<https://helda.helsinki.fi>

FUT2-dependent breast milk oligosaccharides and allergy at 2 and 5 years of age in infants with high hereditary allergy risk

Sprenger, Norbert

2017-04

Sprenger , N , Odenwald , H , Kukkonen , A K , Kuitunen , M , Savilahti , E & Kunz , C 2017 ,
' FUT2-dependent breast milk oligosaccharides and allergy at 2 and 5 years of age in infants
with high hereditary allergy risk ' , European Journal of Nutrition , vol. 56 , no. 3 , pp.
1293-1301 . <https://doi.org/10.1007/s00394-016-1180-6>

<http://hdl.handle.net/10138/236702>

<https://doi.org/10.1007/s00394-016-1180-6>

publishedVersion

Downloaded from Helda, University of Helsinki institutional repository.

This is an electronic reprint of the original article.

This reprint may differ from the original in pagination and typographic detail.

Please cite the original version.

FUT2-dependent breast milk oligosaccharides and allergy at 2 and 5 years of age in infants with high hereditary allergy risk

Norbert Sprenger¹ · Hannah Odenwald^{1,2,5} · Anna Kaarina Kukkonen³ · Mikael Kuitunen⁴ · Erkki Savilahti⁴ · Clemens Kunz²

Received: 2 November 2015 / Accepted: 6 February 2016 / Published online: 24 February 2016
© Springer-Verlag Berlin Heidelberg 2016

Abstract

Purpose Manifestation of allergic disease depends on genetic predisposition, diet and commensal microbiota. Genetic polymorphism of mothers determines their breast milk glycan composition. One major determinant is the fucosyltransferase 2 (*FUT2*, secretor gene) that was shown to be linked to commensal microbiota establishment. We studied whether *FUT2*-dependent breast milk oligosaccharides are associated with allergic disease in breast-fed infants later in life.

Methods We analyzed *FUT2*-dependent oligosaccharides in breast milk samples of mothers ($n = 266$) from the placebo group of a randomized placebo-controlled trial of prebiotics and probiotics as preventive against allergic disease in infants with high allergy risk (trial registry number: NCT00298337). Using logistic regression models, we studied associations between *FUT2*-dependent breast milk oligosaccharides and incidence of allergic disease at 2 and 5 years of age.

Results At 2 years, but not at 5 years of age, we observed a presumed lower incidence ($p < 0.1$) for IgE-associated eczema manifestation in C-section-born infants who were fed breast milk containing *FUT2*-dependent oligosaccharides. By logistic regression, we observed a similar relation ($p < 0.1$) between presence of *FUT2*-dependent breast milk oligosaccharides and IgE-associated disease and IgE-associated eczema in C-section-born infants only. When testing with the levels of breast milk oligosaccharide 2'-fucosyl-lactose as proxy for *FUT2* activity, we observed significant ($p < 0.05$) associations in the C-section-born infants with 'any allergic disease,' IgE-associated disease, eczema and IgE-associated eczema.

Conclusion The data indicate that infants born by C-section and having a high hereditary risk for allergies might have a lower risk to manifest IgE-associated eczema at 2 years, but not 5 years of age, when fed breast milk with *FUT2*-dependent milk oligosaccharides. Further studies with larger cohorts and especially randomized controlled intervention trials are required to build on these preliminary observations.

✉ Norbert Sprenger
Norbert.Sprenger@rdls.nestle.com

✉ Clemens Kunz
Clemens.Kunz@uni-giessen.de

¹ Nestlé Research Center, Nestec S.A., Vers-Chez-Les-Blanc, 1000 Lausanne 26, Switzerland

² Institute of Nutritional Sciences, University of Giessen, Wilhelmstr. 20, 35392 Giessen, Germany

³ Skin and Allergy Hospital, Helsinki University Central Hospital, 00029 Helsinki, Finland

⁴ Children's Hospital, University of Helsinki and Helsinki University Central Hospital, 00029 Helsinki, Finland

⁵ Present Address: Nestle Health Sciences, Biopole, 1066 Epalinges, Switzerland

Keywords Human milk oligosaccharides · Breast-feeding · Allergy · Mode of delivery

Introduction

Allergies are at the rise in the developed world, and besides genetic predisposition, environmental factors such as microbes are discussed to be key determinants for allergy onset [1]. At birth, the infant transits from a highly protected and regulated maternal, basically microbiota-free environment, to the colonization with microbiota from the surrounding. With vaginal birth the microbiota comes

mainly from the mother's intestine, vagina and skin, while with C-section birth the first colonizing microbes are primarily from the hospital environment and the mother's skin [2, 3]. Besides the primary microbial colonizers, the infant's diet is one of the major variables orchestrating the newly acquired microbiota establishment [4, 5]. This process plays an important role in infant health, providing resistance to pathogens, immune stimulation and further developmental cues that likely go beyond the gut mucosa [3, 6]. Milk, the natural and evolutionary adapted early feed of mammalian newborns, provides not only nutrients, protective compounds and growth and development factors, but also microbe orchestrating prebiotics, such as lactose-based oligosaccharides [7, 8].

