can adapt to

Maternal gut
Bifidobacterium
Bacteroides
E. coli

Breastfeeding, vagina
Lactobacillus
Streptococcus

Skin
Staphyloccocus

Environment/food
Clostridium difficile
Klebsiella
Enterobacter



Infant gut

Adlerberth I, Wold AE. The establishment of the gut microbiota in Western infants. Acta Paediatr. 2009; 98:229-238.

Johnson CL, Versalovic J. The human microbiome and its potential importance to Pediatrics. Pediatrics 2012; 129:950-960.

Homeostasis

- Balance between the gut microbiota and the host
- Major functions of gut microbiota
  - *Metabolic*
    - Fermentation of non-digestible dietary residue
  - *Trophic*
    - Gut integrity
    - Development of the immune system
  - *Protective*
    - Protects against pathogens

Dysbiosis

- Gut microbial imbalance
  - *May impact both composition and function*
- Dysbiosis has been associated with development of allergic diseases in infants and children

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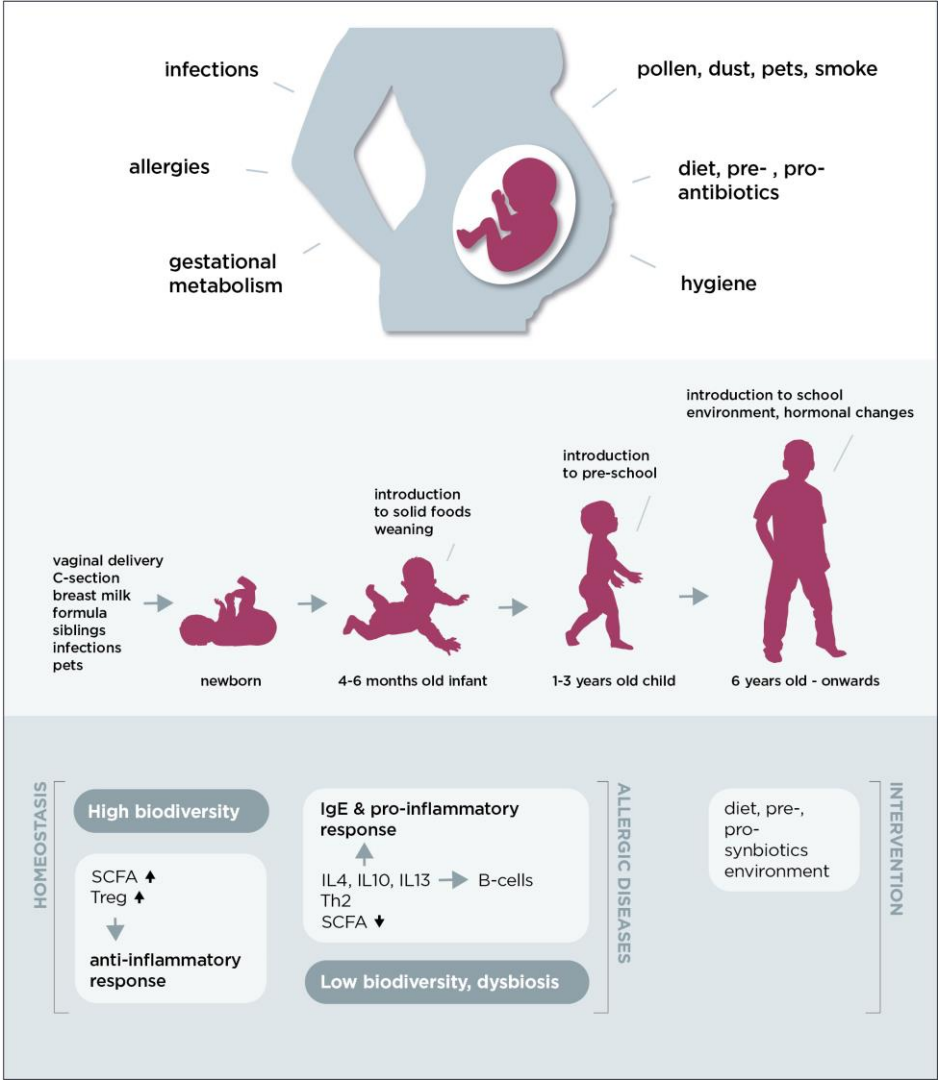
West CE, Renz H, Jenmalm MC et al. The gut microbiota and infammatory non-communicable diseases: Associations and potentials for gut microbiota therapies. JACI 2015;135:3-13.



- Microbiota establishment is influenced by <u>diet</u> and <u>environmental exposures</u> perinatally and in childhood
- Parallells the development of innate and adaptive immune pathways
- High biodiversity
  - Short-chain fatty acid production and induction of Treg cells
- Low biodiversity/dysbiosis
  - IgE production and pro-inflammatory responses

Simonyte Sjödin K, Vidman L, Rydén P, West CE. Emerging evidence of the role of gut microbiota in the development of allergic diseases. Curr Opin Allergy Immunol, 2016;16:390-5.

