

ORIGINAL ARTICLE

Maternal psychosocial factors determining the concentrations of transforming growth factor-beta in breast milk

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Keywords

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Abstract

Background: Cytokines in breast milk may play crucial roles in the beneficial effects of breastfeeding in protecting against allergic and infectious diseases in infants. In particular, breast milk-borne transforming growth factor-beta (TGF- β) has an important potential role in developing the mucosal immune system in infants. However, little is known about what factors influence TGF- β expression in human milk. We investigated whether the behavioral and psychosocial characteristics of mothers affect breast milk TGF- β levels.

Methods: We conducted a survey of all 139 mothers who were lactating between February and October 2010 in Koshu City, Japan. Participants completed a questionnaire and provided breast milk at the health checkups for their 3-month-old child (N = 129, 93%). Breast milk was assayed for total TGF- β 2 levels by ELISA. We took an exploratory approach based on linear and ordered logistic regressions to model TGF- β 2 concentrations with their multiple potential determinants.

Results: Mothers with depression or poor self-rated health had higher TGF- β 2 concentrations than mothers without depression (odds ratio for a higher TGF- β 2 quartile: 3.11, 95% confidence intervals: 1.03–9.37) or those reporting better health (odds ratio: 2.34, 1.21–4.55). Smoking, drinking alcohol, probiotics supplementation, social support, and maternal history of allergic diseases were not associated with milk TGF- β 2 levels. Milk gathered between August and October or later in the afternoon (3–4 PM vs. 12–2 PM) contained less TGF- β 2.

Conclusion: Depression, as the consequence of psychosocial stress, may be a strong determinant of TGF- β levels in breast milk. Seasonal and daily fluctuations in milk TGF- β 2 concentrations warrant further study.

Cytokines in breast milk may play important roles in the development of the infant immune system, and they may explain the mechanisms of the beneficial effects of breastfeeding in protecting against allergic and infectious diseases in infants (1). Transforming growth factor- β (TGF- β) is a central cytokine in the regulation of the immune system (2). In particular, growing evidence suggests that TGF- β may be a key immunoactive substance for the establishment of the mucosal immune response including the production of IgA

and induction of oral tolerance (3). Interestingly, human milk contains a large amount of TGF- β (2). Among the three isoforms of TGF- β (TGF- β 1, TGF- β 2, and TGF- β 3) identified in mammals, TGF- β 2 is the major isoform present in human milk (4).

A recent systematic review found that the concentration of TGF- β in human milk is highly heterogeneous within and between studies, which is potentially explained by a maternal history of allergic disease prevalence (5) or probiotics supplementation (3, 6, 7). However, associations between TGF- β levels in breast milk and other maternal factors, including behavioral and psychosocial characteristics, have not been studied; for example, psychosocial stresses or depressive

Abbreviations:

CI, confidence intervals; OR, odds ratio; TGF- β , transforming growth factor-beta.

symptoms influence the secretion of neurotransmitters and neuroendocrine hormones, and they can modify the functioning of immune cells and cytokines produced by immune cells including TGF- β (8, 9). Moreover, other behavioral and psychosocial factors, including smoking, alcohol drinking, socioeconomic status, and social support, can also alter TGF- β secretion (10) and overall immunological functions (11, 12).

There is a growing body of epidemiologic and animal experimental evidence suggesting the impacts of maternal psychosocial illnesses and behavioral factors on offspring's immunological susceptibility to allergic and infectious diseases. By a large-scale ($n = 13,907$) prospective study in Canada, for example, Kozyskyj and colleagues have reported a 1.25-time higher risk of developing asthma among the children who were chronically exposed to maternal distress, even after adjusted for other traditional asthma risks (13). Potential mechanisms suggested so far have focused on children's psychosocial stress attributed to unfavorable parent-child relationship; for example, sustained corticosteroid secretion because of a chronic exposure to maternal stress could increase the risks for the development of atopic diseases in infants (9, 14). However, given the rich concentration of immune factors in breast milk and their potential critical roles in developing immune systems of infants, an alternative 'breast milk' pathway could also exist; that is, maternal psychosocial factors could alter immune compositions – e.g., key cytokines including TGF- β , especially – of the mothers' breast milk and then affect the immunity of their breastfed infants.

This population-based epidemiologic study was to explore whether TGF- β 2 concentrations in human mature milk were altered by the behavioral and psychosocial characteristics of mothers.