These generally non-digestible oligosaccharides are extensions of the milk sugar lactose brought about by the action of glycosyltransferases such as those transferring *N*-acetyl-glucosamine, galactose, sialic acid or fucose. For the latter, two fucosyltransferases FUT2 (secretor gene) and FUT3 (Lewis gene) are implicated. Both are polymorphic with different alleles being responsible for the nonsecretor (*FUT2*^{-/-}) and Lewis negative (*FUT3*^{-/-}) glyco-types. The consequent varying enzyme activities manifest in measurable variation of specific human milk oligosaccharides and profiles [9, 10].

As a consequence of the genetic polymorphism of mothers, infants naturally receive milk containing different levels and types of oligosaccharides. Nonsecretor mothers, lacking a functional FUT2 enzyme, represent approximately 15–25 % of mothers depending on their ethnic background [11, 12], and their milk is essentially devoid of alpha-2 linked fucose epitopes on oligosaccharides. Recently, Lewis et al. [13] studied the impact of the absence or presence of FUT2-dependent milk oligosaccharides on the establishment of gut microbiota in breast-fed infants and found the establishment of a bifidobacteria-loaded microbiota to be associated with the presence of FUT2-dependent oligosaccharides in milk. Another study did not identify mothers that completely miss FUT2-dependent oligosaccharides; however, levels of FUT2-dependent oligosaccharides, namely 2'-fucosyllactose, were associated with infectious gastroenteritis [14, 15]. Deduced from preclinical studies, FUT2-dependent oligosaccharides are thought (1) to act as decoy affecting pathogens directly and (2) to alter specific commensal bifidobacteria and their metabolism [16–18], thereby altering gut functions. Further, the FUT2-dependent oligosaccharide 2'-fucosyllactose was recently shown to reduce hypothermia and diarrhea in a mouse model on food allergy [19].

Commensal gut microbiota composition and diversity were proposed to be associated with allergic manifestations [20–22]. Possibly, gut commensal microbiota impacts the onset of allergic and other immune system-related

manifestations through its metabolic activity and metabolites [6, 23] that can be modulated by specific oligosaccharides such as the FUT2-dependent oligosaccharides [13].

With the above in mind, we set out to explore whether FUT2-dependent breast milk oligosaccharides are associated with the onset of allergic diseases in infants with a high hereditary risk for allergies. To this end, we did a retrospective analysis of a part of the placebo group of a randomized placebo-controlled trial of prebiotics and probiotics as preventive against allergic disease in infants with hereditary allergy risk (trial registry number: NCT00298337) [24] for which milk samples were available for oligosaccharide analysis. The study findings contribute to better understand biological roles of FUT2-dependent milk oligosaccharides for the breast-fed infant and will guide the formulation of novel working hypotheses to be tested in future prospective studies.

Materials and methods

Clinical data and breast milk samples

Pregnant mothers carrying infants with hereditary risk for allergy [i.e., one or both parents with physician-diagnosed allergic disease (allergic rhinitis, eczema, asthma)] were recruited in the Helsinki suburban area from November 2000 to March 2003 and invited to participate in a large randomized, double-blind, placebo-controlled study [24]. Pre-term infants (≤ 37 weeks of gestation), infants with major malformations and infants from multiple birth (except the first born) were not eligible for the study. The study cohort analyzed here was part of the control (placebo) group for which milk samples were available for milk oligosaccharide analysis. Of the original 464 infants from the placebo group that were followed for at least 2 years, we could analyze 266 mother infant pairs. According to protocol, both mothers and their infants of the placebo group received a daily capsule of microcrystalline cellulose and the infants received additionally 20 drops of sugar syrup (22 % glucose, 20 % fructose, 27 % sucrose).

Diagnostic criteria of allergic diseases were previously described [24, 25]. Briefly, measures of the exploratory analysis presented here included medical doctor-diagnosed parameters, such as (1) any allergic disease, (2) IgE-associated disease (i.e., any allergic disease and sensitization), (3) eczema (i.e., itchy skin condition and at least 3 of the following: familial history of atopic disease, dry skin during the last year, history of eczema, visible eczema involving typical sites), (4) IgE-associated eczema (i.e., eczema and sensitization). Sensitization was defined as any positive skin prick test or serum antigen-specific IgE at a level >0.7 kU/L.

The ethics committee of Children's Hospital at Helsinki University Central Hospital approved the study, and parents gave their written informed consent.

