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Human milk oligosaccharide profiles and food sensitization among infants in the CHILD Study

To the Editor:

Allergies originate early in life and food sensitization is often the first manifestation of allergic disease (1). Breastfeeding has been inconsistently associated with allergic conditions (2). These inconsistencies could reflect differences in human milk composition, which varies across different settings and populations. However, it remains poorly understood which of the bioactive components in human milk contribute to the developmental programming of allergic disease.

Human milk oligosaccharides (HMOs) are the third most abundant component of human milk, yet they are absent from infant formula (3). HMO composition is determined by genetic fucosyltransferase-2 secretor status, and also influenced by lactation stage, gestational age, maternal health, ethnicity, geographic location and breastfeeding exclusivity (3). Among their many functions (3), HMOs act as selective substrates to guide development of the infant gut microbiota (4). We have previously reported that gut microbiota richness in early infancy is associated with subsequent food sensitization, suggesting that HMOs and other determinants of early gut colonization could influence the development of allergic disease (5). This hypothesis is also supported by experimental research in rodents (6) and one small

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clinical study where low concentrations of the HMO lacto-N-fucopentaose (LNFPIII) were associated with higher incidence of cow's milk allergy (7). However, the potential impact of other individual HMOs on food sensitization is not known, and the impact of overall HMO composition has not been studied, yet this may be important because breastfed infants are naturally exposed to complex combinations of HMOs in human milk.

In the current study, among 421 mother-infant dyads from the Canadian Healthy Infant Longitudinal Development (CHILD) cohort (8), we examined the associations of 19 individual HMOs and overall HMO profiles with food sensitization at one year of age using Projection on Latent Structures-Discriminant Analysis (PLS-DA), a multivariate classifier (9). Detailed methods are provided in **Supplementary Materials**.

Overall, 59/421 infants (14.0%) were sensitized to one or more food allergens at one year of age (**Supplementary Table 1**). We did not observe any significant associations for the 19 individual HMOs or total HMOs and food sensitization (**Figure 1a**); however, overall HMO profiles differed significantly in milk consumed by sensitized vs. non-sensitized infants ($p < 0.001$; robust to leave-one-out cross validation) (**Figure 1b**). The discrimination performance was “fair”, with an area-under-the-curve (AUC) of 0.73, 95% Confidence Interval (CI) 0.66-0.79 (robust to permutation testing with 100 replicates; $p = 0.02$) (**Figure 1c**). Similar results were observed in a sensitivity analysis excluding 22 infants with food allergy symptoms prior to milk sample collection (AUC 0.75, 95%CI 0.69-0.81) (**Supplementary Figure 1**).

Restricting our analysis to the top 10 most important HMOs contributing to the PLS-DA score resulted in similar discrimination (AUC 0.71; 95%CI 0.64-0.78), indicating that these 10 HMOs are sufficient to explain the association of HMO profile and food sensitization. The rankings, PLS-DA scaled importance scores, and direction of association for these 10 HMOs are listed in **Supplementary Table 2**. HMO profiles associated with

lower risk of food sensitization were characterized by relatively higher concentrations of fucodisialyllacto-N-hexaose (FDSLNH), lacto-N-fucopentaose II (LNFPII), lacto-N-neotetraose (LNnT), lacto-N-fucopentaose I (LNFPI), sialyl-lacto-N-tetraose c (LSTc) and fucosyllacto-N-hexaose (FLNH), and relatively lower concentrations of lacto-N-hexaose (LNH), lacto-N-tetrose (LNT), 2'-fucosyllactose (2'FL), and disialyllacto-N-hexaose (DSLNH).

Finally, to account for potential confounders and adjust for known allergy risk factors, we evaluated the PLS-DA score in multivariable logistic regression models (**Table 1**). Compared to infants consuming milk with a discriminant score in the highest quintile, those in the lowest quintile had a 90% lower risk of food sensitization (Odds Ratio (OR) 0.10 (95% CI 0.03, 0.34)).

To our knowledge, only one previous study has explored the association of HMOs with food sensitization in children, where infants receiving milk with low LNFPIII concentrations were more likely to develop cow's milk allergy (7). In contrast, we did not observe associations of any individual HMOs with food sensitization, and LNFPIII was not among the most discriminatory HMOs in our analysis. This may reflect differences in study populations (high risk infants (7) vs. our general population cohort), timing of milk collection (one month vs. three-four months), or outcomes assessed (confirmed milk allergy (7) vs. sensitization to food allergens).