Dysbiosis in allergic disease

- Reduced relative abundance (RA) and α-diversity of Bacteroidetes in infancy before onset of IgE- associated eczema and asthma

  Abrahamsson, Jenmalm et al JACI 2012 and Clin Exp Allergy 2014

- Reduced RA of *Ruminococcaceae* at 1 yr, 5 times more likely in food-sensitized infants in the CHILD cohort study

  Azad, Kozyrskyj et al Clin Exp Allergy 2015

- Reduced RA of *Ruminococcaceae* at 1 week preceded onset of IgE-ass eczema; the RA of *Ruminococcus* was inversely correlated with inflammatory responses

  West, Prescott et al, Clin Exp Allergy 2015

Toll-like receptors (TLRs)- ancient ”gate-keepers” in innate immunity

- Expressed on epithelial and endothelial cells, leukocyte subsets in blood
- Sense conserved structural components of microbes
- T regulatory cells (Tregs) recently shown to express TLRs
- TLR activation can increase or decrease the suppressor activity of T regulatory cells, thus providing an important link between innate and adaptive immunity

Innate immune responses in allergic disease

Increased inflammatory responses following Toll-like receptor (TLR)-activation

Tulic JACI 2011;122:391

Immature T-helper 1 (Th1) function

Tulic JACI 2011;122:391

Immature T regulatory function

Smith JACI 2008;121:1460
Schaub JACI 2008;121:1491

Atopic mothers

IgE-associated eczema

6 mo: PBMC cultured with specific microbial ligands for TLR2 (Gram+) and TLR4 (Gram -)

3rd trimester **1 week** **1 mo** **12 mo** **2.5 yrs**

Atopic mothers

Not IgE-sensitized No eczema

West CE, Rydén P, Lundin D, Engstrand L, Tulic MK, Prescott SL. Gut microbiome and innate immune response patterns in IgE-associated eczema. Clin Exp Allergy. 2015;45:1419-29.

Lower α-diversity of Bacteroidetes

At 1 week: Reduced relative abundance of *Ruminococcaceae*

Inverse relationship Ruminococcus

and TLR-2 induced

TNF-α (rs=-0.597)

IL-6 (rs=-0.567)

West CE, Rydén P, Lundin D, Engstrand L, Tulic MK, Prescott SL. Gut microbiome and innate immune response patterns in IgE-associated eczema. Clin Exp Allergy. 2015;45:1419-29.

Lower abundance of *Ruminococcaceae* in food sensitization and IgE-associated eczema

A reduction of potentially immune-modulatory bacteria (ruminococci) is associated with aberrant innate immune responses and increased risk of atopic eczema

West et al, 2015

A reduction in the abundance of *Ruminococcaceae* at 1 yr, was 5 times more likely in food-sensitized infants, independently of

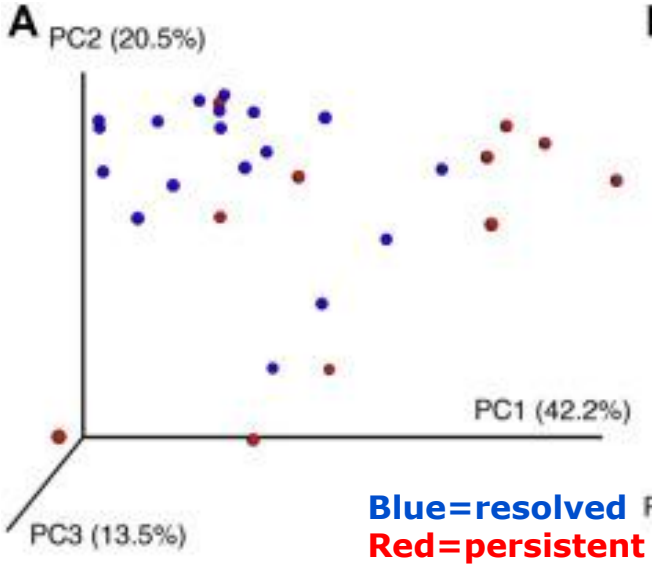
- Delivery mode
- Breastfeeding
- Anitibiotics exposure

Azad et al, 2015

- *Ruminococci produce bacteriocins, e.g. Ruminococcin A, which can inhibit the development of Clostridium species*
- *Ruminococci can degrade fiber, increase SCFA production*