Oligosaccharide analysis of breast milk samples

Milk samples available for analysis were from mean postpartum day 2.6 (95 % CI of mean 2.34–2.73). The samples were immediately frozen upon collection at the hospital and kept at -70°C until analysis. Samples were thawed and centrifuged at $10,000\times g$ 3 times for 15 min each, the fat layer was discarded, and the supernatant without debris served for analyses. Skimmed milk samples were stored at -70°C for over 10 years before analysis, which might have led to water loss of the samples and overestimation of the exact oligosaccharide concentration. However, the likelihood for water loss is minimal, and as all samples were stored in the same conditions, the comparison of the oligosaccharide concentrations among all samples is considered reliable. On the other hand, the analysis of presence or absence of FUT2-dependent oligosaccharides is most reliable and also representative over the whole lactation period. Assignment of milk samples to a group (1) with FUT2-dependent milk oligosaccharides (FUT2 positive) and (2) without FUT2-dependent milk oligosaccharides (FUT2 negative) was done using two independent methods. First, using MALDI-TOF-MS (matrix-assisted laser desorption/ionization-time of flight-mass spectrometry) profiling and second, using liquid chromatography to measure additional specific milk oligosaccharides such as 2'-fucosyllactose, the simplest and most abundant representative of the FUT2-dependent milk oligosaccharides. 2'-Fucosyllactose absence or presence was used to confirm grouping of milk samples. Levels of 2'-fucosyllactose in milk were used as a proxy for FUT2 activity presumed to be valid throughout lactation.

The employed MALDI-TOF-MS approach was described in detail by Blank et al. [26]. Briefly, a 0.05-mL aliquot of milk was diluted $10\times$ and centrifuged for 20 min at $14,000\times g$. The supernatant was then subjected to solid-phase extraction on porous graphitic carbon cartridges (25 mg, Thermo Scientific, Bellefonte PA, USA). Eluted samples were dried in a vacuum centrifuge and suspended in MilliQ water. MALDI-TOF-MS spectra were recorded with an Ultraflex I instrument (Bruker Daltonics, Bremen, Germany), equipped with a nitrogen laser that emits at 337 nm.

For analysis by liquid chromatography, milk samples were defrosted, mixed and centrifuged for 5 min at $10,000\times g$. An aliquot of the skimmed milk was diluted 10 times and then again 10 times with MilliQ water. All samples were analyzed thereafter by high-performance anion

exchange chromatography (HPAEC) coupled to a pulsed amperometry detector (ICS3000, Thermo Fischer Dionex, Sunnyvale, USA) in duplicate and at least at $100\times$ dilution. Some samples were further diluted to allow for quantification. Briefly, samples were loaded onto a CarboPac PA1 analytical column (4×250 mm; Thermo Fischer Dionex) without guard column at room temperature and a flow rate of 1 mL/min. Milk oligosaccharide separation and elution were achieved using the following gradient: For 17 min gradient from 50 mM NaOH to 120 mM NaOH followed by a 4-min wash step with 150 mM NaOH and 250 mM sodium acetate and a 10-min equilibration step at 50 mM NaOH. For identification and quantification, standard curves with authentic external 2'-fucosyllactose standards were employed at concentrations of 10, 25, 50 and 100 mg/L. 2'-Fucosyllactose standards were purchased from Dextra Laboratories (UK). Eluents were prepared from 50 % NaOH (J.T. Baker, UK) and sodium acetate (Fluka, CH). To control the HPAEC and to quantify the oligosaccharides, the Chromeleon software package 6.8 was employed.

Statistical analysis

Descriptive statistics were done to describe the baseline variables and the incidence of allergic disease at 2 years and 5 years of age. Odds ratio with 95 % confidence interval (95 % CI) and two-tailed Fisher exact probability test were calculated for the FUT2-negative phenotype.

Logistic regression analysis was performed to compare the incidence of allergic diseases (response: any allergic disease, IgE-associated disease, eczema, IgE-associated eczema) at the end of the 2-year follow-up period in infants who were breast-fed with milk containing FUT2-dependent oligosaccharides compared to infants fed milk containing no FUT2-dependent oligosaccharides. The following covariates were included in the statistical model: siblings, mode of delivery, gender, both parents allergic, gestational age. Additionally, the statistical model included also the interactions between the milk FUT2 glycan status and the mode of delivery or the presence of siblings.

Further logistic regression models were used to study possible associations between milk 2'-fucosyllactose levels as proxy for FUT2 activity and allergic manifestations at 2 years of age. As before, the following covariates were included in the model: siblings, mode of delivery, gender, both parents allergic, gestational age, and also the interactions between the milk 2'-fucosyllactose levels and the mode of delivery or the presence of siblings.

Statistical models were done using the software package R (<http://www.r-project.org/>). Figures were drawn with GraphPad Prism 6 (GraphPad Software, CA, USA).

Results

Participant characteristics

From the original study population, as previously published [24, 25], 266 breast milk samples from the placebo control group were available for the analysis of this association study. Of these 266 milk samples, 233 samples contained FUT2-dependent oligosaccharides (e.g., 2'-fucosyllactose, lacto-*N*-fucopentaose I, and di-fucosyllactose) and were dubbed 'FUT2-positive milk.' The remaining 33 milk samples did not show any of the FUT2-dependent oligosaccharides listed above and were dubbed 'FUT2-negative milk.' The baseline characteristics for this infant cohort are depicted in Table 1. These characteristics are well distributed between those who received milk without FUT2-dependent glycans (FUT2 negative) and those who received milk with FUT2-dependent glycans (FUT2 positive). In both groups, about 20 % were born by C-section and about 45 % had siblings at birth. Exclusive breast-feeding was about 2.5 months, and total duration of breast-feeding 8.6 months. About 77 % of the mothers were allergic in both groups, and both parents were allergic in 36 % of infants who received FUT2-positive milk, while both parents were allergic for 27 % of infants who received FUT2-negative milk. For infants who received FUT2-negative milk, 33 % of the parents smoked and 30 % of infants had pets in the household, while for infants who received FUT2-positive milk 22 % of parents smoked and 20 % had pets in the household.

