Maternal FUT2 (Secretor) Status Influences Gut Microbial Communities of Breast-fed Infants

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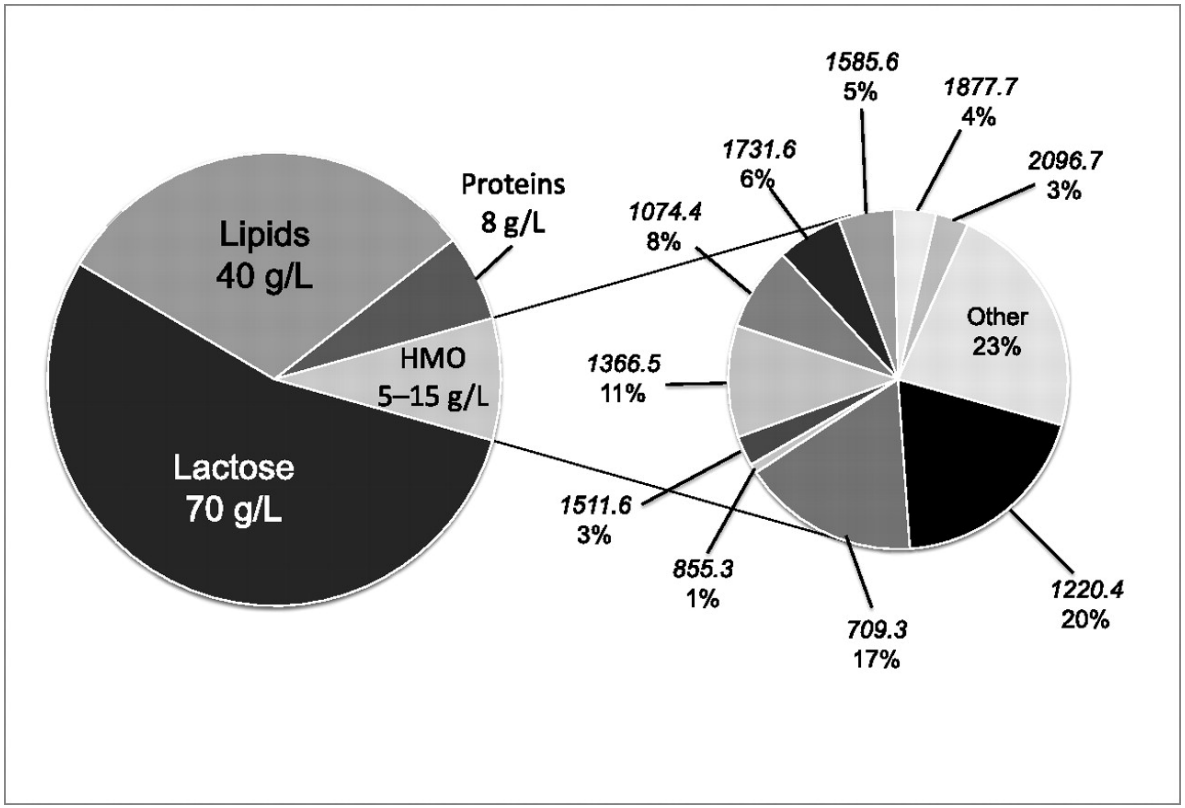
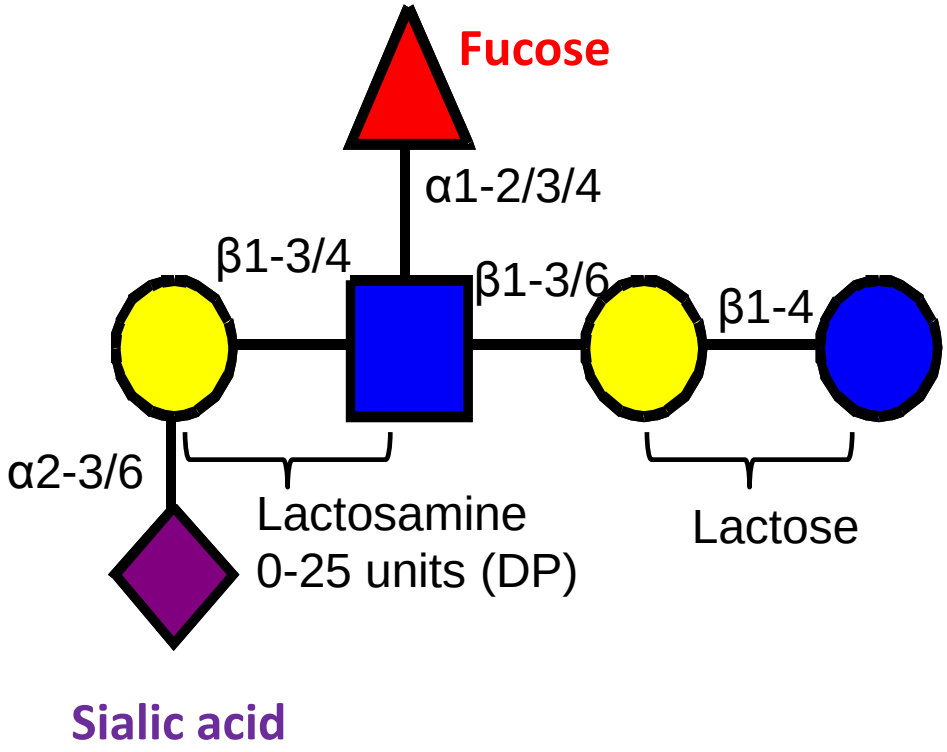
Components of breast milk: The result of millions of years of mammalian evolution