Methods

Study subjects

We conducted an epidemiological survey in Kosu City, Yamanashi, Japan. The city government has provided regular checkups for children at the ages of 3 months, 7 months, 1, 1.5, 3, and 5 yr. All children who resided in the city were required to have these checkups. Our study subjects were mothers whose children were eligible for checkups at the age of 3 months between February and October 2010. All mothers who breastfed were eligible to this study. All participants submitted written informed consent forms for the participation in this study, and the protocol of this study was approved by the Ethics Review Board of the Faculty of Medicine at the University of Yamanashi.

Data acquisition and measurements

We conducted a questionnaire survey and collected breast milk at monthly checkup sessions. At the checkup sessions, participants were required to provide at least 2 ml of breast milk and they submitted a questionnaire asking their age, history of diagnosed allergic diseases (a multiple choice for the options asking whether they had atopic dermatitis, asthma,

allergic rhinitis, allergic conjunctivitis, food allergy, pollen allergy, and other allergic diseases), history of tobacco smoking (never, ever, or current), number of cigarettes and duration of smoking, current alcohol consumption (six options ranging from everyday to never), postpartum depression, self-rated health, social support, and educational attainment. To determine whether mothers had postpartum depression, we used a validated psychometrical scale, the Japanese version of the Edinburgh Postpartum Depression Scale (15). Following the standard criterion of determining potential postpartum depression, we defined mothers who scored 9 or higher as having postpartum depression. We used self-rated health as a general indicator that represented participants' mental and physical health, which is widely used in questionnaire surveys and had a high predictive capacity for contemporaneous and subsequent health statuses (16). We used a single question, 'What is your current health status?' with five options, i.e., extremely good, very good, good, fair, and poor. We dichotomized self-rated health as extremely good/very good and good/fair/poor, as this dichotomization had the best sensitivity to our outcome when we conducted a preliminary analysis. We used three questions to evaluate participants' emotional social support, asking if each participant had someone with whom she could consult about her worries and troubles, someone who could cheer her up, and someone who worries and thinks about her. Participants selected yes or no for each of these questions. We scored social support by the number of 'yes' answers, ranging from 0 to 3. We created these questions by referring to a validated social support scale developed by Noguchi (17). The three items were highly correlated to one another, showing a high internal consistency (Cronbach's $\alpha = 0.74$), supporting the reliability of this measure. The questionnaire was self-administered, but we interviewed mothers upon requests to prevent missing information in their answers.

Measurement of TGF- β 2 concentrations in breast milk

We collected breast milk samples on-site at the community health center where the 3-month checkups were held. Mothers expressed their milk by themselves directly into a vial, and the milk samples were immediately put in a cooler in which the temperature was maintained at $<5^{\circ}\text{C}$ for up to 5 h, and then they were preserved in a frozen state at -80°C . We analyzed the whole milk without a centrifuge separation, because our preliminary analysis certified that the measurement results did not differ between whole and centrifuged samples. The amounts of TGF- β 2 in human milk samples were determined using the human TGF- β 2 ELISA kit (R&D Inc., Minneapolis, MN, USA) according to the manufacturer's instructions. To determine the total TGF- β 2 concentrations in their milk, the samples were activated by an acidification procedure before the ELISA (18).

Statistical analysis

First, we observed descriptive characteristics of our study subjects. Then, we conducted linear regression analysis to

investigate the associations between TGF- β 2 concentrations in breast milk and maternal characteristics. The TGF- β 2 concentration was log-transformed because of its long-tailed skewed distribution. However, because we do not have any *a priori* evidence on the shape of the distribution of TGF- β 2 concentrations in breast milk, we further conducted an alternative analysis by using ordered logistic regression for quartiled TGF- β 2 levels. The ordered logistic models calculated odds ratios (ORs) for 1-step higher TGF- β 2 quartile by maternal characteristics. Next, we modeled TGF- β 2 by multivariate linear regression and ordered logistic regression analyses. We used a stepwise procedure that selected a set of predictive variables best explaining TGF- β 2 concentrations. Stepwise regression technique is suitable to explore when there are a large number of potential explanatory variables and no strong underlying theory on which to base the model selection – like the case of this study. Because self-rated health and postpartum depression scores were moderately correlated, we executed stepwise procedures using 1 of 2 sets of variables including either self-rated health or having depression. Then we created a final multivariate model using all explanatory variables selected by the stepwise procedures. All analyses were performed using SAS version 9.2 (SAS Institute Inc., Cary, NC, USA).