Presence and absence of breast milk FUT2-dependent oligosaccharides and allergies

The incidence proportion of vaginal-born infants who developed any allergic disease was about 33 % at 2 years and

about 52 % at 5 years of age independent of breast milk FUT2 oligosaccharide status. At 2 years, it seemed that already 57 % of infants manifested any allergic disease, when born by C-section and fed breast milk deficient in FUT2-dependent oligosaccharides (Table 2). For IgE-associated eczema, a statistically nonsignificant $p < 0.1$ by two-tailed Fisher exact probability test was obtained with an odds ratio for FUT2-negative milk of 4.75 (95 % CI 0.84–26.71, $p = 0.095$). At 5 years, all infants, vaginal born and C-section born, had a similar and high allergic disease incidence, and no association was observed between the consumption of breast milk with or without FUT2-dependent milk oligosaccharides and allergic diseases and eczema (Table 3).

To corroborate this first observation, regression models were employed to assess possible associations between allergic diseases at 2 years of age and FUT2-dependent milk oligosaccharides. The model included as covariates: siblings at birth, mode of delivery, gender, both parents allergic, gestational age and also tested for the interaction between the milk FUT2-dependent oligosaccharides status and the mode of delivery or the presence of siblings at birth (Table 4).

The regression model revealed no statistically significant relation ($p < 0.1$) when testing for the interaction between C-section birth and FUT2-dependent milk oligosaccharide status for IgE-associated disease ($p = 0.097$) and IgE-associated eczema ($p = 0.083$). Figure 1 depicts the odds ratios from the regression model with upper and lower levels for infants who were fed breast milk with FUT2-dependent oligosaccharides in all and the C-section-born infants.

Independent of the milk FUT2-dependent milk oligosaccharide status, we studied by logistic regression possible associations of both parents allergic with any allergic disease ($p = 0.0644$) and eczema ($p = 0.0466$) and of the mode of delivery with IgE-associated disease ($p = 0.0833$) and IgE-associated eczema ($p = 0.0830$).

Table 1 Baseline variables by milk FUT2-dependent oligosaccharide status

	FUT2 positive		FUT2 negative		<i>p</i> (two-tailed) ^b
	<i>n</i>	%	<i>n</i>	%	
Female sex	115/233	49	21/33	64	0.139
Delivery by C-section	44/233	19	7/33	21	0.813
Siblings at birth	103/233	44	15/33	45	1
Exclusively breast-fed (months) ^a	2.64 ± 1.95		2.75 ± 2.08		
Breast-fed duration (months) ^a	8.78 ± 5.12		8.46 ± 3.79		
Mother allergic	182/233	78	25/33	76	0.823
Both parents allergic	84/233	36	9/33	27	0.339
Parents smoking (0–2 years)	51/233	22	11/33	33	0.185
Pets in household (0–2 years)	46/233	20	10/33	30	0.174

^a Mean ± SD

^b By Fisher exact Chi-squared probability test

Table 2 Allergies at 2 years by FUT2-dependent milk oligosaccharide status and mode of delivery

	Vaginal born				C-section born			
	FUT2 positive	FUT2 negative	OR (95 % CI)	<i>p</i> value	FUT2 positive	FUT2 negative	OR (95 % CI)	<i>p</i> value
	<i>n</i> %	<i>n</i> %			<i>n</i> %	<i>n</i> %		
Any allergic disease	61/189 32 %	7/26 27 %	1.3 (0.52–3.24)	0.658	13/44 30 %	4/7 5 %	0.32 (0.06–1.6)	0.203
IgE-associated allergic disease	38/189 20 %	2/26 8 %	3.0 (0.67–10.2)	0.179	7/44 16 %	3/7 43 %	0.25 (0.05–1.38)	0.126
Eczema	59/189 31 %	7/26 27 %	1.23 (0.49–3.1)	0.821	12/44 27 %	4/7 57 %	0.28 (0.06–1.44)	0.186
IgE-associated eczema	36/189 19 %	2/26 8 %	2.8 (0.64–12.5)	0.182	6/44 14 %	3/7 43 %	0.21 (0.04–1.18)	0.095

Table 3 Allergies at 5 years by milk FUT2-dependent oligosaccharide status and mode of delivery