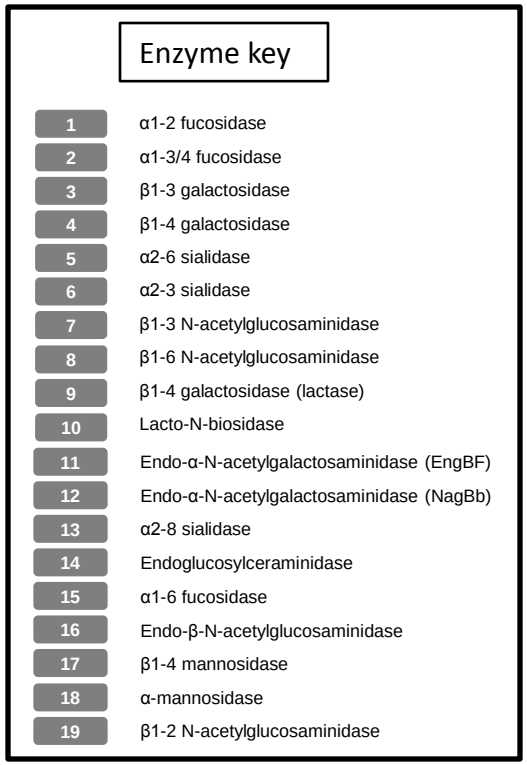
Zivkovic A M et al. PNAS 2011;108:4653-4658

