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Human milk oligosaccharide profiles and child atopic dermatitis up to 2 years of age: The Ulm SPATZ Health Study

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Abstract

Background: Human milk oligosaccharides (HMOs) have several biological functions. Yet, very few studies have investigated the effect of HMOs on the development of allergies and even fewer on their specific associations with atopic dermatitis (AD) during early childhood.

Objective: This study investigated whether individual HMO concentrations, measured at two time points of lactation, were associated with reported diagnosis of AD in children up to two years of age.

Method: Outcome data were available for HMOs measured in human milk samples collected at 6 weeks ($n = 534$) and 6 months ($n = 356$) of lactation. Associations of HMOs with AD, ascertained from parents and pediatricians at ages one and two years, were assessed in crude and adjusted logistic regression models.

Results: Few associations were statistically significant at the conventional level ($p < .05$), for example, 6-week Lacto-N-neotetraose with 2-year AD [OR 95%CI: 0.82 (0.66, 1.00)] and 6-month 3'-sialyllactose among non-secretor mothers with 1-year AD [2.59 (1.53, 6.81)]. Importantly, accounting for multiple testing, these and all further associations were not statistically significant (all $p > .0031$, which is the threshold for statistical significance after correction for multiple testing).

Conclusion: Our findings suggest that the intake of different levels (or even absence) of the individual HMOs measured at 6 weeks and 6 months of lactation, in the current study, is not significantly associated with the development of AD in early childhood. Given the exploratory nature of our study and the limited sample size, these results should be interpreted with caution. The specific HMOs for which we show plausible associations at conventional level may warrant further research and investigation.

KEYWORDS
atopic dermatitis (AD), human milk groups, human milk oligosaccharides (HMOs), targeted LC-MS/MS

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1 | INTRODUCTION

The prevalence of atopic dermatitis (AD) varies globally[1] and yet remains one of the most common atopic disorders in children. The impact and benefits of breastfeeding in general are well recognized in relation to allergy outcomes in infants and children.[2,3] Hence, the importance of early life exposure to human milk has received considerable attention. Human milk contains several bioactive components, including human milk oligosaccharides (HMOs), which have been associated with infant health and early life immune development.[4–7] HMOs are the third most abundant component of human milk.[2] However, the amount and diversity of HMO structures present in human milk differ greatly among mothers and largely depend on the expression of fucosyltransferase enzymes.[8,9] Here, the Secretor and Lewis blood group genes encode for the respective maternal fucosyltransferase enzymes which lead to types of glycosidic linkages between fucose residues and HMO core structures.[9,10]

For instance, the Se-enzyme is encoded by the secretor gene (FUT2) and adds a fucose by an α1-2-linkage thereby producing HMOs such as 2′-fucosyllactose (2′-FL) and lacto-N-fucopentaose I (LNFP I). While Fuc-TIII which is encoded by the Lewis gene (FUT3), adds fucose by α1-3/4-linkages and produces, for example, LNFP II.[11] The presence of structures such as 2′-FL and LNFP I can therefore be used as a proxy to determine FUT2 activity, and LNFP II as a proxy for FUT3 activity. Based on this, human milk can be attributed to four different milk groups in which each group has a distinctive HMO profile and composition. In this case, secretor milk is attributed to groups I and III, while non-secretor milk is attributed to groups II and IV.[10,12–16] As such, HMOs are determined by several factors which include, but are not limited to, maternal secretor status and milk group which are dependent on Secretor and Lewis blood group genes, respectively.[10,16–19] This in turn influences the amount and specificity of certain HMOs produced and supplied to the infant through human milk.[16]

On one hand, not many studies have specifically focused on the associations of certain HMO profiles with allergy.[20–24] Thus, the limited evidence on the impact of HMOs remains inconsistent regarding the development of cow's milk allergy,[21,22] sensitization to food[20,24] and aero allergens,[24] food allergy and asthma,[21,24] wheeze,[24] hay fever,[21] and AD.[21,23,24] The discrepancy in results could be attributed to the different time points during lactation of the HMOs measurements; that is, 2–4 days,[21,23] 1.1–1.4 months,[22] 3–4 months,[20] and day 3–3 months[24] postpartum. Of note, human milk changes dynamically between and within groups of mothers during lactation, and any associations observed at specific time points could just be applicable to a specific group of infants, but not to others.[4]

On the other hand, these studies only investigated individual HMOs,[21,22] structure specific clusters,[21,23] and other statistically defined clusters or classes.[20,22,24] As such, beneficial effects of HMOs are specific to certain HMO structures which can be clearly distinguished between nonsialylated (neutral) and sialylated (acidic) HMOs.[5,25,26] Consequently, results on clinical outcomes will also differ depending on whether absolute or relative proportions of HMOs are measured and whether the statistically defined clusters are generalizable to other groups of mothers.[4] Hence, in the current study, we do not only overcome the limitation of measuring more isomeric HMOs in comparison to previous studies, but the method used offered more sensitivity, accuracy, and more reliable and robust techniques which could also potentially allow us to measure even more HMOs in the future. Moreover, granted that HMO concentrations are greatly influenced by secretor status, milk groups, and time of lactation,[8,9,12,17,19] these factors should be considered when investigating associations with child health outcomes. Considering this, we investigated the associations between several (~15) individual HMOs measured at 6 weeks and 6 months of lactation, with child AD at 1 and 2 years of age using data from the Ulm SPATZ Health Study, an ongoing birth cohort in Southern Germany.

Key Message

The findings from this study suggest that the presence of different levels (or even absence) of the investigated individual human milk oligosaccharides (HMOs) is not implicated in the development of atopic dermatitis (AD) in early childhood. The full presentation of results, including potentially plausible weak associations, will aid in determining candidate selection and sample size planning of future studies investigating similar associations.

It is unclear whether individual HMOs may be implicated in the development of AD during early childhood. However, the potential benefits of HMOs and additional, not yet identified (polyvalent) HMOs for the infants' healthy development do warrant further investigations.

2 | MATERIALS AND METHODS

2.1 | Study design and population

Data were obtained from the Ulm SPATZ Health Study, an ongoing birth cohort study, in which a total of 970 mothers and their 1 006 newborn infants were recruited from the general population, soon after their delivery, during their hospital stay at the University Medical Centre Ulm, southern Germany, between April 2012 and May 2013.[27] Exclusion criteria were insufficient German language skills, outpatient childbirth, maternal age <18 years, postpartum transfer of mother or child to intensive care unit or stillbirth. Participation in the study was voluntary and all study participants gave written informed consent. Ethical approval for the SPATZ study was obtained from the Ethics board of Ulm University (No. 311/11).