	Vaginal born				C-section born			
	FUT2 positive	FUT2 negative	OR (95 % CI)	<i>p</i> value	FUT2 positive	FUT2 negative	OR (95 % CI)	<i>p</i> value
	<i>n</i> %	<i>n</i> %			<i>n</i> %	<i>n</i> %		
Any allergic disease	96/185 52 %	11/26 42 %	1.47 (0.64–3.37)	0.406	25/44 57 %	4/7 57 %	0.98 (0.20–4.94)	1.0
IgE-associated allergic disease	59/184 32 %	4/26 15 %	2.6 (0.86–7.87)	0.109	17/41 39 %	3/7 43 %	0.94 (0.19–4.78)	1.0
Eczema	74/184 40 %	10/26 39 %	1.07 (0.46–2.50)	1.0	18/44 41 %	4/7 57 %	0.52 (.10–2.61)	0.684
IgE-associated eczema	46/184 25 %	3/26 12 %	2.72 (0.78–9.47)	0.182	11/41 25 %	3/7 43 %	0.44 (0.09–2.30)	0.656

Levels of breast milk 2'-fucosyllactose as proxy for FUT2 activity and allergies

Using logistic regression, we also investigated possible associations of 2'-fucosyllactose levels in early milk, as a proxy for FUT2 activity, with any allergic disease, IgE-associated disease, eczema and IgE-associated eczema at the age of 2 years (Table 4). The regression model showed significant associations of 2'-fucosyllactose levels in milk in the interaction with C-section birth for any allergic disease ($p = 0.016$), IgE-associated disease ($p = 0.009$), eczema ($p = 0.005$) and IgE-associated eczema ($p = 0.003$). The higher 2'-fucosyllactose levels seen in early milk consumed by C-section-born infants who did not develop IgE-associated allergic disease or IgE-associated eczema (Fig. 2) indicate the direction of the observed association, with higher 2'-fucosyllactose levels showing a lower risk.

In all infants, a significant association was observed between 2'-fucosyllactose levels and eczema ($p = 0.044$), which might be explained by the covariates such as delivery

by C-section, both parents allergic or siblings at birth as seen in the regression model (Table 4).

Discussion

The onset of allergic manifestations depends on genetic and environmental factors including diet and microbiota. The data presented here indicate, for the first time, that the onset of IgE-associated allergic manifestations, namely eczema, in infants with a high hereditary risk for allergies and born by C-section might be associated with FUT2-dependent oligosaccharides in breast milk that the infants consumed. First, comparing incidence ratios with respective odds ratios, we observed a presumed lower risk, although not statistically significant, to manifest IgE-associated eczema when C-section-born infants received breast milk containing FUT2-dependent milk oligosaccharides. Second, by logistic regression we made a similar observation, indicating that consumption of FUT2-positive

Table 4 Logistic regression outcomes with allergic manifestations at 2 years by milk FUT2-dependent oligosaccharide status (FUT2 positive) and proxy for FUT2 activity level (2'FL levels)

	Any allergic disease			IgE-associated allergic disease			Eczema			IgE-associated eczema		
	Estimate	SE	<i>p</i> value	Estimate	SE	<i>p</i> value	Estimate	SE	<i>p</i> value	Estimate	SE	<i>p</i> value
FUT2 positive	0.25	0.70	0.72	0.09	0.93	0.92	0.174	0.71	0.81	0.013	0.94	0.98
Siblings at birth	0.46	0.84	0.58	−0.76	1.29	0.55	0.466	0.84	0.58	−0.75	1.29	0.56
Delivery by C-section	1.50	0.97	0.12	1.95	1.13	0.08 ^a	1.501	0.97	0.12	1.96	1.31	0.083 ^a
Gender	0.07	0.27	0.79	−0.048	0.33	0.88	0.076	0.27	0.78	0.02	0.34	0.95
Both parents allergic	0.51	0.28	0.064 ^a	0.367	0.33	0.27	0.55	0.27	0.046 ^b	0.50	0.34	0.14
Gestational age	−0.003	0.01	0.71	−0.004	0.03	0.87	−0.003	0.01	0.73	−0.005	0.05	0.90
FUT2 positive × siblings at birth	−0.019	0.89	0.98	1.89	1.33	0.16	−0.027	0.89	0.98	1.86	1.34	0.17
FUT2 positive × C-section birth	−1.49	1.04	0.15	−2.021	1.22	0.097 ^a	−1.533	1.04	0.14	−2.14	1.23	0.083 ^a
2'FL levels	0.00014	0.0001	0.12	0.00015	0.00012	0.25	0.00019	0.0001	0.044 ^b	0.00018	0.00012	0.14
Siblings at birth	0.899	0.61	0.14	1.28	0.76	0.09 ^a	1.167	0.631	0.064 ^a	1.503	0.79	0.06 ^a
Delivery by C-section	1.86	0.74	0.011 ^b	2.34	0.85	0.006 ^b	2.197	0.759	0.004 ^b	2.698	0.88	0.002 ^b
Gender	0.00785	0.275	0.98	−0.040	0.33	0.90	−0.00240	0.280	0.99	0.025	0.35	0.94
Both parents allergic	0.585	0.282	0.038 ^b	0.459	0.340	0.18	0.642	0.287	0.025 ^a	0.615	0.35	0.08 ^a
Gestational age	−0.00257	0.008	0.75	−0.003	0.016	0.86	−0.00253	0.0088	0.77	−0.0023	0.017	0.89
2'FL × siblings at birth	−0.00009	0.0001	0.40	−0.00006	0.0001	0.68	−0.00015	0.00011	0.20	−0.00010	0.00014	0.46
2'FL × C-section birth	−0.00041	0.0002	0.016 ^b	−0.00054	0.0002	0.009 ^b	−0.00050	0.00018	0.005 ^b	−0.00067	0.00023	0.003 ^b