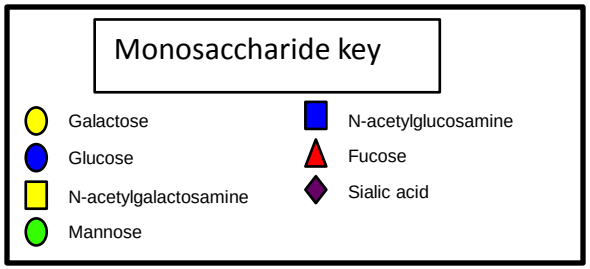
Human Milk Oligosaccharides

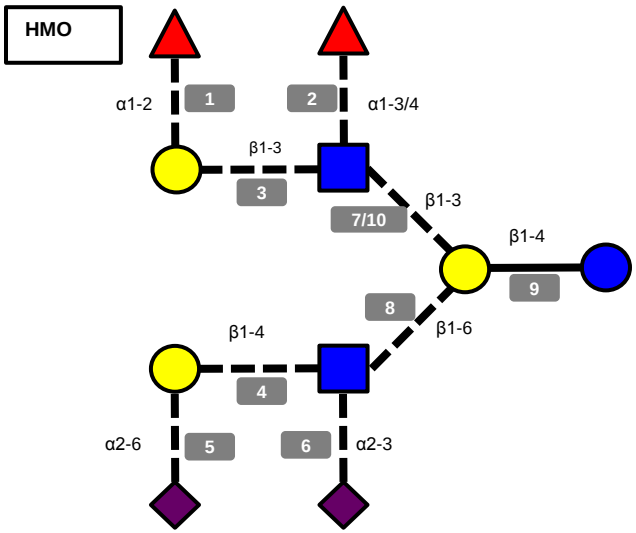
- Complex structure
- Not metabolized by the infant
- Arrive intact in the colon
- Significant nutrient for the gut microbiota

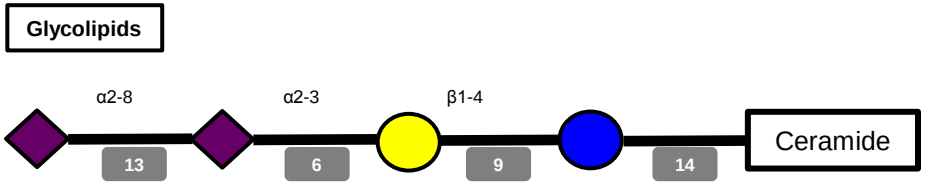


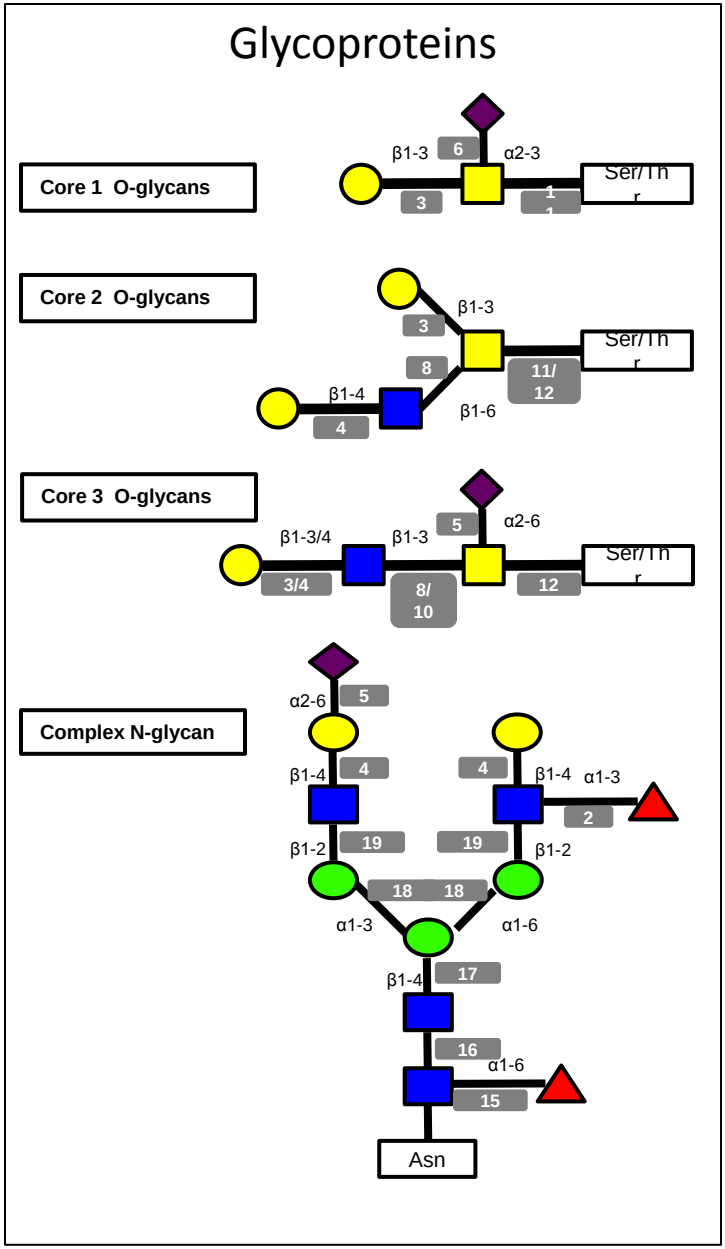
Human milk glycans (HMGs) are diverse in structure