2.2 | Data collection and measurements

The Ulm SPATZ Health Study was conducted at Ulm University. Demographic, lifestyle, and birth-related data including child sex, delivery mode, birth season, birth weight, maternal age, education, parity, pre-pregnancy body mass index (BMI, calculated as weight (kg)/height $(m)^2$), and smoking status in the year prior to and during pregnancy were collected using self-administered questionnaires, hospital charts, and routine screening examinations. AD diagnosed by a doctor was assessed by reports from the parents as well as from the caring pediatricians in separate, self-administered questionnaires at 1 year and again at 2 years of age. In the yearly follow-ups:

Parent reports of AD diagnosis were defined as positive response of "neurodermatitis (endogenous eczema, atopic dermatitis)" to the question "Has a doctor diagnosed your child with one of the following in the past 12 months?" asked in the follow-ups at ages 1 and 2 years. Separately assessed pediatrician reports of AD diagnosis were defined as a positive response of "neurodermatitis (endogenous eczema, atopic dermatitis)" to the question "Has one of the following diseases been found/diagnosed by a doctor until now?" asked in the follow-ups at ages 1 and 2 years. We distinguished between "parent-reported" and "pediatrician-reported," but both reports were for a doctor's diagnosis although not necessarily made by the caring and responding pediatricians themselves. For the purposes of the current analysis, children with a positive report of AD diagnosis by either the parents or the caring pediatrician were defined as AD cases. At 2 years of age, the positive reports of 1 year were also included (ie, cumulative incidence of AD). Additional information was collected on AD severity using the patient-oriented eczema measure (POEM)[28] in the self-administered parental questionnaires at the follow-ups at ages 2 and 3 years.

Mothers who were still breastfeeding their infants and were willing to provide a human milk sample at the time of sample collection were asked how long the child had been exclusively breastfed without giving additional complementary food or liquids. This was confirmed in a second structured and open question, in which mothers were asked what the first complementary food or additional liquid they had given to their child was. A variable for exclusive breastfeeding (EBF) was then derived based on these questions and maternal recall of these feeding practices at each time point. In essence, EBF was considered when the mother gave no additional liquid or solids, and non-EBF when infants were receiving any one of the following in addition to human milk [formula milk (adapted/unadapted), cow's milk, vegetable puree, fruit puree, cereal, or other foods].

Human milk samples were collected at approximately 6 weeks [mean (standard deviation); 5.9 (0.7) weeks], 6 months [25.8 (0.8) weeks], and 1 year [53.9 (4.3) weeks] post-delivery from participating lactating women, who were still breastfeeding at the time of sample collection. They were instructed to manually express or pump human milk between 9am and 12pm, after breakfast and before lunch, but at least one hour after the infants last feed. Where necessary, trained study nurses helped mothers with the expression of their milk. Human milk samples were stored in the refrigerator by the mothers until study nurses collected them from their homes on the same day if milk was expressed between 9am and 12pm (76.9%), or on the next day if milk was expressed—against the protocol—in the evening [after 12pm, 9%; and before 9am, 13.8%)] and delivered it refrigerated to the study center. Additional information was collected at 6 weeks, 6 months, and 1 year post-delivery by telephone interview or self-administered questionnaires sent by post. Due to the limited number of human milk samples available at 12 months ($n = 73$), analyses were restricted to human milk samples collected at 6 weeks and 6 months of lactation.

2.3 | Analysis of HMOs

Human milk samples were stored at −80°C until the analysis of HMOs in 2019 as previously described.[29] HMOs were quantified and measured in the laboratories at Danone Nutricia Research,

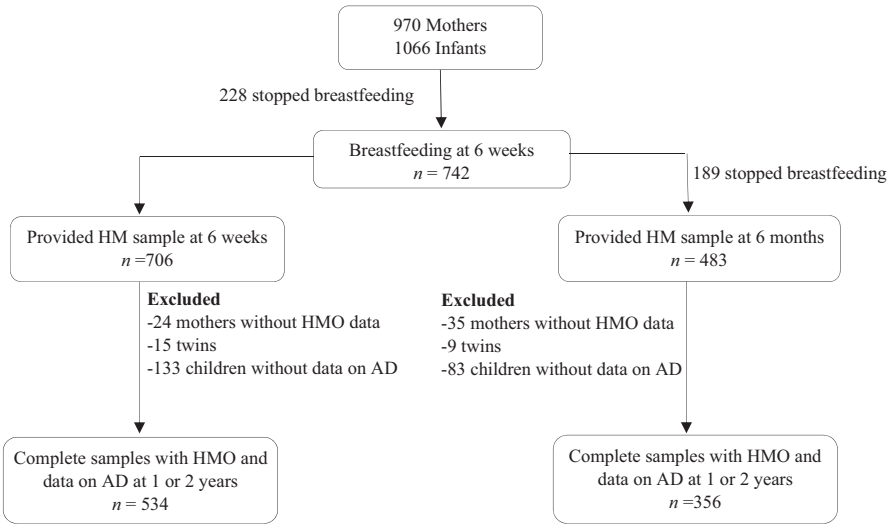

FIGURE 1 Flow chart of study population and final analysis sample size. HM-Human milk; HMO-Human milk oligosaccharides; AD-Atopic Dermatitis

TABLE 1 Characteristics of lactating mothers and children who had a complete set of data on human milk oligosaccharides (HMOs) and atopic dermatitis (AD) available in the Ulm SPATZ Health Study

	6 weeks samples (n = 534)	6 months samples (n = 356)
Mother		
Age (years)	33.2 (4.42)	33.4 (4.16)
Age category (years)		
<30	16 (3.0)	9 (2.5)
30–35	378 (70.8)	249 (69.9)
>35	140 (26.2)	98 (27.5)
BMI category (kg/m^2)		
Underweight (BMI <18.5)	5 (1.0)	8 (2.5)
Normal (18.5 ≤BMI <25)	295 (58.6)	214 (66.0)
Overweight (25 ≤BMI <30)	148 (29.4)	70 (21.6)
Obese (BMI ≥30)	55 (10.9)	32 (9.9)
Parity (n births of foetus ≥24 weeks)		
0 births	287 (53.8)	187 (52.5)
≥1 birth	246 (46.2)	169 (47.5)
Milk group		
I	397 (74.3)	265 (74.4)
II	99 (18.5)	64 (18.0)
II	33 (6.2)	24 (6.7)
IV	5 (0.9)	3 (0.8)
Maternal allergy		
Yes	167 (31.3)	118 (33.2)
No	366 (68.7)	237 (66.8)
Infant		
Female	257 (48.1)	168 (47.2)
Male	277 (51.9)	188 (52.8)
Gestation category (weeks)		
≤36	134 (25.1)	87 (24.4)
36–41	314 (58.8)	208 (58.4)
≥41	86 (16.1)	61 (17.1)
Delivery mode		
Vaginal spontaneous	366 (68.7)	255 (71.6)
Elective caesarean	54 (10.1)	27 (7.6)
Emergency caesarean	61 (11.4)	39 (11.0)
Vaginal assisted	52 (9.8)	35 (9.8)
Exclusive breastfeeding		
Yes	415 (77.7)	249 (83.7)
No	119 (22.3)	107 (16.3)
AD at 1 year of age		
Yes	89 (17.9)	51 (15.5)
No	407 (82.1)	279 (84.5)
AD at 2 years of age		
Yes	133 (24.9)	82 (23.0)
No	401 (75.1)	274 (77.0)

