

ORIGINAL ARTICLE

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Association of maternal allergy with human milk soluble CD14 and fatty acids, and early childhood atopic dermatitis

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Abstract

Background: This study aimed to investigate whether maternal allergy is associated with soluble CD14 (sCD14) and fatty acid composition in different stages of lactation and the onset of atopic dermatitis (AD) in early childhood.

Methods: In total, 443 mother-child groups (445 children) were enrolled in the Prediction of Allergies in Taiwanese Children birth cohort study. Colostrum and mature milk at 2 months postpartum (2-month HM) were collected from lactating mothers. Information regarding parental allergy histories and physician-diagnosed atopic diseases was obtained using age-specific questionnaires (0-2 years). We compared sCD14 levels and the composition of 30 fatty acids in the colostrum and 2-month HM, respectively, between allergic and non-allergic mothers and between children with and without AD by the age of 2 years.

Results: In total, 185 (41.8%) mothers presented with allergies, and 154 (34.6%) children had physician-diagnosed AD by the age of 2 years. Both in the colostrum and 2-month HM of 289 lactating mothers, sCD14 levels were significantly lower in allergic mothers whose children presented with AD compared with children who did not ($P = 0.015$ and 0.044 , respectively). Among the children with AD who were born to non-allergic mothers, sCD14 levels were lower. However, the result was not statistically significant ($P = 0.376$ and 0.264 , respectively). Our data revealed the lack of associations between fatty acid composition and AD ($P > 0.05$).

Conclusion: Decreased sCD14 levels in the colostrum and 2-month HM were associated with AD at 2 years of age, particularly among children born to mothers with allergies.

KEYWORDS

atopic dermatitis, children, colostrum, fatty acids, maternal allergy, mature milk, sCD14

1 | INTRODUCTION

Atopic dermatitis (AD) is often the first manifestation of atopy in early childhood and is associated with food allergy, asthma, and allergic rhinitis concurrently and later in life.¹ Evidence suggests that early nutrition influences immune system development and

affects both short- and long-term health outcomes during infancy.² Breastfeeding has been promoted due to its nutritional, immunological, and psychological benefits.³ However, whether maternal allergy affects the immunologic components of human milk (HM) is debated.^{4,5} Additionally, the HM immune components may vary over time and among individuals.⁶ Therefore, for infants with a hereditary

risk of allergy, the role of breastfeeding in preventing allergies remains unclear.^{4,7}

Soluble CD14 (sCD14) is a lipopolysaccharide (LPS)-associated immune factor.⁵ Breast milk sCD14 is important for monitoring the bacterial load of the gut and for initiating an appropriate immune activity to commensals and pathogens via the toll-like receptor 4, resulting in the high secretion of IL-12, which promotes a strong helper T (Th) 1 response and counteracts the development of IgE-mediated allergic reactions.^{5,8,9} Nevertheless, results from clinical studies on the association between sCD14 and atopic disease have been inconsistent.⁹ By contrast, fatty acids (FAs) have been proposed to have immunoregulatory properties, and they may be important in the etiology of allergic diseases.^{11,12} Previous studies have reported that increased n-3 polyunsaturated fatty acid (PUFA) levels^{13,14} and decreased n-6 PUFA levels are more likely to be associated with eczema in childhood.¹³

First, we investigated whether maternal allergy influences sCD14 and FA composition during different lactation stages. Second, the association between HM immune components in allergic and non-allergic mothers and subsequent AD development in early childhood was assessed.

2 | METHODS

2.1 | Study participants

In total, 799 children were delivered at Chang Gung Memorial Hospital, Keelung, Taiwan, between March 2012 and December 2015 and were enrolled in the Prediction of Allergies in Taiwanese Children cohort study after obtaining written informed consent from their parents soon after birth.^{4,16} Among these, 43 children who dropped out during the 2-year follow-up period; 90 children who did not regularly return for follow-up or whose parents did not complete the questionnaires; 41 infants who had a gestational age <35 weeks, any perinatal insults, or major congenital anomalies; and 140 children whose mothers did not provide HM samples or who were only partially breastfed were excluded. Furthermore, at age 2 years, 40 children with allergic disorders other than AD (allergic rhinitis: 26 and asthma: 14) were excluded because the case numbers did not provide sufficient statistical power. Finally, 443 mother-child groups (445 children, two pairs of twins) were included. This study was approved by the ethics committee of Chang Gung Memorial Hospital (CMRPG 2H0161, 2E0131-134, and 2B0041-43).

2.2 | Questionnaires

Participants returned regularly for follow-up clinic visits and were checked by either one of two pediatricians for general health and atopic manifestations at 2, 4, 6, 12, 18, and 24 months of age. The children were diagnosed with AD if they presented with relapsing itchy skin rashes on the face and/or extensors in infancy or on the flexors (eg, elbows, wrists, and back of knees) and/or creases in the body in toddler years. After evaluation, age-specific

questionnaires were administered to parents under the guidance of well-trained research assistants. The questionnaires recorded information regarding demographic data, history of parental allergies, infant feeding practices (eg, breastfeeding and solid foods), environmental risk factors, medical problems, and any physician-confirmed atopic symptoms that have been clearly described in previous reports.^{4,16}

2.3 | Definitions used in the study

Allergic mothers/fathers: Parents who self-reported having a physician-confirmed allergic disease, such as asthma, rhinoconjunctivitis, eczema, or urticaria or food allergies.