Results

During the study period, we had 139 eligible mothers (i.e., all mothers who had 3-month-old children in the city) and 134 agreed to participate in our study. We used the data of 129 participants who provided their breast milk, while the breast milk samples of five participants could not be assayed because of insufficient amounts. The average age of participants was 30.7 yr. Among them, 49 participants (38%) had an allergic disease, 24 (19%) had multiple allergic diseases, and 14 (11%) were suspected to be depressed. Only two mothers (2%) had 9 yr or fewer of educational attainment (Table 1).

Linear regression analysis showed that the milk collected between August and October contained less TGF- β 2 ($p = 0.09$), as did milk taken after 3 PM (Table 2). Mothers who reported poorer subjective health statuses ($p = 0.003$) and those who were suspected to have postpartum depression ($p = 0.02$) were more likely to have higher TGF- β 2 concentrations in their breast milk, while lower educational attainment (9 yr or less) was associated with lower TGF- β 2 concentrations ($p = 0.03$). Age, allergic diseases history, smoking habits, consumptions of alcohol beverages, and probiotics were not associated with milk TGF- β 2 concentrations.

In a multivariate model with depression, a stepwise procedure selected depression (beta coefficient = 0.55, $p = 0.04$), sampling dates, time of sampling, and educational attainment ($R^2 = 0.15$) as the variables best explaining milk TGF- β 2 concentrations. The results of the stepwise model using another variable set including self-rated health were identical to the model with depression, while the self-rated health model showed a somewhat better model fitness and a

stronger statistical significance (beta coefficient = 0.53 for poorer self-rated health, $p = 0.001$, and $R^2 = 0.19$). These associations were confirmed by ordered logistic regression (Table 3), whereas educational attainment was not selected by stepwise ordered logistic regression. Meanwhile, the stepwise regression using the variable sets with depression did not select sampling dates and times and only selected depression; depressed mothers had a 4.4-fold (95% confidence interval: 1.5–13) higher likelihood to have higher TGF- β 2 concentrations in their breast milk. The final model created based on the stepwise models including both depression and self-rated health attenuated the coefficients for all variables. The attenuation for depression and self-rated health was –11% and –20% in linear regression models and –30% and 13% in ordered logistic models, respectively. Nevertheless, the final models showed similar associations between maternal psychosocial factors and TGF- β 2 concentrations, as well as their seasonal and circadian variations.

Discussion

Our population-based epidemiologic survey found a strong association of poorer self-rated health and postpartum depression with high concentrations of TGF- β 2 in maternal mature milk collected 3 months after delivery. Maternal histories of allergic diseases, smoking habits, and alcohol drinking did not explain the TGF- β 2 concentrations. To our knowledge, this is the first study reporting a significant positive association between high TGF- β 2 concentrations in breast milk and poor psychosocial status represented by depression and poor perceived health. We also found seasonal and hourly variations in milk TGF- β 2 concentrations.

The strong association between depression and milk TGF- β 2 concentrations is supported by the negative association between self-rated health and milk TGF- β 2 concentrations, given that self-rated health reflects the general health status of participants including mental and physical illnesses (16, 19). Meanwhile, unobserved chronic or acute physical illnesses that were in part represented by self-rated health may also be a determinant of milk TGF- β concentrations. Nevertheless, this finding does not directly indicate that breastfeeding by depressive mothers has an extra benefit for offspring immunity. The impact of milk TGF- β concentrations on the subsequent onset of atopic diseases among children is still under debate (3, 4, 6, 20–22); for example, Bottcher and colleagues hypothesized a bell-shaped association between milk TGF- β concentrations and the development of atopy, i.e., TGF- β in milk may be beneficial if the concentration is low or high, but it may be harmful to the sensitization of children if the concentration is intermediate (6). Tomicic et al. suggested the association between low colostral TGF- β 2 levels and sensitization in Swedish infants in a study analyzing 99 breast milk samples (23).

Depression can cause various immunological and endocrinal dysregulation and lead to infectious, allergic, and autoimmune diseases (8). Some of its molecular mechanisms have been revealed (9); for example, it is known that serotonin receptors play important roles in the pathology of depression.