Early gut microbiome composition associated with resolution of CMA

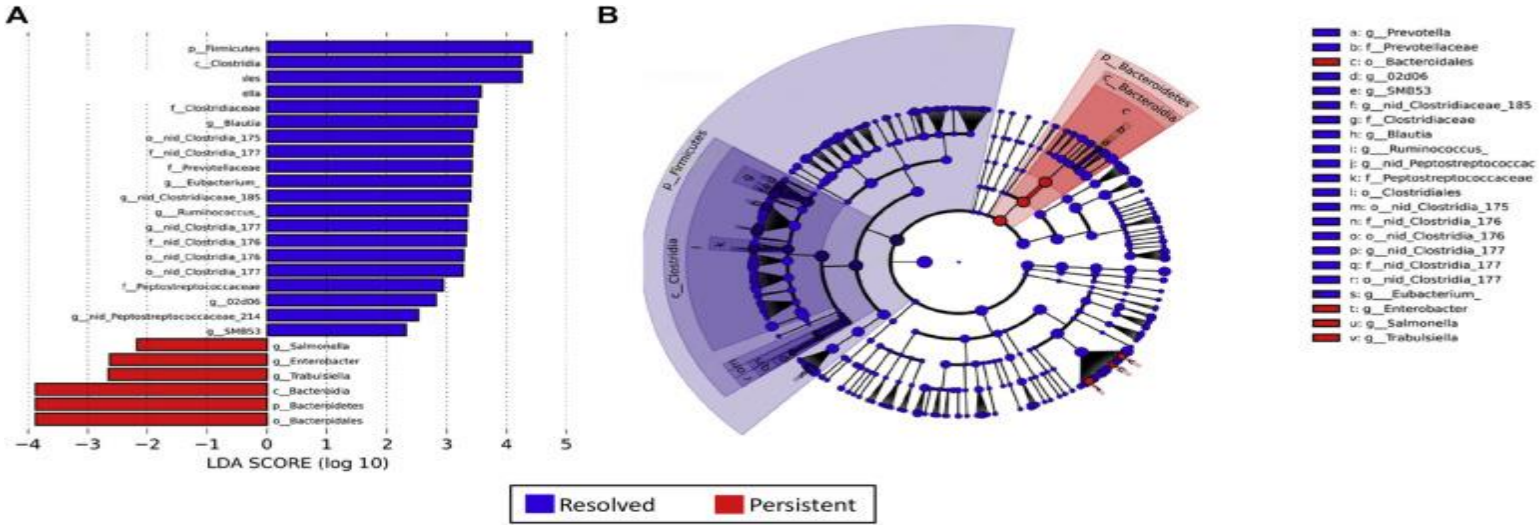
- Consortium of Food Allergy observational study (N=226 children, enrolled in infancy)
- CMA (IgE-mediated)
- Stool sampled at entry
- Followed until 8 years
- Milk allergy resolved in 128 children (56.6%) by 8 years of age



Supinda Bunyavanich, Nan Shen, Alexander Grishin, Robert Wood, Wesley Burks, Peter Dawson, Stacie M. Jones, Donald Y.M. Leung, Hugh Sampson, Scott Sicherer, Jose C. Clemente

Early-life gut microbiome composition and milk allergy resolution

Journal of Allergy and Clinical Immunology, Volume 138, Issue 4, 2016, 1122–1130 http://dx.doi.org/10.1016/j.jaci.2016.03.041



Distinct gut microbiome composition associated with milk allergy resolution. Taxa within Clostridia and Firmicutes were enriched in children sampled at age 3 to 6 months with milk allergy resolution versus milk allergy persistence by age 8 years.

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Early-life gut microbiome composition and milk allergy resolution

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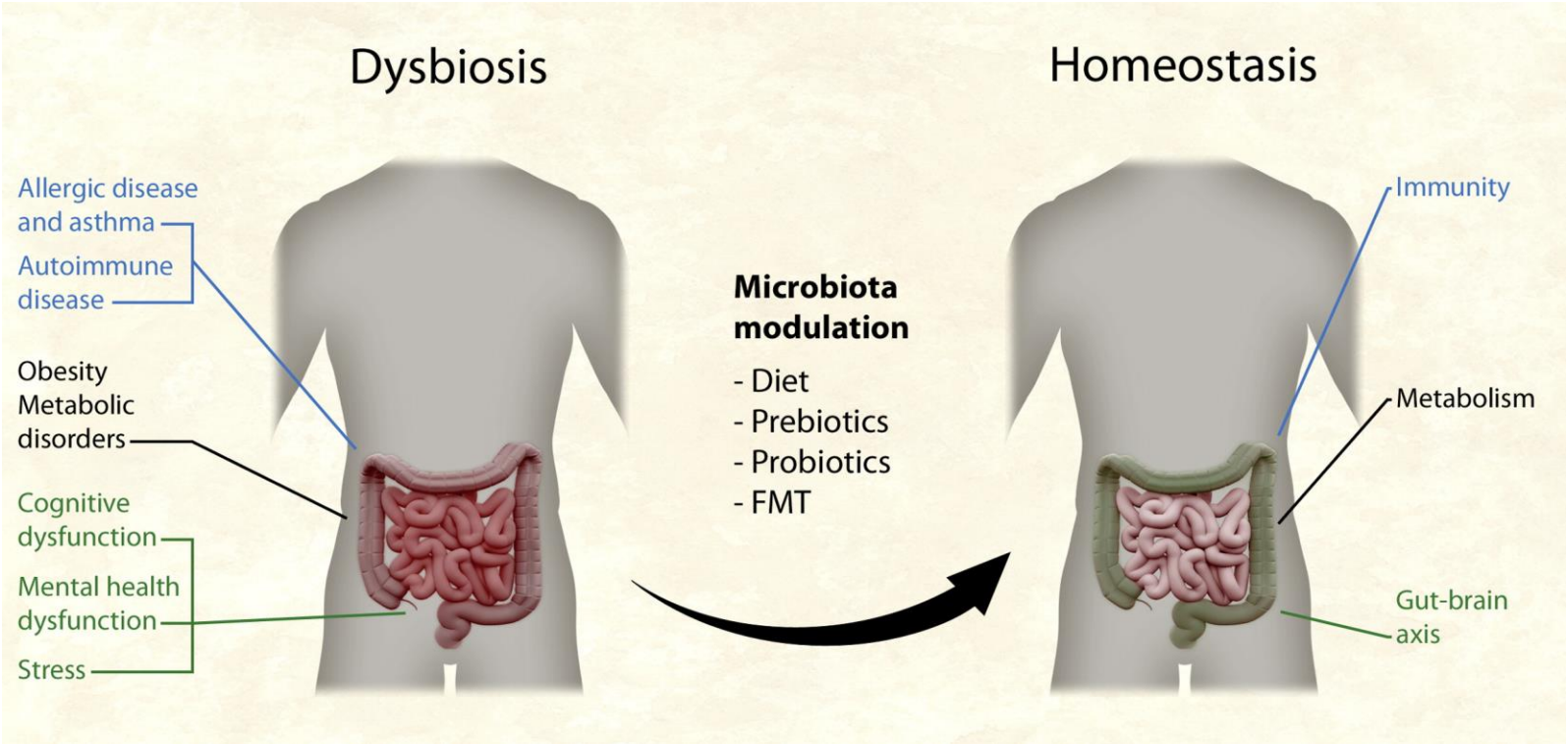
Dysbiosis in non-communicable diseases