Non-allergic mothers/fathers: Parents who self-reported the absence of a physician-confirmed allergic disease, as described above.

Children with atopic dermatitis (AD group): According to the questionnaires, parents who reported that their children had relapsing skin rashes that had been confirmed as AD by any physician by the age of 2 years.

Healthy controls (HC group): According to the questionnaires, parents who reported that their children did not have any confirmed allergic diseases by a physician, as described above, and chronic medical illness at age 2 years.

2.4 | Collection and processing of HM samples

Mothers were asked to provide one colostrum and one mature milk sample at 2 months postpartum (2-month HM). The methods of collecting and processing milk sample have been described in our previous study.⁴ Briefly, mothers were instructed to collect HM (10–15 mL) using a manual breast pump before breastfeeding. The milk-filled tube was kept in the refrigerator (0°C) until it was collected by a research assistant. Thereafter, HM samples were thawed and centrifuged (8000 g, 30 min, no braking, 4°C) to separate the lipid and aqueous fractions. Aqueous fractions were stored at –80°C until the analysis.

2.5 | Determining sCD14 levels using enzyme-linked immunosorbent assays

After thawing the milk sample aqueous fractions, sCD14 concentrations (µg/mL) in the colostrum and 2-month HM were determined using the Quantikine Human sCD14 enzyme-linked immunosorbent assay kits (R&D Systems, catalog no. DC140, USA), according to the manufacturer's instructions.

2.6 | HM fatty acid analysis

Total lipids were extracted from HM samples according to Bligh and Dyer's method¹⁷ and were converted to their methyl esters, which were analyzed with Agilent 7820A GC using flame ionization detection on a SP-2560 polar fused silica capillary column (100 m × 0.25 mm × 0.2 µm; Supelco Inc, PA, USA) with nitrogen as

the carrier gas.¹⁸ The FA peaks were identified by comparing retention times with those of a standard mixture of GLC-68A, GLC-481B, GLC-532, GLC-744 (Nu-Chek Prep), 37 FAME, trans 16:1n-7, trans 18:1n-7, trans 18:1n-9, and cis/trans 18:2n-6 (all from Supelco Inc or Sigma). The FA composition was expressed as a percentage of the weight of carbon 10 to that of carbon 24 FA (wt%).

2.7 | Statistical analysis

Demographic data of the children were collected via questionnaires and analyzed. Numerical variables were presented as mean $\pm$ SD or frequencies and percentages. The associations between categorical variables were assessed using chi-square tests.

TABLE 1 Demographic data and characteristics of the participants

Characteristics	Children with atopic dermatitis by the age of 2 years (AD) (N = 154)	Healthy children (HC) (N = 291)	P value
AD [†] concurrently with other atopic diseases			
Asthma, n (%)	5 (3.2)		
Allergic rhinitis, n (%)	23 (14.9)		
Allergic rhinitis and asthma, n (%)	4 (2.6)		
Neonatal and environmental factors			
Sex of infants, male (%)	88 (57.1)	162 (55.6)	0.841
Gestational age (weeks)	38.1 $\pm$ 1.3	38.6 $\pm$ 1.3	0.458
Cesarean section, n (%)	58 (36.7)	95 (32.3)	0.350
Siblings			0.658
0	82 (53.2)	165 (56.7)	
1	58 (37.7)	97 (33.3)	
≥ 2	14 (9.1)	29 (10.0)	
Birth weight (kg)	3.1 $\pm$ 0.5	3.1 $\pm$ 0.4	0.158
Environmental tobacco smoking within the last year, n (%)	51 (33.1)	105 (36.1)	0.602
Household pets within the last year, n (%)	51 (33.1)	89 (30.6)	0.593
Maternal factors			
Maternal age during delivery (years)	31.1 $\pm$ 4.7	30.8 $\pm$ 4.7	0.570
Maternal education level			0.735
High school or lower, n (%)	39 (25.3)	79 (27.2)	
College or higher, n (%)	115 (74.6)	212 (72.9)	
Maternal tobacco smoking during lactation, n (%)	5 (3.2)	3 (0.1)	0.132
Recent use of medications (<2 weeks) during collection of milk samples			
Antibiotics, n (%)	6 (3.8)	14 (4.8)	0.811
Probiotics, n (%)	10 (6.5)	18 (6.2)	0.999
Parental history of allergy			0.138
Only maternal allergy, n (%)	31 (20.1)	58 (19.9)	
Only paternal allergy, n (%)	36 (23.4)	63 (21.6)	
Both maternal and paternal allergy, n (%)	41 (26.6)	55 (18.9)	
Infant feeding practices			
Duration of exclusive breastfeeding, n (%)			0.131
Never breastfed	63 (40.9)	93 (32.0)	
1–5 months	44 (28.6)	105 (36.0)	
≥ 6 months	47 (30.5)	93 (32.0)	
Duration of exclusive breastfeeding in breastfed infants, months	6.3 $\pm$ 6.2	7.7 $\pm$ 8.4	0.149
Age at which solid foods were introduced, months	5.2 $\pm$ 1.0	5.2 $\pm$ 1.0	0.863

[†]AD, Atopic dermatitis.