Table 1 TGF- β 2 concentration by the characteristics of study subjects (N = 129, mean age = 30.7 $\pm$ 5.1)

		TGF-β2 concentration* (pg/ml)
	n, %	Median [25%, 75%]
Breast milk sample characteristics		
Sampling dates		
Feb–March 2010	30, 23.3	1557.6 [853.3, 1858.0]
Apr–May 2010	31, 24.0	917.8 [705.7, 2036.3]
Jun–Jul 2010	33, 25.6	1186.3 [558.7, 2333.6]
Aug–Oct 2010	35, 27.1	790.2 [472.6, 1262.0]
Time of sampling		
12–2 pm	85, 65.9	1037.2 [691.1, 2036.3]
3–4 pm	44, 34.1	794.6 [466.7, 1813.6]
Maternal characteristics		
Allergic disease		
Atopic dermatitis	6, 4.7	591.1 [456.2, 891.7]
Asthma	2, 1.6	969.1 [380.5, 1557.6]
Allergic rhinitis	19, 14.7	1262.1 [500.9, 2188.2]
Allergic conjunctivitis	5, 3.9	628.8 [457.4, 891.7]
Food allergy	4, 3.1	716.4 [544.0, 1058.0]
Hay fever	63, 48.8	951.7 [628.8, 1923.2]
Other allergic disease	2, 1.6	4406.6 [1014.6, 7798.6]
No. of allergic diseases		
0	56, 43.4	1111.8 [663.0, 2070.6]
1	49, 38.0	881.3 [544.0, 1769.2]
2+	24, 18.6	1085.5 [500.9, 1950.3]
Smoking habits		
No	86, 66.7	929.1 [585.8, 1894.6]
Ex smoker	38, 29.5	1050.1 [599.0, 2188.2]
Current smoker	5, 3.9	872.1 [425.9, 992.3]
Smoking: pack-years		
0	86, 67.7	929.1 [585.8, 1894.6]
0.1–4.5	23, 18.1	1085.5 [549.2, 2218.0]
4.6–23	18, 14.2	949.6 [639.5, 2036.3]
Alcohol drinking		
Fewer/never	115, 89.2	917.8 [584.6, 1950.3]
1 or 2 times a month	8, 6.2	1324.8 [801.6, 1907.3]
3 times a week or more	6, 4.7	992.3 [478.3, 1923.2]
Consumption of probiotics		
1 time a week or less	47, 36.4	992.3 [584.6, 2091.9]
2–6 times a week	36, 27.9	1185.1 [672.6, 1977.0]
Everyday	46, 35.7	891.7 [478.3, 1769.2]
Self-rated health		
Extremely good/very good	71, 55.0	872.1 [483.8, 1711.6]
Good/fair/poor	58, 45.0	1365.8 [790.2, 2188.2]
Postpartum depression		
No (score <9)	115, 89.1	899.3 [568.6, 1807.8]
Yes (score ≥9)	14, 10.9	2177.7 [1186.3, 2282.9]
Social support		
High	124, 96.1	929.1 [584.6, 1894.6]
Low	5, 3.9	2137.5 [1564.2, 2188.2]
Educational attainment (yr)		
9 yr or less	2, 1.6	522.7 [53.0, 992.3]
10–12	35, 27.1	1280.6 [599.0, 2188.0]
13+	92, 71.3	915.7 [568.6, 1894.6]

*Median value for all subjects = 969.7 [585.2, 1936.7].

