The early-life gut microbiome and pediatric asthma

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The global distribution is disproportionate.
Animal models

Ovalbumin Model of Allergic Asthma

Sensitization

<table>
<thead>
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<th>14</th>
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Challenge

<table>
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<th>22</th>
<th>23</th>
<th>24</th>
<th>25</th>
<th>26</th>
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OVA IP OVA IP

Intranasal OVA Sacr.

House Dust Mite Model of Allergic Asthma

<table>
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<tr>
<th>0</th>
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<th>21</th>
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<th>23</th>
<th>25</th>
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HDM IN HDM IN HDM IN Sacr.
Epidemiology

Farms

C-sections

Antibiotics

Helminths

Breastfeeding
Low resilience during critical immune development stage

Experimental evidence

Ovalbumin Model of Allergic Asthma

**Streptomycin or Vancomycin**
(200 mg/L) (200 mg/L)

**Sensitization**
0 7 14

**Challenge**
21 22 23 24 25 * 26

- **Birth (Neonatal)**
- **Neonatal Abx**
- **Adult Abx**
- **7 wks (Adult)**

Intranasal OVA
Sacr.
Neonatal antibiotic treatment affects experimental asthma severity

A

**Neonatal Abx**

- Total cells x 10^6 ml^-1
- PBS, OVA, OVA + Vanco, OVA + Strep
- *p < 0.05

**Adult Abx**

- Total cells x 10^6 ml^-1
- PBS, OVA, OVA + Vanco, OVA + Strep
- n.s.

**Eosinophils**

- cells x 10^6 ml^-1
- PBS, OVA, OVA + Vanco, OVA + Strep
- **p < 0.01
- n.s.

**OVA-specific IgE**

- ng ml^-1
- PBS, OVA, OVA + Vanco, OVA + Strep
- *p < 0.05
- n.d.
CHILD Study:

16S rRNA analysis at 3 months and one year of age

- **Atopy**
  - 87 subjects: Atopy Only
  - Positive allergen skin prick test at 1-year
  - 22 subjects: Atopy + Wheeze
- **Wheeze**
  - 136 subjects: Wheeze Only
  - ≥1 episode of wheezing by 1-year of age

Risk of asthma at school age [Asthma Predictive Index (Odds Ratio)]
Gut bacterial metabolism: short-chain fatty acids (SCFA)

SCFA:
- Used as energy source by intestinal and mucosal immune cells.
- Transported to the liver where they are involved in numerous biochemical pathways of lipid and energy metabolism.
- Involved in immune response regulation.
**Pathway**

- Secondary bile acid metabolism
- Hemoglobin metabolism
- Phenylalanine metabolism
- Histidine metabolism
- Food component

**Urine metabolites**

- Glycolithocholate sulfate
- Glycocholenate sulfate
- Glycohyocholate
- Tauroursodeoxycholate
- I-urobilinogen
- Phenylacetylglutamine
- Imidazole propionate
- Ferulate

From correlation to causation

**Human microbiota transfer** (inoculated with feces from AW subject)

(i) **Control**
- Asthmatic Microbiota (AW)
- Breeding mice
- Offspring
- Birth
- 3 wks
- 7 wks
- Ovalbumin

(ii) **FLVR**
- AW + FLVR
- Breeding mice
- Offspring
- Birth
- 3 wks
- 7 wks
- Ovalbumin

Human microbiota transfer (inoculated with feces from AW subject)
Microbial intestinal colonization in F1 mice

![Graph A: PC 1 and PC 2 scatter plot](image)

**A**
- PC 1 (41.5%)
- PC 2 (24.8%)

![Graph B: Genus abundance](image)

**B**
- Abundance (%)
- Genus: Faecalibacterium, Lachnospira, Rothia, Veillonella

![Graph C: Family abundance](image)

**C**
- Abundance (%)
- Family: Bacteroidaceae, Clostridiaceae, Enterobacteriaceae, Enterococccaceae, Lachnospiraceae, Micrococcaceae, Peptostreptococcaceae, Prevotellaceae, Ruminococcaceae, S24-7, Turicibacteraceae

**Citation:** Arrieta, et al 2015 *Sci. Transl. Medicine.*
FLVR reduces lung inflammation

Bronchoalveolar lavage

**BAL Counts**

**BAL Total Cell Differential**

What about asthma elsewhere?