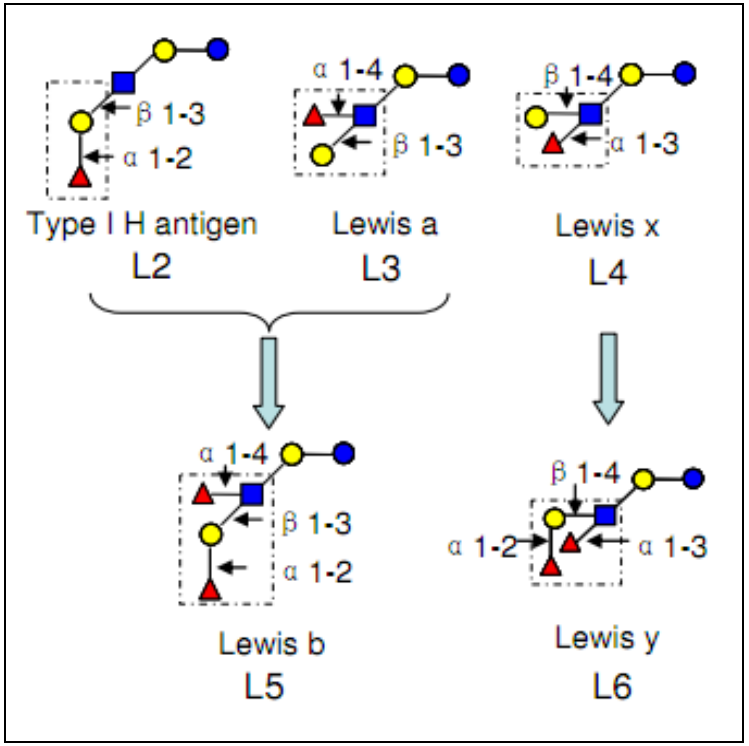






Human Fucosyltransferase (FUT) Genes Add Fucose to HMGs

- FUT2 ("Secretor" gene)
  - Produces the 2' fucosylated precursor to the A, B, H, and Lewis b antigens in secretions, including breast milk
  - 20% of U.S. population are non-secretors
- FUT3 ("Lewis" gene)
  - Fucosyltransferase that is able to make both 3' and 4' linkages, depending on whether it is acting on a type I or type II oligosaccharide (whether the GlcNAc→galactose linkage is β1-3 or β1-4)
  - 8% of U.S. population is Lewis recessive
- Other FUT genes (11 Total)
  - Not involved in HMO glycosylation?



Lewis blood types, from Wu S. et al. Oligosaccharides with an alpha 1-2 fucose linkage are secretor +. Red triangle=fucose. Yellow circle=galactose, blue square=GlcNAc, Blue circle=glucose