Table 2 Univariate regression models for TGF- β 2 concentration in breast milk

	Linear regression			Ordered logistic regression	
	β	s.e.	p	OR	95% CI
Breast milk sample characteristics					
Sampling dates					
Feb–March 2010	0	Referent		1	Referent
Apr–May 2010	0.03	0.25	0.90	1.0	0.4–2.6
Jun–Jul 2010	0.07	0.25	0.78	1.3	0.5–3.1
Aug–Oct 2010	−0.42	0.24	0.09	0.5	0.2–1.2
Time of sampling					
12–2 pm	0	Referent		1	Referent
3–4 pm	−0.31	0.18	0.09	0.5	0.3–1.0
Maternal characteristics					
Age (yr)	−0.01	0.02	0.48	1.0	0.9–1.1
Have allergic disease (vs. not have)					
Atopic dermatitis	−0.43	0.41	0.30	0.26	0.1–1.2
Asthma	−0.29	0.70	0.68	0.43	0.1–5.6
Allergic rhinitis	0.06	0.25	0.80	1.59	0.7–3.9
Allergic conjunctivitis	−0.36	0.45	0.42	0.42	0.1–2.2
Food allergy	−0.32	0.57	0.57	0.49	0.1–3.9
Hay fever	−0.03	0.17	0.87	1.00	0.5–1.9
Other allergic disease	1.03	0.70	0.14	4.51	0.3–70.1
No. of allergic diseases					
0	0	Referent		1	Referent
1	−0.21	0.19	0.28	0.7	0.4–1.4
2+	−0.02	0.24	0.94	1.0	0.4–2.3
Smoking habits					
No	0	Referent		1	Referent
Ex smoker	0.03	0.19	0.87	1.3	0.7–2.5
Current smoker	–	–	–	–	–
Smoking: pack-years					
0	0	Referent		1	Referent
0.1–4.5	0.18	0.23	0.44	1.2	0.5–2.7
4.6–23	0.02	0.26	0.92	1.3	0.5–3.1
Alcohol drinking					
Fewer/never	0.00	Referent		1	Referent
1 or 2 times a month	0.35	0.36	0.33	1.8	0.5–6.5
3 times a week or more	0.13	0.45	0.78	0.9	0.2–4.3
Consumption of probiotics					
1 time a week or less	0	Referent		1	Referent
2–6 times a week	−0.08	0.22	0.73	1.1	0.5–2.4
Everyday	−0.32	0.20	0.11	0.7	0.4–1.5
Self-rated health: good/fair/poor (vs. extremely good/very good)	0.50	0.17	0.003	2.5	1.3–4.7
Depression (+) (score ≥ 9) (vs. <9)	0.65	0.27	0.02	4.4	1.5–13.0
Low social support	0.36	0.45	0.42	4.2	0.7–23.9
Educational attainment (yr)					
9 yr or less	−1.52	0.69	0.03	0.5	0.04–6.2
10–12	−0.01	0.19	0.94	1.4	0.7–2.8
13+	0	Referent		1	Referent

s.e., standard error; OR, odds ratio; CI, confidence intervals. For linear regression analyses, continuous TGF- β 2 was log-transformed.

Ordered logistic regression calculated OR for one step higher TGF- β 2 quartile by maternal characteristics. OR higher than 1 represents a positive association between maternal characteristics and TGF- β 2 concentration.

Li and colleagues suggested the possibility of a significant interaction between serotonin receptors and the malfunction of immune systems among depressive individuals (24).

Empirical studies have also suggested that TGF- β plays important roles in the association between depression and immune deficiency, whereas the findings of the studies using

Table 3 Multivariate regression models for TGF- β 2 concentration in breast milk

	Stepwise model with depression				Stepwise model with self-rated health				Final model			
	Linear regression		Ordered logistic		Linear regression		Ordered logistic		Linear regression		Ordered logistic	
	β	s.e.	p	OR*	β	s.e.	p	OR	β	s.e.	p	OR
Breast milk sampling dates												
Feb–Jul 2010	0	Ref.	0.01		0	Ref.	0.002	1	0	Ref.	0.008	1
Aug–Oct 2010	–0.48	0.18			–0.57	0.18		0.35	–0.49	0.18		0.38
Time of sampling												
12–2 pm	0	Ref.	0.03		0	Ref.	0.04	1	0	Ref.	0.08	1
3–4 pm	–0.39	0.17			–0.34	0.17		0.50	–0.30	0.17		0.49
Self-rated health: good/fair/poor	–	–	–	–	0.53	0.16	0.001	2.69	0.47	0.17	0.006	2.34
(vs. extremely good/very good)												
Depression (+) (score ≥ 9) (vs. <9)	0.55	0.26	0.04	4.45	–	–	–	–	0.44	0.27	0.09	3.11
Educational attainment (yr)												
9 yr or less	–1.72	0.66	0.01		–1.82	0.64	0.005					
10+ yr	0	Ref.			0	Ref.						
Intercept	7.16	0.12	<0.0001		7.00	0.13	<0.0001		6.91	0.14	<0.0001	
R^2	0.15				0.19				0.21			

s.e., standard error; OR, odds ratio; CI, confidence intervals.