**Las Esmeraldas, Ecuador**

Forno et al. *Thorax* 2015

Prof. Phil Cooper
Rural to urban transition
## Ecuador cohort: epidemiology

<table>
<thead>
<tr>
<th>Categorical variable</th>
<th>Odds ratio</th>
<th>P</th>
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<tbody>
<tr>
<td>Maternal antibiotics during pregnancy</td>
<td>2.39</td>
<td>0.02</td>
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<tr>
<td>Vaginal birth</td>
<td>-2.42</td>
<td>0.03</td>
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<tr>
<td>Breastfeeding over 5 months</td>
<td>-0.17</td>
<td>0.02</td>
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<tr>
<td>Child Ascaris infection</td>
<td>3.82</td>
<td>0.05</td>
</tr>
<tr>
<td>Child Trichuris infection</td>
<td>-8.18</td>
<td>0.004</td>
</tr>
<tr>
<td>Respiratory infections</td>
<td>2.21</td>
<td>0.001</td>
</tr>
<tr>
<td>Potable water</td>
<td>2.49</td>
<td>0.02</td>
</tr>
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</table>
Microbiota in babies at risk of asthma

A

Decreased in CTRL

Increased in CTRL

B

Pichia kudriavzevii

Fungal relative abundance

Fungal 18S

Atopic wheeze

Healthy

3 months

Bacteria

\[\uparrow\text{Streptococcus sp.}\]
\[\uparrow\text{Bacteroides sp.}\]
\[\downarrow\text{Faecalibacterium sp.}\]
\[\downarrow\text{Lachnospira sp.}\]
\[\downarrow\text{Ruminococcus gnavus}\]
\[\downarrow\text{Bifidobacterium sp.}\]

Fungi

\[\uparrow\uparrow\text{Pichia kudriavzevii}\]
\[\downarrow\text{Saccharomycetales}\]

Microbial metabolites

\[\downarrow\text{Acetate}\]
Antibiotic-driven alterations in the fungal intestinal microbiota affect immune development and asthma.
Can fecal fungal overgrowth influence atopy?

A

- Abx
- Abx + C. albicans and P. kudriavzevii
- untreated

B

Fecal fungal growth

C

IL-10+

CD4+ IL-13+

CD4+ IL-5+

CD4+ IL-6+

CD4+ IL-4+

* P=0.1

P=0.06

P=0.01

P=0.1
Does fungal gut colonization influence immune development?

A. Diagram showing the addition of bacteria (B) and fungi (F) to create a mixture (B + F).

B. Graph showing FITC-Dextran Colon Fluorescence (AFU) with B and B+F conditions.

C. Bar graph showing Colon Culture with IL-10, IL-9, and TNF-α levels for B and B+F conditions.

D. Graph showing Jejunum culture with IFN-γ, IL-17E/IL-25, IL-4, IL-9, IL-10, IL-17F, and TNF-α levels and p values.
Does fungal gut colonization influence immune development?

**SPLENOCYTES**

- **B cells (CD19+)**
  - % Parental: B: 55, B+F: 60
  - *p-value*

- **T cells (CD3+)**
  - % Parental: B: 40, B+F: 45
  - *p-value*

- **CD25+ FoxP3+ T regs**
  - % Parental: B: 1.5, B+F: 1.0
  - *p-value*

- **CD8+ T cells**
  - % Parental: B: 35, B+F: 30
  - *p-value*

- **IL-6 + cells**
  - % Parental: B: 0.8, B+F: 0.6
  - *p-value*

- **IL-4 + cells**
  - % Parental: B: 0.15, B+F: 0.10
  - *p-value*

- **IL-13 + cells**
  - % Parental: B: 0.15, B+F: 0.10
  - *p-value*

- **IL-10 + cells**
  - % Parental: B: 0.15, B+F: 0.10
  - *p-value*
Summary

• Changes in the gut microbiome can influence asthma development.
• Early life is the most critical stage for the development of the microbiome.
• The first 100 days of human life represent an early ‘critical window’ in which microbial dysbiosis enhances the risk of asthma and allergic disease.
Summary

• Microbial dysbiosis can change with geographical location.
• Asthma-related microbial dysbiosis is both bacterial and fungal.
• Fungal overgrowth may also explain immune alterations that lead to asthma.
• Prospective observational microbiome studies are critical to study dysbiotic markers that precede and may be involved in asthma pathogenesis.
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Ecuador infant study
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Mohn Lab
Turvey Lab
Ecuador infant study
Wegener-Parfrey Lab

Children's Hospital
Research Institute
for Child and Maternal Health

University of Calgary

Ecuador infant study

CHILD Study
HELP CHILDREN GROW UP HEALTHY

UBC

Canadian Institutes of Health Research

IRSC