Note: Data represent n and column (%). Continuous variables (ie, years) presented as mean (SD).

Abbreviations: AD, atopic dermatitis, BMI, body mass index; SD, standard deviation.

blinded to demographic variables and clinical outcomes. Briefly, individual native HMOs were identified and quantified using targeted liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI-MS^2) in negative ion mode. Quantification of absolute HMO concentrations was done for lactose and for 16 of the most abundant HMOs comprising: 2'-fucosyllactose (2'-FL); 3-fucosyllactose (3-FL); 3'-sialyllactose (3'-SL); 4'-galactosyllactose (4'-GL); 6'-galactosyllactose (6'-GL); 6'-sialyllactose (6'-SL); difucosyllactose (DFL); lacto-N-tetraose (LNT); lacto-N-neotetraose (LNnT); lacto-N-fucopentaose-I (LNFP I); lacto-N-fucopentaose-II (LNFP II); lacto-N-fucopentaose-III (LNFP III); lacto-N-fucopentaose-V (LNFP V); lacto-N-difucohexaose I (LNDFH I); and the sum of lacto-N-difucohexaose II and lacto-N-neodifucohexaose II (LNDFH II +LNnDFH II, standard containing both). Secretor status and milk group were determined as previously described.[29] Briefly, lactating mothers whose milk presented both α1,2- and α1,4-fucosylated HMOs (like 2'-FL and LNDFH I, respectively) were attributed to group I. Women whose milk samples did not contain LNFP I and LNDFH I [below the lower limit of quantification (<LLOQ)] were classified as group II. Women whose milk samples lacked LNFP II and LNDFH I (<LLOQ) were classified as group III. Those whose human milk sample lacked LNFP I, LNDFH I, and LNFP II (<LLOQ) were classified as type IV. For the categorization according to secretor phenotypes, milk groups I and III were grouped as secretors, while milk groups II and IV were grouped as non-secretors. HMO concentrations were presented as absolute values (g/l). Human milk samples with a complete set of HMO data and any report of AD outcomes (at 1 year only or up to 2 years) were available from 534 mothers at 6 weeks and 356 mothers at 6 months (**Figure 1**). The reports of AD at two years were irrespective of whether a 1-year report was affirmative.

2.4 | Statistical analysis

Data for 4'-GL were excluded because >90% of values were found to be <LLOQ. For the remaining HMOs, all values <LLOQ were

TABLE 2 Absolute concentrations of human milk oligosaccharides (HMOs, g/l) measured at 6 weeks post-delivery stratified by child atopic dermatitis (AD) at 1 and 2 years of age in the Ulm SPATZ Health Study

	6 weeks human milk samples					
	AD at 1 year			AD at 2 years		
	Yes ($n = 89$)	No ($n = 407$)	p^*	Yes ($n = 133$)	No ($n = 401$)	p^*
Lactose						
Mean (SD)	66.0 (3.55)	66.8 (3.82)	.081	66.3 (3.49)	66.7 (3.82)	.146
Median [Min, Max]	67.0 [56.0, 73.0]	67.0 [52.0, 77.0]		67.0 [56.0, 74.0]	67.0 [52.0, 77.0]	
2'-FL						
Mean (SD)	2.20 (1.39)	2.29 (1.40)	.347	2.16 (1.42)	2.34 (1.38)	.092
Median [Min, Max]	2.40 [0.13, 6.6]	2.50 [0.13, 6.3]		2.30 [0.13, 6.6]	2.50 [0.13, 6.00]	
3'-FL						
Mean (SD)	0.69 (0.53)	0.67 (0.54)	.353	0.71 (0.57)	0.64 (0.52)	.142
Median [Min, Max]	0.54 [0.04, 2.2]	0.48 [0.03, 2.9]		0.53 [0.04, 2.40]	0.46 [0.03, 2.9]	
6'-GL						
Mean (SD)	0.02 (0.01)	0.02 (0.01)	.091	0.02 (0.01)	0.02 (0.01)	.129
Median [Min, Max]	0.02 [0.004, 0.06]	0.02 [0.01, 0.15]		0.02 [0.004, 0.06]	0.02 [0.004, 0.15]	
3'-SL						
Mean (SD)	0.16 (0.04)	0.15 (0.04)	.364	0.15 (0.04)	0.15 (0.04)	.719
Median [Min, Max]	0.15 [0.08, 0.26]	0.15 [0.05, 0.32]		0.15 [0.07, 0.27]	0.15 [0.05, 0.32]	
6'-SL						
Mean (SD)	0.26 (0.10)	0.26 (0.10)	.498	0.26 (0.11)	0.26 (0.10)	.673
Median [Min, Max]	0.25 [0.05, 0.52]	0.24 [0.06, 0.73]		0.24 [0.05, 0.61]	0.24 [0.06, 0.73]	
DFL						
Mean (SD)	0.19 (0.16)	0.18 (0.17)	.371	0.18 (0.16)	0.18 (0.17)	.901
Median [Min, Max]	0.18 [0.01, 1.0]	0.17 [0.01, 1.8]		0.17 [0.01, 1.0]	0.17 [0.01, 1.80]	
LNT						
Mean (SD)	0.93 (0.52)	0.94 (0.49)	.598	0.94 (0.50)	0.93 (0.49)	.986
Median [Min, Max]	0.81 [0.31, 2.90]	0.85 [0.14, 3.1]		0.84 [0.15, 2.9]	0.84 [0.14, 3.1]	
LNnT						
Mean (SD)	0.08 (0.06)	0.09 (0.06)	.183	0.07 (0.06)	0.09 (0.06)	.047
Median [Min, Max]	0.06 [0.01, 0.33]	0.07 [0.01, 0.35]		0.06 [0.01, 0.33]	0.08 [0.01, 0.35]	
LNFP I						
Mean (SD)	0.47 (0.49)	0.49 (0.44)	.479	0.47 (0.47)	0.50 (0.46)	.286
Median [Min, Max]	0.35 [0.04, 3.0]	0.39 [0.04, 2.4]		0.35 [0.04, 3.0]	0.39 [0.04, 2.7]	
LNFP II						
Mean (SD)	0.34 (0.37)	0.35 (0.41)	.553	0.37 (0.4)	0.33 (0.39)	.117
Median [Min, Max]	0.18 [0.04, 1.9]	0.18 [0.04, 2.5]		0.19 [0.04, 1.9]	0.17 [0.04, 2.5]	
LNFP III						
Mean (SD)	0.19 (0.08)	0.18 (0.07)	.614	0.18 (0.08)	0.18 (0.07)	.611
Median [Min, Max]	0.18 [0.06, 0.39]	0.18 [0.04, 0.48]		0.17 [0.05, 0.43]	0.18 [0.04, 0.48]	
LNFP V						
Mean (SD)	0.04 (0.04)	0.04 (0.04)	.362	0.04 (0.04)	0.03 (0.04)	.086
Median [Min, Max]	0.02 [0.01, 0.21]	0.02 [0.01, 0.24]		0.02 [0.01, 0.21]	0.02 [0.01, 0.24]	
LNDFH I						
Mean (SD)	0.57 (0.42)	0.53 (0.38)	.655	0.55 (0.42)	0.53 (0.36)	.977

