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Human milk oligosaccharides in Chinese lactating mothers and relationship with allergy development in offspring

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Abstract

Background: The health benefits of breastfeeding are partly contributed by human milk oligosaccharides (HMOs), but there is limited data on breast milk (BM) HMO composition in Chinese.

Objective: This study investigated the association between early-life HMO intake and allergy occurrence in Chinese children.

Methods: 103 healthy Chinese pregnant women regardless of allergy history were recruited into this birth cohort. Their babies were followed until 24 months old. Concentrations of 2′-fucosyllactose (2′-FL), lacto-N-neotetraose (LNnT), 3′-sialyllactose (3′-SL) and 6′-sialyllactose (6′-SL) in BM collected at 1-month postpartum were measured by liquid chromatography-mass spectrometry. The associations between these HMOs and allergy occurrence by 24 months were analyzed by multivariate regression analyses.

Results: Twenty-nine percent and 19% of participants had eczema at 12 and 24 months old respectively. Eighty BM samples were analyzed, with 2′-FL being the most abundant HMO (median 1447 ppm, interquartile range [IQR] 291-1906 ppm), and median (IQR) levels of LNnT, 6′-SL and 3′-SL in ppm were 738 (580-950), 20.5 (12.7-38.8) and 23.0 (17.8-27.6) respectively. Participants with eczema by 24 months consumed BM with higher 2′-FL concentration at 1-month (P = 0.008), and also lower 6′-SL concentration in exclusively breastfed infants (P = 0.012) but higher 6′-SL concentration for those with mixed feeding at 1 month (P = 0.043). Food allergic children at 12 months consumed BM with higher 2′-FL concentrations at 1 month (P = 0.048).

Conclusions: BM 2′-FL concentration is higher in children who develops eczema by 24 months and food allergy during infancy. The relationship for 6′-SL is divergent depending on mode of feeding in infants.

Key words: Birth cohort, breastfeeding, eczema, human milk oligosaccharide, prevention

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Introduction

Eczema is the most common chronic inflammatory skin disease in children, which is also linked to the subsequent development of asthma, allergic rhinitis and conjunctivitis (i.e. atopic march). Breastfeeding has health benefits for the mother and child. Exclusive breastfeeding for the first 6 months of an infant's life is recognized as the "gold" standard

for infant feeding. Breast milk (BM) contains immunological components that protect against infections and allergic disease in infancy.[1] The composition of human BM is complex, containing factors that interact with the infant immune system and intestinal milieu including allergens, cytokines, immunoglobulins, polyunsaturated fatty acids, and chemokines. Microbial diversity of BM is intrinsic to healthy immune maturation of the breastfed infants. Besides, early-life nutrition exerts significant influences on the evolution and composition of stool microbiome.[2]

Apart from the passive transfer of maternal antibodies, the presence of diverse human milk oligosaccharides (HMOs) in BM exerts potent influences on early-life immune development. HMOs, forming the so-called milk glycobiome, are the third most abundant class of biomolecules found in BM after lactose and lipids, reaching between 5 and 20 g/L in mature human milk.[3] HMOs are composed out of only 5 different monosaccharides, namely glucose, galactose, N-acetyl-glucosamine, fucose, and sialic acid, being conjugated via several linkage types (i.e. glycosidic bonds).[4] The presence of this unique diversity of HMOs in BM suggests different biological functions and mechanisms by which they may influence the infant's microbiome and immune maturation. HMOs have the following main functions: (a) antimicrobial and antiviral effects;[5,6] (b) prebiotic effects;[7] (c) mucosal barrier maturation;[8] (d) modulation of pathogen recognition;[9] and (e) immunomodulatory effects.[10,11]

Fucosylated HMOs and N-glycans on milk proteins are also beneficial for the development of healthy gut microbiota. Bai et al. reported results of longitudinal research on paired milk and stool samples from 56 Chinese mothers and their breast-fed children.[12] Alterations in the levels of fucosylated HMOs and milk N-glycans were highly correlated with the growth of *Bifidobacterium* spp. and *Lactobacillus* spp. in the gut of infants during early and later lactation, respectively. Nonetheless, it remains unclear how the diverse HMO structures may protect against infections and allergies.

Based on the above observations, we hypothesized that dietary HMO intake would influence the postnatal development of eczema and allergy in Chinese infants. This study aimed to characterize HMO composition in BM from Chinese lactating mothers and to examine any association between exposure to major HMOs and occurrence of allergic diseases by 24 months of age.

Methods

Participants

This SMART Gen HK, an acronym for InteStinal Microbiota on Allergy, GRowth and DevelopmenT of the Next Generation in Hong Kong, is a mother-child cohort that recruited healthy Chinese pregnant women from November 2018 to May 2019. All newborns were delivered in our university-affiliated teaching hospital.[13] These pregnant mothers were not selected in relation to allergy status (i.e. standard-risk for allergy). Inclusion criteria included: (i) parents were ethnic Chinese by self reporting; (ii) singleton born at 37-41 weeks' gestation; (iii) no persistent dermatitis within four weeks after birth; and (iv) no congenital anomalies or syndromal diagnosis. Pregnant women gave informed written consent to participate. Joint Chinese University of Hong Kong-New Territories East Cluster Clinical Research Ethics Committee approved this study (reference CRE-2018.252).

