Gut microbial metabolomics to understand allergies in early life

Savova, M.V.

Appendix

Summary

Samenvatting

Curriculum Vitae

List of Publications

Acknowledgements

Summary

The rise in allergy incidence, especially in Western countries, is a growing concern, as allergies can significantly diminish quality of life and, in severe cases, be life-threatening. At the core of allergies is a breakdown in immune tolerance; the inability of the immune system to regulate responses to harmless environmental antigens appropriately. Multiple hypotheses have been proposed to explain the increase in allergies in Western countries with a leading hypothesis proposing that reduced microbial exposure can lead to an ill-trained immune system, incapable of recognizing a friend from a foe, resulting in allergen sensitization and thus allergies.

Our guts harbor a large proportion of our body's immune cells and support one of the largest microbial ecosystems, consisting of trillions of microorganisms. Gut microbial colonization occurs rapidly in early life, particularly during the first year, until the gut microbiome (GM) starts resembling that of an adult by approximately three years of age. This process is shaped by multiple factors, including mode of delivery (vaginal birth versus cesarean section), antibiotic exposure, and diet. Notably, breastfeeding promotes a GM rich in bifidobacteria, which utilize sugars in breastmilk and play a key role in supporting immune system development during early life.

Disruptions in gut microbial composition during early life, particularly reduction in bifidobacteria, have been linked to immune-mediated diseases, including allergies. Because of that, the first three years of life are often seen as a critical period during which GM disturbances can lead to disease, but also as a window of opportunity in which the GM's dynamic nature can be used to prevent or treat diseases such as allergies. However, there is a considerable knowledge gap in understanding the link between the GM and allergies, and how microbial-based interventions can be used for allergy management. As the GM impacts host physiology through the production of diverse metabolites, examining these small molecules offers direct mechanistic insights into host–GM interactions. Those metabolites can be measured non-invasively in fecal samples using a technique called liquid chromatography – mass spectrometry.

The research is based on the hypothesis that the GM in early life influences the development and resolution of allergies via the production of metabolites and that metabolomics can be used to study the function of the GM. The aim of this research is to study the links between allergy and intestinal health, the GM, and external factors by exploring the metabolome in longitudinal clinical studies and in vitro models.

Chapter 2 gives a comprehensive overview of current knowledge on the role of the GM in the most common food allergy in early life: IgE-mediated cow's milk allergy (CMA). The review shows that IgE-mediated CMA is consistently associated with a reduction in *Bifidobacterium* spp., and that probiotic *Bifidobacterium* interventions effectively induce bifidobacterial growth in the gut. Notably, the chapter highlights that research in this area

has predominantly focused on microbial compositional analysis, with a limited number of studies exploring the metabiota and none investigating other omics layers, such as proteomics. Furthermore, metabolomics research has been limited by the narrow range of metabolites examined, primarily short-chain fatty acids (SCFAs), amino acids, and organic acids. The review emphasizes the urgent need for metabolomics studies encompassing a broader range of gut microbial metabolites to better understand the microbiome–host interplay. It also strongly underscores the importance of multi-omics approaches to uncover mechanistic insights into the relationship between the GM and CMA in early life.

Chapter 3 and **Chapter 4** address the limited metabolomic scope reported in the literature by covering a wide range of host- and microbiota-derived metabolites, including microbial amino acid metabolites, bile acids, and SCFAs. In **Chapter 3**, infants at risk of developing allergies, exclusively breastfed for at least 16 weeks, were followed during the first year of their lives, a period of rapid microbial colonization and potential onset of the atopic march. Alteration in the fecal metabolome and key microbial members were examined in relation to age, diet, delivery mode, and allergy development during this period. The findings revealed significant age-related metabolomic shifts likely driven by simultaneous alterations of the host metabolism, feeding practices, and microbial composition. These included increases in amino acid metabolites, bile acids, B vitamins, SCFAs, and phenolic acids, along with decreases in long-chain fatty acids and acylcarnitines. C-section was found to significantly associate with fecal metabolome alterations up to six months of age. Owing to the prospective nature of the study, it was possible to identify that infants who developed allergies within the study period had lower levels of *Bifidobacterium* spp. and significantly higher levels of long-chain fatty acids prior to onset of clinical manifestations. Even though the findings of this research require validation in a larger cohort, the study advanced our understanding of the fecal metabolome development in early life and factors that shape it during this critical period of immune system and microbiome development.

