

Breastfeeding promotes egg white sensitization in early infancy

To the Editor

A recent systematic review indicates that food sensitization in early childhood is a risk factor, not only for food allergies, but also for developing an allergic march which can lead to airway allergic diseases.¹ Over the past few decades, many groups have studied risk factors for allergic diseases, including sensitization. These risks include eczema, gut microbiome, timing of introduction of solid food, vitamin D, etc. Among these, eczema is thought to be one of the most important factors for food sensitization and developing food allergy.^{2,3} However, the prophylactic use of emollients could not prevent food allergen sensitization in infants even if it decreases eczema prevalence,⁴ indicating that other factors are involved. The WHO recommends exclusive breastfeeding for at least 6 months in all infants. However, the possible effects of breastfeeding on childhood allergic sensitization and allergic diseases are controversial.⁵ This study aimed to analyze the risk factors producing hen's egg sensitization at the age of 1 year, including feeding methods, in a high-risk birth cohort in Chiba, an urban area near Tokyo.

A birth cohort study of newborn infants with a family history of allergies was jointly arranged in the Seikei-kai Chiba Medical Center and the Chiba University Hospital from January 2010 to December 2011. The study protocols were approved by the Bioethics Review Committee, Faculty of Medicine, Chiba University, Japan (approval no. 754). Informed consent was obtained from each study subject's guardian. After the study subjects' births, we reconfirmed their

eligibility. We asked the guardians to take the study subjects to their designated clinic at 1 month, 6 months, and 1 year of age to take physical examinations. Other details of this study are shown in Appendix S1. The detailed schedule of this CHIBA study is shown in Figure S1.

In total, 306 pregnant women were recruited in the CHIBA study at 36 weeks of gestation. After providing information about the nature of the study to the pregnant women, 21 subjects were excluded at birth because of insufficient eligibility. We confirmed that the remaining 285 babies met the eligibility criteria, and all the babies were enrolled in the CHIBA study. Of these subjects, 18 were excluded by 1 year of age. The study flowchart is shown in Figure 1.

Positive sensitization rates of egg white (EW), cow's milk, *Dermatophagoides farinae*, cat dander, and Japanese cedar pollen were 33.2%, 13.1%, 6.7%, 3.0%, and 0%, respectively. The sensitization rate to EW was the highest of allergens analyzed.

We compared subjects who were sensitized to EW to those who were not. Those infants who were male, had a high birthweight, had a high gestational age, were breastfed by 4 months of age, had a presence of eczema by 6 months of age, and had maternal history of allergic diseases had significantly higher positive rates of sensitization to EW as detailed in Table S1. In the framework of our multivariate analysis, we found that the following variables were significantly related to EW sensitization: presence of eczema by 6 months of age (OR, 5.59; $P < .0001$), breastfed infants (this included those in the

FIGURE 1 Flowchart of study participants showing until 1 y of age

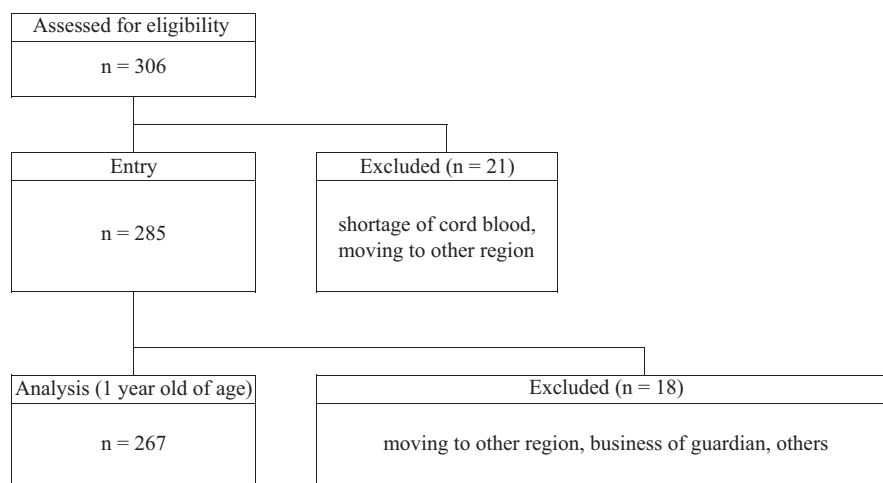


TABLE 1 Factors related to egg white (EW) sensitization (multivariate analysis)

	OR	95% CI	P value
Eczema by 6 mo	5.59	2.88-10.83	<.0001
Breast feeding	2.64	1.45-4.81	.001
Male	2.04	1.12-3.69	.01
Maternal allergic disease	2.68	1.01-7.11	.03