Participants' perinatal history, family history of allergies, socioeconomic background and environmental exposures were recorded.[14] They attended study visits at 6 ± 1, 12 ± 1 and 24 ± 2 months, when occurrence of eczema was diagnosed by pediatricians according to Hanifin and Rajka criteria.[15] Food allergy was diagnosed by pediatricians based on suspicious clinical manifestations together with positive skin prick test (SPT) and/or oral food challenges.

All participants underwent SPT with locally relevant allergens (cow's milk, hen's egg, soybean, wheat, peanut, mixed fish (cod fish, flounder, halibut, mackerel, tuna) and *D. pteronyssinus* (ALK Abelló AS, Hørsholm, Denmark)[14,16,17] as well as positive (histamine 10 mg/mL) and negative (normal saline) controls at 12 months old. Wheal ≥ 3 mm larger than negative control was regarded as positive. Participants were atopic when they had positive result to at least one of the above allergens.

Collection and processing of BM samples

Home visits were arranged at 4 ± 1 weeks postpartum to collect 10 mL BM samples expressed either manually or by breast pump from mothers who were breastfeeding their babies. These samples were transferred to laboratory on ice and stored at -80°C until analysis. Four HMOs, namely 2′-fucosyllactose (2′-FL), lacto-N-neotetraose (LNnT), 6′-sialyllactose (6′-SL), and 3′-sialyllactose (3′-SL), with commercially available standards (Glycom A/S, Horsholm, Denmark) were selected for measurement in this study. Briefly, the aqueous layer of raw milk was obtained after removing the fat and protein, followed by drying using rotary evaporation.[18,19] The dried samples were dissolved in distilled water and mixed with equal volume of 1 M $NaBH_4$ at 65°C for 1.5 hour, followed by solid phase extraction (SPE) using graphitized carbon cartridges. The SPE cartridges were washed, and HMOs eluted then lyophilized. The samples were reconstituted in 0.05 M NaCl solution and diluted before mass spectrometry (MS) analysis. Standards of the above four HMOs were reduced by $NaBH_4$, eluted by SPE and diluted by NaCl solution to plot calibration curves.

HMO detection and analysis

The extracted HMO samples were analyzed using Agilent 6460 electrospray ionization triple quadruple mass spectrometer equipped with Agilent 1290 liquid chromatography (LC) system. A porous graphitic carbon column (Hypercarb, ThermoFisher) was applied to elute different HMOs.[18] The column temperature was 40°C. Ten mM ammonium in 0.1% ammonia and 0.1% ammonia (v/v) in ACN were used as solvent A and B, respectively. LC gradient was set for a total run of 60 minutes with flow rate ranged from 0.1 to 0.15 ml/ml. MS analysis was operated in positive mode. Fragmentor voltage and collision energy were optimized based on different HMO standards.

Data processing and analysis

Univariate analyses by Mann-Whitney U test were conducted to compare HMO concentrations between different clinical outcome groups (e.g. eczema ever, atopy, food allergy) within the first 24 months of life. Multivariate analyses by logistic regression were further performed to confirm HMOs that were independently associated with eczema and other allergy outcomes, adjusting for covariates such as sex and mode of delivery. All analyses were performed using R version 4.1.3.

Results

Study population

Eighty-six BM samples were collected from the 103 mother-child dyads enrolled in this birth cohort. Six samples were subsequently excluded because the infants were found to be exclusively formula-fed before sample collection. **Table 1** summarises the demographics, feeding patterns, allergy diagnoses, and SPT results of 80 participants who were included in the current analysis. Sixty-seven (84%) of them were born by vaginal delivery, and two-fifths were exposed to intrapartum antibiotics. At one month of age, 51% of participants had exclusive breastfeeding while 49% had mixed feeding. Sixty, 63 and 54 subjects contributed allergy data at 6, 12 and 24 months old respectively. All these characteristics did not differ between children with and without eczema by 24 months old (see **Table S1**).

HMO concentrations in 1-month BM samples

Table S2 provides the distribution of four HMOs in individual BM samples. In our cohort, 2′-FL was the most abundant HMO (median [interquartile range, IQR]: 1447 [291-1906] ppm) while BM samples contained lower levels of LNnT (median [IQR]: 738 [580-950] ppm), 6′-SL (median

Table 1. Demographics and clinical characteristics of study participants

Characteristics (N = 80)	Result
Maternal characteristics	
Received higher than secondary school education, n/N (%)	56/80 (70.0)
History of allergy, n/N (%)	10/80 (12.5)
Paternal characteristics	
Received higher than secondary school education, n/N (%)	47/79 (59.5)
History of allergy, n/N (%)	10/79 (12.7)
Child characteristics	
Male, n/N (%)	36/80 (45.0)
Gestational age (weeks)	39.1 ± 1.2
Birth weight (g)	3060 ± 393
Born by vaginal delivery, n/N (%)	67/80 (83.8)
Received intrapartum antibiotics, n/N (%)	32/80 (40.0)
Exclusive breastfeeding at 1 month, n/N (%)	41/80 (51.2)
Mixed breast and formula feeding at 1 month, n/N (%)	39/80 (48.8)
Exposure to household smoking at 1 month, n/N (%)	16/79 (20.3)
Furry pets at home at 1 month, n/N (%)	17/79 (21.5)
Allergy diagnosis, n/N (%)	
Eczema at 6 months	24/60 (40.0)
Eczema at 12 months	18/63 (28.6)
Eczema at 24 months	10/54 (18.5)
Food allergy at 6 months	0/60 (0.0)
Food allergy at 12 months	4/62 (6.5)
Food allergy at 24 months	4/54 (7.4)
Allergic rhinitis or asthma by 12 months	0/74 (0.0)
Wheeze episode by 12 months	5/74 (6.8)
Atopy revealed by skin prick test at 12 months, n/N (%)	11/58 (19.0)