^a Trend *p* = 0.05–0.1^b Significant *p* < 0.05 **Fig. 1** Risk to show IgE-associated allergic disease and IgE-associated eczema in C-section-born infants at 2 years of age who consumed FUT2-positive breast milk. Odds ratio with 95 % confidence interval (95 % CI) in all infants and C-section infants at 2 years, to manifest IgE-associated allergic disease and IgE-associated eczema when they consumed FUT2-positive breast milk. Odds ratio and 95 % CI were calculated from estimates of the logistic regression analysis presented in Table 4

milk might be associated with lower risk to manifest IgE-associated allergic disease and IgE-associated eczema in the C-section-born infants only. Third, we corroborated these observations using 2'-fucosyllactose levels as a proxy for FUT2 activity in a logistic regression model that highlighted significant interactions between IgE-associated allergic disease and IgE-associated eczema with higher 2'-fucosyllactose levels in breast milk consumed by C-section-born infants who were not allergic at 2 years. Collectively, although based on weak associations, these observations indicate that FUT2-dependent milk oligosaccharides like 2'-fucosyllactose might be involved in the onset of IgE-associated eczema in C-section-born infants at 2 years of age, while at 5 years of age no indication for any association was found.

Using FUT2-positive versus FUT2-negative milk in the logistic regression model, we observed a weak and not statistically significant association between the consumption of FUT2-dependent milk oligosaccharides and

Fig. 2 Breast milk consumed by C-section-born infants who did not show IgE-associated allergic disease and IgE-associated eczema contained higher levels of 2'-fucosyllactose a proxy for FUT2-dependent oligosaccharides. 2'-Fucosyllactose (2'FL) levels in early breast milk (2.6 ± 1.1 days *postpartum*) that was consumed by C-section-born infants who showed IgE-associated allergic disease (a) or IgE-associated eczema (b) at 2 years or not. Box and whisker plots are shown with median and minimal and maximal values. Two-tailed *p* values from unpaired *t* test are indicated

IgE-associated eczema at 2 years in the C-section-born infants. The number of C-section-born infants who consumed milk without FUT2-dependent milk oligosaccharides was relatively low ($n = 7$). The small sample size could have resulted in a reduced power of this study to detect the magnitude of the association. To validate our initial observation, we also used regression analysis to study the association of 2'-fucosyllactose levels (the major FUT2-dependent milk oligosaccharide) determined in very early milk with allergic diseases diagnosed at 2 years of age. In this case, we assumed (1) that the highly variable levels of 2'-fucosyllactose in milk are due to genetic polymorphisms in the FUT2 gene and consequent activity levels, as previously proposed [27], and (2) that the single time point measured low or high amounts are representative also for relative lower or higher levels throughout lactation.

Interestingly, these presumed associations of IgE-associated eczema and consumption of FUT2-dependent milk oligosaccharides were observed only in infants born by C-section, a delivery mode that is considered a risk factor for atopic disease at least up to 2 years through its effect

on early gut microbiota establishment [28, 29]. In the same cohort of children, we demonstrated previously a delayed colonization of bifidobacteria in infants born by C-section, which was corrected by supplementation with a mixture of galacto-oligosaccharides and probiotic mixture containing two lactobacilli, a bifidobacteria and a propionibacteria [25]. Equally in this study, an extension of allergy preventive effects until 5 years was shown in C-section-born children but not in vaginally delivered children. Of note, a clear allergy preventive effect by the intervention was shown in the whole cohort at age 2 years [24]. Recently, FUT2-dependent milk oligosaccharides were associated with the establishment of a bifidobacteria-dominated microbiota in breast-fed infants [13]. Further, milk oligosaccharide like 2'-fucosyllactose stimulate specific bifidobacteria like *Bifidobacterium longum ssp infantis* [17, 18] known for its gut barrier promoting and anti-inflammatory properties [30–32]. In a preclinical food allergy mouse model, 2'-fucosyllactose feeding reduced allergic diarrhea scores and hypothermia [33]. The authors discussed that one likely route was through the stimulation of bifidobacteria.

Individuals with a null mutation in the *FUT2* gene (non-secretors) show higher susceptibility for a number of inflammatory and infectious diseases and on the other hand show protection from other infective agents and increased vitamin B12 uptake [34]. Hence, the *FUT2* polymorphism represents an evolutionary trade-off, to which also the breast milk FUT2-dependent oligosaccharides contribute in the early neonatal development. In the study presented here, we do not know the *FUT2* genotype of the infants and, therefore, cannot fully dissect *FUT2* genetic background effects in the infant from dietary FUT2-dependent maternal effectors.