West CE, Renz H, Jenmalm MC et al. The gut microbiota and infammatory non-communicable diseases: Associations and potentials for gut microbiota therapies. JACI 2015;135:3-13.

Definitions

- **Prebiotics**- “A dietary prebiotic is a selectively fermented ingredient that results in specific changes, in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health” (ISAAP 2008)

  Commonly used prebiotics in infant formula: galacto- and fructooligosaccharides and their combination 9:1

Prebiotics

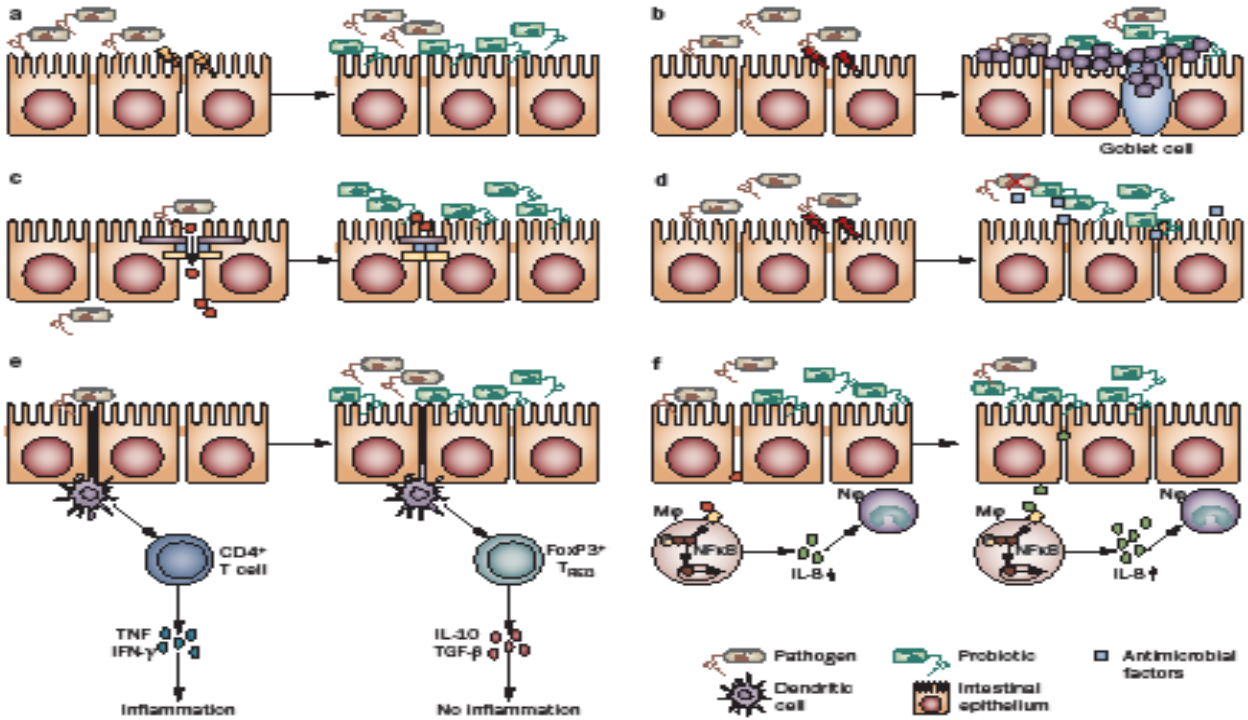
- Oligosaccharides are the third largest fraction in human milk and confer many potential benefits in breastfed infants
- Current commercially available prebiotics are less complex
- Prebiotics have the potential to promote gut colonization with bifidobacteria; some studies have demonstrated immune-stimulating effects
- Increases the production of short-chain fatty acids, with nutritive and anti-inflammatory effects

West CE. Prebiotics in infancy and childhood: clinical research warranted. Br J Nutr, 2011.
Frei R et al. Prebiotics, probiotics, synbiotics, and the immune system: experimental data and clinical evidence. Curr Opin Gastroenterol, 2015.