IgE-mediated CMA is typically managed through elimination diets, including the use of amino acid-based formulas (AAF) in formula-fed infants. Given the growing evidence of the beneficial role of *Bifidobacterium* spp. for the immune development and their association with allergy, shown in **Chapter 2** and **Chapter 3**, bifidogenic supplementation of AAF has emerged as a promising strategy in the management of IgE-mediated CMA. **Chapter 4** explores the link between IgE-mediated CMA, the GM, and bifidobacteria-promoting synbiotic supplementation by means of fecal metabolomics. For this, infants diagnosed with IgE-mediated CMA who received either standard AAF or AAF supplemented with synbiotic blend of probiotic *Bifidobacterium breve* M-16 V and prebiotic inulin and oligofructose were followed for a year. The longitudinal data analysis revealed minor metabolome alterations associated with tolerance acquisition to cow's milk protein, including alterations to the branched-chain SCFAs, bile acids, and amino acid levels. Notably, infants who developed tolerance exhibited significantly elevated citrulline levels, suggesting reduced gut permeability, as well as insignificantly lower levels of serotonin and 5-hydroxytryptophan,

which are involved in inflammation. One of the study's key findings is that the impact of synbiotic supplementation on the fecal metabolome was most pronounced after six months of intervention, with changes largely diminishing by 12 months, suggesting that early intervention is required to maximize the effect of synbiotics. Specifically, synbiotic supplementation led to increased levels of aromatic lactic acids, purine metabolites, long-chain fatty acids, and bile acids, reflecting changes in GM activity. Among these, indolelactic acid and 4-hydroxyphenyllactic acid, aromatic amino acid metabolites of infant-type *Bifidobacterium*, were significantly elevated and positively correlated with the abundance of the *Bifidobacterium* genus. These findings complement the microbiome and proteomics findings from the same cohort, further supporting the efficacy of the synbiotic intervention in promoting *Bifidobacterium* growth and activity in the gut. While the synbiotic had no statistically significant effect on tolerance acquisition, the observed increase in anti-inflammatory indolelactic acid suggests that synbiotic supplementation may still confer immunological benefits.

3D gut-on-a-chip platforms are promising in vitro models that replicate key aspects of intestinal physiology. Such models can be used to study the intestinal barrier, the dysfunction of which plays a role in a variety of diseases, such as allergies. Using an intestinal epithelium tubules-on-a-chip model, **Chapter 5** demonstrates how pro-inflammatory cytokines exposure impacts intestinal barrier integrity and the secretion of signaling lipids under serum-containing and serum-free medium conditions. Pro-inflammatory cytokine exposure significantly increased intestinal permeability, cellular permeability, and induced structural changes to the intestinal barrier under both conditions. While the intestinal permeability and cellular permeability alterations were comparable between media conditions, structural damage, assessed by actin remodeling, was significantly lower in serum-free compared to serum conditions. The impaired gut barrier was associated with elevated prostaglandin levels in the apical (luminal), but not in the basolateral compartment, with this effect being more pronounced under serum-free conditions. The developed integrated model offers a valuable framework for exploring the interplay between inflammation, barrier integrity, and lipid metabolism in intestinal pathophysiology which is also relevant for allergy research.

Chapter 6 summarizes the key findings of the thesis and discusses perspectives for future research. Using fecal metabolomics and *in vitro* modelling, this thesis has advanced our understanding of the complex interactions between allergy and intestinal health, the GM, and external factors. The field would benefit from well-designed longitudinal clinical studies integrating multi-omics approaches and advanced physiologically relevant *in vitro* models. In addition, alternative allergy prevention and treatment strategies that mimic healthy breastfed infant gut environment should be explored. Such efforts require multidisciplinary collaboration, ultimately paving the way for microbiome-based nutritional strategies to prevent and treat allergies.