P value based on chi-square test and student t-test.

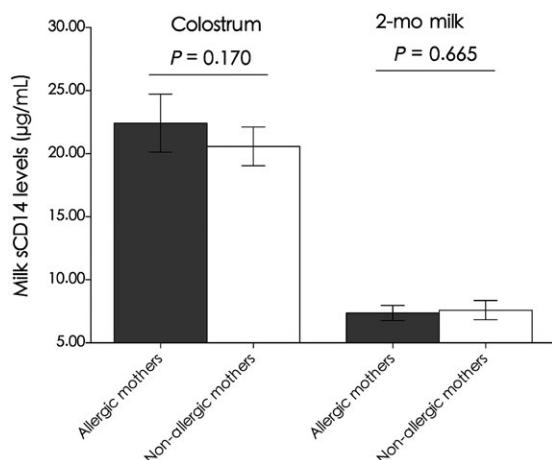


FIGURE 1 Mean and 95% confidence interval of sCD14 levels (µg/mL) in the colostrum and 2-month human milk samples in terms of maternal history of allergy. The sCD14 levels were significantly higher in the colostrum than in the 2-month human milk (21.40 ± 12.03 µg/mL vs 7.53 ± 4.34 µg/mL, $P < 0.001$). P value based on ANOVA test. * P values < 0.05 were considered statistically significant.

Analysis of variance (ANOVA) was used to compare sCD14 (µg/mL) and FA composition (%) in the colostrum and 2-month HM samples, respectively, between allergic and non-allergic mothers and between children with AD and HC. We conducted a multivariate logistic regression analysis to examine the association between sCD14 levels (colostrum and 2-month HM separately) and physician-diagnosed AD at age 2 years. The following factors were also used as variables: maternal age and allergy, cesarean section, number of siblings, the presence of pets in the household, environmental tobacco smoking within the last year, and recent use (< 2 weeks) of antibiotics/probiotics during HM sample collection. Histograms were prepared using mean values and 95% confidence interval. P values < 0.05 were considered statistically significant. Statistical analyses were performed using the Statistical Package for the Social Sciences software version 20.0 for Mac (Chicago, IL, USA).

3 | RESULTS

In total, 443 mother-child groups (445 children, two pairs of twins) were included. Among 289 lactating mothers, 170 (58.8%) women provided colostrum samples, and 265 (91.7%) provided 2-month HM samples; 146 provided both samples. sCD14 levels were measured in all HM samples, whereas FA composition was analyzed in 157 (92.3%) colostrum and 227 (85.6%) 2-month HM samples (including 146 paired samples). The participants' demographic characteristics are shown in Table 1. In total, 185 (41.8%) mothers presented with allergies, and 258 mothers (58.2%) did not. By the age of 2 years, 154 (34.6%) children had AD, and 291 (65.4%) presented with HC. A lower AD incidence was observed in children who were breastfed than in those who were not (31.5% vs 40.3%; $P = 0.061$). Overall, no significant difference was observed in sex, birth history, environmental tobacco smoking, household pets, maternal characteristics

(including recent antibiotics/probiotics exposure during HM collection), and prevalence of parental allergies. Moreover, infant feeding practices were similar between the AD and HC groups (Table 1).

3.1 | sCD14 levels and fatty acid composition in the colostrum and 2-month HM in terms of maternal history of allergies

As shown in Figure 1, sCD14 levels were significantly higher in the colostrum than in the 2-month HM. Nevertheless, between allergic and non-allergic mothers, sCD14 levels did not significantly differ in the colostrum (22.42 ± 13.38 µg/mL vs 20.56 ± 11.03 µg/mL, $P = 0.170$) and 2-month HM samples (7.37 ± 3.35 µg/mL vs 7.59 ± 4.97 µg/mL, $P = 0.665$).

The FA compositions in the colostrum and 2-month HM are shown in Table 2. Between allergic and non-allergic mothers, none of the FA profiles in the colostrum or 2-month HM significantly differed ($P > 0.05$; Table 2).

3.2 | sCD14 levels in the colostrum and 2-month HM in terms of maternal history of maternal and physician-diagnosed AD

In the entire study group, mothers whose children presented with AD by the age of 2 years had lower sCD14 levels in the colostrum than the remaining mothers (18.22 ± 9.56 µg/mL vs 22.07 ± 13.04 µg/mL, $P = 0.032$; Figure 2A). When the study groups were divided according to maternal history of allergies, sCD14 levels were still significantly lower in the colostrum of allergic mothers whose children presented with AD (16.67 ± 7.86 µg/mL vs 24.27 ± 16.97 µg/mL, $P = 0.015$), whereas among children born to non-allergic mothers, the differences were not significant (18.74 ± 9.55 µg/mL vs 20.67 ± 10.83 µg/mL, $P = 0.376$) (Figure 2A).

In the entire study group, children with AD received 2-month HM with significantly lower sCD14 concentrations than HC (6.44 ± 3.07 µg/mL vs 7.47 ± 3.93 µg/mL, $P = 0.029$; Figure 2B), and this result is consistent with the findings on colostrum. In the allergic mother group, children with AD had also received 2-month HM with significantly low sCD14 levels (6.53 ± 2.08 µg/mL vs 7.70 ± 3.79 µg/mL, $P = 0.044$), whereas among children with AD who were born to non-allergic mothers, although the sCD14 level is more likely to be lower, the differences were not significant (6.35 ± 3.96 µg/mL vs 7.25 ± 4.02 µg/mL, $P = 0.264$; Figure 2B).