Definitions

- **Probiotics** ”Live microorganisms, which when administered in adequate amounts confer a health benefit on the host” (FAO/WHO 2002)

Commonly used probiotics are strains of bifidobacteria and lactobacilli



Probiotics- ”live microorganisms which, when administered in adequate amounts confer a health benefit on the host” WHO/FAO

Gareau MG, Sherman PM, Walker WA. Probiotics and the gut microbiota in human health and disease. Nat Rev Gastro-enterol Hepatol 2010; 7:503-14.

a) Block pathogen entry b) Create a mucus barrier
c) Maintain intestinal integrity d) Produce antimicrobial factors
e) Stimulate innate immune system via dendritic cells
f) Stimulate/dampen innate immune response

Synbiotics

- Synbiotics- combination of pre- and probiotics
- Anticipated to have more global effects on colonization
- Meta-analysis eczema management (six studies, 369 children): SCORAD -6.56 (95% CI -11.43 to -1.68; P=0.008)
- "the effect most evident in children >1 yr and if the synbiotic contained a mix of bacterial strains"

Chang YS, et al. Synbiotics for Prevention And Treatment of Atopic Dermatitis: A Meta-analysis of Randomized Clinical Trials. JAMA Pediatr. 2016;170(3):236-242.

Current recommendations

International expert bodies do not generally recommend pre- pro- or synbiotics for management or prevention of allergic disease

Probiotics: ”there is a likely net benefit from using probiotics resulting primarily from prevention of eczema” in infants at high risk of allergic disease *WAO guideline panel 2015*

Prebiotics: ”suggests using prebiotic supplementation in not-exclusively breastfed infants and not using prebiotic supplementation in exclusively breastfed infants” for allergy prevention
WAO guideline panel 2016

Recommendations conditional, based on very low certainty of the evidence

Significant heterogeneity between studies

- **The gut microbiota develops to a complex and diverse ecosystem during the first years of life**
- **Aberrant intestinal colonization and reduced biodiversity have been associated with allergic diseases**
- **Pre- pro- and synbiotics have the potential to promote healthy gut colonization and immune maturation in allergic disease**
- **To date, significant heterogeneity between conducted studies precludes general recommendation on their use**

SYNBIOTICS ARE HYPOTHESISED TO PLAY A ROLE IN MANAGEMENT OF ALLERGIC DISEASE

Studies suggest eHF + probiotics and/or prebiotics may have beneficial effect in allergy management *(Berni Canani 2012, Gruber 2010, Van de Aa 2010 & 2011)*

Very limited data regarding Non-IgE mediated allergic infants receiving AAF

Aim: Clinical study to assess the effect of an Amino Acid based Formula (AAF) with a synbiotic blend on gut microbiota and clinical symptoms in suspected GI Non-IgE mediated CMA

- Control product: Commercially available AAF
- Test product: AAF + synbiotics*; mixture of short and longchain fructo-oligosaccharides, *Bifidobacterium breve* M-16V

**Michaelis et al. 2016*

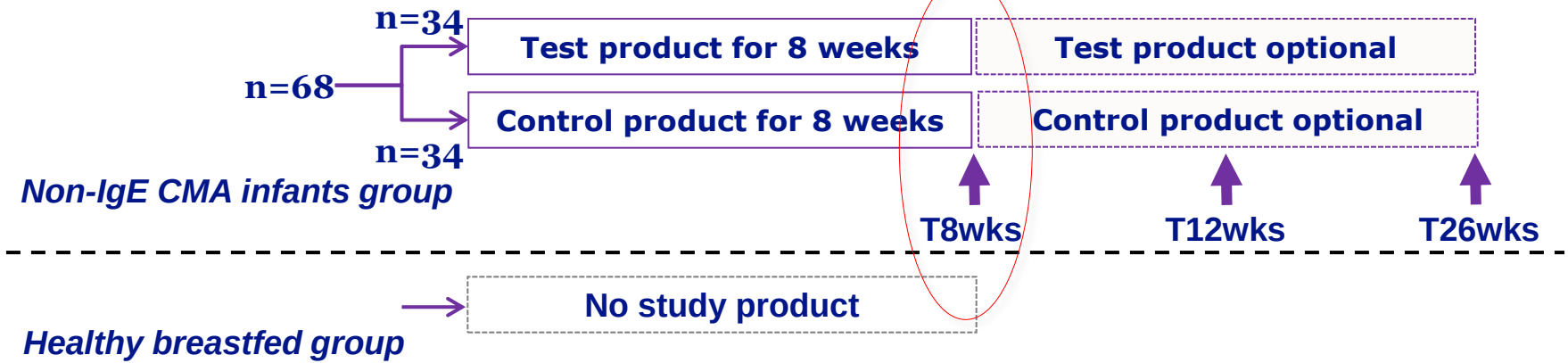
Previous studies including the specific synbiotic ingredients