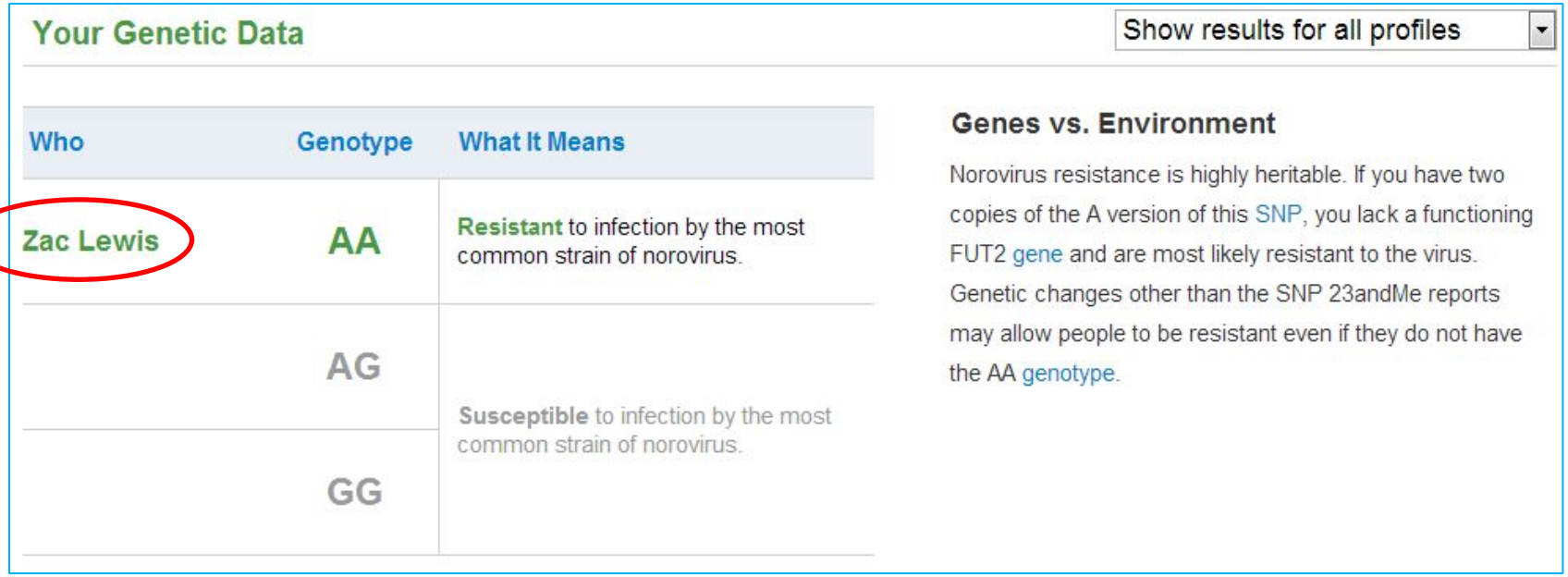
Effects of having a non-secretor FUT2 genotype: Evolutionary trade-offs

"Pros"

- Norovirus resistant
- HIV resistant
- Lower risk of *H. pylori* infection
- Reduced angiogenisis (implicated in rheumatoid arthritis and tumor growth, but also in wound healing)

"Cons"

- Higher risk of *E. coli* UTI's
- Higher risk of flu virus infection
- Higher risk of rheumatic fever
- Higher risk of cholera
- More susceptible to Crohn's disease
- Higher risk of Type 1 Diabetes
- Breast-fed infants of non-secretor mothers more susceptible to *Campylobacter* infection



Human milk glycans (HMGs) are diverse in structure

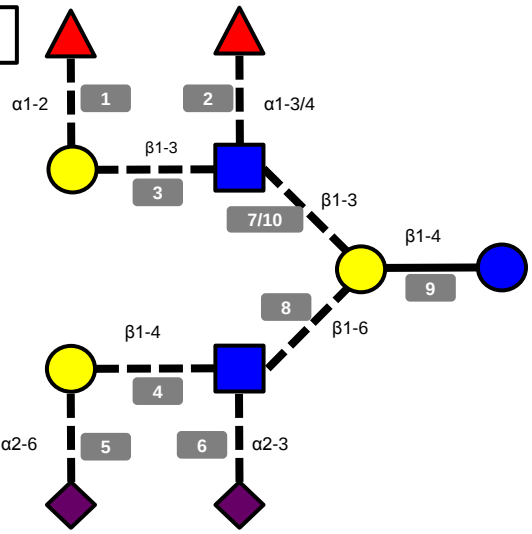
Enzyme key

1	α1-2 fucosidase
2	α1-3/4 fucosidase
3	β1-3 galactosidase
4	β1-4 galactosidase
5	α2-6 sialidase
6	α2-3 sialidase
7	β1-3 N-acetylglucosaminidase
8	β1-6 N-acetylglucosaminidase
9	β1-4 galactosidase (lactase)
10	Lacto-N-biosidase
11	Endo-α-N-acetylgalactosaminidase (EngBF)
12	Endo-α-N-acetylgalactosaminidase (NagBb)
13	α2-8 sialidase
14	Endoglucosylceraminidase
15	α1-6 fucosidase
16	Endo-β-N-acetylglucosaminidase
17	β1-4 mannosidase
18	α-mannosidase
19	β1-2 N-acetylglucosaminidase