In the logistic regression model, we observed that the sCD14 levels in neither colostrum nor 2-month HM samples were associated with physician-diagnosed AD by the age of 2 years or any of the variables listed in the Methods section (data not shown).

3.3 | Fatty acid composition in the colostrum and 2-month HM in terms of maternal history of allergies and physician-diagnosed AD

Tables 3 and 4 show the FA composition of colostrum in terms of maternal history of allergy and atopic outcome in children at age

TABLE 2 Fatty acid composition in the colostrum and 2-month human milk samples of mothers with allergic histories

	Colostrum (N = 157)			2-month human milk (N = 227)		
	Allergic mothers (N = 67)	Non-allergic mothers (N = 90)	P value	Allergic mothers (N = 101)	Non-allergic mothers (N = 126)	P value
	Mean (%) $\pm$ SD	Mean (%) $\pm$ SD		Mean (%) $\pm$ SD	Mean (%) $\pm$ SD	
SFA	40.97 $\pm$ 6.17	40.49 $\pm$ 5.92	0.643	54.76 $\pm$ 10.39	54.56 $\pm$ 11.15	0.897
C10:0	0.21 $\pm$ 0.13	0.23 $\pm$ 0.32	0.574	1.20 $\pm$ 1.05	1.15 $\pm$ 0.99	0.658
C12:0	1.90 $\pm$ 1.51	2.00 $\pm$ 1.81	0.741	5.12 $\pm$ 2.02	4.70 $\pm$ 2.00	0.139
C14:0	3.97 $\pm$ 2.28	4.06 $\pm$ 2.24	0.806	5.97 $\pm$ 2.59	5.67 $\pm$ 2.11	0.370
C16:0	22.63 $\pm$ 4.41	22.09 $\pm$ 3.21	0.395	30.16 $\pm$ 8.00	30.50 $\pm$ 8.65	0.770
C17:0	0.27 $\pm$ 0.07	0.26 $\pm$ 0.08	0.861	0.33 $\pm$ 0.12	0.36 $\pm$ 0.13	0.140
C18:0	10.35 $\pm$ 2.63	10.14 $\pm$ 2.62	0.652	10.72 $\pm$ 2.33	10.95 $\pm$ 2.49	0.490
C20:0	0.46 $\pm$ 0.19	0.49 $\pm$ 0.21	0.525	0.40 $\pm$ 0.11	0.41 $\pm$ 0.13	0.600
C22:0	0.54 $\pm$ 0.35	0.57 $\pm$ 0.35	0.636	0.47 $\pm$ 0.27	0.46 $\pm$ 0.24	0.820
C24:0	0.64 $\pm$ 0.43	0.64 $\pm$ 0.42	0.988	0.38 $\pm$ 0.22	0.36 $\pm$ 0.19	0.351
MUFA	32.60 $\pm$ 4.28	32.40 $\pm$ 4.35	0.788	25.69 $\pm$ 5.27	26.00 $\pm$ 5.80	0.694
C16:1n-9	0.40 $\pm$ 0.10	0.41 $\pm$ 0.11	0.619	0.21 $\pm$ 0.07	0.23 $\pm$ 0.09	0.065
C16:1n-7	1.25 $\pm$ 0.42	1.39 $\pm$ 0.47	0.063	1.32 $\pm$ 0.44	1.40 $\pm$ 0.51	0.192
C18:1n-9	27.39 $\pm$ 3.70	26.98 $\pm$ 4.04	0.541	22.36 $\pm$ 4.75	22.49 $\pm$ 5.18	0.851
C18:1n-7	1.72 $\pm$ 0.32	1.79 $\pm$ 0.36	0.226	1.19 $\pm$ 0.22	1.24 $\pm$ 0.25	0.134
C20:1n-9	0.88 $\pm$ 0.39	0.84 $\pm$ 0.31	0.519	0.35 $\pm$ 0.12	0.36 $\pm$ 0.13	0.549
C24:1n-9	0.96 $\pm$ 0.49	0.99 $\pm$ 0.51	0.766	0.27 $\pm$ 0.18	0.28 $\pm$ 0.16	0.903
Trans-FA	0.50 $\pm$ 0.12	0.54 $\pm$ 0.38	0.476	0.64 $\pm$ 0.23	0.68 $\pm$ 0.39	0.431
T16:1	0.08 $\pm$ 0.02	0.08 $\pm$ 0.03	0.928	0.08 $\pm$ 0.04	0.10 $\pm$ 0.05	0.052
T18:1	0.24 $\pm$ 0.08	0.28 $\pm$ 0.36	0.470	0.39 $\pm$ 0.18	0.42 $\pm$ 0.34	0.407
T18:2	0.18 $\pm$ 0.05	0.18 $\pm$ 0.05	0.922	0.17 $\pm$ 0.08	0.16 $\pm$ 0.06	0.314
n6-PUFA	22.86 $\pm$ 4.11	23.35 $\pm$ 3.64	0.459	17.23 $\pm$ 5.64	17.08 $\pm$ 6.01	0.860
C18: 2n-6	17.20 $\pm$ 3.27	17.90 $\pm$ 2.65	0.159	15.67 $\pm$ 5.10	15.54 $\pm$ 5.42	0.862
C18:3n-6	0.04 $\pm$ 0.02	0.03 $\pm$ 0.02	0.379	0.05 $\pm$ 0.04	0.06 $\pm$ 0.04	0.564
C20:2n-6	1.21 $\pm$ 0.32	1.20 $\pm$ 0.31	0.935	0.45 $\pm$ 0.12	0.45 $\pm$ 0.11	0.931
C20:3n-6	0.86 $\pm$ 0.28	0.83 $\pm$ 0.28	0.663	0.25 $\pm$ 0.13	0.25 $\pm$ 0.14	0.987
C20:4n-6	2.17 $\pm$ 1.11	2.08 $\pm$ 1.09	0.614	0.57 $\pm$ 0.40	0.55 $\pm$ 0.41	0.680
C22:2n-6	0.22 $\pm$ 0.10	0.22 $\pm$ 0.07	0.628	0.05 $\pm$ 0.02	0.05 $\pm$ 0.02	0.642
C22:4n-6	0.97 $\pm$ 0.45	0.89 $\pm$ 0.05	0.356	0.13 $\pm$ 0.08	0.13 $\pm$ 0.08	0.923
C22:5n-6	0.20 $\pm$ 0.09	0.18 $\pm$ 0.09	0.369	0.06 $\pm$ 0.04	0.07 $\pm$ 0.05	0.594
n3-PUFA	3.07 $\pm$ 0.80	3.23 $\pm$ 0.96	0.324	1.68 $\pm$ 0.65	1.68 $\pm$ 0.64	0.979
C18:3n-3	0.80 $\pm$ 0.33	0.90 $\pm$ 0.33	0.090	1.06 $\pm$ 0.40	1.03 $\pm$ 0.41	0.597
C20:5n-3	0.37 $\pm$ 0.28	0.41 $\pm$ 0.32	0.506	0.13 $\pm$ 0.13	0.13 $\pm$ 0.09	0.809
C22:5n-3	0.55 $\pm$ 0.22	0.56 $\pm$ 0.29	0.742	0.11 $\pm$ 0.07	0.12 $\pm$ 0.08	0.489
C22:6n-3	1.34 $\pm$ 0.52	1.35 $\pm$ 0.60	0.934	0.38 $\pm$ 0.25	0.41 $\pm$ 0.29	0.534
n6+ n3-PUFA	25.93 $\pm$ 4.61	26.65 $\pm$ 4.19	0.338	18.91 $\pm$ 6.22	18.76 $\pm$ 6.56	0.870
n6/n3-PUFA	7.81 $\pm$ 1.79	7.66 $\pm$ 1.79	0.632	10.64 $\pm$ 2.21	10.48 $\pm$ 2.13	0.597