Only the variables selected by a stepwise procedure on either linear regression or ordered logistic regression are shown. For linear regression, continuous TGF- β 2 was log-transformed.Ordered logistic regression calculated OR for one step higher TGF- β 2 quartile by maternal characteristics. OR higher than 1 represents a positive association between maternal characteristics and TGF- β 2 concentration. Because self-rated health and postpartum depression scores were moderately correlated, we developed stepwise multivariate models using a set of variables including either self-rated health or having depression. Then we created the final models consisting of the explanatory variables selected by the stepwise models. Education was excluded from the final models because of too small number in the '9 yr or less' category ($n = 2$).

human subjects have been mixed in terms of the directions of the association between physical and mental illnesses and serum TGF- β levels. Kim et al. compared the concentrations of multiple cytokines in plasma between a group of 48 patients with major depressive disorders and a group of 63 non-depressive adults and found elevated TGF- β 1 levels among the depressed individuals (25). Similar findings were observed among patients with chronic fatigue syndrome (26). On the contrary, Li et al. reported rather lower serum TGF- β levels among the patients of major depression disorder (24). However, these studies so far are likely to have limitations such as not adjusting for confounding factors sufficiently and a small sample size. Nevertheless, these findings do not explain the mechanisms linking depression and high TGF- β concentrations in breast milk. Because it has been demonstrated by Hawkes et al. that TGF- β in breast milk is directly excreted from the mammary gland and that the level of TGF- β in breast milk is not correlated with that in plasma, a novel explicit explanation of the regulatory systems of TGF- β in breast milk is required (27).

In our study, TGF- β 2 concentrations were low in the milk sampled between August and October or during later hours in the afternoon. Although further observations with additional milk samples from additional seasons and hours would be needed, the seasonal and circadian changes may be attributable to the seasonal changes in climatic factors such as air temperature and sunshine hours, daily activities including sleeping and eating, and hormonal circadian changes. To our knowledge, this is the first demonstration that the concentrations of a cytokine in human milk exhibit seasonal or circadian variations.

Our study results do not support the findings of studies suggesting associations between maternal allergic history and milk TGF- β concentrations (3); for example, Laiho et al. compared 43 atopic and 51 non-atopic lactating women and found lower TGF- β 2 concentrations in breast milk among atopic persons (5), whereas Rigotti et al. found lower TGF- β 1 concentrations in mature milk among 13 mothers with allergies than among nine normal controls (20). Using a larger study sample in Japan, our present study revealed new evidence of the non-association between maternal allergic history and TGF- β 2 levels in breast milk. An alternative cause of our null result may be the information bias attributable to the self-reporting nature of our information on maternal allergy history. However, by asking the history of formal diagnosis of allergic diseases by physicians rather than the participants' perceived illnesses, we attempted to minimize the possibility of such biases.

Although a number of studies have suggested that probiotics supplementation is another potential factor affecting TGF- β concentrations in breast milk (3, 7, 28), this was not shown in the present study. The results of recent studies are mixed, and the study heterogeneity may be explained by the differences in study populations, types of probiotics, TGF- β isoforms, and other unobserved factors among studies. A randomized controlled trial in Sweden conducted by Bottcher et al. showed lower levels of TGF- β 2 in colostrum among 54 mothers taking *Lactobacillus reuteri* supplementation

compared with 55 placebo controls, while another randomized trial in Finland provided *Bifidobacterium lactis* or *Lactobacillus rhamnosus* to atopic mothers and found higher rather than lower milk TGF- β 1 levels in the intervention group than in placebo controls (28). Rautava et al. also conducted a randomized trial with 159 atopic Finnish mothers and reported that TGF- β 2 concentrations in breast milk were significantly higher among mothers receiving *L. rhamnosus* supplementation, while the differences in TGF- β 1 levels in breast milk were not statistically significant (7). Alternatively, the nonsignificant association in our study may be due to the design of our questionnaire in which we only asked respondents about their frequency of probiotics consumption in general and not about the types of probiotics consumed.

In addition, although our analysis demonstrated a statistically significant crude association between poor educational attainment and low concentrations of TGF- β 2 in breast milk, this was not confirmed by our sensitivity analysis, perhaps because of the limited sample size or the crude association observed was just by chance.