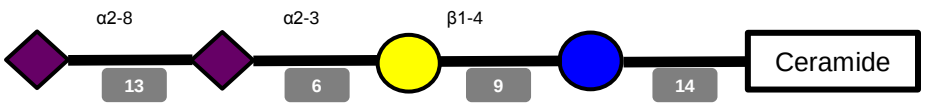
Monosaccharide key

- Galactose
- Glucose
- N-acetylgalactosamine
- Mannose
- N-acetylglucosamine
- Fucose
- Sialic acid

HMO

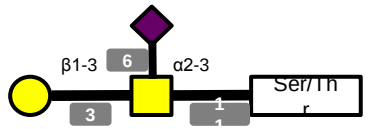


Glycolipids

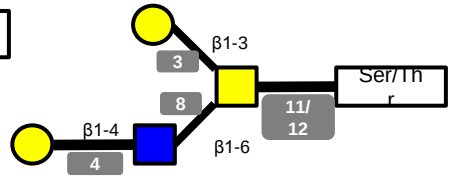


Glycoproteins

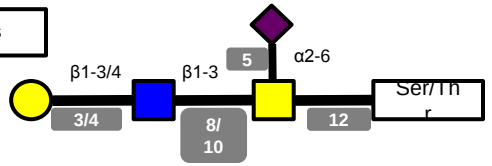
Core 1 O-glycans



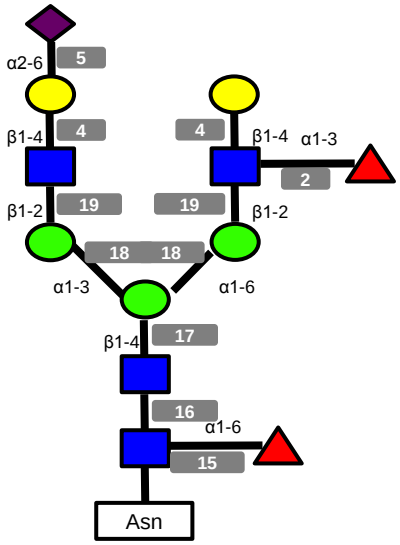
Core 2 O-glycans



Core 3 O-glycans



Complex N-glycan



Fucosidase classes according to CAzY

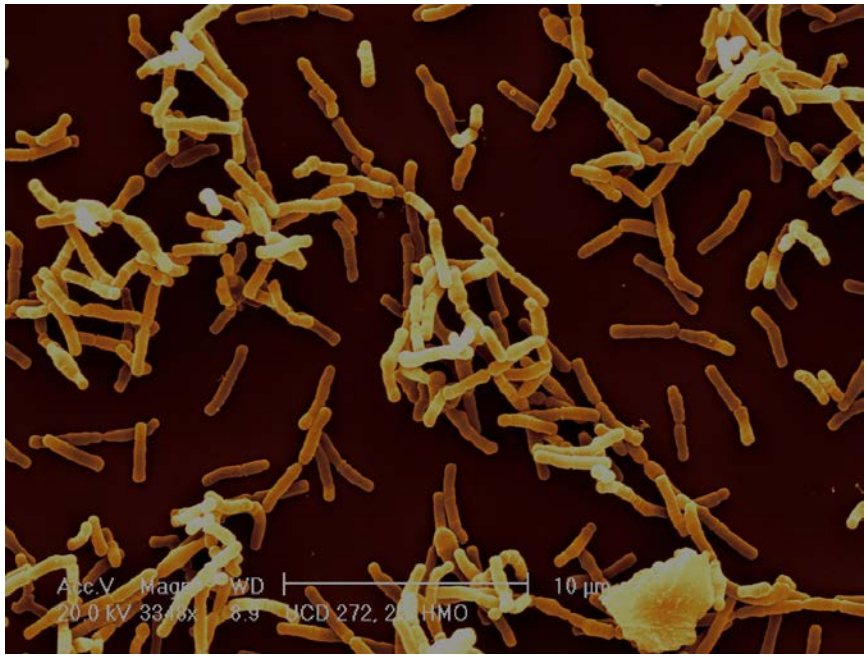
- GH 95
  - Prefers 2’ linkage
- GH 29
  - Active on 3/4’ linkage
- A combination of the two may be necessary for growth on 2-fucosyllactose, at least in some species (Ruiz-Moyano et al. 2013, *AEM*)