P value based on ANOVA test. MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; Trans-FA, trans-fatty acids. Comparison of fatty acid composition between study groups via the ANOVA test.

2 years. Although insignificant, in the colostrum, the proportions of trans-fatty acids showed an upward trend in children with AD compared to HC, regardless of whether their lactating mothers had

allergies (Table 3). Otherwise, both in the colostrum and 2-month HM, neither saturated FA nor monounsaturated FA or individual n-6 series or n-3 series of PUFA or n6/n3 PUFA ratio showed significant

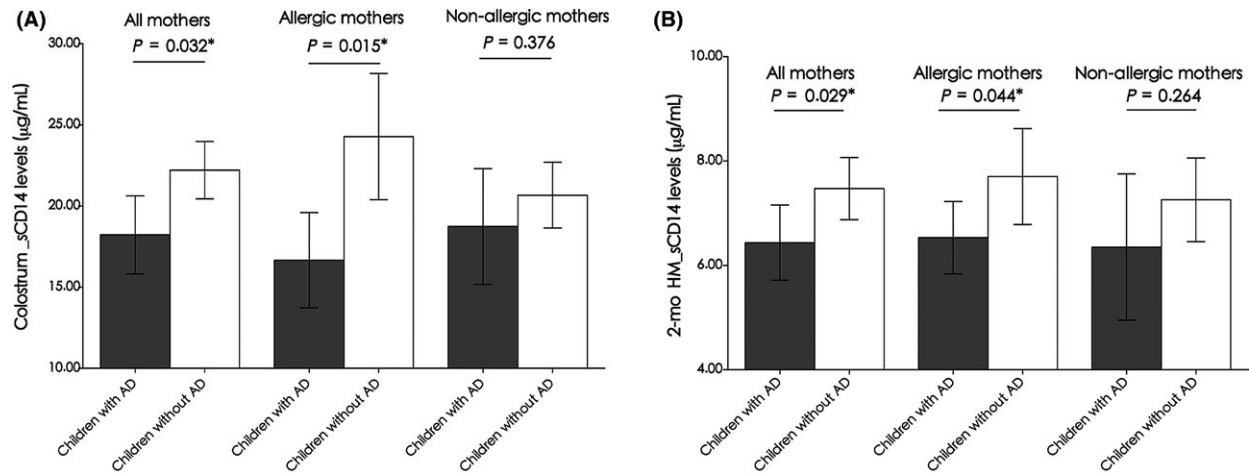


FIGURE 2 Mean and 95% confidence interval of sCD14 levels (μg/mL) in the (A) colostrum and (B) 2-month human milk in terms of maternal history of allergy and physician-diagnosed atopic dermatitis by the age of 2 years. *P* value based on ANOVA test. **P* values <0.05 were considered statistically significant.

deviations between children in the AD and HC groups, irrespective of maternal allergic classifications ($P > 0.05$; Tables 3 and 4).