Results expressed in number (percentage) or mean ± standard deviation.

Table 2. Relationship between HMO concentrations and selected demographic factors and perinatal exposures.

Variable	No. of subjects	Total HMO		2′-FL		LNnT		6′-SL		3′-SL	
		Median (IQR)	*P*	Median (IQR)	*P*	Median (IQR)	*P*	Median (IQR)	*P*	Median (IQR)	*P*
Maternal educational level											
Secondary school	24	2228 (1877-2673)	0.705	1369 (28.3-1862)	0.469	682 (553-1040)	0.867	29.4 (16.4-42.8)	0.061	24.8 (19.8-29.9)	0.115
Higher than secondary school	56	2298 (1922-2809)		1536 (839-1906)		758.6 (602-914)		19.4 (10.6-35.2)		21.3 (16.7-26.9)	
Maternal history of allergy											
No	70	2258 (1907-2786)	0.716	1429 (42.4-1929)	0.694	724 (562-1005)	0.476	20.2 (12.2-39.2)	0.565	22.3 (17.5-27.4)	0.816
Yes	10	2455 (1992-2595)		1601 (1336-1841)		900 (658-920)		26.3 (16.1-36.8)		24.7 (18.9-27.5)	
Intrapartum antibiotics											
No	48	2256 (1905-2594)	0.473	1429 (753-1814)	0.371	735 (554-909)	0.768	21.1 (13.1-37.9)	0.694	24.4 (19.1-27.8)	0.243
Yes	32	2324 (1921-2924)		1565 (43.5-1998)		761 (594-980)		19.8 (10.5-38.8)		20.9 (17.1-26.4)	
Sex of child											
Male	36	2229 (1928-2614)	0.542	1377 (290-1817)	0.575	686 (586-991)	0.656	21.1 (10.3-35.7)	0.908	22.7 (18.0-26.9)	0.656
Female	44	2354 (1889-2883)		1545 (556-1938)		802 (580-914)		19.4 (13.1-39.4)		23.2 (17.8-27.9)	
Mode of delivery											
Vaginal birth	67	2238 (1798-2631)	0.067	1390 (41.3-1832)	**0.035**	726 (552-928)	0.681	21.2 (12.5-39.0)	0.506	23.5 (18.3-27.7)	0.422
Caesarean section	13	2497 (2168-2939)		1872 (1378-2171)		758 (632-968)		17.8 (14.2-23.2)		21.3 (16.8-25.2)	

2′-FL, 2′-fucosyllactose; HMO, human milk oligosaccharide; IQR, interquartile range; LNnT, lacto-N-neotetraose; 3′-SL, 3′-sialyllactose; 6′-SL, 6′-sialyllactose.
HMO concentrations were expressed in parts per million (ppm).
P-value < 0.05 was highlighted in bold.

[IQR]: 20.5 [12.7-38.8] ppm) and 3′-SL (median [IQR]: 23.0 [17.8-27.6] ppm). Mothers delivering babies by caesarean section had a higher level of 2′-FL in their BM (P = 0.035) (**Table 2**), while demographic factors and other perinatal exposures were not associated with the concentrations of these four HMOs. **Figure 1** shows heatmap on Spearman correlations between maternal age and levels of four individual and total HMOs. Total HMO level correlated significantly with 2′-FL level (P < 0.001). On the other hand, there was no significant correlation among four HMO levels or between maternal age and their levels.

Eczema development and early-life HMO exposures

Twenty-two (41%) of 54 participants seen at 24 months of age had eczema within the first two years. **Table 3** summarizes HMO concentrations in participants with and without eczema in the first two years of life. Compared to those never diagnosed with eczema, participants who developed eczema had consumed BM at 1-month that contained significantly higher 2′-FL concentration (P = 0.006). This finding remained significant by logistic regression analysis following adjustment for mode of delivery and feeding pattern at 1 month (odds ratio [OR] 1.001 and 95% confidence interval [CI] 1.000-1.002; adjusted P = 0.010). **Table S3** compared HMOs in 26 children who lost to follow up and those with and without eczema. Those lost to follow up had similar HMOs as children with eczema. **Table 4** describes HMO concentrations in infant subgroups with different feeding patterns. The difference in BM 2′-FL levels in participants with and without eczema by 24 months was significant only in those exclusively breastfed at 1 month (P = 0.046). In addition, participants with eczema ever consumed BM with lower 6′-SL concentration among the subgroup of infants who were exclusively breastfed (P = 0.012). On the other hand, participants with mixed feeding at 1 month who developed eczema within two years consumed BM that contained higher levels of 6′-SL (P = 0.043). The total HMO concentration was higher in those with eczema by 24 months only in participants who received mixed feeding at 1 month (P = 0.040).