(Continues)

TABLE 2 (Continued)

	6 weeks human milk samples					
	AD at 1 year			AD at 2 years		
	Yes ($n = 89$)	No ($n = 407$)	p^*	Yes ($n = 133$)	No ($n = 401$)	p^*
Median [Min, Max]	0.58 [0.02, 1.80]	0.58 [0.02, 1.9]		0.57 [0.02, 1.8]	0.58 [0.02, 1.9]	
LNDFH II +LNnDFH II						
Mean (SD)	0.06 (0.11)	0.07 (0.11)	.325	0.07 (0.12)	0.06 (0.10)	.091
Median [Min, Max]	0.02 [0.01, 0.49]	0.02 [0.01, 0.73]		0.02 [0.01, 0.70]	0.02 [0.01, 0.73]	
Total HMOs						
Mean (SD)	6.19 (1.46)	6.23 (1.24)	.441	6.17 (1.43)	6.23 (1.24)	.294
Median [Min, Max]	6.02 [3.02, 11.6]	6.17 [2.41, 11.1]		5.92 [3.02, 11.6]	6.18 [2.41, 11.1]	

Abbreviations: 2'-FL, 2'-fucosyllactose; 3'-SL, 3'-sialyllactose; 3-FL, 3-fucosyllactose; 6'-SL, 6'-sialyllactose; 6'-GL, 6'-galactosyllactose; AD, atopic dermatitis; DFL, 3,2'-difucosyllactose; HMO, human milk oligosaccharides; LNDFH I, lacto-N-difucohexaose I; LNDFH II, lacto-N-difucohexaose II; LNFP I, lacto-N-fucopentaose-I; LNFP II, lacto-N-fucopentaose-II; LNFP III, lacto-N-fucopentaose-III; LNFP V, lacto-N-fucopentaose-V; LNnDFH II, lacto-N-neodifucohexaose II; LNnT, lacto-N-neotetraose; LNT, lacto-N-tetraose; SD, standard deviation.

*p values derived from Wilcoxon sum-rank test comparing HMO concentrations between children with and without AD. Bonferroni-adjusted level of statistical significance is $\alpha = 0.05/16 = 0.0031$.

TABLE 3 Associations between human milk oligosaccharides (HMOs) measured at 6 weeks of lactation with reports of atopic dermatitis (AD) at 1 or 2 years in the Ulm SPATZ Health Study

	AD at 1 year			AD at 2 years		
	OR	95% CI	p^*	OR	95% CI	p^*
Lactose	0.80	(0.63, 1.01)	.06	0.88	(0.72, 1.08)	.24
2'-FL	0.92	(0.72, 1.17)	.51	0.88	(0.71, 1.08)	.22
3'-FL	1.11	(0.88, 1.41)	.38	1.17	(0.95, 1.43)	.14
6'-GL	1.19	(0.94, 1.50)	.15	1.17	(0.96, 1.43)	.11
3'-SL	1.12	(0.88, 1.43)	.35	1.05	(0.85, 1.29)	.66
6'-SL	1.02	(0.81, 1.28)	.89	0.98	(0.80, 1.19)	.82
DFL	1.10	(0.87, 1.39)	.44	0.99	(0.81, 1.22)	.93
LNT	0.98	(0.78, 1.25)	.90	1.01	(0.83, 1.24)	.91
LNnT	0.86	(0.68, 1.09)	.22	0.82	(0.66, 1.00)	.05
LNFP I	0.94	(0.73, 1.21)	.64	0.91	(0.74, 1.13)	.41
LNFP II	1.04	(0.82, 1.32)	.72	1.14	(0.93, 1.40)	.19
LNFP III	1.10	(0.87, 1.40)	.43	1.12	(0.91, 1.37)	.30
LNFP V	1.10	(0.87, 1.40)	.41	1.18	(0.96, 1.44)	.11
LNDFH I	1.09	(0.86, 1.38)	.48	1.04	(0.85, 1.28)	.68
LNDFH II + LNnDFH II	1.10	(0.86, 1.41)	.43	1.20	(0.97, 1.49)	.09
Total HMOs	0.95	(0.75, 1.22)	.71	0.95	(0.78, 1.17)	.65

Abbreviations: 2'-FL, 2'-fucosyllactose; 3'-SL, 3'-sialyllactose; 3-FL, 3-fucosyllactose; 6'-SL, 6'-sialyllactose; 6'-GL, 6'-galactosyllactose; AD, atopic dermatitis; CI, confidence interval; DFL, 3,2'-difucosyllactose; HMO, human milk oligosaccharides; LNDFH I, lacto-N-difucohexaose I; LNDFH II, lacto-N-difucohexaose II; LNFP I, lacto-N-fucopentaose-I; LNFP II, lacto-N-fucopentaose-II; LNFP III, lacto-N-fucopentaose-III; LNFP V, lacto-N-fucopentaose-V; LNnDFH II, lacto-N-neodifucohexaose II; LNnT, lacto-N-neotetraose; LNT, lacto-N-tetraose; OR, odds ratio.