4 | DISCUSSION

This study explored the dynamic changes in sCD14 levels and FA composition during different lactation stages. Furthermore, we evaluated the influence of maternal allergy on HM immune components and its relationship with subsequent AD development in breastfed infants. We found that sCD14 levels and FA composition significantly varied between the colostrum and 2-month HM. However, it did not differ between allergic and non-allergic mothers (Figure 1, Table 2). By the age of 2 years, a lower AD incidence was observed in breastfed children than in non-breastfed children (31.5% vs 40.3%; $P = 0.061$). Additionally, decreased sCD14 levels in the colostrum and 2-month HM were associated with physician-diagnosed AD in breastfed children. This association was more apparent among children with AD who were born to allergic mothers than to non-allergic mothers (Figure 2A and B), indicating that sCD14 may have immunomodulating effects on AD in early childhood, particularly among children who are at high risk of allergies.

Previous studies have indicated that orally ingested sCD14 is considered the primary pattern recognition receptor of LPS specific to gram-negative bacteria in the gastrointestinal tract.¹⁹ Moreover, it has been implicated in the development of resistance to potentially harmful bacteria and influences immune response programming.²⁰ However, the association between sCD14 and atopic diseases remains unclear in clinical studies.⁹ Some reports are in accordance with our findings.^{8,10,21} Nonetheless, Savilahti et al have found a positive association between higher sCD14 levels in 3-month HM and the risk of allergy in children with hereditary AD by the age of 5 years.²² A random trial on probiotics in the preselected atopic hereditary population may result in differences in the result.²² Rothenbacher

et al indicated that a longer duration of breastfeeding with increased levels of sCD14 in 6-week HM might decrease the risk of atopic diseases in early childhood.⁸ However, we did not observe such effects (data not shown). As the sCD14 level in the colostrum is significantly higher than that in the mature milk (Figure 1), we proposed that higher sCD14 concentrations in early infancy, rather than longer duration of breastfeeding, may be a more important factor that influences the development of an infant's immune system, gut microbiome, and possible atopic outcomes later in life.²⁰ Inconsistent results obtained from other studies regarding the associations between sCD14 and allergic manifestations^{5,23,24} may be related to varying HM collection periods (from colostrum to 3-month HM), differences in atopy classification methods (eg, parent or physician's report), wide range of assessed atopic outcomes (eg, atopic dermatitis, wheeze, or asthma), and varying follow-up periods (from 1 to 5 years).

Although several observational studies have indicated that n-3 and n-6 PUFA may play a role in atopic eczema development in breastfed infants and toddlers, it is worth noting that in the most updated review article,¹² only three of nine studies found decreased n-3 PUFA levels,^{13,14} and one of eight studies found that increased levels of n-6 PUFA were associated with an increased risk of eczema during childhood.¹⁴ Thus, there is insufficient evidence showing that HM PUFA influences the risk of eczema during childhood.¹² Consistently, our data showed that neither total or individual n-6 series or n-3 series of PUFA nor n6/n3 PUFA ratio in the colostrum and mature milk was relevant to AD development by the age of 2 years (Tables 3 and 4).

Some limitations of this study must be considered. First, we cannot exclude some misclassifications of maternal history of allergies and AD in children, which were reported by parents via questionnaires, despite regular follow-ups by our pediatricians and the specification of any physician-confirmed AD in the questionnaire. Second, 34.2% of children who were either lost to follow-up, only partially breastfed, or whose mothers did not provide any HM

TABLE 3 Fatty acid composition in the colostrum in terms of maternal histories of allergy and atopic outcome in children at age 2 years