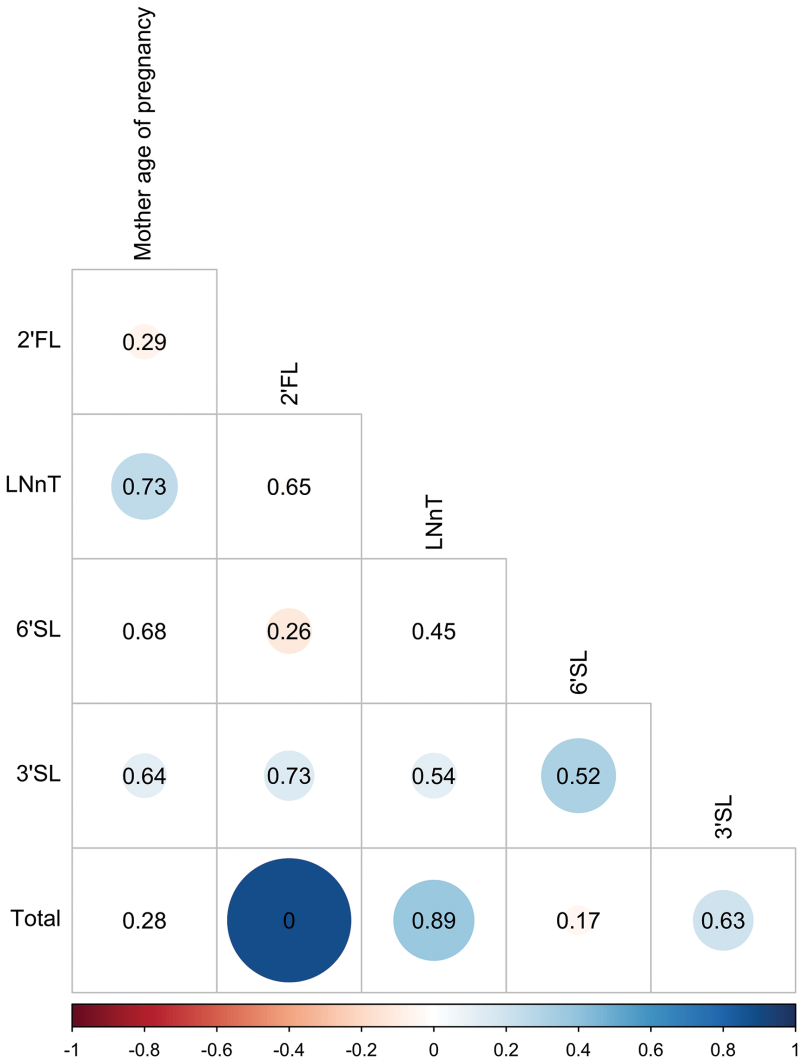

Figure 1. Heatmap on the Spearman correlation between maternal age and levels of four individual and total HMOs. A darker and larger circle represents that higher Spearman correlation coefficient, while the corresponding *P*-value is shown in the centre of each circle. 2′-FL, 2′-fucosyllactose; LNnT, lacto-N-neotetraose; 3′-SL, 3′-sialyllactose; 6′-SL, 6′-sialyllactose.

Table 3. Concentrations of HMOs in breast milk consumed by participants with and without eczema development in the first two years of life.

HMO	Never eczema within 24 months (N = 32)	Eczema ever within 24 months (N = 22)	*P*
2′-FL	1030 (25.4-1627)	1613 (1330-1951)	**0.006**
LNnT	631 (483-984)	859 (731-912)	0.175
6′-SL	26.8 (10.6-39.4)	19.4 (14.3-37.8)	0.705
3′-SL	21.2 (17.0-26.2)	23.3 (18.1-27.0)	0.573
Total	2048 (1057-2427)	2467 (2021-2877)	**0.013**

2′-FL, 2′-fucosyllactose; HMO, human milk oligosaccharide; LNnT, lacto-N-neotetraose; 3′-SL, 3′-sialyllactose; 6′-SL, 6′-sialyllactose.
Results expressed in median (interquartile range).
HMO concentrations were expressed in parts per million (ppm).
P-value < 0.05 was highlighted in bold.

Table 4. HMO concentrations in breast milk among participants with different early-life feeding patterns at one month who had or did not have eczema by 24 months.

HMO	Exclusive breastfeeding			Mixed feeding		
	Never eczema (N = 17)	Eczema ever (N = 11)	*P*	Never eczema (N = 15)	Eczema ever (N = 11)	*P*
2′-FL	1177 (15.8-1810)	1872 (1462-1948)	**0.046**	883 (28.1-1470)	1561 (1214-1826)	0.052
LNnT	632 (550-1103)	795 (561-902)	0.981	631 (421-926)	864 (798-1009)	0.092
6′-SL	35.2 (21.2-40.6)	19.3 (11.4-21.4)	**0.012**	10.7 (4.72-26.8)	35.2 (14.6-45.1)	**0.043**
3′-SL	24.8 (20.9-27.7)	19.1 (14.9-25.8)	0.359	18.7 (13.8-22.7)	25.1 (20.2-28.0)	0.073
Total	2218 (1679-2435)	2549 (2072-2788)	0.138	1991 (478-2306)	2356 (2060-2887)	**0.040**

2′-FL, 2′-fucosyllactose; HMO, human milk oligosaccharide; LNnT, lacto-N-neotetraose; 3′-SL, 3′-sialyllactose; 6′-SL, 6′-sialyllactose.
Results expressed in median (interquartile range).
HMO concentrations were expressed in parts per million (ppm).
P-value < 0.05 was highlighted in bold.