*Associations determined by logistic regression. ORs reflect the odds of atopic dermatitis per standardized score (Blom transformed) of HMO concentrations. Models adjusted for secretor status, milk group, child sex, maternal allergy, delivery mode, exclusive breastfeeding and parity. Bonferroni-adjusted level of statistical significance is $\alpha = 0.05/16 = 0.0031$.

TABLE 4 Associations between human milk oligosaccharides (HMOs) in secretor milk measured at 6 weeks of lactation with reports of atopic dermatitis (AD) at 1 and 2 years in the Ulm SPATZ Health Study

	Secretor milk					
	AD at 1 year			AD at 2 years		
	OR	95% CI	*p**	OR	95% CI	*p**
Lactose	0.88	(0.67, 1.15)	.36	0.93	(0.74, 1.18)	.56
2'-FL	0.88	(0.62, 1.25)	.50	0.85	(0.63, 1.15)	.30
3'-FL	1.27	(0.90, 1.81)	.19	1.19	(0.89, 1.60)	.25
6'-GL	1.16	(0.89, 1.50)	.28	1.18	(0.95, 1.49)	.14
3'-SL	1.15	(0.86, 1.54)	.36	1.07	(0.83, 1.37)	.61
6'-SL	1.01	(0.78, 1.31)	.93	0.97	(0.77, 1.20)	.75
DFL	1.23	(0.87, 1.72)	.23	1.06	(0.78, 1.42)	.72
LNT	0.97	(0.73, 1.29)	.84	1.05	(0.82, 1.34)	.72
LNnT	0.79	(0.57, 1.09)	.15	0.75	(0.56, 0.99)	.05
LNFP I	0.93	(0.65, 1.32)	.68	0.93	(0.69, 1.26)	.66
LNFP II	1.23	(0.86, 1.77)	.26	1.26	(0.93, 1.72)	.14
LNFP III	1.05	(0.81, 1.38)	.70	1.05	(0.84, 1.32)	.67
LNFP V	1.19	(0.83, 1.70)	.35	1.28	(0.94, 1.75)	.12
LNDFH I	1.18	(0.86, 1.63)	.30	1.15	(0.87, 1.52)	.33
LNDFH II + LNnDFH II	1.32	(0.89, 1.94)	.16	1.33	(0.95, 1.86)	.10
Total HMOs	1.01	(0.74, 1.38)	.94	0.98	(0.75, 1.28)	.88

*Associations determined by logistic regression in a stratified analysis. ORs reflect the odds of atopic dermatitis per standardized score (Blom transformed) of HMO concentrations. Models adjusted for milk group, child sex, maternal allergy, delivery mode, exclusive breastfeeding and parity. Bonferroni-adjusted level of statistical significance is $\alpha = 0.05/16 = 0.0031$.

Abbreviations: 2'-FL, 2'-fucosyllactose; 3-FL, 3-fucosyllactose; 6'-SL, 6'-sialyllactose; 6'-GL, 6'-galactosyllactose 3'-SL, 3'-sialyllactose; AD, atopic dermatitis; CI, confidence interval; DFL, 3,2'-difucosyllactose; HMO, human milk oligosaccharides; LNDFH I, lacto-N-difucohexaose I; LNDFH II, lacto-N-difucohexaose II; LNFP I, lacto-N-fucopentaose-I; LNFP II, lacto-N-fucopentaose-II; LNFP III, lacto-N-fucopentaose-III; LNFP V, lacto-N-fucopentaose-V; LNnDFH II, lacto-N-neodifucohexaose II; LNnT, lacto-N-neotetraose; LNT, lacto-N-tetraose; OR, odds ratio.

replaced by LLOQ / √ (2) and values higher than the upper limit of quantification (>ULOQ) were extrapolated for 3-FL, 3'-SL, 6'-GL, 6'-SL, DFL, LNT, LNFP I, LNFP II, LNDFH I, LNDFH II +LNnDFH II.[29] Absolute HMO concentrations measured at 6 weeks and 6 months of lactation stratified by AD at 1 or 2 years are presented as mean (SD) and median [min, max]. An unpaired two samples Wilcoxon sum rank test was used to evaluate differences of HMO and lactose concentrations between children with or without AD. Absolute HMO concentrations were standardized to normal scores using Blom transformation[30] prior to analysis. Fisher's exact test was used to evaluate associations between maternal secretor status and milk groups I and II with AD. Logistic regression was used to assess associations between HMOs and AD at 1 and 2 years of age. These associations were assessed in crude and adjusted models. Adjustment variables were child sex, maternal allergy, exclusive breastfeeding, delivery mode, secretor status, milk group, and parity. These covariates were selected to be included in the models comparable to previous studies evaluating similar associations between HMOs and allergy, as well as their very close association with HMOs.[23,24,26] A stratified analysis was also done to investigate these associations among children receiving secretor or non-secretor milk and those receiving milk attributed to milk group I or II. The small number of mothers whose milk were attributed to milk groups III and IV would limit conclusions; thus, this analysis was not done. Statistical level of significance was based on Bonferroni-corrected P values (α threshold = 0.0031) to account for multiple testing. This was applied to all analyses. Although this approach reduces the probability of observing statistically significant results, it reduces the risk of drawing conclusions based on overestimated assertions.[31] Potential associations considered significant at conventional level of significance ($p < .05$) were further investigated in a subset of children who had AD at 2 years and consistent scores of POEM at both 2 and 3 years. Scores were dichotomized into two levels: clear (0–2), and mild to severe (3–28). Children who had reported no AD at 2 years in addition to POEM scores of 0–2 at both 2 and 3 years consistently were used as the reference group for the sub-analysis. Children who had a positive report of AD in addition to POEM scores of 3–28 at both 2 and 3 years consistently were used as cases for the sub-analysis. All statistical analyses were performed with R (version 3.5.1; R Foundation for Statistical Computing).