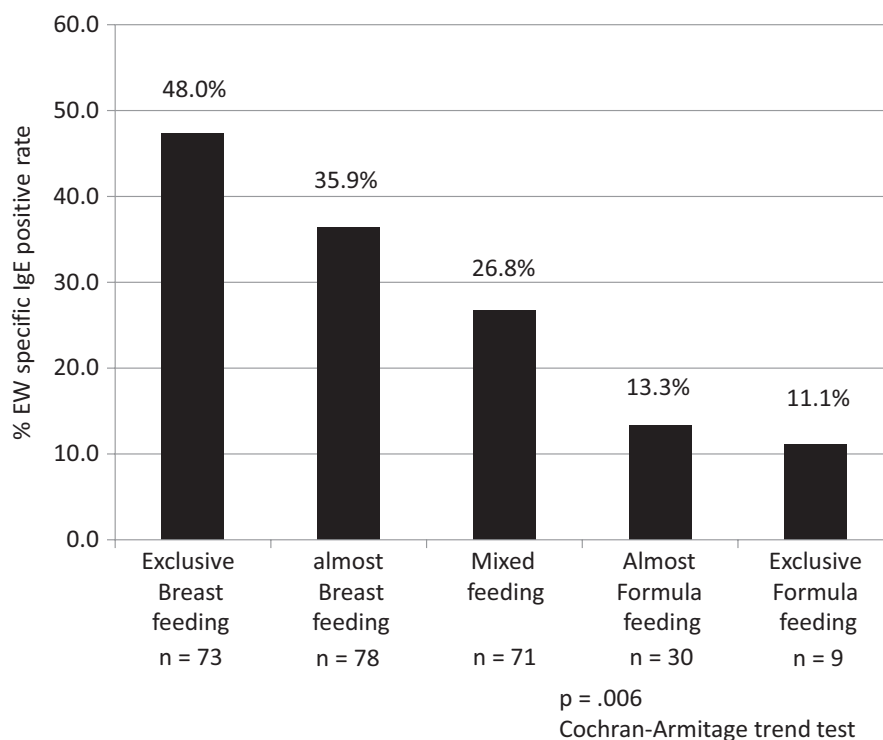
Note: Explanatory variable: eczema by 6 mo, breastfeeding, male, maternal allergic disease, gestational age, birthweight. Logistic regression analysis was used for multivariate analysis. We classified breastfeeding as "exclusively breast feeding group" and "almost breast feeding group."

exclusively breastfeeding group and also those in the almost all breastfeeding group; OR, 2.64; $P = .001$), male gender (OR, 2.04; $P = .01$), and maternal history of allergic diseases (OR, 2.68; $P = .03$), as shown in Table 1. The Hosmer-Lemeshow test was used to examine goodness of fit of the models ($P = .6669$). In addition, we analyzed the odds ratios of breastfeeding for egg white sensitization in babies with and without eczema. In babies with eczema, the odds ratio of breastfeeding for egg white sensitization was 5.44 (95% CI: 1.73-17.17). In babies without eczema, the odds ratio of breastfeeding for egg white sensitization was 1.96 (95% CI: 1.01-3.78). These results suggest that breastfeeding is an independent risk factor for egg white sensitization regardless of eczema. Furthermore, sensitization rates of EW were significantly correlated with feeding method. Infants exclusively fed breastmilk were the most sensitized to EW, and those exclusively fed formula milk were the least sensitized to EW, as illustrated in Figure 2.

To check whether a high positive rate of EW sensitization in the breastfed group was due to the amount of EW protein in breastmilk, we measured the amount of EW in the breastmilk when the infants were 1 month of age. This was found to be 9 ng/mL (interquartile range: 9, 20.5) in the EW non-sensitized babies group; remarkably, it was also 9 ng/mL (interquartile range: 9, 9) in the EW-sensitized babies group (Figure S2). There was no relation between the amount of EW in the mothers' breastmilk and EW sensitization at 1 year of age.

In the present study, our multivariate analysis indicated that not only eczema by 6 months of age, but also breastfeeding by 4 months of age was a significant independent risk factor for EW sensitization at 1 year of age. We also found a clear dose dependency between breastfeeding and EW sensitization. These results strongly indicate that breastfeeding is a risk factor for EW sensitization. Although the effect of breastfeeding on childhood allergic sensitization and atopic diseases remain controversial,⁵ our present findings are in accordance with those of a previous birth cohort study in Japan.⁶ Some studies conducted in countries other than Japan have also reported that breastfed infants had a high rate of sensitization to EW compared with infants who were fed formula milk.⁷