Two double-blind randomized controlled clinical trials evaluated safety and growth (AAF vs AAF with synbiotics) and hypoallergenicity

Subjects	N	Study length	Conclusions AAF with synbiotics	Ref.
Healthy infants full term (0 - 15 days)	**115**	**16 weeks**	- Safe - Adequate growth in healthy infants	Harvey et al. 2014
CMA infants (0-8 months) confirmed IgE and/or non-IgE mediated	**110**	**16 weeks**	- Safe - Adequate growth in CMA infants - Suitable for management of CMA - Exploratory outcome suggests less reported infections, less antibiotics	Burks et al. 2015
CMA infants/children (0-3years) confirmed IgE with DBPCFC	**30**	**7 days** As per American Academy of Pediatrics criteria	- Hypoallergenic (no allergic reaction to AAF with specific synbiotic ingredients in 30 CMA infants)	Harvey et al. 2014

ASSIGN – CLINICAL study

Confidential



Methods:

- DB-RCT, 68 non-IgE mediated CMA infants (0-13 months) 8 wks intervention

Safety:

- Adverse events, medication use, growth

Primary parameters:

- Faecal bifidobacteria and *E. rectale / C. coccoides* group (FISH)

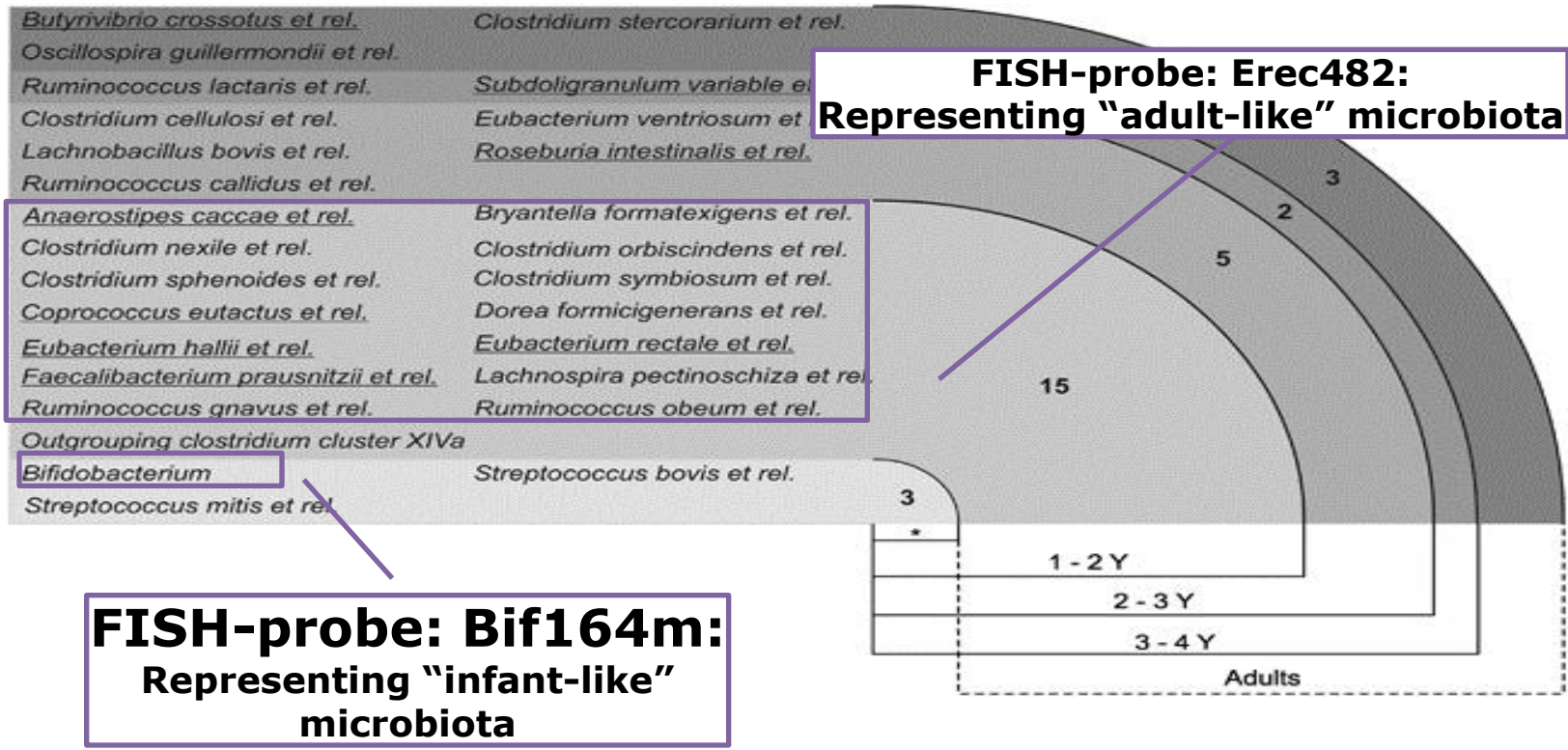
Secondary parameters:

- Stool characteristics, gut immune health markers

Exploratory parameters:

- Sequencing of faecal bacteria, clinical symptoms (SCORAD + parent reported, follow-up at 12 & 26 wks

Development of core microbiota – A gradual transition from infant to adult-like



Cheng et al., ISME J. 2016;10(4):1002-14

Demographics - Intention to treat

	Test (N = 35)	Control (N = 36)	Total CMA (N = 71)	Healthy subjects (N = 51)
Age at baseline (months)				
Mean (SD)	5.67 (3.24)	6.33 (2.71)	6.00 (2.98)	7.84 (3.25)
Min - Max	1.8 - 12.8	1.2 - 11.6	1.2 - 12.8	2.6 - 14.2
Sex (%)				
Female	28.6	25.0	26.8	45.1
Male	71.4	75.0	73.2	54.9
Race (%)				
Asian	5.7	2.8	4.2	0.0
Black	2.9	0.0	1.4	0.0
Caucasian / White	88.6	88.9	88.7	92.2
Combination Of Above / Other	2.9	8.3	5.6	7.8
Mode of delivery (%)				
Caesarean section	20.0	41.7	31.0	13.7
Vaginal	80.0	58.3	69.0	86.3
Country of residence (%)				
Belgium	17.1	13.9	15.5	0.0%)
United Kingdom	60.0	69.4	64.8	29.4
Italy	17.1	13.9	15.5	11.8
Sweden	5.7	2.8	4.2	58.8

N is number of subjects. Denominator for % is number of subjects in treatment group with non-missing data.