In conclusion, we observed a possible association between the onsets of IgE-associated allergic disease, primarily eczema, in C-section-born infants with a high hereditary risk for allergies with FUT2-dependent oligosaccharides in the breast milk they consumed. This presumed association was seen at 2 years, but not anymore at 5 years of age. We speculate that the FUT2-dependent breast milk oligosaccharides transiently modulated mucosal immunity, possibly through the early microbiota establishment, which appeared to delay the IgE-associated eczema manifestation. Future association studies in larger cohorts and more importantly randomized controlled intervention trials are required to further build on our preliminary observation, taking into account also further environmental cues and genetic predisposition, such as infant *FUT2* status, to fully dissect effects of FUT2-dependent breast milk oligosaccharides on the onset of allergic manifestations and tolerance induction.

Acknowledgments We thank the study participants and their parents for participation, Dr. Rajat Mukherjee for help with the statistical analysis and Drs. Aristeia Binia, Carine Gaelle Blanchard, Chiara

Nembrini, and Susan Wernimont for critical discussion and reviewing of the manuscript. The study got financial support from the Nestle Research Center, Nestec S.A., Switzerland.

Compliance with ethical standards

Conflict of interest NS and HO are employees of Nestec S.A., and CK received a research Grant from Nestec S.A.

References

- Legatzki A, Rösler B, von Mutius ME (2014) Microbiome diversity and asthma and allergy risk. *Curr Allergy Asthma Rep* 14:466
- Macfarlane GT, Macfarlane LE (2009) Acquisition, evolution and maintenance of the normal gut microbiota. *Dig Dis* 27(Suppl 1):90–98
- Mackie RI, Sghir A, Gaskins HR (1999) Developmental microbial ecology of the neonatal gastrointestinal tract. *Am J Clin Nutr* 69:1035S–1045S
- Nauta AJ, Ben AK, Knol J, Garssen J, van der Beek EM (2013) Relevance of pre- and postnatal nutrition to development and interplay between the microbiota and metabolic and immune systems. *Am J Clin Nutr* 98:586S–593S
- Fouhy F, Ross RP, Fitzgerald GF, Stanton C, Cotter PD (2012) Composition of the early intestinal microbiota: knowledge, knowledge gaps and the use of high-throughput sequencing to address these gaps. *Gut Microbes* 3:203–220
- Trompette A, Gollwitzer ES, Yadava K, Sichelstiel AK, Sprenger N, Ngom-Bru C, Blanchard C, Junt T, Nicod LP, Harris NL, Marsland BJ (2014) Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. *Nat Med* 20:159–166
- Kunz C, Rudloff S, Baier W, Klein N, Strobel S (2000) Oligosaccharides in human milk: structural, functional, and metabolic aspects. *Annu Rev Nutr* 20:699–722
- Jantscher-Krenn E, Bode L (2012) Human milk oligosaccharides and their potential benefits for the breast-fed neonate. *Minerva Pediatr* 64:83–99
- Stahl B, Thurl S, Henker J, Siegel M, Finke B, Sawatzki G (2001) Detection of four human milk groups with respect to Lewis-blood-group-dependent oligosaccharides by serologic and chromatographic analysis. *Adv Exp Med Biol* 501:299–306
- Blank D, Dotz V, Geyer R, Kunz C (2012) Human milk oligosaccharides and Lewis blood group: individual high-throughput sample profiling to enhance conclusions from functional studies. *Adv Nutr* 3:440S–449S
- Erney RM, Malone WT, Skelding MB, Marcon AA, Kleman-Leyer KM, O’Ryan ML, Ruiz-Palacios G, Hilty MD, Pickering LK, Prieto PA (2000) Variability of human milk neutral oligosaccharides in a diverse population. *J Pediatr Gastroenterol Nutr* 30:181–192
- Castanys-Munoz E, Martin MJ, Prieto PA (2013) 2'-fucosyllactose: an abundant, genetically determined soluble glycan present in human milk. *Nutr Rev* 71:773–789
- Lewis ZT, Totten SM, Smilowitz JT, Popovic M, Parker E, Lemay DG, Van Tassell ML, Miller MJ, Jin YS, German JB, Lebrilla CB, Mills DA (2015) Maternal fucosyltransferase 2 status affects the gut bifidobacterial communities of breastfed infants. *Microbiome* 3:13
- Newburg DS, Ruiz-Palacios GM, Morrow AL (2005) Human milk glycans protect infants against enteric pathogens. *Annu Rev Nutr* 25:37–58
- Morrow AL, Ruiz-Palacios GM, Jiang X, Newburg DS (2005) Human-milk glycans that inhibit pathogen binding protect breast-feeding infants against infectious diarrhea. *J Nutr* 135:1304–1307
- Chaturvedi P, Warren CD, Altaye M, Morrow AL, Ruiz-Palacios G, Pickering LK, Newburg DS (2001) Fucosylated human milk oligosaccharides vary between individuals and over the course of lactation. *Glycobiology* 11:365–372
- Yu ZT, Chen C, Newburg DS (2013) Utilization of major fucosylated and sialylated human milk oligosaccharides by isolated human gut microbes. *Glycobiology* 23:1281–1292
- Yu ZT, Chen C, Kling DE, Liu B, McCoy JM, Merighi M, Heidtman M, Newburg DS (2013) The principal fucosylated oligosaccharides of human milk exhibit prebiotic properties on cultured infant microbiota. *Glycobiology* 23:169–177
- Castillo-Court Han S, Lee S, Mian FM, Buck R, Forsythe P (2015) Attenuation of food allergy symptoms following treatment with human milk oligosaccharides in a mouse model. *Allergy* 70:1091–1102
- Kalliomaki M, Kirjavainen P, Eerola E, Kero P, Salminen S, Isolauri E (2001) Distinct patterns of neonatal gut microflora in infants in whom atopy was and was not developing. *J Allergy Clin Immunol* 107:129–134
- Abrahamsson TR, Jakobsson HE, Andersson AF, Björkstén B, Engstrand L, Jenmalm MC (2012) Low diversity of the gut microbiota in infants with atopic eczema. *J Allergy Clin Immunol* 129:434–440
- Ismail IH, Oppedisano F, Joseph SJ, Boyle RJ, Licciardi PV, Robins-Browne RM, Tang ML (2012) Reduced gut microbial diversity in early life is associated with later development of eczema but not atopy in high-risk infants. *Pediatr Allergy Immunol* 23:674–681
- Venkatesh M, Mukherjee S, Wang H, Li H, Sun K, Benechet AP, Qiu Z, Maher L, Redinbo MR, Phillips RS, Fleet JC, Kortagere S, Mukherjee P, Fasano A, Le VJ, Nicholson JK, Dumas ME, Khanna KM, Mani S (2014) Symbiotic bacterial metabolites regulate gastrointestinal barrier function via the xenobiotic sensor PXR and Toll-like Receptor 4. *Immunity* 41:296–310
- Kukkonen K, Savilahti E, Hahtela T, Juntunen-Backman K, Korpela R, Poussa T, Tuure T, Kuitunen M (2007) Probiotics and prebiotic galacto-oligosaccharides in the prevention of allergic diseases: a randomized, double-blind, placebo-controlled trial. *J Allergy Clin Immunol* 119:192–198
- Kuitunen M, Kukkonen K, Juntunen-Backman K, Korpela R, Poussa T, Tuure T, Hahtela T, Savilahti E (2009) Probiotics prevent IgE-associated allergy until age 5 years in cesarean-delivered children but not in the total cohort. *J Allergy Clin Immunol* 123:335–341
- Blank D, Gebhardt S, Maass K, Lochnit G, Dotz V, Blank J, Geyer R, Kunz C (2011) High-throughput mass finger printing and Lewis blood group assignment of human milk oligosaccharides. *Anal Bioanal Chem* 401:2495–2510
- Newburg DS, Ruiz-Palacios GM, Altaye M, Chaturvedi P, Meinzen-Derr J, Guerrero ML, Morrow AL (2004) Innate protection conferred by fucosylated oligosaccharides of human milk against diarrhea in breastfed infants. *Glycobiology* 14:253–263
- Negele K, Heinrich J, Borte M, von Berg A, Schaaf B, Lehmann I, Wichmann HE, Bolte G (2004) Mode of delivery and development of atopic disease during the first 2 years of life. *Pediatr Allergy Immunol* 15:48–54
- Penders J, Gerhold K, Stobberingh EE, Thijs C, Zimmermann K, Lau S, Hamelmann E (2013) Establishment of the intestinal microbiota and its role for atopic dermatitis in early childhood. *J Allergy Clin Immunol* 132:601–607
- Chichlowski M, De LG, German JB, Raybould HE, Mills DA (2012) Bifidobacteria isolated from infants and cultured on