Table 5. Concentrations of HMOs in breast milk consumed by participants with and without atopy or food allergy at the age of 12 months.

HMO	Atopy*			Food allergy		
	Yes (N = 11)	No (N = 47)	*P*	Yes (N = 4)	No (N = 58)	*P*
2′-FL	1561 (1206-1851)	1463 (39.2-1903)	0.532	2005 (1849-2223)	1447 (471-1863)	**0.032**
LNnT	759 (548-952)	712 (590-900)	0.866	899 (830-980)	682 (549-886)	0.122
6′-SL	17.8 (11.3-31.9)	19.5 (12.5-38.4)	0.545	31.8 (19.7-37.7)	19.5 (13.0-34.9)	0.774
3′-SL	24.9 (20.6-27.3)	21.9 (16.6-27.4)	0.388	21.9 (16.8-25.8)	24.0 (18.3-27.5)	0.753
Total	2218 (1924-2912)	2276 (1773-2618)	0.627	2983 (2823-3184)	2233 (1894-2578)	**0.012**

2′-FL, 2′-fucosyllactose; HMO, human milk oligosaccharide; LNnT, lacto-N-neotetraose; 3′-SL, 3′-sialyllactose; 6′-SL, 6′-sialyllactose.
Results expressed in median (interquartile range).
HMO concentrations were expressed in parts per million (ppm).
P-values < 0.05 were highlighted in bold.
* Defined by ≥ 1 positive skin prick tests.

Food allergy and HMO intake at 1 month

At 12 months of age, 11 (19%) of participants were atopic as revealed by SPT. **Table 5** illustrates HMO concentrations in relation to atopy and food allergy. No significant association was detected between atopy and concentrations of four HMOs in BM at 1 month. Four participants had food allergy at 12 months (see **Table S4**). These food allergic participants consumed BM that contained higher 2′-FL concentrations at 1 month by univariate (P = 0.032) and logistic regression analyses (OR 1.003 and 95%CI 1.000-1.005; adjusted P = 0.048). The total HMO concentration was also higher in infants with food allergy (P = 0.012).

Discussion

In this birth cohort, we measured the levels of four HMOs namely 2′-FL, LNnT, 3′-SL and 6′-SL in 1-month BM sample from 80 healthy Chinese pregnant women regardless of allergy history and followed their offspring until 24 months old. 2′-FL was the most abundant HMO followed by LNnT, 3′-SL and 6′-SL. Participants who developed eczema by 24 months consumed BM with higher 2′-FL concentration. Eczema was associated with lower 6′-SL level in exclusively breastfed infants but higher 6′-SL concentration for those with mixed feeding. Besides, food allergic children at 12 months consumed BM with higher 2′-FL level. These results supported some allergy-modifying effects of early-life HMO exposure.

Over 150 HMOs have been identified and their concentrations in human milk vary depending on Secretor and Lewis blood group status, environmental and geographical factors, lactation stage, gestational period, and maternal health. These non-digestible HMOs are vital for healthy neonatal microbial colonization, immune development, immunomodulatory effects and development of the neonatal mucosa.[5-11] In this study, we found 2′-FL to be much higher than LNnT, 3′-SL and 6′-SL (respective medians 1447 ppm, 738 ppm, 23.0 ppm and 20.5 ppm). Among Caucasians, Conze and coworkers reported the mean reference levels of 2′-FL, 3′-SL and 6′-SL to be 2580 ppm, 280 ppm and 390 ppm.[20] Another study from Australia measuring 19 HMOs found median levels of 2′-FL, LNnT, 3′-SL and 6′SL to be 2193 ppm, 490 ppm, 242 ppm and 408 ppm respectively.[21] While LNnT level appeared to be higher in the BM of our Chinese mothers (738 ppm vs 490 ppm), the BM levels of 2′-FL, 3′-SL and 6′-SL reported in these two Caucasian studies were much higher than those of our Chinese mothers. Knowing the ethnic-specific reference HMO levels would help to determine the level of appropriate HMO supplementation in infant formulae.