Gut bacteria fucosidase genotypes

	GH 95+	GH 95-
GH 29+	Infant associated Bifidobacteria, Bacteroidetes, Select Clostridia, Some Streptococci	Lactobacilliales, Adult-type Bifidobacteria
GH 29-	Some Bacillales	Human Gut Gammaproteobacteria, Fusobacteria

Bifidobacteria

- Gram-positive high-GC bacterial genus
- Very common in infants, often the major group present
- Used as a probiotic due to immunomodulatory effects, beneficial metabolite production, pathogen inhibition



The genome sequence of *Bifidobacterium longum* subsp. *infantis* reveals adaptations for milk utilization within the infant microbiome

D. A. Sela[a,b], J. Chapman[c], A. Adeuya[d], J. H. Kim[b,e], F. Chen[c], T. R. Whitehead[f], A. Lapidus[c], D. S. Rokhsar[c], C. B. Lebrilla[g], J. B. German[b,h], N. P. Price[d], P. M. Richardson[c,1], and D. A. Mills[b,e,2]

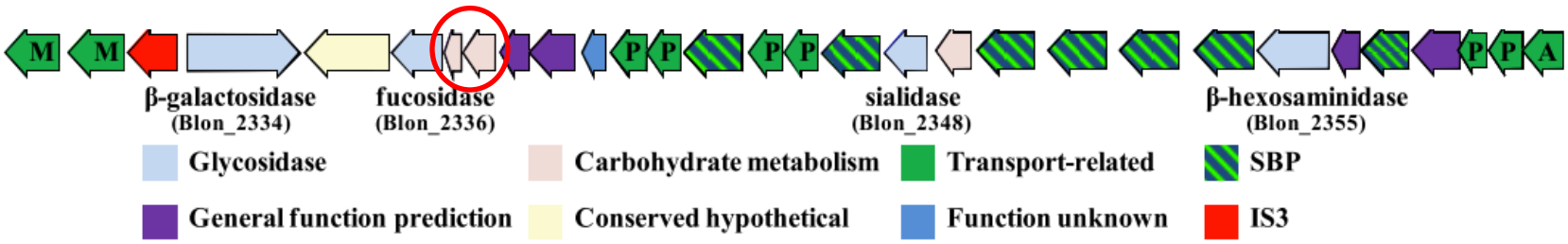
B. longum subsp. *infantis* has several fucosidases

Blon_2335
- Extracellular
- GH 95
- Prefers 2' fucose linkages

Blon_2336
- Intracellular
- GH29 (pfam01120)
- More active on 3/4' fucose linkages

Blon_0248 and Blon_0426.
- Paralogs
- Intracellular
- GH29
- Low/No activity on HMO-like structures

Blon_0346
- Beta-galactosidase (COG1874)
- Possesses a pfam 01120 fucosidase domain at the 5' terminus
- Low/No activity on HMO-like structures

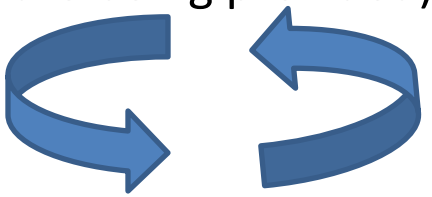


Do the species present in an infant correlate with the specific oligosaccharides found in the mother's breast milk?

Do secretor mothers enrich their infants for microbes with fucosidases?

