Fucosyltransferase 2 (FUT2) in infant health: Implications for human milk oligosaccharides

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Major points

1. Deep evidence base for human milk protection
2. Milk is a fundamental linkage of the dyad: its role involves mother, milk, infant and their environment
3. Research on OS & genomics are leading to exciting new insights and opportunities for interventions in high risk populations
Overview

1. **FUT2**
   - Neutral OS biosynthesis
   - Polymorphisms
   - Role in child health outcomes

2. Role of the human milk oligosaccharides (HMOS)

3. The mother-infant dyad
FUT2:
- Role in HMOS and host histo-blood group biosynthesis
- Polymorphisms
Human Milk Oligosaccharides

- Major line of defense of breastfeeding infants
- Most common solid constituent after lactose and lipid ~8-10 g/L (colostrum ~20 g/L)
- ~200 different HMOS by glycomic analysis (3 - 32 sugars)
- Quantity of HMOS varies between mothers and over lactation
Neutral Fucosylated Oligosaccharide Structures

Type 1

LNT Galβ1-3GlcNAc β1-R

Se → Le

LNFI, H-1
Galβ1-3GlcNAc β1-R
Fucα1,2
Fucα1,4

LNF II, Leα
Galβ1-3GlcNAc β1-R
Fucα1,4

Le

LDFH I, Leb
Galβ1-3GlcNAc β1-R
Fucα1,2 Fucα1,4

Type 2

Lactose Galβ1-4Glc (for 2'FL, 3FL, LDFT)
LNneoT Galβ1-4GlcNAc β1-R (for LNF III)

Se → Le

FUT4,5,6,7

2'FL, H-2ga
Galβ1-4Glc
Fucα1,2

3FL, LeXga, LNF III, LeX
Galβ1-4Glc-R1R2
Fucα1,3

Le

FUT4,5,6,7

LDFT, LeYga
Galβ1-4Glc
Fucα1,2 Fucα1,3

Newburg, 2006
Biosynthesis of Neutral Human Milk OS

SECRETORS (active FUT2 allele)
75% of European, Asian, African
97% of Mexican

NON-SECRETORS (lack active FUT2 allele)

“Secretor” oligosaccharide contains α1,2-linked fucose

“Lewis” oligosaccharide contains α1,3/4-linked fucose
Oligosaccharides also found as histo-blood group antigens (HBGAs)

- In the child and adult, oligosaccharides are also expressed in secretions and many tissues, including GI and respiratory tracts known as HBGAs produced by enzymes of the histo-blood group genes (ABO; FUT2; FUT3,4,5,6,7)
A or B blood group antigen

SECRETORS (active FUT2 allele)

FUT2

H-1
H-2

FUT3

Leb
Le\textsuperscript{a}
Le\textsuperscript{x}
Le\textsuperscript{y}

NON-SECRETORS (lack active FUT2 allele)

~25% of European, Asian, African;
<3% of Amerindian, Polynesian

“Secretor” or Lewis status can be measured genetically or phenotypically (milk, saliva).
**FUT2 (Secretor gene)**

- Major histo-blood group gene, chromosome 19q13
- *FUT2* (and *FUT1*) produce transferases that link fucose to acceptor molecules in $\alpha_1,2$ linkage. *FUT2* activity is responsible for expression at mucosal surfaces and *secretions (milk, saliva, etc)*
- Null mutation occurs in 20-25% in most populations, but absent in Amerindians and Polynesians
- Null mutation in European or African descent is 428G>A. Primary Asian mutation is 385A>T
- Recent selection pressure on *FUT2* promoter region. More polymorphisms in “pathogen rich” environments
HMOS variation by maternal genotype

(mg/L)

Overall Slope
Salivary histo-blood group antigens (neutral OS) by infant secretor genotype
FUT2 in health and disease
Oligosaccharide histo-blood group antigens (HBGAs)

- Risk of infection with some pathogens is influenced by individual phenotype of oligosaccharide HBGAs being expressed on mucosal surfaces

- Humans differ in blood group type due to evolutionary pressures of host response to pathogens (balancing selection): pathogens adapt to use available opportunities to bind and infect, hosts adapt to protect survival
Cell surface glycans in pathogen-host cell interaction

“Secretor” OS binding to major enteric pathogens:

- Noroviruses
- Invasive Campylobacter
- Some diarrheagenic E. coli
- V. cholerae
- H. pylori
Adherence of *V. cholerae* to $\alpha_{1,2} \text{FUT}^+$ CHO cells