Although infant formulae are successful in providing adequate nutrition for those infants who cannot receive BM, these formulae lack many components such as HMOs and specific antibodies that are tailor made by each mother for the immune imprinting of her baby. Thus, many formula milk companies have been trying to replenish some specific prebiotic oligosaccharides such as short chain galacto- and long chain fructooligosaccharides (scGOS/lcFOS) and HMOs such as 2′-FL. Firstly, several studies confirmed that such HMO supplementation was safe.[22-24] On the other hand, our recent single-center, randomized, double-blind controlled trial failed to detect any benefit of 2′-FL-supplemented growing-up milk formula in preventing respiratory and gastrointestinal infections in Chinese toddlers over a 6-month study period.[25] It remained unclear if 2′-FL is ineffective, its dose inadequate or that such strategy should better be applied in young infants when formula milk is the main source of nutrient intake.[26,27] A multi-center observational cohort named "Protecting against Respiratory tract infections through human Milk Analysis" (PRIMA) is underway to identify novel functions of components in human milk that are protective against respiratory tract infections and allergic diseases early in life.[28]

The effects of various HMOs on allergy prevention have been inconsistently observed. Siziba and coworkers investigated the effect of HMOs, measured at 6 weeks and 6 months postnatally during lactation, on the development of allergies by two years of age.[29] They found 6-week LNnT to be associated with eczema at 2 years and 6-month 3′-SL among non-secretor mothers to be associated with eczema at 1 year. However, these associations became insignificant after adjusting for multiple testing. A study of BM from 266 mothers who participated in the placebo group of a randomized placebo-controlled trial of prebiotics and probiotics showed significant association between eczema and caesarean section at 2 years when adjusted for BM 2′-FL levels.[30] However, such association became insignificant when children reached 5 years old. Lodge et al. examined the relationship between HMO profiles from 285 mothers and allergic diseases of offspring up to 18 years of age in the high-allergy-risk Melbourne Atopy Cohort Study.[21] Nineteen HMOs were measured, and latent class analysis revealed seven distinct maternal milk profiles. Compared with offspring exposed to the neutral Lewis HMO profile, exposure to acidic Lewis HMOs was associated with a higher risk of asthma at 18 years. Exposure to the acidic-predominant profile was associated with a reduced risk of food sensitization at 12 years. The present observational study supported allergy-modifying effects of HMO exposure at 1-month by finding positive association between 2′-FL level and eczema by 24 months and divergent associations between eczema and 6′-SL level depending on infants' mode of feeding. Food allergy at 12 months was also associated with higher 2′-FL level. Nonetheless, we found that elevated levels of total HMO and 2′-FL in 1-month BM were associated with increased risks of eczema and food allergy. This counterintuitive association might be due to the lack of confounding genotyping data on maternal ABO secretory blood groups which modulated such relationship. Our findings for HMOs will need to be replicated in larger cohort (e.g. PRIMA[28]) and in randomized controlled trials.

Microbes are found in abundance in BM, and recent studies have suggested an entero-mammary route of microbial transfer. Pannaraj et al. studied possible vertical transfer of bacteria from mother to infant by breastfeeding.[31] They performed 16S rRNA sequencing of BM, areolar skin and infant stool samples in 107 healthy US mother-infant pairs. The infant gut microbial communities were closely related to own mother's BM and skin. Bacterial diversity and composition changes in infant gut microbiome were associated with the proportion of daily BM intake in a dose-dependent manner, even after the introduction of solid foods. This observation opens up the possibility of infant gut microbiota modulation through maternal probiotic supplementation. Nonetheless, another study yielded contradictory results.[32] Another possibility was the modulation of infant gut microbiota by HMOs. Different types of HMOs would influence the proliferation and increase the percentage of particular microbial strains in the gut. For example, increase in 2′-FL and decrease in 6′-SL affected gut microbiota composition that modulated the risk of allergy development.

Some reports suggested association between BM microbiota and allergic diseases. A study involving 202 mother-infant dyads found that the abundance and evenness of BM microbiota and the number of differential bacteria were higher in children without food allergy than in those from the food allergy group.[33] Importantly, changes in *Bifidobacterium* abundance were consistent with those in the BM flora. Grönlund et al. also assessed the association between maternal BM and fecal Bifidobacteria at 30-35 weeks of gestation and infants' fecal Bifidobacteria at one month of age in 61 mother-infant pairs.[34] *Bifidobacterium longum* was the most frequently detected species in BM. Allergic mothers had lower amounts of Bifidobacteria in BM compared with non-allergic mothers, and their infants had concurrently lower counts of Bifidobacteria in feces. Dzidic et al. determined BM microbiota in relation to allergy development in 40 children participating in an intervention trial with pre- and post-natal *Lactobacillus reuteri* supplementation.[35] They reported that BM fed to children developing allergic manifestations by 7 years old had lower bacterial richness when compared to that given to children who remained healthy. Overall, the results suggested that consumption of BM with a reduced microbial richness in early-life plays an important role in allergy development during childhood.

This study has several limitations. First, this study has not determined participants' polymorphic alleles of two fucosyltransferases FUT2 (secretor gene) and FUT3 (Lewis gene) that are responsible for transferring fucose and determining variations of specific HMOs and profiles.[30] FUT2 gene encodes fucosyltransferase (FucT) II, which catalyze the formation of α-1-2-fucosylated HMOs (majority will be 2′FL, followed by LNFP I, DFL and Tetra-LND III in rather low abundance). Thus, mothers with high expression of FULT2 gene would be categorized as secretor while the low expression is non-secretor. Nonetheless, our approach of measuring individual HMO levels would be a direct way for testing their associations with various clinical allergy phenotypes that obviates the need to determine these confounding fucosyltransferase genotypes. In addition, this study cannot evaluate the longitudinal effects of postnatal HMO exposures on allergy outcomes as we only collected one BM sample at 1-month after birth. Furthermore, we measured the levels of only four HMOs which precluded the analyses for various secretor and Lewis HMO profiles identified by an extended array of HMOs as reported in the Melbourne Atopy Cohort.[21] Despite this, this study can still detect significant associations between eczema and 2′-FL and 6′-SL levels in 1-month BM samples among Chinese infants. Another drawback relates to the lack of BM microbiota data that our participants were exposed in early-life.[33-35] It would be interesting to analyze the possible relationship between HMOs and microbiota in our BM samples and if both might expert synergistic effects on allergy development in the offspring.