We hypothesized that egg white in breastmilk may enhance sensitization through eczematous skin with disrupted barrier function and that the amount of EW in breastmilk would be important in sensitization. Although we only checked the EW in the breastmilk at one point, its concentration in breastmilk was not related to EW sensitization. Therefore, it seems that some factor(s) other than the amount of EW in the breastmilk may be responsible for EW sensitization in infancy. Breastmilk contains many immunologically active substances, including a wide variety of cytokines, inflammatory mediators, signaling molecules, and soluble receptors.⁵ These immune components in

**FIGURE 2** Relationship between egg white (EW) sensitization and method of feeding by 4 mo of age. Barplots showing the percentages of EW specific IgE-positive rates. Cochran-Armitage trend test was used for analysis

breastmilk might be important for sensitization. In addition, breastmilk contains only minimal amounts of vitamin D, which is thought to have a protective effect against allergic diseases.⁸

There are some limitations in our study. Since we used a high-risk cohort, it is not clear that we can generalize our results to the normal population. However, we think that it is important to prevent allergic diseases from affecting high-risk groups. As mentioned above, we could not determine the exact amount of EW intake from infant foods to check the effect of early intake of hen's egg. However, the timing of the introduction of infant foods did not affect the EW sensitization in the present study (data not shown).

In conclusion, although the effects of breastmilk on allergic diseases remain controversial, our results showed that breastfeeding is a risk factor for egg white sensitization. Immune components in the breastmilk might affect the sensitization at 1 year of age. However, the mechanism through which breastmilk induces allergic sensitization is currently unknown. We are planning to investigate the breastmilk components that might affect allergic sensitization.

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CONFLICT OF INTERESTS

None related to this work.

AUTHOR CONTRIBUTIONS

TN analyzed data and wrote the manuscript. KN did statistical analysis and wrote the manuscript. SO, SS, FY, YM, YI, TA, HK, and HS examined children and collected the data and biospecimens. NM measured and analyzed egg white antigen in the breastmilk. AH, MS, YS, MT, TT, YK, and NS designed the study. NS coordinated the cohort study. All authors contributed extensively to the work presented in this paper.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

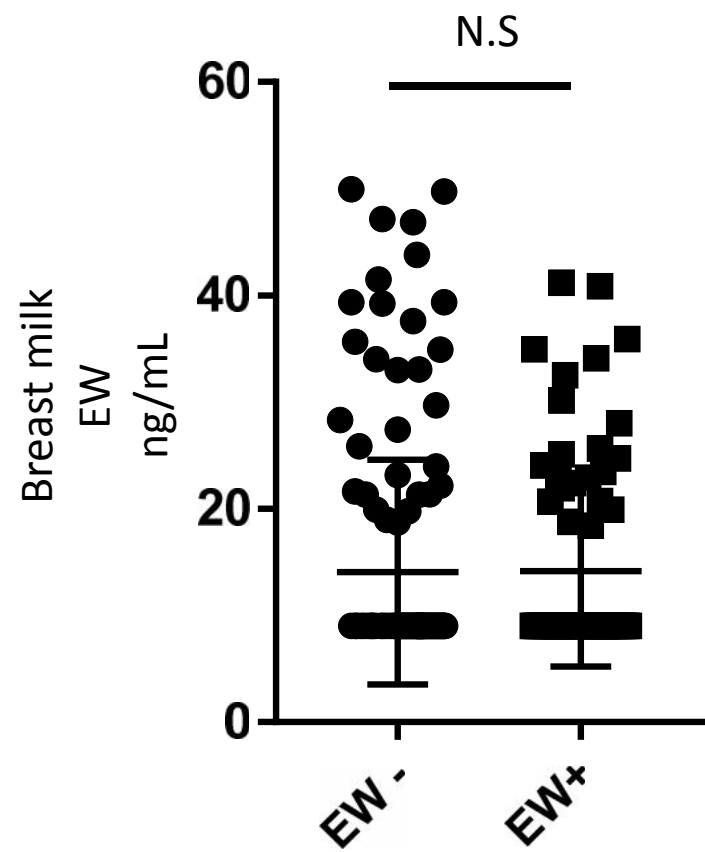


Table S1. Comparison of EW negative group and EW positive group.

Chi-square and Mann–Whitney tests were used for univariate analysis

We classified breastfeeding as “exclusively breastfeeding group” and “almost breastfeeding group.”