Recently, a randomized trial reported that infants receiving formula supplemented with 2'FL had more similar immune responses to breastfed controls, compared to infants receiving formula without 2'FL (10). In addition, a rodent study showed that 2'FL and 6'SL can reduce symptoms of food allergy (6). In contrast, we did not find an association of 2'FL or 6'SL or any other individual HMO with infant food sensitization. Instead, overall HMO

composition was associated with food sensitization, reflecting the complexity of human milk and its evolution to supply the breastfed infant with many different HMOs.

While the causality of these associations remains to be determined, there are several plausible mechanisms by which HMO profiles could influence food sensitization. For example, HMOs modulate immune development through their prebiotic effects on gut bacteria, and by influencing lymphocyte maturation (3). Further research is needed to determine if the “beneficial” HMO profile we have identified can optimally stimulate these developmental processes, and to identify the maternal and environmental factors that promote a “beneficial” HMO profile.

To our knowledge, this is the largest study to examine the association of HMOs and allergy development in infants, and the first to evaluate overall HMO profiles. Key strengths include the prospective design within a large population-based cohort, and standardized skin testing to assess food sensitization. Our methods allowed absolute quantification of HMOs, and we applied a novel multivariate approach to account for the natural occurrence of HMOs in complex combinations within human milk. The main limitation of our study is the lack of external validation; however, our PLS-DA results were robust to cross-validation. Finally, we acknowledge that food sensitization during infancy does not always persist into later childhood; however, it is an important clinical outcome and a strong predictor of future atopic disease (1).

In conclusion, our results demonstrate that HMO composition is associated with the development of food sensitization in the first year of life, and emphasize that overall profiles should be considered when examining the health effects of HMOs or considering their utility for therapeutic interventions. Further research is warranted to confirm our findings in other populations, explore the underlying biological mechanisms, and establish the long term consequences of HMO composition on confirmed allergic disease later in childhood.

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Table 1. Association of HMO profiles at 3-4 months with food sensitization at 1 year in the CHILD cohort (N=421)

HMO profile: PLS-DA score quintile (range)	Food sensitization at 1 year	
	Basic model OR (95% CI) N=421	Adjusted model OR (95% CI) N=369
Quintile 1 (-1.69, 0.63)	0.12 (0.04, 0.37)**	0.10 (0.03, 0.34)**
Quintile 2 (0.63, 1.18)	0.12 (0.04, 0.37)**	0.10 (0.03, 0.32)**
Quintile 3 (1.18, 1.57)	0.32 (0.14, 0.72)*	0.26 (0.10, 0.67)*
Quintile 4 (1.57, 2.17)	0.59 (0.29, 1.22)	0.62 (0.26, 1.46)
Quintile 5 (2.17, 5.16)	<i>Reference</i>	<i>Reference</i>
<i>P for trend</i>	<i><0.001</i>	<i><0.001</i>

Values are odds ratios (OR) and 95% confidence interval (CI). Basic models are adjusted for child's sex and age. Multivariable adjusted models are basic models additionally adjusted for maternal ethnicity, education, reported maternal food allergy, lactation time and infant birth weight and gestational age at birth, breastfeeding duration, breastfeeding exclusivity at 6 months, timing of introduction of solid food, household pets and study site.

Abbreviations: CHILD, Canadian Healthy Infant Longitudinal Development; HMOs, human milk oligosaccharides; PLS-DA Projection on Latent Structures-Discriminant Analysis.

P for trend is obtained by using HMOs discriminant score as an ordinal variable in the regression models.