In conclusion, this study found levels of major HMOs in BM from Chinese mothers to be different from those of Caucasians. Besides, the levels of 2′-FL and 6′-SL in 1-month BM samples are associated with risks of eczema and food allergy in Chinese children. Additional studies that measured the full HMO composition over multiple postnatal time points are necessary to validate their relationship with childhood allergic diseases.

Author Contributions

- TFL obtained research funding, designed this study, collected clinical data and drafted this manuscript.
- YC collected clinical data, collected and processed BM samples, analyzed study data and drafted this manuscript.
- ASYL followed subjects, collected clinical data and analyzed study data.
- AJC and MSW designed and performed HMO measurement.
- KCCC, MKC and NSC collected clinical data.
- PKSC and WHT obtained research funding, designed this study and supervised research staff.
- All authors reviewed and approved the manuscript.

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Conflict of Interest

All authors declared no competing interest.

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Supplemental material

Table S1. Demographics and clinical characteristics presented for participants with and without eczema development within the first 2 years of life.

Characteristics	Eczema ever (N = 22)	Never eczema (N = 32)	*P*
Maternal characteristics			
Received higher than secondary school education, n/N (%)	18/22 (81.8)	22/32 (68.8)	0.447
History of allergy, n/N (%)	4/22 (18.2)	3/32 (9.4)	0.593
Paternal characteristics			
Received higher than secondary school education, n/N (%)	14/22 (63.6)	17/32 (53.1)	0.626
History of allergy, n/N (%)	3/22 (13.6)	3/32 (9.4)	0.961
Child characteristics			
Male, n/N (%)	7/22 (31.8)	20/32 (62.5)	0.053
Gestational age (weeks)	39.2 ± 1.0	39.1 ± 1.2	0.785
Birth weight (g)	2952 ± 289	3070 ± 460	0.291
Born by vaginal delivery, n/N (%)	18/22 (81.8)	29/32 (90.6)	0.593
Received intrapartum antibiotics, n/N (%)	9/22 (40.9)	13/32 (40.6)	1.000
Exclusive breastfeeding at 1 month, n/N (%)	11/22 (50.0)	17/32 (53.1)	1.000
Mixed breast and formula feeding at 1 month, n/N (%)	11/22 (50.0)	15/32 (46.9)	1.000
Exposure to household smoking at 1 month, n/N (%)	6/22 (27.3)	7/31 (22.6)	0.946
Furry pets at home at 1 month, n/N (%)	7/22 (31.8)	6/31 (19.4)	0.475

Results expressed in number (percentage) or mean ± standard deviation.

Table S2. Summary of four HMOs in 1-month BM samples from 86 mothers who participated in our birth cohort.

Specimen no.	2′-FL	LNnT	6′-SL	3′-SL	Total
0002	1872	795	23.2	15.9	2706
0003	6.37	2272	12.9	20.6	2312
0005	2866	482	35.2	26.3	3410
0007	12.2	1339	-	12.3	1364
0009	3.45	2119	18.1	20.2	2161
0011	1810	632	21.0	21.3	2484
0013	1550	599	-	18.7	2168
0014	1390	944	20.1	18.3	2372
0017	2171	968	3.16	16.8	3159
0019	1767	625	21.2	21.2	2435

Specimen no.	2′-FL	LNnT	6′-SL	3′-SL	Total
0021	1585	611	21.2	23.4	2240
0023	15.8	2141	32.9	27.7	2218
0025	1937	433	14.2	12.2	2396
0026	2898	1062	-	16.6	3976
0028	6.44	1875	40.1	12.4	1933
0030	1941	891	19.3	19.1	2870
0032	1177	842	13.0	29.4	2061
0034	19.8	1970	26.6	18.4	2035
0036	1186	436	30.8	25.7	1679
0042	1065	721	61.4	42.8	1890

Table S2. (Continued)