Ruiz-Palacios et al, JBC, 2004
Secretors* are at risk of Norwalk virus infection

Table 1  Infection$^a$ among Se+ and Se− volunteers by Norwalk virus dose

<table>
<thead>
<tr>
<th>PDU NV</th>
<th>≤10⁴</th>
<th>10⁵</th>
<th>10⁶</th>
<th>10⁷</th>
<th>10⁸</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Se+</td>
<td>9/13$^b$</td>
<td>3/6</td>
<td>2/3</td>
<td>7/14</td>
<td>13/19</td>
<td>34/55</td>
</tr>
<tr>
<td></td>
<td>(69%)</td>
<td>(50%)</td>
<td>(67%)</td>
<td>(50%)</td>
<td>(68%)</td>
<td>(62%)</td>
</tr>
<tr>
<td>Se−</td>
<td>0/6</td>
<td>0/4</td>
<td>0/2</td>
<td>0/6</td>
<td>0/4</td>
<td>0/22</td>
</tr>
<tr>
<td></td>
<td>(0%)</td>
<td>(0%)</td>
<td>(0%)</td>
<td>(0%)</td>
<td>(0%)</td>
<td>(0%)</td>
</tr>
</tbody>
</table>

NV, Norwalk virus. $^a$Viral RNA detected in stool ($n = 34$) or a ≥4-fold increase in Norwalk virus–specific serum IgG ($n = 32$). $^b$Number of volunteers infected/number of volunteers dosed. PDU, PCR-detectable units.

Histo-Blood Group Antigens Bind to Variable P-domain of NV Capsid Protein

Red – conserved residues
Blue – variable positions
Oligosaccharide-mediated adhesion of Pseudomonas aeruginosa in cystic fibrosis

Galactose and Fucose-binding lectins (PA-IL and PA-IIL) contribute to virulence.

Relevance to Preterm Infants

- Immature intestinal tract is highly permeable and hyper-inflammmatory

- Sepsis and necrotizing enterocolitis are major causes of morbidity and mortality, caused by many different pathogens
**FUT2** gene upregulated with early bacterial colonization

Meng, Newburg et al (2005)
Secretor Genotype and Phenotype as Biomarkers of Risk in Preterm Infants

**Secretor Genotype**
- GG: 2/96 (2.1%)
- AG: 11/203 (5.4%)
- AA: 12/95 (12.6%)

**Deaths by Genotype**
- GG: 2/96 (2.1%)
- AG: 11/203 (5.4%)
- AA: 12/95 (12.6%)

**Secretor Phenotype**
- High H: 6/272 (2.2%)
- Low H: 20/138 (14.5%)

**Deaths by Phenotype**
- High H: 6/272 (2.2%)
- Low H: 20/138 (14.5%)

**Log Rank p-value**
- Secretor Genotype: 0.008
- Secretor Phenotype: < 0.001
Secretor Phenotype and Risk of Necrotizing Enterocolitis

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Low Salivary Hn</th>
<th>High Salivary Hn</th>
<th>Odds Ratio 95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEC case</td>
<td>15</td>
<td>12</td>
<td>2.7 (1.1, 6.4)</td>
<td>0.019</td>
</tr>
<tr>
<td>Surgical NEC</td>
<td>9</td>
<td>5</td>
<td>3.8 (1.1, 15)</td>
<td>0.018</td>
</tr>
<tr>
<td>NEC deaths</td>
<td>9</td>
<td>2</td>
<td>9.6 (1.9, 92)</td>
<td>0.001</td>
</tr>
<tr>
<td>Controls</td>
<td>112</td>
<td>251</td>
<td>1.0</td>
<td></td>
</tr>
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Biosynthesis Pathway of blood group antigens

A or B blood group antigen

SECRETORS (active FUT2 allele)

FUT2

H-1
H-2

FUT3

Le\(^b\)
Le\(^y\)

FUT3*

Le\(^a\)
Le\(^x\)

NON-SECRETORS (lack active FUT2 allele)

~25% of European, Asian, African; <3% of Amerindian, Polynesian

“Secretor” or Lewis status can be measured genetically or phenotypically (milk, saliva).
Salivary oligosaccharide phenotype is a biomarker of gram negative sepsis risk in preterm infants

Gram Negative Status Comparison

Median of EDF Percentile

Status Comparison

- Healthy Control
- gramneg
Human Milk Oligosaccharide Protection

- Inhibits pathogen binding
- Prebiotic
Human Milk Oligosaccharides and Glycoconjugates (Glycans)

- **Free** (unbound, monovalent) OR

- **Bound** to major proteins (e.g., lactoferrin, lactadherin, bile salt stimulating lipase), mucins, lipids, and other
  - Linkages may be N-linked or O-linked – helps evade digestion or specific immune and other functions
The Mother-Infant Dyad
Diarrhea by child "secretor" status

