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Østfold Hospital Trust, Kalnes, Norway), Sandra G. Tedner, MD (Astrid Lindgren Children's Hospital, Karolinska University Hospital, Stockholm, Sweden; Department of Women´s and Children´s Health, Karolinska Institutet, Stockholm, Sweden), Magdalena R. Værnesbranden, MD (University of Oslo, Faculty of Medicine, Institute of Clinical Medicine, Oslo, Norway; Institute of Biotechnology, University of Helsinki, Helsinki, Finland), Johanna Wiik, MD (Institute of Biotechnology, University of Helsinki, Helsinki, Finland; Department of Obstetrics and Gynecology, Institute of Clinical Sciences, Sahlgrenska Academy, Gothenburg, Sweden)

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

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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 most infant formulas.[3] HMO composition is influenced by genetic fucosyltransferase-2 secretor status and also 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 a small clinical study where low concentrations of the HMO lacto-N-fucopentaose III (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 this 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 1 year of age using Projection on Latent Structures-Discriminant Analysis (PLS-DA).[9] Detailed methods are provided in Supplementary Materials.

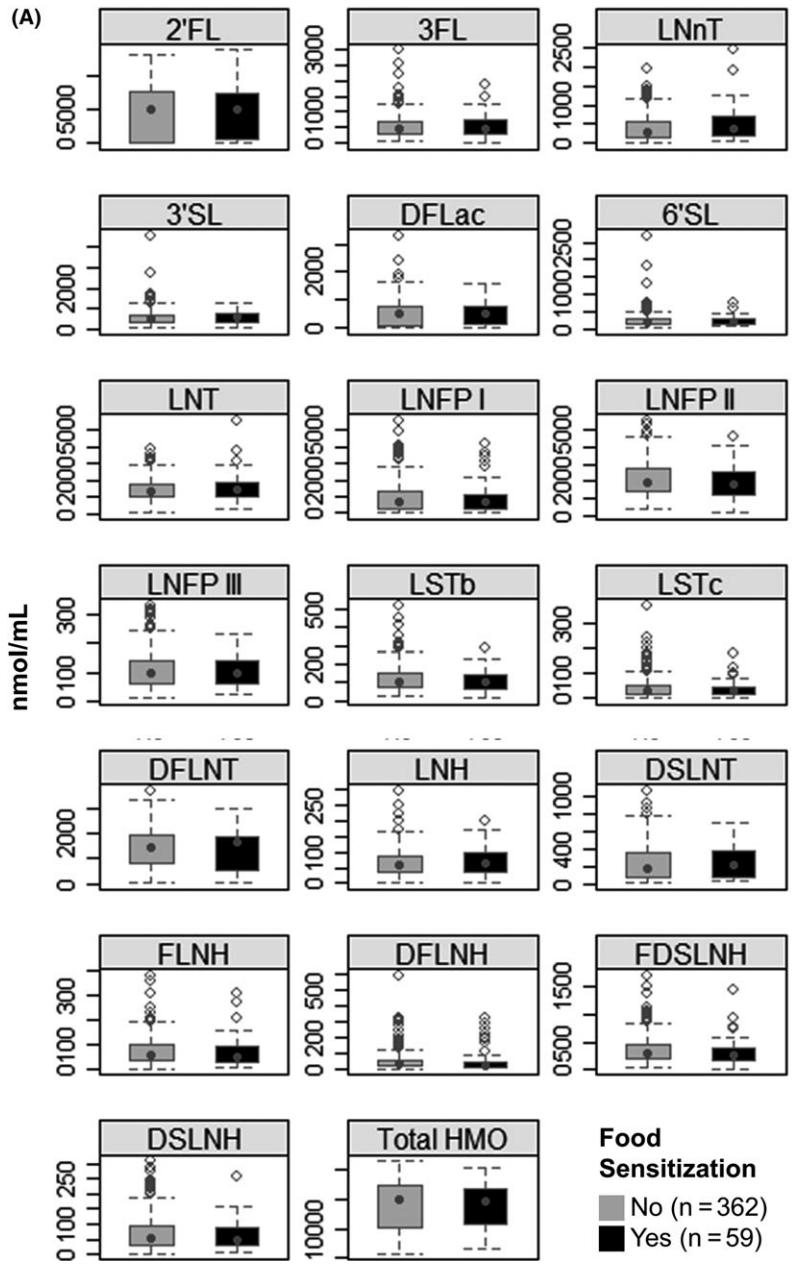
Overall, 59/421 infants (14.0%) were sensitized to 1 or more food allergens at 1 year of age (Table S1). We did not observe any significant associations for the 19 individual HMOs or total HMOs and food sensitization (Figure 1A); however, overall HMO profiles differed significantly in milk consumed by sensitized vs nonsensitized infants ($P < .001$; 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; $P = .02$) (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) (Figure S1).