Specimen no.	2′-FL	LNnT	6′-SL	3′-SL	Total
0044	2214	682	16.8	26.3	2939
0046	20.6	1785	11.9	21.9	1840
0048	2192	1106	35.2	27.0	3360
0050	1954	627	19.5	24.7	2625
0052	1620	892	8.61	27.6	2549
0054	1905	827	39.4	16.0	2788
0055	1510	555	13.1	27.1	2105
0056	2109	913	26.2	24.8	3073
0058	1754	1183	-	17.3	2945
0060	1958	855	48.6	18	2879
0062	1360	1338	42	37.9	2778
0064	41.8	2075	74.2	46.9	2238
0066	37.7	2151	46.6	40.9	2276
0067	2950	1047	18.4	17.2	4033
0069	1378	1037	40.2	41.7	2497
0071	1694	1181	-	19.0	2894
0073	727	1239	14.3	47.9	2029
0075	761	908	-	24.1	1693
0077	2.69	657	45.8	20.9	727
0079	1463	712	74.9	33.9	2283
0081	2067	1018	-	18.3	3103
0083	3190	659	-	27.6	3877
0085	1.29	1103	40.6	22.0	1167
0087	1046	864	47.0	25.1	1982
0089	1319	548	48.2	26.9	1942
0091	1579	726	41.7	28.8	2376
0093	2308	582	39.5	34.1	2963
0095	1909	922	62.7	25.2	2920
0099	374	64.8	8.13	20	467
0100	1975	631	10.0	21.9	2638
0102	1227	636	17.8	24.9	1906
0104	1606	548	14.5	34.1	2202
0106	2279	630	15.2	28.5	2953
0108	1206	749	4.6	31.5	1991
0110	864	517	8.03	26.1	1415
0112	1838	550	10.4	26.2	2425
0114	1427	837	43.2	26.1	2333
0115	1901	661	37.4	10.2	2610
0116	1660	468	32.6	27.9	2188
0118	2008	871	26.4	23.9	2929
0120	28.2	51.8	4.79	-	84.7
0121	22.2	265	12.0	-	299
0123	883	809	10.7	3.20	1706
0125	1582	911	14.9	29.4	2537
0127	1363	758	15.0	1.46	2138
0131	40.8	307	31.1	20.8	399
0133	1254	622	28.5	5.15	1910
0135	28.0	359	10.6	-	398
0137	1319	574	19.5	13.8	1926
0139	1432	759	34.3	3.08	2228
0141	44.1	297	27.1	22.6	391
0143	3287	621	18.8	30.3	3958
0145	2564	886	38.6	29.0	3517
0147	1825	683	45.4	34.7	2588
0149	28.4	371	55.3	23.5	478
0151	13.9	252	31.7	10.4	308
0153	14.7	233	79.4	24.8	352
0155	26.4	484	27.1	18.9	556
0157	1561	759	14.4	21.3	2356
0159	1726	636	14.2	16.1	2393
0001*	8.86	1730	3.73	20.6	1763
0015*	1179	1063	28.9	21.9	2292
0038*	1971	1058	-	12.9	3020
0040*	2584	574	-	15.0	3174
0097*	6.23	997	66.3	22.1	1092
0129*	1682	752	11.0	4.47	2450

2′-FL, 2′-fucosyllactose; HMO, human milk oligosaccharide; LNnT, lacto-N-neotetraose; 3′-SL, 3′-sialyllactose; 6′-SL, 6′-sialyllactose.
HMO concentrations were expressed in parts per million (ppm).
* Samples were excluded because infants were reported to be exclusively formula-fed before BM collection.

Table S3. Assessment for possible participation bias by comparing HMO comparison of subjects who were followed and those lost to follow-up at 24 months.

HMO	Subjects lost to follow up at 24 months (N = 26)	Never eczema within 24 months (N = 32)	Eczema ever within 24 months (N = 22)	*P*
2′-FL	1619 (955 - 2130)	1030 (25.4-1627)	1613 (1330-1951)	**0.007**
LNnT	682 (624 - 956)	631 (483-984)	859 (731-912)	0.303
6′-SL	18.3 (13.4, 36.6)	26.8 (10.6-39.4)	19.4 (14.3-37.8)	0.916
3′-SL	25.1 (20.0, 28.4)	21.2 (17.0-26.2)	23.3 (18.1-27.0)	0.349
Total	2384 (2119, 2936)	2048 (1057-2427)	2467 (2021-2877)	**0.016**

2′-FL, 2′-fucosyllactose; HMO, human milk oligosaccharide; LNnT, lacto-N-neotetraose; 3′-SL, 3′-sialyllactose; 6′-SL, 6′-sialyllactose.
Results expressed in median (interquartile range).
HMO concentrations were expressed in parts per million (ppm).
P-value < 0.05 was highlighted in bold.

Table S4. Medical history of participants diagnosed with food allergy at 12 months.

Subject	Clinical history	Skin prick tests at 12 months	Allergy diagnosis
A	Avoids eggs and pumpkin, some skin rash after taking egg and pumpkin.	Histamine 6 mm Egg 6 mm Peanut 4.5 mm	Egg
B	Tried egg at 7 months old, vomited afterwards, but then gradually better, very slight rash around mouth.	Histamine 6 mm Egg 5 mm	Egg
C	Still exclusively breastfed with no regurgitation with no dairy product. Tried egg yolk since 6 months old and now eating half yolk for 1-2 times every month. Had generalized hives 1 hour after first trial of 2-3 cm size of egg white at 8 months old, and avoiding egg white since then. Avoiding beef since birth due to eczema.	histamine 6 mm Egg 4.5 mm Cow's milk 4.5 mm	Egg white
D	Adverse food reaction to Chinese yam (cinnamon-vine): Tried Chinese yam with immediate occurrence of itchy skin rash at 7 months. Skin itchiness after taken egg and chicken before, but improved now. No angioedema or respiratory involvement.	Chinese yam not tested	Chinese yam