TABLE 5 Associations between human milk oligosaccharides (HMOs) in non-secretor milk measured at 6 weeks of lactation with reports of atopic dermatitis (AD) at 1 and 2 years in the Ulm SPATZ Health Study

	Non-secretor milk					
	AD at 1 year			AD at 2 years		
	OR	95% CI	*p**	OR	95% CI	*p**
Lactose	0.55	(0.29, 0.99)	.05	0.75	(0.45, 1.24)	.27
3'-FL	0.73	(0.34, 1.60)	.40	1.25	(0.65, 2.63)	.53
6'-GL	1.27	(0.76, 2.21)	.37	1.10	(0.71, 1.72)	.68
3'-SL	1.09	(0.67, 1.81)	.73	0.95	(0.62, 1.46)	.81
6'-SL	1.00	(0.57, 1.73)	.99	0.97	(0.60, 1.54)	.89
LNT	0.92	(0.51, 1.68)	.79	0.72	(0.43, 1.19)	.20
LNnT	1.11	(0.47, 2.51)	.81	1.04	(0.51, 2.09)	.90
LNFP II	0.60	(0.31, 1.13)	.10	0.90	(0.53, 1.58)	.70
LNFP III	1.38	(0.73, 2.66)	.33	1.45	(0.84, 2.55)	.19
LNFP V	1.02	(0.39, 2.65)	.97	0.95	(0.41, 2.14)	.89
LNDFH II + LNnDFH II	0.63	(0.28, 1.42)	.25	1.12	(0.57, 2.36)	.75
Total HMOs	0.73	(0.38, 1.36)	.34	0.95	(0.56, 1.59)	.84

*Associations determined by logistic regression in a stratified analysis. ORs reflect the odds of atopic dermatitis per standardized score (Blom transformed) of HMO concentrations. Models adjusted for milk group, child sex, maternal allergy, delivery mode, exclusive breastfeeding and parity. Bonferroni-adjusted level of statistical significance is $\alpha = 0.05/16 = 0.0031$.

Abbreviations: 3'-SL, 3'-sialyllactose; 3-FL, 3-fucosyllactose; 6'-SL, 6'-sialyllactose; 6'-GL, 6'-galactosyllactose; AD, atopic dermatitis; CI, confidence interval; HMO, human milk oligosaccharides; LNDFH II, lacto-N-difucohexaose II; LNFP II, lacto-N-difucohexaose II; LNFP III, lacto-N-fucopentaose-III; LNFP V, lacto-N-fucopentaose-V; LNnDFH II, lacto-N-neodifucohexaose II; LNnT, lacto-N-neotetraose; LNT, lacto-N-tetraose; OR, odds ratio.

3 | RESULTS

A total of 534 lactating women (55.1% of women participating in SPATZ) with HMO data available at 6 weeks and 356 (36.7% of women participating in SPATZ) at 6 months with outcome data on child AD at either 1 or up to 2 years were included in the current analysis (**Table 1**). Lactating mothers who provided human milk samples were higher educated, less likely to have had a history of smoking and less likely to have been overweight or obese compared to the SPATZ full cohort sample size.[28] The majority (75%) of infants were receiving human milk exclusively at 6 weeks. Regarding positive reports of AD by either parent or pediatrician, 18% and a quarter (25%) had at least one positive report of AD at 1 or up to 2 years, respectively.

Maternal secretor status and milk group were not statistically significantly correlated with child AD at 1 or 2 years (all $p > .50$, Table S1). Absolute HMO concentrations of human milk samples collected at 6 weeks and 6 months were compared between children with and without AD at 1 or 2 years. There were no statistically significant (α threshold = 0.0031 after Bonferroni adjustment) differences in the concentration of HMOs measured at 6 weeks (Table 2) and 6 months (Table S2) by children who developed AD at 1 or 2 years and those that did not. Further stratification of 6 weeks and 6 months samples by secretor status and milk groups I and II also did not show any statistically significant differences in the milk of children with or without AD at 1 or 2 years (α threshold = 0.0031 after Bonferroni adjustment, Tables S3–S10).

Some associations [odds ratio (OR) (95% confidence interval (CI))] were observed between individual HMOs and AD at 1 and 2 years; however, these were not statistically significant (α threshold = 0.0031 after Bonferroni adjustment) in neither crude nor adjusted models (Table 3, Table S11–S12). We further investigated the associations of individual HMOs with AD at 1 and 2 years in a stratified analysis. Some of these associations (adjusted/unadjusted ORs, 95% CIs) were statistically significant at conventional level ($p < .05$), but were not statistically significant following adjustments for multiple testing (α threshold = 0.0031, Table 4 and Table 5, Table S13–S16).

There were also no statistically significant associations between individual HMOs measured at 6 weeks and 6 months within milk groups I and II (Table 6 and Table 7, Table S17–S20) with odds of AD at 1 or 2 years. We further investigated individual candidate HMOs whose associations were significant at conventional level ($p < .05$) in a restricted subset of children with consistent POEM scores at ages 2 and 3 years (data not shown). There were no statistically significant associations and differences in the concentrations of these selected HMOs at 6 weeks and 6 months for children who developed AD ($n = 17$ and $n = 13$, respectively) compared to those that did not ($n = 122$, $n = 83$, respectively).

4 | DISCUSSION

In the present study, we investigated associations between individual HMOs measured at 6 weeks and 6 months of lactation with

TABLE 6 Adjusted associations between human milk oligosaccharides (HMOs) in group I milk measured at 6 weeks of lactation with reports of atopic dermatitis (AD) at 1 and 2 years in the Ulm SPATZ Health Study

	Group I milk					
	AD at 1 year			AD at 2 years		
	OR	95% CI	*p**	OR	95% CI	*p**
Lactose	0.91	(0.69, 1.20)	.52	0.96	(0.75, 1.22)	.72
2'-FL	0.87	(0.58, 1.29)	.50	0.78	(0.55,,1.11)	.17
3'-FL	1.28	(0.84, 1.97)	.26	1.30	(0.91, 1.81)	.16
6'-GL	1.20	(0.92, 1.58)	.19	1.24	(0.98, 1.57)	.07
3'-SL	1.12	(0.83, 1.50)	.46	1.09	(0.84, 1.41)	.51
6'-SL	1.05	(0.80, 1.39)	.71	1.04	(0.82, 1.31)	.76
DFL	1.15	(0.79, 1.66)	.47	1.03	(0.74, 1.43)	.84
LNT	0.94	(0.69, 1.26)	.66	1.05	(0.81, 1.37)	.73
LNnT	0.74	(0.52, 1.03)	.08	0.75	(0.56, 1.01)	.06
LNFP I	0.88	(0.59, 1.31)	.54	0.87	(0.61, 1.22)	.41
LNFP II	1.20	(0.80, 1.84)	.39	1.39	(0.97, 2.00)	.08
LNFP III	1.00	(0.756, 1.33)	.98	1.05	(0.82, 1.34)	.69
LNFP V	1.18	(0.81, 1.75)	.39	1.38	(0.99, 1.95)	.06
LNDFH I	1.16	(0.80, 1.69)	.44	1.25	(0.90, 1.75)	.18
LNDFH II + LNnDFH II	1.29	(0.86, 1.93)	.22	1.36	(0.96, 1.94)	.08
Total HMOs	0.97	(0.70, 1.34)	.84	0.97	(0.73, 1.29)	.85

Abbreviations: 2'-FL, 2'-fucosyllactose; 3'-SL, 3'-sialyllactose; 3-FL, 3-fucosyllactose; 6'-SL, 6'-sialyllactose; 6'-GL, 6'-galactosyllactose; AD, atopic dermatitis; CI, confidence interval; DFL, 3,2'-difucosyllactose; HMO, human milk oligosaccharides; LNDFH I, lacto-N-difucohexaose I; LNDFH II, lacto-N-difucohexaose II; LNFP I, lacto-N-fucopentaose-I; LNFP II, lacto-N-fucopentaose-II; LNFP III, lacto-N-fucopentaose-III; LNFP V, lacto-N-fucopentaose-V; LNnDFH II, lacto-N-neodifucohexaose II; LNnT, lacto-N-neotetraose; LNT, lacto-N-tetraose.