- human milk oligosaccharides affect intestinal epithelial function. *J Pediatr Gastroenterol Nutr* 55:321–327
31. Ewaschuk JB, Diaz H, Meddings L, Diederichs B, Dmytrash A, Backer J, Looijer-van LM, Madsen KL (2008) Secreted bioactive factors from *Bifidobacterium infantis* enhance epithelial cell barrier function. *Am J Physiol Gastrointest Liver Physiol* 295:G1025–G1034
 32. Ganguli K, Meng D, Rautava S, Lu L, Walker WA, Nanthakumar N (2013) Probiotics prevent necrotizing enterocolitis by modulating enterocyte genes that regulate innate immune-mediated inflammation. *Am J Physiol Gastrointest Liver Physiol* 304:G132–G141
 33. Castillo-Courtade L, Han S, Lee S, Mian FM, Buck R, Forsythe P (2015) Attenuation of food allergy symptoms following treatment with human milk oligosaccharides in a mouse model. *Allergy* 70:1091–1102
 34. Maroni L, van de Graaf SF, Hohenester SD, Oude Elferink RP, Beuers U (2014) Fucosyltransferase 2: a genetic risk factor for primary sclerosing cholangitis and Crohn's disease—a comprehensive review. *Clin Rev Allergy Immunol* 48:182–191