Strengths and limitations

The primary advantages of our study are that we widely explored maternal behavioral and psychosocial factors and that we used highly representative inventory data of our study area, providing the maximum potential of eliminating sampling bias. Moreover, the utilization of standardized psychometric scales confirms the accuracy of the information of psychosocial and physical health statuses. The major limitations of this study are that we did not evaluate other potential determinants of TGF- β concentrations, including maternal genetic traits (3), local infections of the mammary gland (29), and the concentrations of other nutrients in milk such as fatty acid (5). However, Hawkes et al. demonstrated no correlation between milk TGF- β levels and fatty acid content (30). Second, information on depression and behavioral factors was self-reported. However, the Edinburgh Postpartum Depression Scale is the most widely used psychometric screening scale for postpartum depression with good validity and reliability (15). Third, although the sample size of our study was relatively large compared with other recent studies, we could not evaluate the contributions of other potential determinants of milk TGF- β 2 concentrations; for example, the findings that education and social support – both known as predictors of high psychological stresses and depression – were not associated with milk TGF- β 2 levels may be due to the insufficient statistic power. For the same reason, we also failed to provide a robust evaluation of the association between milk TGF- β 2 concentrations and specific maternal allergic diseases such as atopic dermatitis and asthma. Finally, causal associations between maternal depression and TGF- β 2 concentrations cannot be evaluated because of the cross-sectional design of this study. However, it is difficult to make any logically plausible explanations for some reverse causal pathways, i.e., the milk TGF- β concentration determines maternal depression.

Conclusion

Currently, most public health authorities strongly recommend breastfeeding to all family because of its potential health benefits, including the prevention of atopic and infectious diseases. Nevertheless, the findings of our study may have important implications for public health and clinical practices, that is, variations in maternal levels of TGF- β , a critical cytokine controlling the immunological functions in breast milk, may be attributable to the physical and psychological health of mothers. Given this, further investigations exploring the complex pathways linking maternal psychosocial stresses, genetic traits, diet, and behavior to cytokines in breast milk and the roles of these cytokines in the bodies of their children are warranted.

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References

- Goldman AS. The immune system of human milk: antimicrobial, antiinflammatory and immunomodulating properties. *Pediatr Infect Dis J* 1993; **12**: 664–71.
- Deryck R, Miyazono K. The TGF-Beta Family. Woodbury, NY: Cold Spring Harbor Laboratory Press, 2007.
- Oddy WH, Rosales F. A systematic review of the importance of milk TGF-beta on immunological outcomes in the infant and young child. *Pediatr Allergy Immunol* 2010; **21**: 47–59.
- Kalliomaki M, Ouwehand A, Arvilommi H, Kero P, Isolauri E. Transforming growth factor-beta in breast milk: a potential regulator of atopic disease at an early age. *J Allergy Clin Immunol* 1999; **104**: 1251–7.
- Laiho K, Lampi AM, Hamalainen M, et al. Breast milk fatty acids, eicosanoids, and cytokines in mothers with and without allergic disease. *Pediatr Res* 2003; **53**: 642–7.
- Bottecher MF, Abrahamsson TR, Fredriksson M, Jakobsson T, Bjorksten B. Low breast milk TGF-beta2 is induced by Lactobacillus reuteri supplementation and associates with reduced risk of sensitization during infancy. *Pediatr Allergy Immunol* 2008; **19**: 497–504.
- Rautava S, Kalliomaki M, Isolauri E. Probiotics during pregnancy and breast-feeding might confer immunomodulatory protection against atopic disease in the infant. *J Allergy Clin Immunol* 2002; **109**: 119–21.
- Calcagni E, Elenkov I. Stress system activity, innate and T helper cytokines, and susceptibility to immune-related diseases. *Ann N Y Acad Sci* 2006; **1069**: 62–76.
- Haczku A, Panettieri RA Jr. Social stress and asthma: the role of corticosteroid insensitivity. *J Allergy Clin Immunol* 2010; **125**: 550–8.
- Ryder MI, Saghizadeh M, Ding Y, Nguyen N, Soskolne A. Effects of tobacco smoke on the secretion of interleukin-1 β , tumor necrosis factor- α , and transforming growth factor- β from peripheral blood mononuclear cells. *Oral Microbiol Immunol* 2002; **17**: 331–6.
- Cohen S, Tyrrell DA, Smith AP. Psychological stress and susceptibility to the common cold. *N Engl J Med* 1991; **325**: 606–12.
- Cohen S, Doyle WJ, Skoner DP, Rabin BS, Gwaltney JM Jr. Social ties and susceptibility to the common cold. *JAMA* 1997; **277**: 1940–4.
- Kozyrskyj AL, Mai XM, McGrath P, Hayglass KT, Becker AB, Macneil B. Continued exposure to maternal distress in early life is associated with an increased risk of childhood asthma. *Am J Respir Crit Care Med* 2008; **177**: 142–7.
- von Hertzen LC. Maternal stress and T-cell differentiation of the developing immune system: possible implications for the development of asthma and atopy. *J Allergy Clin Immunol* 2002; **109**: 923–8.
- Okano T, Murata M, Masuji S, et al. Reliability and validity of the Japanese version of the Edinburgh Postpartum Depression Scale (in Japanese: nihon ban ejinbara sango utsu byou jikohyouka hyou (EPDS) no shinraisei to datousei). *Seishinka Shindangaku* 1996; **7**: 525–33.
- Idler EL, Benyamini Y. Self-rated health and mortality: a review of twenty-seven community studies. *J Health Soc Behav* 1997; **38**: 21–37.
- Noguchi Y. Social Support of the elderly: its concept and measurement (in Japanese: koureisha no sosharu sapohto: sono gainen to sokutei). *Ronen Shakai Gaku* 1991; **34**: 37–48.
- Nakamura Y, Miyata M, Ando T, et al. The latent form of transforming growth factor-beta administered orally is activated by gastric acid in mice. *J Nutr* 2009; **139**: 1463–8.
- Chang-Quan H, Xue-Mei Z, Bi-Rong D, Zhen-Chan L, Ji-Rong Y, Qing-Xiu L. Health status and risk for depression among the elderly: a meta-analysis of published literature. *Age Ageing* 2010; **39**: 23–30.
- Rigotti E, Piacentini GL, Ressa M, Pigozzi R, Boner AL, Peroni DG. Transforming growth factor-beta and interleukin-10 in breast milk and development of atopic diseases in infants. *Clin Exp Allergy* 2006; **36**: 614–8.
- Oddy WH, Halonen M, Martinez FD, et al. TGF-beta in human milk is associated with wheeze in infancy. *J Allergy Clin Immunol* 2003; **112**: 723–8.
- Nakao A. The role and potential use of oral transforming growth factor-beta in the prevention of infant allergy. *Clin Exp Allergy* 2010; **40**: 725–30.