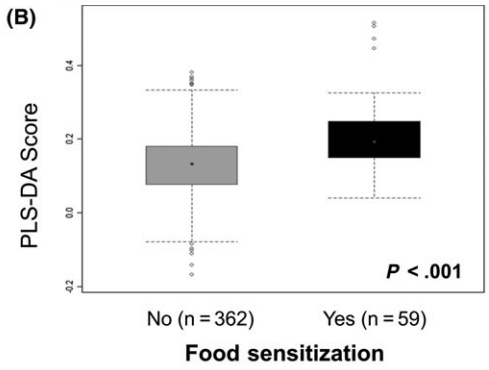
Restricting our analysis to the top 10 most important HMOs contributing to the PLS-DA score resulted in similar discrimination (AUC 0.71; 95% CI: 0.64-0.78), indicating that these 10 HMOs are sufficient to explain the association of HMO profile and food sensitization. The rankings, PLS-DA scaled importance scores, and direction of association for these 10 HMOs are shown in Figure 1D. 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).

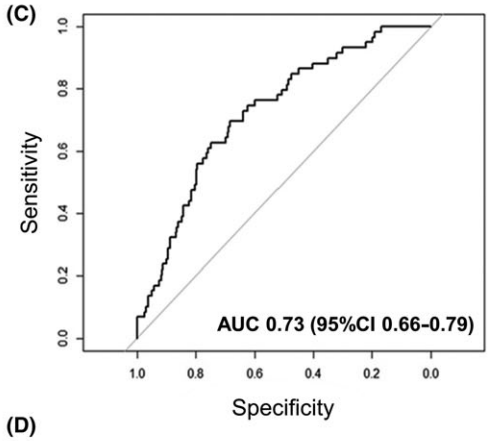
Finally, to account for potential confounders and adjust for known allergy risk factors, we evaluated the PLS-DA score in multivariable logistic regression models (Table 1). Compared to infants consuming milk with a discriminant score in the highest quintile, those in the lowest quintile had a 90% lower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

To our knowledge, only 1 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 (1 month vs 3-4 months), or outcomes assessed (confirmed milk allergy[7] vs sensitization to various 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







(D)

HMO	Higher (+) or Lower (–) in milk consumed by healthy infants	PLS-DA Scaled Importance Score	Rank
FDSLNH	+	100	1
LNH	–	78	2
LNFPII	+	73	3
LNnT	+	73	4
LNT	–	67	5
LNFPI	+	67	6
LSTc	+	63	7
FLNH	+	60	8
2'FL	–	60	9
DSLNH	–	57	10

Figure 1. A-B, Association of individual and total HMOs (A) and overall HMO profile (B) at 3-4 months with food sensitization at 1 year in the CHILD cohort (N = 421). C, Receiver operating characteristic (ROC) curve. D, Scaled importance scores, ranking and direction of association for the top 10 HMOs contributing to the overall PLS-DA score. Boxes indicate interquartile range; white dots indicate median values; whiskers indicate range. Mann-Whitney *U* test ($P < .001$). Abbreviations: PLS-DA, Projection on Latent Structures-Discriminant Analysis, AUC, area under the curve. 2'FL, 2'-fucosyllactose; 3FL, 3-fucosyllactose; LNnT, lacto-N-neotetraose; 3'SL, 3'-sialyllactose; DFLac, difucosyllactose; 6'SL, 6'-sialyllactose; LNT, lacto-N-tetrose; LNFP I, lacto-N-fucopentaose-I; LNFP II, lacto-N-fucopentaose-II; LNFP III, lacto-N-fucopentaose-III; LSTb, sialyl-lacto-N-tetraose b; LSTc, sialyl-lacto-N-tetraose c; DFLNT, difucosyllacto-N-tetrose; LNH, lacto-N-hexaose; DSLNT, disialyllacto-N-tetraose; FLNH, fucosyllacto-N-hexaose; DFLNH, difucosyllacto-N-hexaose; FDSLNH, fucodisialyllacto-N-hexaose; DSLNH, disialyllacto-N-hexaose.

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, in our study, 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 whether the "beneficial" HMO profile we have identified can optimally stimulate these developmental

TABLE 1 Association of HMO profiles at 3-4 mo with food sensitization at 1 y in the CHILD cohort (N = 421)

	Food sensitization at 1 y	
HMO profile: PLS-DA score quintile (range)	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)	*Reference*	*Reference*
P for trend	<.001	<.001

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, self-reported maternal food allergy, lactation stage (weeks postpartum), infant birthweight and gestational age at birth, breastfeeding duration, breastfeeding exclusivity at 6 mo, timing of introduction of solid food, household pets, and study site.

