Background - allergy

- Breastfeeding has been linked to a reduction in the prevalence of allergy and asthma – more so in mothers with an existing allergy

- House dust mite allergy:
  - House dust mite (HDM) allergy is the most common sources of allergens, affecting up to 20% of the population of industrialised countries
  - Exposure to HDM allergens cannot be fully prevented
  - HDM allergens activate both innate and adaptive immune system
Human milk proteins:

- Human milk contains, besides nutrients, immune modulating components, like proteins
- Immune-modulating proteins in human milk:
  - Innate immunity (complement proteins)
  - Adaptive immunity (immunoglobulins)
  - Antimicrobial proteins

Are these proteins linked to prevention of allergies?

Objective

The objective of this study was to explore differences in the breast milk proteome from individual allergic and non-allergic mothers
Approach

Protein analysis:
- Non-targeted, label-free proteomics technology (LC/MSMS)

Data analysis:
- Maxquant for identifying & quantifying proteins
- DAVID for Gene Ontology Annotation
- Perseus for statistical analyses
Milk proteome of (non)allergic mothers

- Proteins in the milk serum:
  - Total of 351 proteins
  - 98% overlap, 2% unique to one group

Milk proteome of (non)allergic mothers

- Functional analysis

<table>
<thead>
<tr>
<th>GO annotation cluster</th>
<th>Protein count</th>
<th>Non-allergic</th>
<th>Allergic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Response to wounding</td>
<td>53</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>Carbohydrate catabolic process</td>
<td>29</td>
<td>28</td>
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<tr>
<td>Homeostasis</td>
<td>20</td>
<td>20</td>
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<tr>
<td>Coenzyme metabolic process</td>
<td>21</td>
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<tr>
<td>Cellular carbohydrate biosynthetic process</td>
<td>14</td>
<td>13</td>
<td></td>
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<tr>
<td>Regulation of apoptosis</td>
<td>45</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>Response to extracelluar stimulus (nutrients)</td>
<td>17</td>
<td>15</td>
<td></td>
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<tr>
<td>Inflammatory response</td>
<td>35</td>
<td>36</td>
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<tr>
<td>Hydrogen peroxide metabolic process</td>
<td>16</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Response to organic substance</td>
<td>44</td>
<td>42</td>
<td></td>
</tr>
</tbody>
</table>

- No differences between both groups: on a qualitative level both groups are similar
Milk proteome of (non)allergic mothers

- Variation within groups
  - Signal intensity ranges ($10^{\text{log intensity}}$)
    - Non-allergic mothers: 3.5 - 9.2
    - Allergic mothers: 2.9 - 9.1
  - Relative standard deviation ranges from 1-35%

Milk proteome of (non)allergic mothers

- Differences between groups (all proteins)
Milk proteome of (non)allergic mothers

Variation between individual mothers

Significantly different proteins (functionality)

- Cystatin-3
- S100 A1
- Apolipoprotein D
- Nucleobindin-2
- Cytokine-like protein 2-21
- Inter-alpha-trypsin inhibitor heavy chain H4
- Apolipoprotein B
- Cathepsin C
- Ras-related protein Rab-11B
- Ras-related protein Rab-1A
- Ras-related protein Rab-2A
- Inter-alpha-trypsin inhibitor heavy chain H2
- Serpin peptidase inhibitor D
- Chitinase-3-like protein 2
- Inter-alpha-trypsin inhibitor heavy chain H1
- Transferrin
- EPH homology kinase 3
- Ezrin-radixin-moesin-binding phosphoprotein 50
- Calretinin

Protease inhibitor (5)
Transport protein (3)
GDP-binding (3)
Calcium binding (3)
The major HDM allergen, Der p 1, is a protease.

Related to sensitivity to atopic dermatitis.
The major HDM allergen, Der p 1, is a protease
- Its proteolytic activity is relevant to the pathogenesis of asthma and allergy.
- It degrades antiprotease-based defence proteins, which protect mucosal barriers
- Serine proteases and its inhibitors are involved in the maintenance of the epithelial barriers: an imbalance allows easier penetration of allergens.

Protease inhibitors have therefore been suggested as potential therapeutics for allergy and asthma.

Milk proteome of (non)allergic mothers

Conclusions

- Milk proteome of allergic and non-allergic mothers similar from qualitative perspective
- Significant quantitative differences in the proteome between allergic and non-allergic mothers:
  - Transport proteins, GDP- & calcium-binding proteins
  - Protease inhibitors: Breast milk of mothers with an allergy may thus naturally provide components to the offspring that protect against the allergen.
On a philosophical note

What is the *magic* milk protein?

Food is not pharma

Milk already contains all *magic ingredients*

Changes in milk composition are often concerted changes of groups of components

Processing should aim at retaining goodness of milk
Thanks for your attention

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