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Conflict of interest

None.

Authors' contributions

NK had a primary role in study design, collection of data, analysis, and drafting of the manuscript. YS contributed to the conception and collection of data. AN and KO participated in the conception and drafting of the manuscript. KS, MS, TT, and AN contributed to the management of our epidemiological survey and collection of data. KI assayed milk samples. ZY supervised our survey and participated in drafting of the manuscript. All authors approved the final version of our manuscript to be published in *Pediatric Allergy and Immunology*.

23. Tomicic S, Johansson G, Voor T, Bjorksten B, Bottcher MF, Jenmalm MC. Breast Milk Cytokine and IgA Composition Differ in Estonian and Swedish Mothers-Relationship to Microbial Pressure and Infant Allergy. *Pediatric Research* 2010; **68**: 330–4.
24. Li Y, Xiao B, Qiu W, et al. Altered expression of CD4(+)CD25(+) regulatory T cells and its 5-HT(1a) receptor in patients with major depression disorder. *J Affect Disord* 2010; **124**: 68–75.
25. Kim YK, Na KS, Shin KH, Jung HY, Choi SH, Kim JB. Cytokine imbalance in the pathophysiology of major depressive disorder. *Prog Neuropsychopharmacol Biol Psychiatry* 2007; **31**: 1044–53.
26. Bennett AL, Chao CC, Hu S, et al. Elevation of bioactive transforming growth factor-beta in serum from patients with chronic fatigue syndrome. *J Clin Immunol*. 1997; **17**: 160–6.
27. Hawkes JS, Bryan DL, Gibson RA. Variations in transforming growth factor beta in human milk are not related to levels in plasma. *Cytokine* 2002; **17**: 182–6.
28. Prescott SL, Wickens K, Westcott L, et al. Supplementation with *Lactobacillus rhamnosus* or *Bifidobacterium lactis* probiotics in pregnancy increases cord blood interferon-gamma and breast milk transforming growth factor-beta and immunoglobulin A detection. *Clin Exp Allergy* 2008; **38**: 1606–14.
29. Bannerman DD, Paape MJ, Chockalingam A. *Staphylococcus aureus* intramammary infection elicits increased production of transforming growth factor-alpha, beta1, and beta2. *Vet Immunol Immunopathol* 2006; **112**: 309–15.
30. Hawkes JS, Bryan DL, Neumann MA, Makrides M, Gibson RA. Transforming growth factor beta in human milk does not change in response to modest intakes of docosahexaenoic acid. *Lipids* 2001; **36**: 1179–81.