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

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

P-value <.001** <.05*.

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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CONFLICT OF INTEREST

None.

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ORCID

K. Miliku http://orcid.org/0000-0002-9614-7191
M. B. Azad http://orcid.org/0000-0002-5942-4444

K. Miliku[1,2,3]
B. Robertson[4]
A. K. Sharma[5]
P. Subbarao[6]
A. B. Becker[1,2]
P. J. Mandhane[7]
S. E. Turvey[8]
D. L. Lefebvre[9]
M. R. Sears[9]
the CHILD Study Investigators[10]
L. Bode[4]
M. B. Azad[1,2,*]

[1]*Manitoba Developmental Origins of Chronic Diseases in Children Network (DEVOTION), Children's Hospital Research Institute of Manitoba, Winnipeg, MB, Canada*
[2]*Department of Pediatrics and Child Health, University of Manitoba, Winnipeg, MB, Canada*
[3]*The Generation R Study Group, Departments of Pediatrics and Epidemiology, Erasmus University Medical Center Rotterdam, Rotterdam, The Netherlands*
[4]*Department of Pediatrics and Larsson-Rosenquist Foundation Mother-Milk-Infant Center of Research Excellence, University of California San Diego, La Jolla, CA, USA*
[5]*George & Fay Yee Centre for Healthcare Innovation, University of*

Manitoba, Winnipeg, MB, Canada
[6]*Departments of Pediatrics & Physiology, University of Toronto, Toronto, ON, Canada*
[7]*Department of Pediatrics, University of Alberta, Edmonton, AB, Canada*
[8]*Department of Pediatrics, University of British Columbia, Vancouver, BC, Canada*
[9]*Department of Medicine, McMaster University, Hamilton, ON, Canada*
[10]*CHILD Study Investigators are listed in Appendix 1*

Correspondence
Meghan Azad, Children's Hospital Research Institute of Manitoba, Winnipeg, MB, Canada.
Email: meghan.azad@umanitoba.ca

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

APPENDIX 1

CHILD investigators include the following: Subbarao P (Director), The Hospital for Sick Children & University of Toronto; Turvey SE, University of British Columbia (co-Director), Anand SS, McMaster University; Azad M, University of Manitoba; Becker AB, University of Manitoba; Befus AD, University of Alberta; Brauer M, University of British Columbia; Brook JR, University of Toronto; Chen E, Northwestern University, Chicago; Cyr M, McMaster University; Daley D, University of British Columbia; Dell SD, The Hospital for Sick Children & University of Toronto; Denburg JA, McMaster University; Duan Q, Queen's University; Eiwegger T, The Hospital for Sick Children & University of Toronto; Grasemann H, The Hospital for Sick Children & University of Toronto; K HayGlass, University of Manitoba; Hegele RG , The Hospital for Sick Children & University of Toronto; Holness DL, University of Toronto; Hystad P, Oregon State University; Kobor M, University of British Columbia; Kollman TR, University of British Columbia; Kozyrskyj AL, University of Alberta; Laprise C, Université du Québec à Chicoutimi; Lou WYW, University of Toronto; Macri J, McMaster University; Mandhane PJ, University of Alberta; Miller G, Northwestern University, Chicago; Moraes TJ, The Hospital for Sick Children & University of Toronto; Paré, University of British Columbia; Ramsey C, University of Manitoba; Ratjen F, The Hospital for Sick Children & University of Toronto; Sandford A, University of British Columbia; JA Scott, University of Toronto; Scott J, University of Toronto; Sears MR, (Founding Director), McMaster University; Silverman F, University of Toronto; Simons E, University of Manitoba; Takaro T, Simon Fraser University; Tebbutt S, University of British Columbia; To T, The Hospital for Sick Children & University of Toronto.

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Updosing of bilastine is effective in moderate to severe chronic spontaneous urticaria: A real-life study

To the Editor,

We report an open-label study of the effects of bilastine at 20, 40 and 80 mg daily for 2 weeks on the signs and symptoms of chronic spontaneous urticaria (CSU) in patients who had not responded sufficiently to licensed doses of other H_1-antihistamines. The study was designed to mimic the real-life situation in which the antihistamine dose is increased gradually up to fourfold the licensed dose, depending on the effectiveness of the previous dose. Its limitations include the relatively small number of patients and uncontrolled design.