OR, odds ratio;

*Associations determined by logistic regression in a stratified analysis. ORs reflect the odds of atopic dermatitis per standardized score (Blom transformed) of HMO concentrations. Models adjusted for child sex, maternal allergy, delivery mode, exclusive breastfeeding and parity. Bonferroni-adjusted level of statistical significance is $\alpha = 0.05/16 = 0.0031$.

parent or pediatrician reported child AD at 1 or up to 2 years in the Ulm SPATZ Health Study in Southern Germany. There were no statistically significant differences in the absolute HMO concentrations measured at 6 weeks and 6 months, in the milk for children with or without AD at 1 or up to 2 years. We showed some associations that were significant at conventional level ($p < .05$). Of note, there were no statistically significant associations following adjustments for milk group or secretor status, child sex, maternal allergy, delivery mode, exclusive breastfeeding, and parity as well as correction for multiple testing (α threshold = 0.0031).

The novelty of this study is the investigation of individual and total HMOs measured at two time points of lactation (6 weeks and 6 months) in the milk for children with and without AD at 1 or 2 years. Previous studies[21,23,24] investigating similar associations used human milk sampled between 2 days and 3 months postpartum. In addition, we evaluated these associations and differences in HMO content separately for secretor, non-secretor, group I, and group II milks. There were no statistically significant differences in the absolute content of HMOs at 6 weeks and 6 months between children who developed AD at 1 or 2 years, compared to those that did not; irrespective of secretor and milk group status, but also within secretors, non-secretors, and milk groups I and II. Thus, these results do not support the hypothesis that infants with AD receive different amounts of HMOs in comparison to their non-allergic counterparts, irrespective of secretor status or milk group, but also within the investigated secretor and human milk groups. It is suggested that many infants develop AD symptoms within the first year of life, a period during which exposure to HMOs is highest.[32] We previously showed that while the total content of HMOs decreased at 6 months, and up to 12 months of lactation, some individual HMO structures increased.[29] In addition, the data showed large variations within secretor and milk groups at 6 weeks, 6 months and 12 months. This taken together suggests that the composition of human milk sampled at one point of lactation may not depict the overall composition of HMOs during prolonged lactation.

TABLE 7 Adjusted associations between human milk oligosaccharides (HMOs) in group II milk measured at 6 weeks of lactation with reports of atopic dermatitis (AD) at 1 and 2 years in the Ulm SPATZ Health Study

	Group II milk					
	AD at 1 year			AD at 2 years		
	OR	95% CI	*p**	OR	95% CI	*p**
Lactose	0.59	(0.31, 1.09)	.10	0.82	(0.48, 1.36)	.45
3'-FL	1.01	(0.28, 3.42)	.99	1.90	(0.68, 5.30)	.22
6'-GL	1.43	(0.81, 2.64)	.23	1.22	(0.76, 1.97)	.41
3'-SL	1.01	(0.59, 1.76)	.96	0.87	(0.55, 1.37)	.55
6'-SL	1.06	(0.59, 1.90)	.84	1.02	(0.62, 1.64)	.95
LNT	0.75	(0.39, 1.43)	.39	0.65	(0.37, 1.09)	.11
LNnT	1.24	(0.47, 3.14)	.65	1.14	(0.53, 2.40)	.74
LNFP II	0.68	(0.19, 2.17)	.53	1.04	(0.39, 2.71)	.94
LNFP III	1.13	(0.55, 2.28)	.73	1.29	(0.73, 2.31)	.38
LNFP V	0.86	(0.25, 2.70)	.80	0.77	(0.28, 1.99)	.60
LNDFH II + LNnDFH II	0.79	(0.23, 2.54)	.69	1.46	(0.53, 4.18)	.47
Total HMOs	0.78	(0.37, 1.56)	.49	0.95	(0.53, 1.68)	.86

Abbreviations: 3-FL, 3-fucosyllactose; 6'-SL, 6'-sialyllactose; 6'-GL, 6'-galactosyllactose, 3'-SL, 3'-sialyllactose; AD, atopic dermatitis; CI, confidence interval; HMO, human milk oligosaccharides; LNDFH II, lacto-N-difucohexaose II; LNFP II, lacto-N-difucohexaose I; LNFP III, lacto-N-fucopentaose-III; LNFP V, lacto-N-fucopentaose-V; LNnDFH II, lacto-N-neodifucohexaose II; LNnT, lacto-N-neotetraose; LNT, lacto-N-tetraose; OR, odds ratio.

*Associations determined by logistic regression in a stratified analysis. ORs reflect the odds of atopic dermatitis per standardized score (Blom transformed) of HMO concentrations. Models adjusted for child sex, maternal allergy, delivery mode, exclusive breastfeeding and parity. Bonferroni-adjusted level of statistical significance is $\alpha = 0.05/16 = 0.0031$.

Furthermore, we did not find statistically significant associations between individual HMOs and AD at 1 or 2 years. No statistically significant associations were observed when the analyses were stratified by secretor status or milk groups I and II either. Despite these null findings, it is important to note that only three other studies[21,23,24] have investigated similar associations with AD. On one hand, our results are in line with Sjögren et al.[21] who also did not find statistically significant associations between individual concentrations of nine neutral HMOs in colostrum and the risk of AD up to 18 months. However, that study also reported potential associations of an increase in the total concentration of those nine neutral HMOs with a risk of allergic disease (ie, bronchial asthma, allergic rhino conjunctivitis, AD, and food allergy). Furthermore, Miliku et al.[20] and Seppo et al.[22] reported plausible associations between some clusters of HMOs with food sensitization and cow's milk allergy, respectively.