	EW negative	EW positive	p value
Sample size	n=178	n=89	
Male (%)	44.9%	58.4%	0.038
Gestational age (week) (mean $\pm$ SD)	38.8 $\pm$ 1.4	39.1 $\pm$ 1.4	0.033
Birth weight (g) (mean $\pm$ SD)	2987.7 $\pm$ 358.9	3102.25 $\pm$ 355.3	0.017
Maternal allergic diseases (%)	83.2%	92.1%	0.037
Paternal allergic diseases (%)	70.2	80.9	0.07
Cesarean section (%)	30.9%	26.97%	0.51
Maternal IgE (mean $\pm$ SD)	183.3 $\pm$ 539.7	265.8 $\pm$ 436.5	0.0032
Breast milk feeding before 4 months	49.4%	70.8%	0.0009
Eczema by 6 months of age	12.4%	41.6%	<0.0001
Introduction of baby food before 5 months	64.0%	60.7%	0.59

Online Supplementary

Methods

The Chiba High-risk Birth cohort for Allergy (CHIBA) study

A birth cohort study of newborn infants with a family history of allergies was jointly arranged in the Seikei-kai Chiba Medical Center and the Chiba University Hospital in Chiba, one of the prefectures near the Tokyo capital region, from January 2010 to December 2011. The study protocols were approved by the Bioethics Review Committee, Faculty of Medicine, Chiba University, Japan (approval no. 754). Scheduled clinical visits to each hospital were planned at one month, six months, one year of age, and have now been extended up to seven years of age. The detailed schedule of this CHIBA study is shown in supplemental Figure S1.

Participants

The eligibility criteria for the CHIBA study were as follows: (1) Japanese ethnicity, (2) mother, father, or siblings currently or previously had any allergic diseases, and (3) not having (or expecting) any severe congenital abnormalities such as congenital heart diseases. We used a poster to invite all pregnant women registered at the participating hospitals who were at 34 weeks gestation if they, their partners, and/or their children had any allergic diseases, such as atopic dermatitis (AD), asthma, allergic rhinitis, and/or food allergies. Subject to the above three exclusion criteria, we enrolled their expected babies into our study. Informed consent was obtained from each study subject's guardian. After the study subjects' births, we reconfirmed their eligibility. Children with any severe congenital or perinatal abnormalities were excluded from the study. We asked the guardians to take the study subjects to their designated clinic at one month, six months, and one year of age to take physical examinations.

Questionnaire

Perinatal information, such as gestational age at birth, birth weight, mode of delivery, and APGAR scores, was obtained from the mothers' medical records. At three to five days after delivery, and during their stay in the hospital, questionnaires were given to the mothers, addressing the number of siblings, family history of allergy, use of antibiotics or tobacco products, exposure to pets such as dogs or cats, and dietary habits during pregnancy. At one month, six months, and one year of age, the guardians of the study subjects reported on their children's health (allergic diseases and symptoms/diagnoses), feeding methods, timing of starting baby foods, exposure to second-hand tobacco smoke at home, and exposure to pets such as dogs or cats.

Feeding pattern

Mothers were asked about their feeding methods by four months of age via a questionnaire. We divided children into the following five groups: exclusively breastfeeding group, infants who had never given any formula milk; almost breastfeeding group, infants who had given formula only for few times; mixed feeding group, infants who had often given formula milk; almost formula milk feeding group, infants who had given breast milk only for few times; exclusively formula milk feeding group, infants who had never given breast milk. For analysis, we defined breastfeeding as "exclusively breastfeeding group" and "almost breastfeeding group."

Physical examinations

At one month and six months of age, physical examination was performed by pediatric allergists at Chiba University Hospital or by pediatricians at Seikei-kai Chiba Medical Center. The subject was diagnosed with eczema at six months s/he had a history of itchy skin rash chronically relapsing for more than two months.

At one year of age, all subjects were examined by pediatric allergists at Chiba University Hospital, and any diagnoses of AD were made using AD management guidelines published by

the Japanese Dermatological Association.

Blood sampling and measurement of total and allergen-specific IgE

Blood samples were collected from mothers at 36 weeks of gestation and infants at one year of age. The samples were stored at -30°C within 24 h until use for measuring total IgE and/or allergen-specific IgEs. We used the ImmunoCAP® system to analyze the specific IgE levels of egg white, cow's milk, *Dermatophagoides farinae*, cat dander and Japanese cedar pollen in the children's sera at one year of age. A specific IgE level of $0.7 \text{ U}_A/\text{mL}$ or greater was considered positive.

Measurement of hen's egg protein in breast milk

Breast milk samples were collected from the mothers when their children were one month of age. These samples were stored at -80°C within 24 h until they were used to measure EW. Levels of hen's egg protein in breast milk were determined using a commercially available ELISA (FASTKIT ELISA ver.III® hen's egg; Nihon ham, Tsukuba, Japan) coated with antibodies specific to the major allergens of hen's egg. The range of quantification was $0.78\text{--}50 \text{ ng/mL}$.

Statistical analysis

The chi-square and Mann–Whitney tests were used for univariate analysis. Logistic regression analysis was used for multivariate analysis, in which we used as explanatory variables: eczema by six months, breastfeeding, male gender, maternal allergic disease, gestational age, and birth weight. Continuous variables were expressed as medians (interquartile range). The software package JMP pro 13.0. was used for statistical analysis.