P-value<0.001** <0.05*

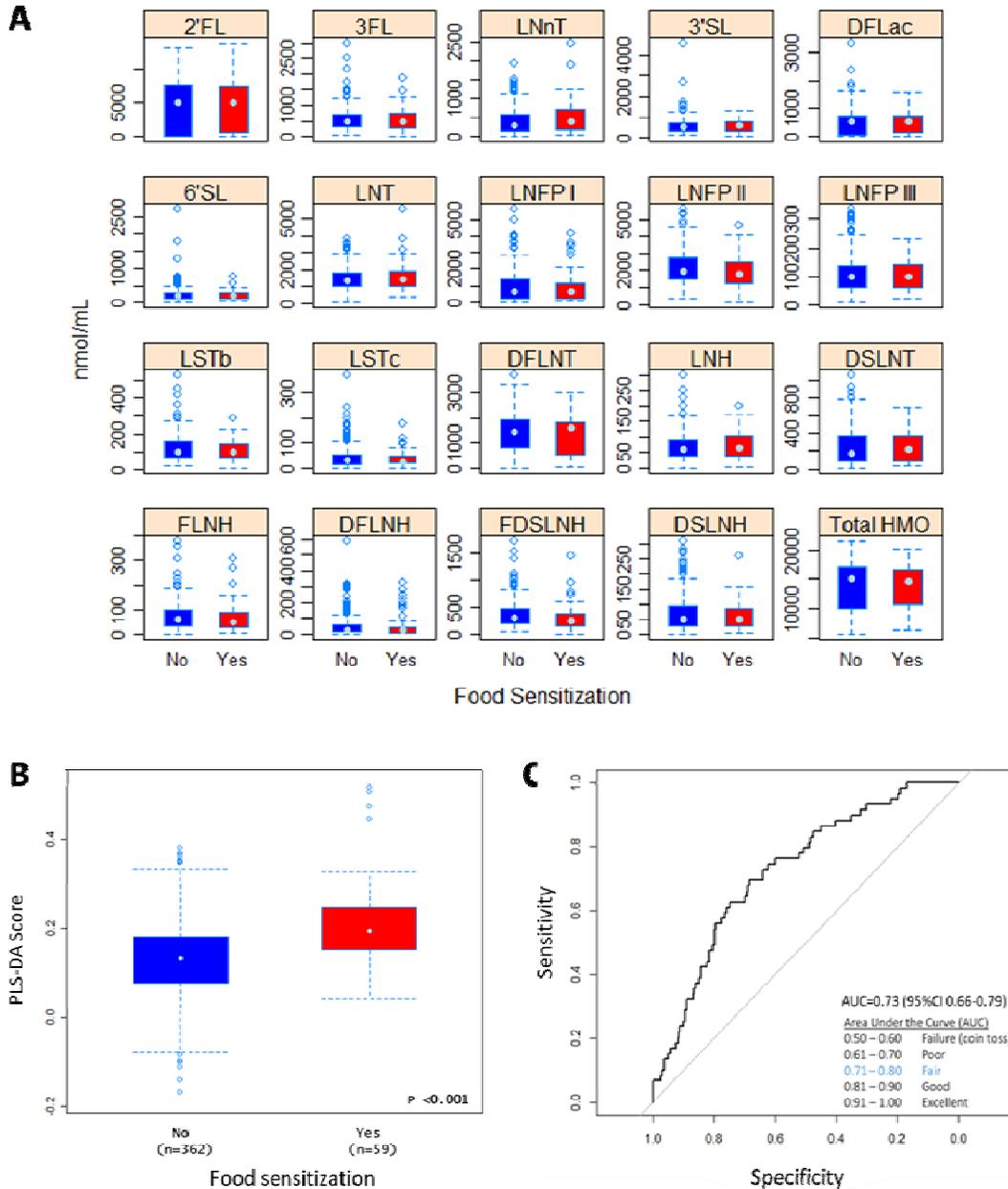


Figure 1a-c. Association of individual and total HMOs (a) overall HMO profile (b) (PLS discriminant score) at 3-4 months and food sensitization at 1 year in the CHILD cohort and (c) the receiver operating characteristics (ROC) curve (N=421)

Boxes indicate interquartile range; white dots indicate median values; whiskers indicate range. Mann-Whitney test ($p < 0.001$).

Abbreviations: PLS-DA, Projection on Latent Structures-Discriminant Analysis.

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MBA conceived and managed the study. The CHILD Study Founding Director (MRS) and site leaders (ABB, PS, PJM and SET) conceived the CHILD cohort design, managed study recruitment and oversaw clinical assessments of study participants. DLL managed the CHILD Study database. LB oversaw HMO analysis of breast milk samples; BR performed these analyses. AKSH conducted the PLS discriminant analysis; KM conducted all other statistical analyses. KM and MBA interpreted the data and drafted the manuscript. All co-authors provided feedback and approved the final version. KM had full access to the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Conflict of interest

None.