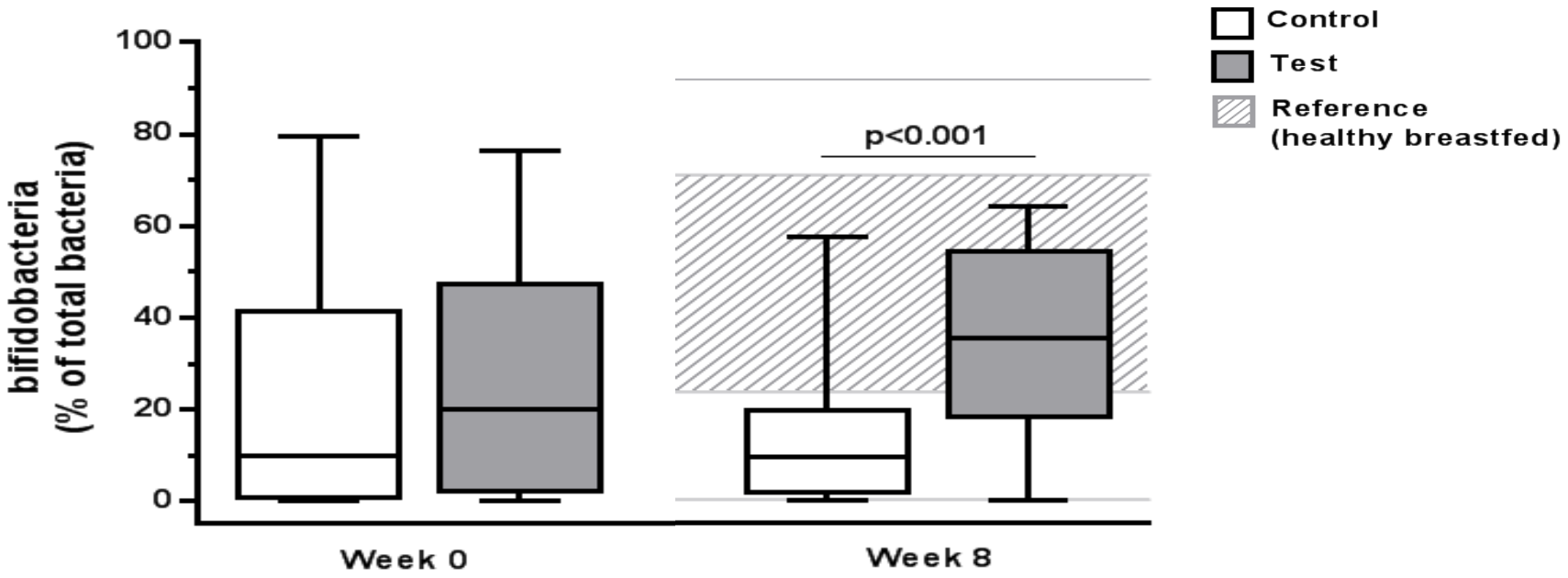
PI ASSIGN: Dr. Louise Michaelis

Consultant Paediatrician in Immunology and Allergy

Great North Children's Hospital, UK

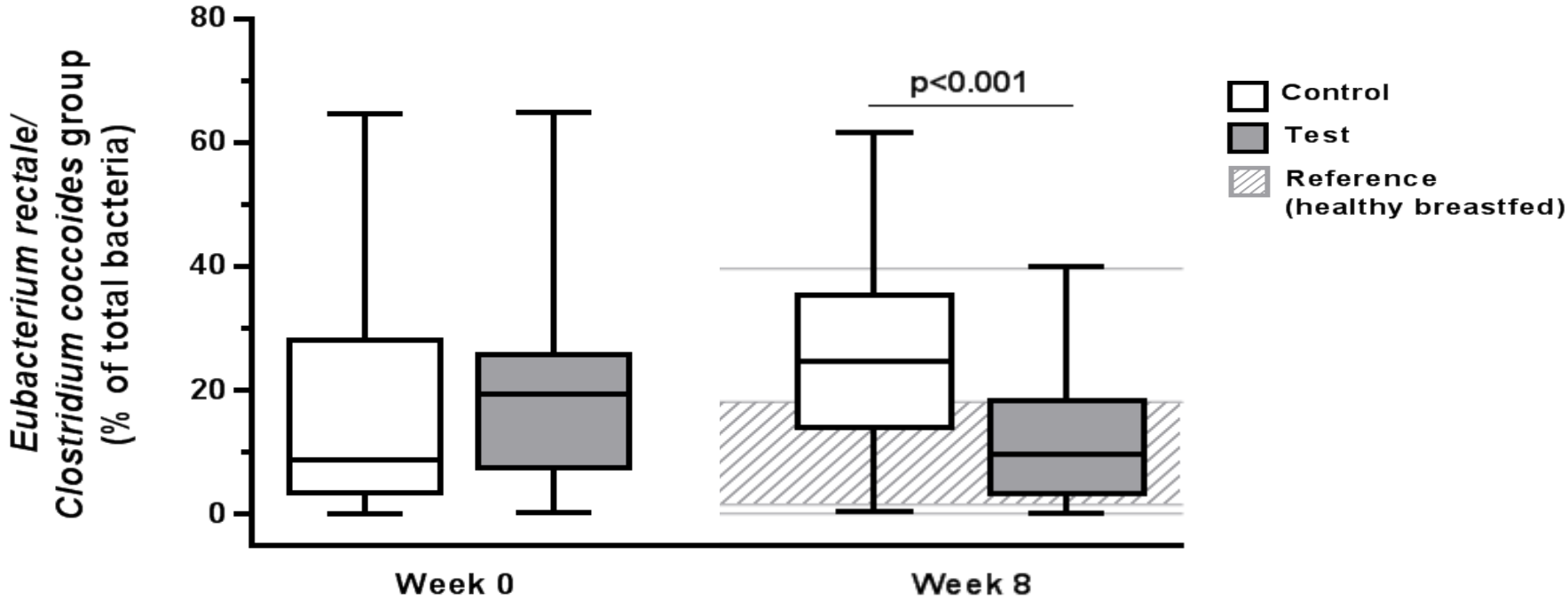
Primary outcomes in intention to treat analysis (ITT)

Increased level of – infant-like – Bifidobacteria



Statistics are based on ANCOVA comparing Test vs Control with Week 8 values as outcome, stratification factor (skin or gastrointestinal symptoms) and imputed baseline values as covariate and treatment as fixed effect.

Decreased level of – adult-like –
E. Rectale / C. coccoides group



Statistics are based on ANCOVA comparing Test vs Control with Week 8 values as outcome, stratification factor (skin or gastrointestinal symptoms) and imputed baseline values as covariate and treatment as fixed effect.

ASSIGN – Conclusions

- The investigated AAF with synbiotics is safe and suitable for dietary management of infants with suspected Non-IgE mediated CMA

- Primary outcome met: Eight weeks use of AAF with specific synbiotics results in:
  - ✓ Increase of infant like bifidobacteria
  - ✓ Decrease of adult like *E. rectale / C. coccoides* group
  - ✓ Bifidobacteria and *E. rectale / C. coccoides* levels in the test group are close to levels seen in age matched healthy subjects

- **Further analysis** ongoing for wk12 and wk26 clinical and fecal data; Wk 8 in depth microbiota (by sequencing method)

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University of Western Australia

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CAIR research team

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Question & Answer Section

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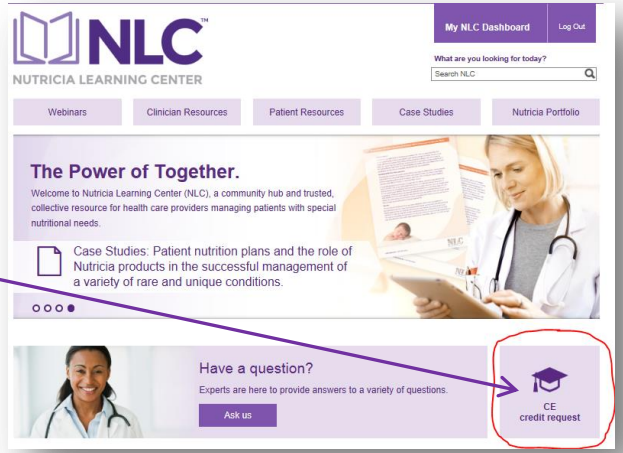

EVENT CODE: **GWRGM1**

EVENT DATE: 11/22/2016