Colostrum	All mothers (N = 157)		Allergic mothers (N = 67)		Non-allergic mothers (N = 90)	
	Children with AD by the age of 2 y (N = 56)		Children with AD by the age of 2 y (N = 26)		Children with AD by the age of 2 y (N = 30)	
	Mean (%) $\pm$ SD	Mean (%) $\pm$ SD	Mean (%) $\pm$ SD	Mean (%) $\pm$ SD	Mean (%) $\pm$ SD	Mean (%) $\pm$ SD
SFA	40.63 $\pm$ 5.65	40.73 $\pm$ 6.30	41.52 $\pm$ 7.60	40.49 $\pm$ 5.78	39.76 $\pm$ 2.96	40.81 $\pm$ 6.64
MUFA	32.56 $\pm$ 4.33	32.50 $\pm$ 4.53	33.01 $\pm$ 5.15	32.37 $\pm$ 4.10	31.86 $\pm$ 3.42	32.66 $\pm$ 4.73
Trans-FA	0.59 $\pm$ 0.54	0.49 $\pm$ 0.13	0.52 $\pm$ 0.12	0.48 $\pm$ 0.11	0.67 $\pm$ 0.77	0.49 $\pm$ 0.14
n6-PUFA	23.00 $\pm$ 4.11	23.15 $\pm$ 3.70	21.89 $\pm$ 5.11	23.48 $\pm$ 3.44	24.26 $\pm$ 2.58	22.93 $\pm$ 3.85
C18: 2n-6	17.73 $\pm$ 3.22	17.59 $\pm$ 2.79	16.68 $\pm$ 3.85	17.58 $\pm$ 2.88	18.87 $\pm$ 2.14	17.58 $\pm$ 2.79
C18:3n-6	0.04 $\pm$ 0.02	0.03 $\pm$ 0.02	0.04 $\pm$ 0.02	0.03 $\pm$ 0.02	0.04 $\pm$ 0.02	0.03 $\pm$ 0.02
C20:2n-6	1.21 $\pm$ 0.29	1.21 $\pm$ 0.32	1.13 $\pm$ 0.27	1.26 $\pm$ 0.34	1.28 $\pm$ 0.30	1.19 $\pm$ 0.32
C20:3n-6	0.84 $\pm$ 0.30	0.84 $\pm$ 0.28	0.83 $\pm$ 0.39	0.86 $\pm$ 0.23	0.85 $\pm$ 0.20	0.82 $\pm$ 0.30
C20:4n-6	1.89 $\pm$ 0.97	2.14 $\pm$ 1.12	1.91 $\pm$ 1.27	2.31 $\pm$ 1.08	1.92 $\pm$ 0.60	2.05 $\pm$ 1.14
C22:2n-6	0.23 $\pm$ 0.12	0.22 $\pm$ 0.07	0.23 $\pm$ 0.16	0.22 $\pm$ 0.06	0.24 $\pm$ 0.05	0.22 $\pm$ 0.07
C22:4n-6	0.88 $\pm$ 0.44	0.92 $\pm$ 0.46	0.88 $\pm$ 0.56	1.01 $\pm$ 0.41	0.89 $\pm$ 0.32	0.87 $\pm$ 0.49
C22:5n-6	0.19 $\pm$ 0.10	0.19 $\pm$ 0.08	0.19 $\pm$ 0.13	0.21 $\pm$ 0.07	0.18 $\pm$ 0.05	0.18 $\pm$ 0.09
n3-PUFA	3.23 $\pm$ 0.81	3.13 $\pm$ 0.89	3.06 $\pm$ 0.94	3.17 $\pm$ 0.72	3.45 $\pm$ 0.62	3.11 $\pm$ 0.97
C18:3n-3	0.93 $\pm$ 0.30	0.85 $\pm$ 0.34	0.82 $\pm$ 0.33	0.82 $\pm$ 0.34	1.04 $\pm$ 0.25	0.87 $\pm$ 0.34
C20:5n-3	0.33 $\pm$ 0.20	0.41 $\pm$ 0.33	0.35 $\pm$ 0.23	0.40 $\pm$ 0.30	0.30 $\pm$ 0.18	0.41 $\pm$ 0.32
C22:5n-3	0.59 $\pm$ 0.25	0.54 $\pm$ 0.26	0.55 $\pm$ 0.28	0.56 $\pm$ 0.21	0.64 $\pm$ 0.23	0.53 $\pm$ 0.29
C22:6n-3	1.39 $\pm$ 0.55	1.33 $\pm$ 0.55	1.34 $\pm$ 0.66	1.39 $\pm$ 0.47	1.47 $\pm$ 0.42	1.29 $\pm$ 0.59
n6+ n3-PUFA	26.23 $\pm$ 4.52	26.35 $\pm$ 4.21	24.95 $\pm$ 5.60	26.66 $\pm$ 3.90	27.71 $\pm$ 2.75	26.14 $\pm$ 4.39
n6/n3-PUFA	7.44 $\pm$ 1.80	7.78 $\pm$ 1.72	7.58 $\pm$ 2.21	7.71 $\pm$ 1.57	7.23 $\pm$ 1.39	7.81 $\pm$ 1.82

Comparison of human milk fatty acid composition between groups via the ANOVA test. MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; Trans-FA, trans-fatty acids.

TABLE 4 Fatty acid composition of the 2-month human milk in terms of maternal histories of allergy and atopic outcome in children at age 2 years