On the other hand, our results are in contrast to Sprenger et al.[23] who reported a lower risk of IgE-associated AD at the age of two years, but not at five years in high-risk infants born by cesarean section and had been fed human milk with FUT2-dependent HMOs. Although 2'-FL levels were used as a proxy for FUT2 activity (secretor status), they also did not find any statistically significant associations between secretor status and eczema. Also, another study[22] reported that infants who received human milk with lower levels of some HMOs including 6′-SL were more likely to develop AD. In a more recent study,[24] infants who consumed human milk containing HMOs in the acidic Lewis cluster (comprising high 3-FL, 3'-SL, LNFP II, and low LNFP I, among others) were at higher risk of AD and respiratory allergy compared to infants consuming human milk containing HMOs in the neutral Lewis class (comprising high LNFP II, and low LNFP I among others). However, the researchers from that study propose that some of these findings could have been due to chance because of the multiple associations tested.

Moreover, there is no direct evidence of plausible correlations between maternal secretor status or milk group with AD. However, only two of the above-mentioned studies[22,23] evaluated these correlations. Both studies[22,23] used 2'FL (and LNFP I[22]) as proxies of FUT2 activity (secretor status) and did not show any statistically significant correlations between maternal secretor status and eczema[23] or cow's milk allergy.[22] Nonetheless, similarly to the current study, only Seppo et al.[22] adjusted for secretor status in their regression models. On one hand, analysis stratified by maternal secretor status are valuable in HMO-related research, yet there is still notable variability in HMO profiles even within the same secretor phenotypes.[15,29] On the other hand, other studies,[33–35] although not necessarily investigating associations with allergy, have also previously assessed maternal secretor status as a confounding variable. Considering all this, maternal secretor status and milk group were included as covariates in the current study because of their strong association with HMOs. Still, inspite of our null findings, we provide much larger sample size and take both secretor and milk group status into account in comparison to the previous studies investigating associations of HMOs with AD. Further research is therefore needed to determine the extent, if any, to which maternal secretor status and milk group affect other infant health outcomes.

It is also worth mentioning that allergic manifestations in early childhood may be transient and not indicative of long-term allergic disease.[24] Thus, POEM stratification offers researchers a tool to capture longitudinal patient AD symptoms and long-term control of flares.[28] Nonetheless, using POEM data available for a subset of children, there were no statistically significant differences ($p > .05$) in the concentration of these selected HMOs at 6 weeks and 6 months. Of note, we tested these specific individual HMOs in order to avoid potential effects of multiple testing. Although the sample size of children included in this sub-analysis was considerably small for the AD cases, it is clear that a single diagnosis may not always be reliable. Thus, it is plausible that any associations that were significant at conventional level ($p < .05$) may have resulted from misclassification, especially since the outcomes were partly based on self-report of a doctor's diagnosis by parents. As such, using parent report of a doctor's diagnosis of AD could have been subject to recall bias. Hence, the need to distinguish between milder and severe cases of diseases by using additional assessment measures related to AD.

We cannot rule out the possible effect or influence of the secretor status of the infant, or child, as well as additional immunological components in human milk, which were not measured in this study. Secretor status of the infant could also modify the associations between HMOs and clinical outcomes.[26] We also acknowledge that while we quantified absolute HMO concentrations using a more accurate, robust, and extensively validated method for HMO quantification in comparison to other studies, there are over 200 structurally unique HMOs that have not yet been identified.[4,26] Therefore, it is plausible that the other important oligosaccharides including long-chain HMOs with polyvalent epitopes and higher activity for specific immune receptors that have not been identified in the current study as well as previous studies may have important biological effects. Another limitation to note in this study is that participants were recruited from the general population in Ulm, southern Germany, subsequently leading to smaller numbers of children with parent or pediatrician reported AD, compared to what a high-risk cohort would report. Nonetheless, the strengths of this study include human milk samples available at two time points (6 weeks and 6 months) of lactation and the classification of human milk according to secretor status and milk groups. Additionally, we had a larger sample size of AD cases in comparison to previous studies.

In conclusion, the findings from this study suggest that individual HMOs are not associated with development of AD at 1 or 2 years. However, given the exploratory nature of this study, more research is needed to determine whether any HMOs and additional, not yet identified (polyvalent) HMOs are indeed implicated in childhood AD. Moreover, little is known about low abundant short chain HMOs like 3'-GL, but also more complex, long-chain HMO structures beyond the size of hexaoses, to date. Their potential benefits for the infants' healthy development may warrant more detailed investigation. We hope that the full display the results from this study will aid in designing future investigations with regard to both candidate selection and sample size planning.

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CONFLICT OF INTEREST

J. Ge. is the project manager of unrestricted grants from Danone Nutricia Research to Ulm University and Leipzig University for research on other aspects of human milk composition in the Ulm birth cohort studies. M.M., B.S., J. Go., and B.B. are employees of Danone Nutricia Research. M.M and B.S. from Danone Nutricia Research contributed to the conceptualization and management of the overall project for advanced compositional human milk analysis. J. Go. and B.B. from Danone Nutricia contributed to the analysis of HMOs in the laboratory. All authors (including authors from Danone Nutricia Research) critically reviewed the final version of the manuscript. However, the principal investigators (J. Ge. and D.R.) along with the first author (L.P.S.) made final decisions on the interpretation and dissemination of results. These authors (J. Ge., D.R. and L.P.S.) therefore accept full accountability for ensuring integrity and accuracy of the research presented. None of the other researchers has any conflict of interest.

AUTHOR CONTRIBUTIONS

Linda P. Siziba: Conceptualization (equal); Formal analysis (lead); Investigation (lead); Writing – original draft (lead); Writing – review & editing (lead). **Marko Mank:** Methodology (lead); Project administration (equal); Resources (equal); Validation (lead); Writing – review & editing (supporting). **Bernd Stahl:** Methodology (supporting); Project administration (supporting); Resources (equal); Validation (supporting); Writing – review & editing (supporting). **Deborah Kurz:** Investigation (supporting); Writing – review & editing (supporting). **John Gonsalves:** Methodology (equal); Resources (supporting); Validation (equal); Writing – review & editing (supporting). **Bernadet Blijenberg:** Methodology (equal); Resources (supporting); Validation (equal); Writing – review & editing (supporting). **Dietrich Rothenbacher:** Conceptualization (supporting); Data curation (equal); Funding acquisition (equal); Investigation (equal); Project administration (supporting); Resources (equal); Writing – review & editing (supporting). **Jon Genuneit:** Conceptualization (equal); Data curation (equal); Formal analysis (supporting); Funding acquisition (lead); Investigation (equal); Project administration (equal); Resources (equal); Writing – original draft (supporting); Writing – review & editing (supporting).

PEER REVIEW

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SUPPORTING INFORMATION

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