Ruiz-Palacios, Morrow et al

<table>
<thead>
<tr>
<th>Secretor</th>
<th>Partial secretor</th>
<th>Non-secretor</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;5 nmol/ml</td>
<td>&gt;0-5 nmol/ml</td>
<td>0 nmol/ml</td>
</tr>
</tbody>
</table>

No. at Risk
- Non-secretor: 7
- Partial secretor: 17
- Secretor: 273

Cumulative frequency of diarrhea episodes

Age (years)

P = 0.04
P = 0.01
Higher Secretor Oligosaccharide in Mother’s Milk: Lower Infant Risk of Moderate-to-Severe Diarrhea

2-Linked Fucosyloligosaccharides (% of Total)

Morrow et al, 2004
Specific milk fucosyloligosaccharides and protection against campylobacter and human calicivirus-associated diarrhea

<table>
<thead>
<tr>
<th>Model No.</th>
<th>Milk Oligosaccharide</th>
<th>Homologous Lewis Antigen</th>
<th>Campylobacter β (SE)</th>
<th>P</th>
<th>Caliciviruses β (SE)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LNT</td>
<td>Type 1 precursor</td>
<td>5.76 (3.68)</td>
<td>0.118</td>
<td>0.45 (5.68)</td>
<td>0.936</td>
</tr>
<tr>
<td>2</td>
<td>LNF-I</td>
<td>H-1</td>
<td>-0.51 (1.75)</td>
<td>0.772</td>
<td>3.30 (2.66)</td>
<td>0.215</td>
</tr>
<tr>
<td>3</td>
<td>LDFH-I</td>
<td>Le&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.87 (2.95)</td>
<td>0.047</td>
<td>-13.32 (5.33)</td>
<td>0.012</td>
</tr>
<tr>
<td>4</td>
<td>LNF-II (and-III)</td>
<td>Le&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.20 (3.84)</td>
<td>0.274</td>
<td>-8.71 (6.79)</td>
<td>0.199</td>
</tr>
<tr>
<td>5</td>
<td>LNneoT</td>
<td>Type 2 precursor</td>
<td>10.99 (11.52)</td>
<td>0.340</td>
<td>-7.95 (18.13)</td>
<td>0.661</td>
</tr>
<tr>
<td>6</td>
<td>2'-FL</td>
<td>H-2</td>
<td>-5.60 (1.93)</td>
<td>0.004</td>
<td>3.77 (2.14)</td>
<td>0.078</td>
</tr>
<tr>
<td>7</td>
<td>LDFT</td>
<td>Le&lt;sup&gt;y&lt;/sup&gt;</td>
<td>3.09 (4.74)</td>
<td>0.514</td>
<td>-16.82 (11.00)</td>
<td>0.126</td>
</tr>
<tr>
<td>8</td>
<td>3-FL</td>
<td>Le&lt;sup&gt;x&lt;/sup&gt;</td>
<td>4.39 (3.17)</td>
<td>0.165</td>
<td>-10.29 (9.00)</td>
<td>0.253</td>
</tr>
</tbody>
</table>
Human Milk sIgA against Different Strains of Noroviruses Depends on Maternal FUT2 genotype

<table>
<thead>
<tr>
<th>Virus</th>
<th>Non-Secretor N=20</th>
<th>Secretor N=33</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-387</td>
<td>.02 (.001, .04)</td>
<td>.07 (.01, 2.66)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>207</td>
<td>.04 (.01, .34)</td>
<td>.07 (.01, 1.19)</td>
<td>0.53</td>
</tr>
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</table>

Sun, Jiang, Morrow
The Match of the Mother-Infant Dyad: Does it Matter to Human Milk Protection?

<table>
<thead>
<tr>
<th>Status</th>
<th>Nonsecretor</th>
<th>Secretor</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonsecretor</td>
<td>12.5%</td>
<td>12.5%</td>
<td>25%</td>
</tr>
<tr>
<td>Secretor</td>
<td>12.5%</td>
<td>62.5%</td>
<td>75%</td>
</tr>
<tr>
<td>Total</td>
<td>25%</td>
<td>75%</td>
<td>100%</td>
</tr>
</tbody>
</table>
Summary

• Human milk provides a generous “cocktail” of oligosaccharides (predominantly “secretor”) that bathe mucosal surfaces and protect infants against infectious and inflammatory diseases

• Bringing genomic tools to study human milk biology promises to refine medical management of human milk feeding in high risk circumstances and lead to development of prebiotic agents relevant to prevention and treatment of infectious and inflammatory diseases

• Human milk glycan match to need of the infant or population at risk is a key concept requiring further research
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