2-month human milk	All mothers (N = 227)		Allergic mothers (N = 101)		Non-allergic mothers (N = 126)	
	Children with AD by the age of 2 y (N = 85)		Children with AD by the age of 2 y (N = 46)		Children with AD by the age of 2 y (N = 39)	
	Mean (%) $\pm$ SD	Mean (%) $\pm$ SD	Mean (%) $\pm$ SD	Mean (%) $\pm$ SD	Mean (%) $\pm$ SD	Mean (%) $\pm$ SD
SFA	54.39 $\pm$ 10.18	55.21 $\pm$ 11.26	54.52 $\pm$ 9.73	55.46 $\pm$ 10.69	53.77 $\pm$ 10.69	54.80 $\pm$ 11.49
MUFA	26.20 $\pm$ 5.57	25.60 $\pm$ 5.66	26.10 $\pm$ 5.20	25.43 $\pm$ 5.46	26.53 $\pm$ 6.03	25.84 $\pm$ 5.72
Trans-FA	0.69 $\pm$ 0.44	0.66 $\pm$ 0.29	0.62 $\pm$ 0.23	0.64 $\pm$ 0.24	0.77 $\pm$ 0.59	0.67 $\pm$ 0.32
n6-PUFA	17.01 $\pm$ 5.46	16.88 $\pm$ 5.98	17.04 $\pm$ 4.91	16.83 $\pm$ 5.69	17.20 $\pm$ 6.08	17.03 $\pm$ 6.14
C18: 2n-6	15.48 $\pm$ 5.01	15.35 $\pm$ 5.37	15.22 $\pm$ 5.02	15.59 $\pm$ 4.51	15.55 $\pm$ 5.57	15.53 $\pm$ 5.55
C18:3n-6	0.06 $\pm$ 0.04	0.06 $\pm$ 0.04	0.05 $\pm$ 0.03	0.05 $\pm$ 0.05	0.06 $\pm$ 0.04	0.06 $\pm$ 0.04
C20:2n-6	0.44 $\pm$ 0.10	0.45 $\pm$ 0.12	0.45 $\pm$ 0.13	0.42 $\pm$ 0.10	0.46 $\pm$ 0.11	0.44 $\pm$ 0.11
C20:3n-6	0.24 $\pm$ 0.12	0.25 $\pm$ 0.14	0.26 $\pm$ 0.14	0.23 $\pm$ 0.11	0.25 $\pm$ 0.13	0.25 $\pm$ 0.14
C20:4n-6	0.56 $\pm$ 0.36	0.55 $\pm$ 0.41	0.59 $\pm$ 0.46	0.52 $\pm$ 0.32	0.61 $\pm$ 0.39	0.52 $\pm$ 0.37
C22:2n-6	0.05 $\pm$ 0.02	0.05 $\pm$ 0.02	0.05 $\pm$ 0.02	0.05 $\pm$ 0.02	0.05 $\pm$ 0.02	0.05 $\pm$ 0.02
C22:4n-6	0.12 $\pm$ 0.07	0.13 $\pm$ 0.08	0.13 $\pm$ 0.09	0.12 $\pm$ 0.06	0.14 $\pm$ 0.07	0.12 $\pm$ 0.08
C22:5n-6	0.07 $\pm$ 0.04	0.06 $\pm$ 0.05	0.06 $\pm$ 0.04	0.05 $\pm$ 0.03	0.08 $\pm$ 0.05	0.06 $\pm$ 0.05
n3-PUFA	1.71 $\pm$ 0.59	1.65 $\pm$ 0.65	1.71 $\pm$ 0.58	1.64 $\pm$ 0.64	1.73 $\pm$ 0.60	1.67 $\pm$ 0.66
C18:3n-3	1.05 $\pm$ 0.44	1.02 $\pm$ 0.40	1.10 $\pm$ 0.44	0.99 $\pm$ 0.34	1.02 $\pm$ 0.42	1.04 $\pm$ 0.43
C20:5n-3	0.14 $\pm$ 0.11	0.13 $\pm$ 0.11	0.13 $\pm$ 0.14	0.14 $\pm$ 0.13	0.13 $\pm$ 0.07	0.13 $\pm$ 0.10
C22:5n-3	0.11 $\pm$ 0.06	0.12 $\pm$ 0.08	0.11 $\pm$ 0.06	0.12 $\pm$ 0.08	0.12 $\pm$ 0.07	0.12 $\pm$ 0.08
C22:6n-3	0.41 $\pm$ 0.25	0.38 $\pm$ 0.28	0.36 $\pm$ 0.20	0.39 $\pm$ 0.28	0.46 $\pm$ 0.29	0.38 $\pm$ 0.28
n6+ n3-PUFA	18.72 $\pm$ 5.98	18.53 $\pm$ 6.55	18.75 $\pm$ 5.41	18.46 $\pm$ 6.27	18.93 $\pm$ 6.62	18.70 $\pm$ 6.69
n6/n3-PUFA	10.32 $\pm$ 2.42	10.58 $\pm$ 2.10	10.49 $\pm$ 2.81	10.62 $\pm$ 1.84	10.11 $\pm$ 1.98	10.59 $\pm$ 2.25

Comparison of human milk fatty acid composition between groups via the ANOVA test. MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; Trans-FA, trans-fatty acids.

samples for analysis were excluded from the study; this may have partially influenced statistical power. Finally, we did not evaluate whether maternal allergies were active or in remission, nor did we examine dietary intake during lactation; this may have influenced sCD14 levels and some FA profiles.^{25,26} Thus, our data should be interpreted with caution.

In conclusion, decreased sCD14 levels in colostrum and 2-month HM were associated with the development of physician-diagnosed AD by the age of 2 years, particularly among children born to mothers with allergies.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to disclose.

AUTHORSHIP

All authors were involved in the study design, recruitment of participants, and writing of consent. MC Hua, HM Su, and JL Huang were involved in the laboratory work and statistical analysis and interpretation of results. MC Hua wrote the first draft of the manuscript, and JL Huang, HM Su, and TC Yao edited the manuscript. All authors reviewed the manuscript and approved its final version.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the research ethics committee of Chang Gung Memorial Hospital (104-7100C, 106-3610C, and 201601904A3C601) and was conducted in accordance with the declaration of Helsinki. A written informed consent was obtained from the parents.

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