Model

Positive feedback
(as long as HMGs
are being provided)



Mother produces secretor oligos in her milk

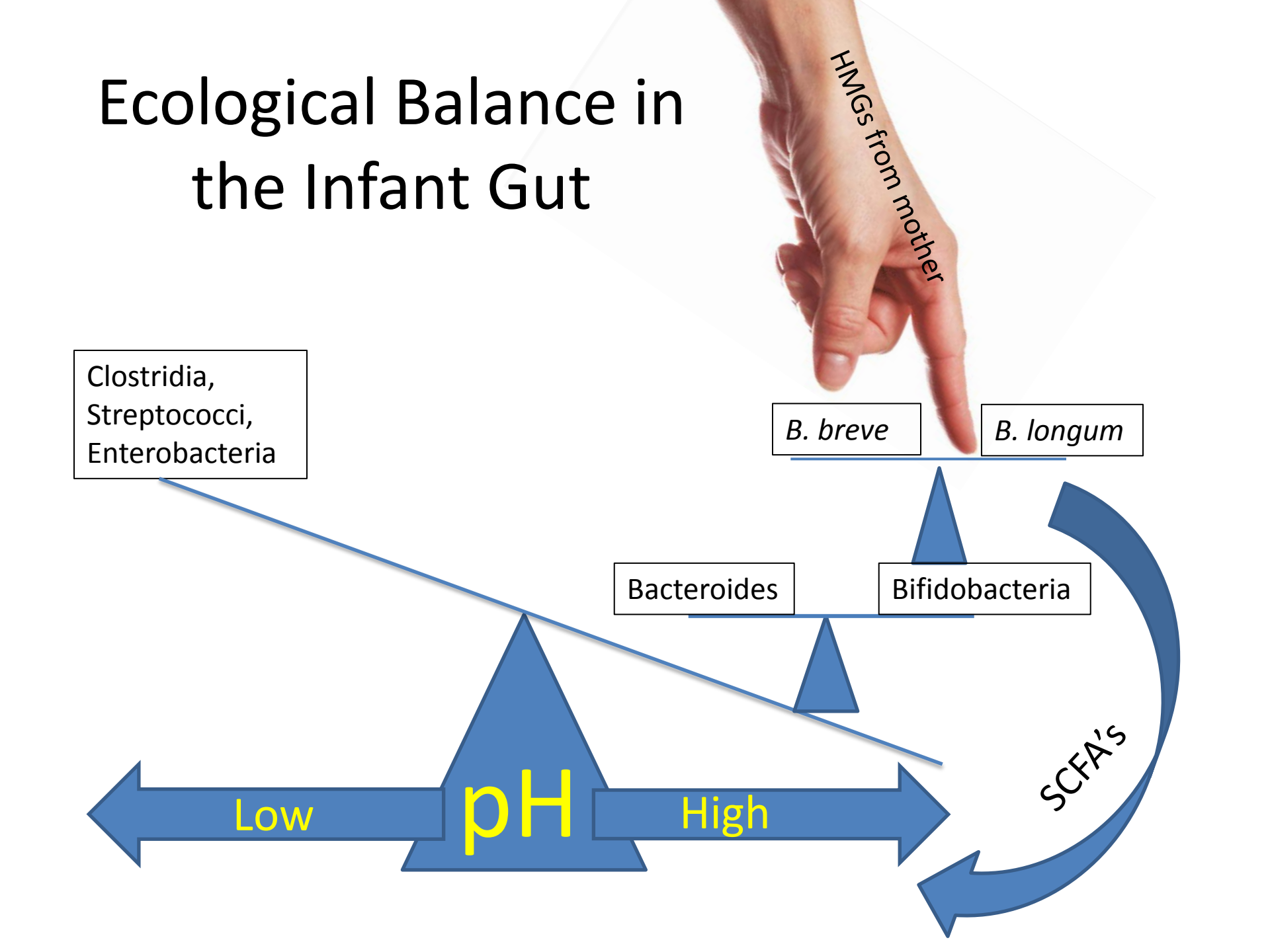
Secretor oligos enrich Bifs (those that can consume the HMGs) within infant

Bifs produce short-chain fatty acids, bacterocins, etc.

Microbiota settles in to alternative stable state with high bifs

Requires presence of Bifidobacterial species that is suited to the types of glycans produced by mother (secretor vs. non-secretor)

Ecological Balance in the Infant Gut



Conclusions

- High and low bifidobacterial communities may be alternative stable states.
  - May be caused be pH shifts driven by short-chain fatty acid production
- Non-secretor-fed infants exhibit a deficit in bifidobacterial colonization.
  - This deficit may be caused by a mismatch between mother's glycan type and bifidobacterial species present.

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